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Original article

Investigation of the Synthesis and Biological Activity of A New Imine Compound Containing Ferrocene and Benzothiazol

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Abstract

In this study, a new Schiff base containing ferrocene and benzothiazol was synthesized from the reaction of ferrocene-2-carboxialdehyde and 6-methoxybenzo[d]thiazole-2-amine. The structure of the synthesized original imine compound 2-((6-methoxybenzo[*d*]thiazol-2-ylimino)methyl) ferrocene was illuminated by UV-VIS, FTIR, MS and NMR spectroscopy. The antimicrobial activity of the compound was determined by the minimum inhibition concentration (MIC) method against various bacterial and yeast cultures, and the antioxidant activity was determined by the free radical scavenging activity of 2,2-diphenyl-1-picrilhydrazyl (DPPH). DNA cleavage activity of Schiff base was investigated hydrolytically and oxidatively by agarose gel electrophoresis method, and binding to DNA was investigated by UV-Visible field spectroscopy method. It was found that the studied ferrocene and benzothiazole imine compound had more effect on *Pseudomonas aeruginosa* (ATCC 27853) and *Bacillus subtilis* (ATCC 6633) bacteria. The compound was found to exhibit good antioxidant activity compared to the standardized butylated hydroxy toluene (BHT). Agarose gel electrophoresis studies showed that the compound cleaved DNA without any external agent, and UV-Vis spectroscopy studies showed that it interacted electrostatically with CT-DNA.

Keywords: Ferrocene-2-carboxyaldehyde, Benzothiazol, Schiff base, Antimicrobial activity, Antioxidant activity, DNA cleavage, DNA binding.

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INTRODUCTION

Imine compounds (Schiff bases) are recently intensely studied compounds due to their easy synthesis and interesting chemical and biological properties. Schiff bases are known to have a wide range of biological activities such as antibacterial, antifungal, antiviral, antioxidant, anticancer, and antibiofilm (Yıldız et al., 2015; Zeyrek et al., 2018; Sykula et al., 2019; Azam et al., 2019; Patil et al., 2018).

Schiff bases were synthesized by German chemist H. Schiff in 1864 and are products formed by primary amines with ketones and aldehyde (Schiff 1869). Chelation with trace elements are important features of Schiff bases for their activities in biological systems. Depending on this, they have very broad pharmacological activity (Sahu et al., 2012). Schiff bases play a role in the oxidation of important molecules such as ascorbic acid, catechol, amino acids, free oxygen, etc. (Niederhoffer et al., 1984).

Ferrocene is a neutral, stable and non-toxic aromatic molecule. Due to the Fe (II) ions it contains, it is very rich in electrons and can easily react. Ferrocene derivatives were also demonstrated to show pharmacologically similar activities to Schiff bases in studies (Meunier et al., 1991; Itoh et al., 2000; Biot et al., 2000).

Infectious diseases, especially involving some bacteria, are more resistant to antibiotics and increasing this effect seriously affects human health. Antimicrobial agents are very important for reducing the burden of infectious diseases. Today, the need for new antimicrobial drugs is increasing day by day. Many studies have reported that Schiff bases show antimicrobial activity (Yıldız et al., 2015; Ünver et al., 2018).

Research into some metal-derived antioxidants has shown the need to identify high-capacity compounds to ameliorate diseases associated with oxidative damage caused by free radicals and reactive oxygen species (ROS). The antioxidant activity of a number of Schiff base metal complexes was investigated to effectively resolve oxidative damage today. Therefore, it is thought that the new Schiff bases to be synthesized will have many biological activities and may also be effective for cancer and microbial diseases.

Cancer is an important public health problem that ranks second among the known causes of death both around the world and in our country (Global Burden of Disease Cancer Collaboration, 2015). For this reason, scientists have conducted extensive studies on the identification or synthesis of new drug molecules that will be effective against cancer and also not harm human health (Göçmen, 2014). Ferrocene derivatives have gained great importance today in studies about the design and biological activities of new drug types. The use of ferrocene derivatives in the structures of various anticancer drugs has shown that this compound is effective in the structure of these drugs (Peter & Aderibigbe, 2019).

