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Research Article

INVESTIGATION OF BIOLOGICAL ACTIVITIES OF 4-HYDROXY-3-(2-HYDROXY-5-METHYLBENZYLIDEAMINO) BENZENESULPHONIC ACID

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ABSTRACT

In this study, the antimicrobial activity, DNA cleavage, DNA binding and antioxidant properties of a sulfonic acid-based imine compound were investigated. The antimicrobial activity of the compound was investigated for minimum inhibitory concentration (MIC) against some bacteria and yeast cultures. The DNA cleavage activity of the compound was investigated as hydrolytic and oxidative with the gel electrophoresis method. H2O2 was used as an oxidizing agent for detection of the cleavage activity mechanism. The Ultraviolet-Visible (UV-Vis) field absorption spectroscopy method was used to determine the binding effect to DNA. The sulfonic acid-based imine compound reacted with Calf Thymus DNA (CT-DNA) which was examined by UV-Vis absorption spectroscopy. The free radical scavenging activity was determined using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method.

The studied compound was found to be effective on yeast and bacteria at different concentrations. The compound was found to be more effective on Staphylococcus aureus ATCC 25923 and Enterococcus faecalis ATCC 29212 bacteria. DNA cleavage study showed that the compound cleaved DNA without any external agents in hydrolytic and oxidative manner. UV-Vis spectroscopy studies of the interactions between the compound and CT-DNA showed that the compound interacts with CT-DNA via electrostatic binding. The compound to be tested was compared with the butylated hydroxytoluene (BHT) solution used as standard. It was found that the compound exhibits good antioxidant activity.

Keywords: *Sulfonic acid, antimicrobial activity, antioxidant activity, DNA cleavage, DNA binding.*

1. INTRODUCTION

Imine compounds (Schiff base) are highly studied chemicals due to their biological and structural importance. They are used extensively as pigments in dye industry, catalysts and polymer stabilizers in organic synthesis (Branchaud, 1983; Silva *et al*., 2011). Nowadays, in studies their use in biological research in the pharmaceutical industry and medicine has increased the interest in these bases due to being reported as having antibacterial, antifungal, antiulcer, antimalarial, and antitumoral activities (Akocak *et al*., 2019; *Sridhar et al*., 2001; Panneerselvam *et al.,* 2005; Gupta *et al.,* 2015; Akocak *et al*., 2017; Sarıkaya *et al.,* 2014).

Infectious diseases cause deaths around the world. In particular, the fact that some bacteria are more resistant to antibiotics and the increase in this effect seriously affects human health (Taşkın, 2012). However, despite the need for new antimicrobial drugs, the development of antimicrobial agents is unfortunately decreasing gradually (Yıldırım, 2016).

Cancer is an significant important public health problem that ranks second after cardiovascular diseases as known cause of death in the world and in our country (Global Burden of Disease Cancer Collaboration, 2015). For this reason, scientists are conducting extensive studies about the identification or synthesis of new drug molecules that can be effective against cancer and at the same time do not harm human health (Göçmen, 2014).

Compounds targeting deoxyribonucleic acid (DNA) have significant theoretical and application value in the field of biology, chemistry and medicine (Li *et al*., 2011). DNA has become the primary target for many therapeutic agents ranging from anticancer drugs to antibiotics since the clarification of its structure (Sobha *et al*., 2012).

Sulfonic acids are used in the synthesis of organic materials, and salts of sulfonic acids are used as components of phenol compounds, detergents, ion exchangers, rust inhibitors, various dyes and sulfonamide drugs.

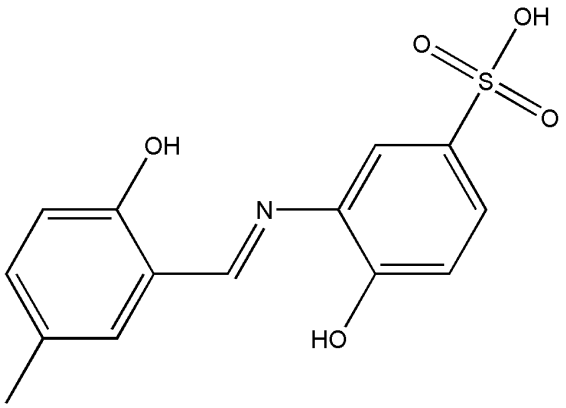
In this study, the sulfonic acid-based imine compound was firstly synthesized by the reaction of 3-amino-4-hydroxybenzenesulfonic with 2-hydroxy-5-methylbenzaldehyde. The structure of the compound was investigated for biological activities such as antimicrobial activity, DNA cleavage, DNA binding and antioxidant activity.