Researching and designing new molecules that can interact with DNA is one of the most promising ways to discover anticancer drugs that target DNA and can be used in chemotherapy (Quiao et al., 2011). Schiff bases are also one of the chemicals on which are studied due to their biological and structural importance (Birbiçer, 1998). Nucleic acids are important targets for small molecule therapeutics because they play an important role in critical cellular events such as protein synthesis and cell division (Wilson, 1996). DNA has become the primary target for many therapeutic agents, from anticancer drugs to antibiotics (Sobha et al., 2012). Compounds targeting DNA have an important theoretical and practical value in biology, chemistry and medicine (Li et al., 2011).

Schiff bases and ferrocenes are widely studied due to their interesting biological and chemical properties. Therefore, the main purpose of the study is to test the idea that the newly synthesized ferrocene-thiazole imine compound has both ferrocene and imine properties, which may have the potential to be used as a more effective biological agent. For this purpose, the ferrocene-imine compound was characterized after synthesis (Figure 1). Then, some biological activities of the compound were investigated by various methods.

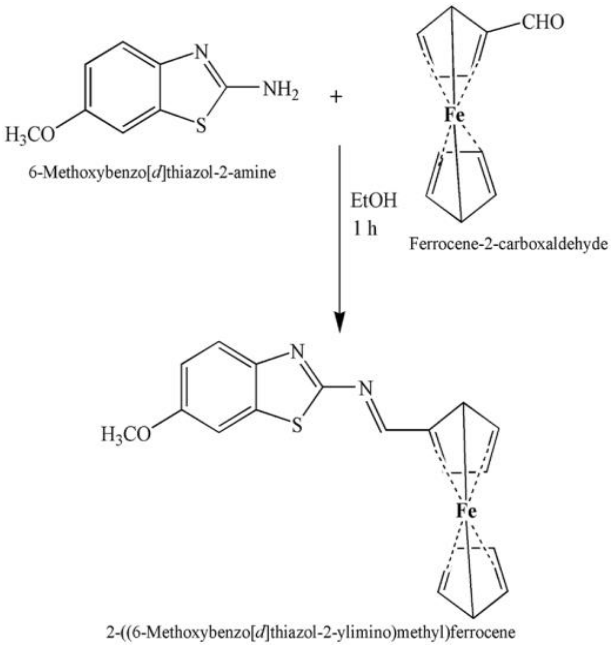


Figure 1. Synthesis of Schiff base.

MATERIALS and METHODS

Materials

The melting point of the compound was determined with Electrothermal IA 9100 melting point device using capillary tube. Infrared (IR) absorption spectra were obtained from a Perkin Elmer BX II FT model spectrometer in KBr discs and were reported in cm^{-1} units. The ^{1}H- and ^{13}C-NMR spectra

were recorded on a Bruker DPX FT-NMR spectrometer operating at 400 and 101.6 MHz, and. UV-VIS spectrum was taken on T+80 UV/Vis Jerrum 2009 spectrophotometer. Ferrocene-2-carboxaldehyde (98%), 6-methoxy-2-aminobenzothiazole (98%), DMSO (99%) and ethanol (99%) (Merck, Germany). All chemicals were used as received.

Methods

Synthesis of 2-((6-methoxybenzo[*d*]thiazole-2-ylimino)methyl)ferrocene

6-Methoxybenzo[*d*]thiazole-2-amine (0.20 g; $1.1x10^{-3}$ mol) and ferrocene-2-carboxaldehyde (0.24 g; $1.1x10^{-2}$ mol) were placed in a single-mouth flask. EtOH (50 mL) was then added to the flask. The mixture was boiled under reflux for 1 hour. The hot solution was filtered and the filtrate was crystallized. Red crystals, m.p. 135 ° C, yield 0.35 g (83%). IR (KBr, cm^{-1}) νCp-H; 3382 m, νAr-H; 3087 m, νC-H; 2937-2835-2744 w, νC = N; 1642 s, νC = C; 1602-1544 s, νC-N; 1464 m, νC-O-Ar; 1276-1261-1206-1178, νCpC = C 1105-825 s. Mass Spectrum (MS, m / z) 377, 376, 362, 353, 352, 339, 338, 337, 244, 228.

Antimicrobial Activity

To investigate the antimicrobial effects of the ferrocene-imine compound, Gram (-) bacteria *Escherichia coli* ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus vulgaris* (ATCC 13315); Gram (+) bacteria *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Bacillus subtilis* (ATCC 6633), and yeast *Candida albicans* (ATCC 60193) were used. Antimicrobial activity was studied with the broth microdilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2006). It determines the minimum inhibitory concentration (MIC) value where the antimicrobial effects of the compounds are observed.

Bacterial cultures were activated in Mueller-Hinton Agar (MHA) at 37 °C for 24 hours, and yeasts in Sabouraud Dextrose Agar (SDA) at 37 °C for 48 hours. After incubation, serial dilutions were made by adding compounds to 96-well "U" based microplates with 100 µL of Mueller Hinton Broth (MHB) and RPMI medium, respectively. The cultures were incubated for 18-24 hours at 35-37 ºC for bacteria and 48 hours at 28-30 ºC for yeast and the lowest concentration without visible growth was recorded as MIC. The experiment was performed in triplicate. Standard antibiotics gentamicin and ampicillin were used as antibacterials, and fluconazole was used as positive controls to determine antifungal activity.

Free Radical Scavenging Activity (DPPH Test)

Free radical scavenging activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical by Brand-Williams et al. (1995) method. In this method, the DPPH molecule is reduced by giving H^+ to the antioxidant molecules in the environment and causes a decrease in absorbance. This situation eliminates the harmful effects of free radicals circulating in the body and delays oxidative damage

(Pisoschi & Pop, 2015). The lower the absorbance value, the greater the free radical scavenging activity of the tested substance.

Different concentrations of the compound were created with ethanol in cuvettes so that the total volume was 2 mL. After adding 0.5 mL of the DPPH solution in ethanol to them, they were incubated at room temperature in the dark for 30 min. The absorbance of the samples at 517 nm was measured with a spectrophotometer against the blank. Butyl hydroxytoluene (BHT) solution used as standard instead of the compound as positive control and solvent (ethanol) used as blank. The experiment was repeated three times and the arithmetic mean of the values was taken.

The radical scavenging activity of each sample was calculated using the equation below, the results were determined as % inhibition.

Inhibition (%) = $[(A_0 - A_1) / A_0] \times 100$

A_0: Compound or standard-free control absorbance; A_1: Compound or standard absorbance.

DNA Cleavage Activity (Agarose Gel Electrophoresis Method)

When the original supercoiled form (Form I) of the pBR322 plasmid DNA is opened with damage, the open circular form (Form II) occurs, and with more fractures, the linear form (Form III) can also be found. When DNA motion in gel electrophoresis, Form I progresses faster in gel than others, while Form II moves slower and Form III moves between Form I and Form II.

In the study, the hydrolytic and oxidative activities of the compound were examined. For hydrolytic activity, plasmid pBR322 DNA was prepared in Tris-HCl buffer (10mM, pH: 7.2) by treatment with compounds; for oxidative activity, hydrogen peroxide (H_2O_2), an inducing agent, was added in addition to the DNA, buffer and compound mixture. After the prepared samples were incubated at 37 ^oC for 3 hours, 4 µL of 6X loading dye (0.25% bromphenol blue, 0.25% xylene cyanol, 30% glycerol, 10mmol EDTA) was added in 1% agarose gel. The gel was run in electrophoresis in 1X TAE buffer (40mM Tris-20 mM acetic acid, 1mM EDTA pH: 8.2) for 1 hour at 60 V. Each experimental condition was tested in triplicate. Later, the bands were obtained and photographed with a gel imaging system under UV light (Quantum ST4 gel imaging system, Vilbar Lourmat) (Qiao et al., 2011).

DNA Binding Activity (UV-VIS Absorption Spectroscopy Method)

DNA binding is an important step in the activity of a compound with DNA. The UV-Vis absorption titration technique was used to determine the DNA binding properties of the compounds (Jenkins, 1997). The basis of this technique is the gradual addition of DNA to it, keeping the amount of compound constant. In this way, whether the compound binds to DNA is determined by observing the change in absorption in the absence and presence of DNA.

Calf thymus-DNA (CT-DNA) concentrations were prepared by dilution in TNE (8 mM Tris-HCl, 50 mM NaCI and 1 mM EDTA, pH: 7.4) buffer. In the study, the concentration of CT-DNA was gradually increased by increasing the concentration of the compound and incubated for 5 minutes at room temperature, after which measurements were taken at 200-600 nm with a UV-Vis spectrophotometer (T + 80 UV/Vis spectrophotometer) (Jerrum, 2009).

RESULTS and DISCUSSION

Spectral Comments

Mass Spectrum

The mass spectrum of the compound was recorded at 240 ºC on the Agilent 1100 MSD device by Electron Impact (LC/MS) technique. For the compound, the M + 1 peak was observed at 377 and the molecular ion peak at 339 was 100% (Figure 2).

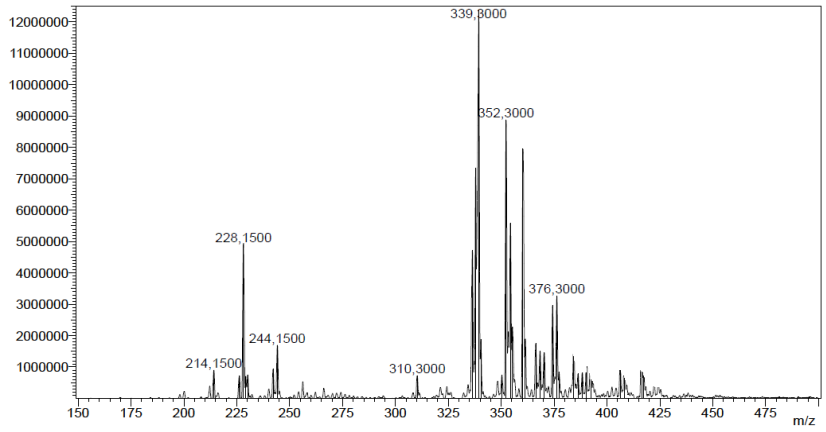


Figure 2. Mass spectrum of the compound.

FTIR Spectrum

The FTIR (KBr disk) spectrum was recorded on the Perkin Elmer BX II FT model spectrometer with a range of 16 scans at a resolution of 1 cm^{-1} at a range of 4000-400 cm^{-1} (Figure 3). Cp-H, Ar-H, C-H, C = N, C = C and CpC = C vibrations were observed in the compound. FT-IR values for the compound are given in the experimental section. The observation of C = N vibration on the Schiff basis at 1642 cm^{-1} shows that the ferrocene group attracts electrons very strongly from the amine group and the double bond character of the imine bond is stronger. In addition, the band at 3385 cm^{-1} corresponds to C-H vibrations in the cyclopentadiene ring, C = C groups in the cyclopentadiene ring in strong bands at 1105 cm^{-1} and 825 cm^{-1}.

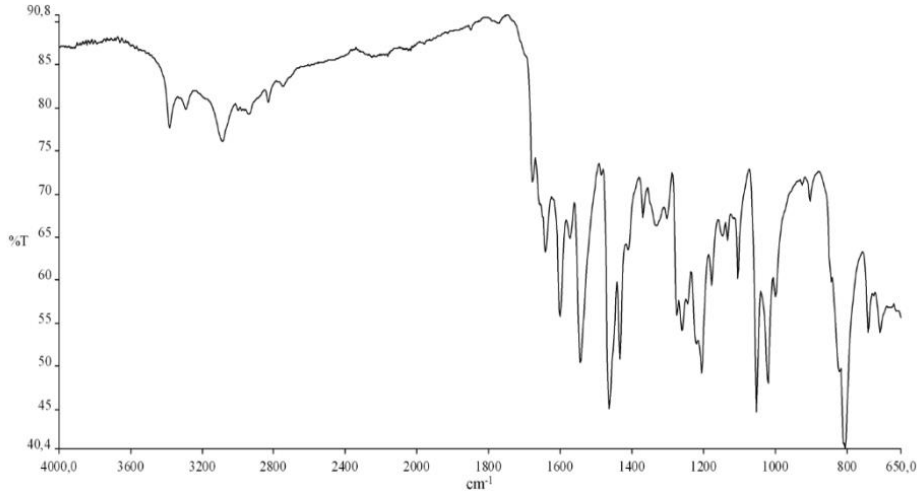


Figure 3. FTIR spectrum of the compound.

[1]H-NMR Spectrum

The ^{1}H-NMR spectrum (400 MHz, DMSO-d_6) was recorded by Bruker DPX FT NMR spectrometer. The -C<u>H</u>=N- proton are observed at 10.01 ppm for the compound. The aramatic protons of the compound gave multiplets at 6.92-7.35 ppm. Cyclopentadienyl protons were observed as broad peak at 4.43 ppm. The methoxy OCH_3 proton was found as single at δ = 3.46 ppm (Figure 4).

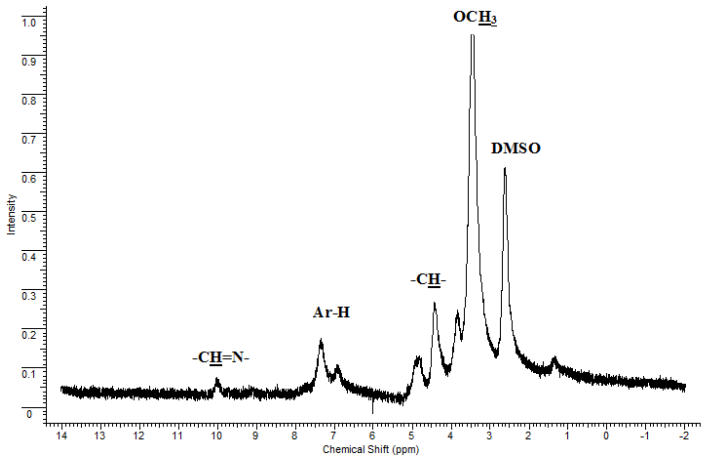


Figure 4. ^{1}H-NMR spectrum of the compound.

[13]C-NMR Spectrum

According to the ^{13}C-NMR spectra, the compound has 17 signals. These peaks are compatible with the structure of the compound. The 6 and 7 and 8 and 10 carbons in the compound are equivalent carbons. The ^{13}C-NMR spectrum with the numbers of the carbon atoms in the compound is given Figure 5. The imine carbons Ar-CH=N-Ar and Ar-N=C(S)-N in the compound were observed at 166.39 ppm and 191.51 ppm as C11 and C12. Other carbons in the compound are (δppm) 165.15 (s, 1C, C16), 163.51 (s, 1, C13), 154.70 (s, 1C, C18), 147.26 (s, 2C, C8 and 10), 132.62 (s, 1C, C3), 123.46 (s, 2C, C6 and

7), 118.50 (s, 1C, C4), 1116.63 (s, 1C, C14), 113.81 (s, 1C, C5), 108.54 (s, 1C, C1), 107.89 (s, 1C, C15), 105.95 (s, 1C, C17), 101.19 (s, 1C, C19), 56.18 (s, 1C, C9) and 55.97 (s, 1C, C2), respectively (Figure 5).

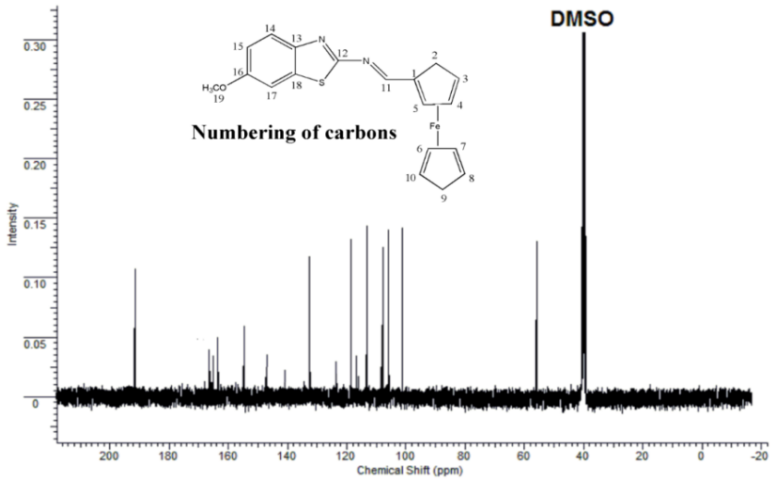


Figure 5. ^{13}C-NMR spectrum of the compound.

Biological Activities

Antimicrobial Activity

MIC values (µg/mL) were determined for the ferrocene-imine compound for antimicrobial activity. The compound was found to be effective on different bacteria and yeast culture *C. albicans* (ATCC 60193). It was shown to be more effective on *P. aeruginosa* (ATCC 27853) and *B. subtilis* (ATCC 6633) bacteria (Table 1).

Table 1. MIC values of compound (µg/mL).

Microorganisms	**Compound**	**Antibiotics**		
		Gentamicin	**Ampisilin**	**Fluconazole**
E. coli ATCC 25922	1	0.125	0	-
P. aeruginosa ATCC 27853	0,0078	0.08	2	-
P. vulgaris ATCC 13315	1	0.125	0.06	-
S. aureus ATCC 25923	0,25	1	0.016	-
E. faecalis ATCC 29212	0,5	1	0.016	-
B. subtilis ATCC 6633	0,078	0.008	0.06	-
C. albicans ATCC 60193	0,5	-	-	0.063

Free Radical Scavenging Activity (DPPH)

In the study, antioxidant activity was calculated using six different concentrations (10, 20, 40, 60, 80 and 100 µg/mL) of the compound by measuring the absorbance values at 517 nm with the method of free radical scavenging activity (DPPH). The antioxidant activities of 55.8, 57.4, 59.27, 59.94, 64.26

and 61.89% are obtained at the compound concentrations of 10, 20, 40, 60, 80 and 100 µg/mL, respectively (Figure 6). The compound shown an antioxidant effect almost same activity observed between the lowest and highest concentrations (from 10 to 100 µg/mL).

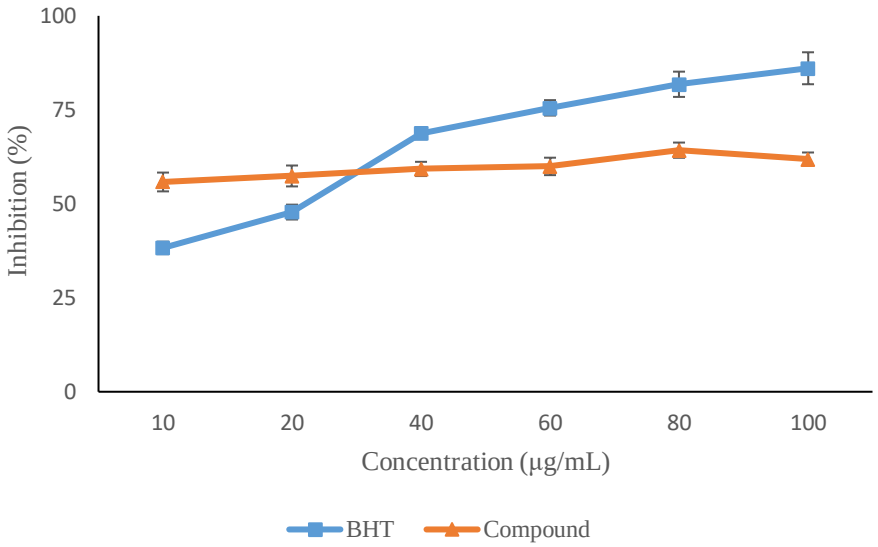


Figure 6. Free radical scavenging activity of the compound.

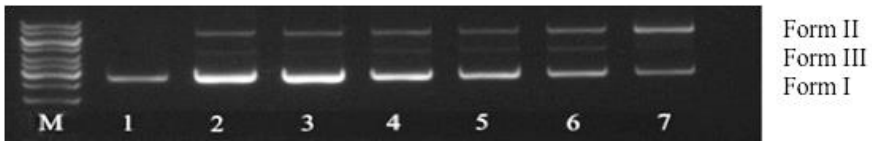
DNA Interactions

DNA Cleavage Activity of the Compound

Six different concentrations (31.25, 62.5, 125, 250, 500 and 1000 µM) of the ferrocene-imine compound were prepared and loaded into the agarose gel device to determine the DNA cleavage activity. Hydrolytic and oxidative cleavage results were examined from images of bands on agarose gel.

Hydrolytically, the compound was found to cleave pBR322 plasmid DNA at a concentration of 62.5 µM (Figure 7A). In the presence of H_2O_2 as the oxidizer, the compound cleaved the DNA at a lower concentration of 31.25 µM, forming a double fracture (Form III and Form II) and after concentration of 250 µM, compound destroyed the supercoiled structure (Form I) and at 1000 µM it was observed to completely denature the DNA (Figure 7B).

A: Hydrolytic



B: Oxidative

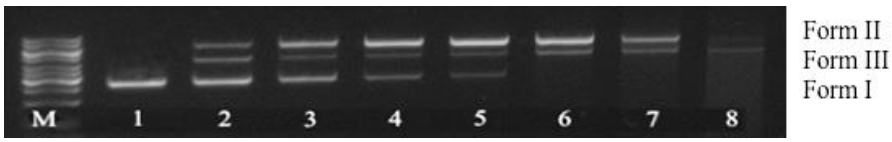


Figure 7. M: Marker, 1. Plasmid DNA, A: 2-7. DNA + compound (31.25, 62.5, 125, 250, 500, 1000 µM, respectively); B: 2. DNA + H_2O_2, 3-8. DNA + H_2O_2 + compound (31.25, 62.5, 125, 250, 500, 1000 µM, respectively).

DNA Binding Activity

The interaction of ferrocene-imine compound with CT-DNA was investigated by the UV-Vis absorption spectroscopy method. In order to determine the changes after the interaction between the compound and DNA, the differences between the free molecule and the maximum absorbance of the molecule attached to DNA were compared. Binding of the compound to DNA causes changes in its spectroscopic properties. These changes are indicated as a decrease or increase in absorption (hypochromic and hyperchromic) and higher wavelength shift bathochromic (red shift) or lower wavelength shift hypochromic (blue shift).

When the UV-Vis spectrum of the compound was examined, it was observed that the change in absorbance intensity was increased in the direction of hyperchromism with increasing CT-DNA concentration added gradually. The compound showed 8-370% hyperchromism and 1-3 nm hypochromic shift (blue) in absorption at 258 nm (Figure 8). As a result of the data obtained, it was determined that the compound interacted electrostatically with CT-DNA.

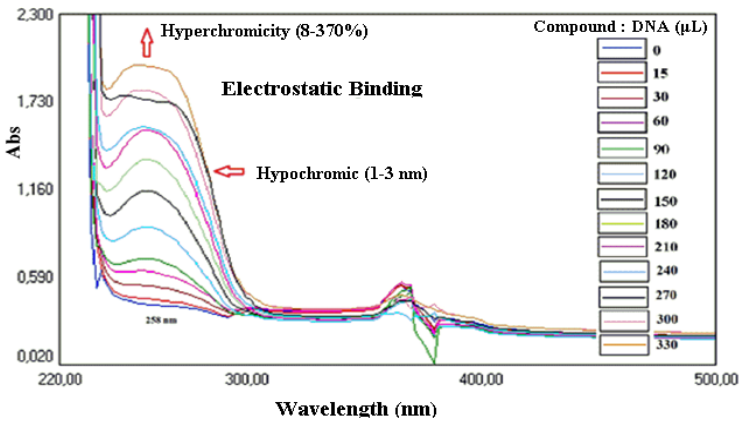


Figure 8. UV-Vis titration spectrum of compound.

Conclusion

The synthesis of new compounds with improved pharmacological properties has been the purpose of scientific research in recent years. Schiff bases are compounds with biological importance and are used as ligands (Özşahin & Bozhan, 2018).

The specific binding properties of Schiff bases and their different applications in cancer therapy were reported and these compounds are suitable candidates for antimicrobial, artificial nuclease, DNA probe and antitumor drugs (Kiran et al., 2015). Therefore, it is very important to investigate the interaction of molecules with DNA to develop effective anticancer and antibacterial drugs with effective chemotherapeutic agents.

The broad spectrum of antimicrobial drugs is one of the most important features. In this study, when the antibacterial activity results of the original ferrocene-imine compound are analyzed, it is more effective against Gram (+) bacteria than Gram (-) bacteria.

Living organisms have antioxidant systems against oxidative damage, but they are often insufficient to prevent this damage (Simic, 1988). In this regard, natural or synthetic antioxidant substances are needed to protect cellular DNA from this damage. The compound's free radical scavenging activity results had good antioxidant properties.

DNA cleavage studies are important for the development of chemotherapeutic agents and antimicrobial drugs (Addison et al., 1988). While hydrolytic cleavage occurs in the phosphodiester bonds of DNA, oxidative cleavage activity occurs in sugar or nucleobases. When the DNA shear activity was examined, the compound was found to cleave the plasmid DNA hydrolytically without any agent. This shows that it has a future use as a therapeutic agent.

Understanding the interactions between the drug and drug molecules with DNA is important for the acquisition of new and effective drugs. DNA is the intracellular target of anticancer drugs because it carries genetic information (Ganji et al., 2018). Since the compounds containing the ferrocene group have antitumoral activity, they are promising for the treatment of diseases such as cancer (Razafimahefa et al., 2005). Electrostatic interactions cover most of the interactions with groups on the outer surface of DNA. An electrical interaction occurs between the negatively-charged phosphate groups on the outer surface of the DNA and the positively-charged compound in the medium. As a general rule, there should be an electrostatic component in the molecular structure for the design of a molecule that can strongly bound to DNA (Anastassopoulou, 2003; Strekowski & Wilson, 2007). At the end of DNA binding studies, it was determined that the compound was electrostatically bound to CT-DNA, which is weaker than other types of binding but is frequently preferred in drug design.

Considering the results obtained from the ferrocene-imine compound used in our study, these and similar compounds that can cleave DNA from certain regions, or bind to DNA, and which can be good antioxidants, will make great contributions to the design and development of new drugs in the future.

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