2. MATERIAL AND METHODS

2.1. Material

The sulfonic acid based imin compound used in this study is given below (Figure 1).

Figure 1. The chemical formula of 4-Hydroxy-3-(2-hydroxy-5-methylbenzylideamino)benzenesulphonic acid.



2.2. Methods

2.2.1. Biological activities of compound

2.2.1.1. Determination of antimicrobial activity

In vitro antimicrobial activity was examined for the compound. The antibacterial activities of the compound were tested against two gram-negative *Pseudomonas aeruginosa* (ATCC 27853) and *Proteus vulgaris* (ATCC 13315) bacteria and three gram-positive *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), and *Bacillus subtilis* (ATCC 6633) bacteria. The antifungal activities were examined for *Candida albicans* (ATCC 60193) and *Candida tropicalis* (ATCC 13803) yeasts. Antimicrobial activity studies were performed by the microdilution method according to the procedure shown in Essential Procedures for Clinical Microbiology (Isenberg, 1998). The minimum inhibitory concentration (MIC) value in which the antimicrobial effects of compound was determined.

2.2.1.2. DNA interactions of compound

The agarose gel electrophoresis and Ultraviolet-Visible (UV-Vis) field absorption spectroscopy method were used to determine if there was any damage caused by binding of sulfonic acid based imine compound to DNA.

2.2.1.2.1 Agarose gel electrophoresis method

The agarose gel electrophoresis method was used to identify DNA cleavage products. When the original supercoiled form of plasmid DNA is opened with damage, an open circular loose form is formed, and more fractures may occur and the linear form may also be present. When gel electrophoresis is carried out for DNA, Form I proceeds relatively faster than others, while Form II proceeds more slowly. If both strands are cleaved, Form III which migrates between Forms I and II is found.

Within the scope of the study, plasmid pBR322 DNA was placed in Tris-HCl buffer (10mM, pH=7.4), treated with compound and prepared samples were incubated at 37 $^{\circ}$C for 3 hours, then a loading buffer was added to the mixture, loaded onto 1% agarose gel and run for 1 hour, at 60 V in tris-acetic acid-EDTA (TAE) buffer (400mM Tris-200 mM acetate, 10 mM EDTA, pH=8.2). Then, the bands were visualized under a UV illuminator and photographed (Quantum ST4 gel imagining system, Vilbar Lourmat) (Qiao *et al*., 2011).

2.2.1.2.2. UV-Visible field absorption titration spectroscopy

Binding of the compound to DNA causes changes in its spectroscopic properties. These changes may be in the form of decreased or increased absorption (hypochromicity and hyperchromicity). This causes higher or lower wavelength shifts (shift to blue or red).

Electrostatic interactions cover almost all interactions with groups on the outer surface of DNA. Because the phosphate groups on the outer surface of DNA are negatively charged, they may interact with metal cations such as Na^{+2} or Mg^{+2} in the intracellular environment. This interaction of metal ions with DNA neutralizes the negative charge of the phosphate groups, and the counter ions are released. This may result in changes in the structure of DNA. As a general rule, an electrostatic component must be present in the molecular structure for the design of a molecule capable of binding strongly to DNA. Other types of interaction with DNA may also involve electrostatic interactions (Strekowski and Wilson, 2007).

In this way, whether the compound binds to DNA was determined by observing the change of absorption in the absence and presence of DNA. For this purpose, Calf-Thymus DNA (CT-DNA) was used. The CT-DNA and studied compound were placed in TNE buffer (10 mM Tris-HCl, 50 mM NaCl and 1 mM EDTA at pH: 7.4). The DNA solution was prepared and

DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient at 260 nm. UV-Vis field spectrum titrations were performed by adding equal amounts of DNA to both the compound and control solution to eliminate the absorbance of DNA itself. After each DNA addition, the measurements were recorded after waiting for 5 minutes at room temperature. UV-Vis measurements were then taken between 200-600 nm.

2.2.1.3. Free radical scavenging activity (DPPH)

The free radical scavenging activity was determined using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical according to the Blois (1958) method with some modifications. In this method, the DPPH molecule is reduced by donating H^+ to the antioxidant molecules present in the medium, which leads to a decrease in absorbance. The lower the absorbance value, the greater the free radical removal activity of the substance being tested.

DPPH solution was prepared in methanol and 1 mL of the prepared DPPH solution was added to the compound at different concentrations (10, 20, 40, 60, 80 and 100 µg/mL). The reaction mixture was stirred vigorously and incubated in the dark for 30 min. at room temperature. The color of DPPH changed from purple to yellow. BHT was used for the positive control and a solvent (methanol) was used as a negative control. Then, the absorbance was measured at 517 nm by using a spectrophotometer (Spectro UV-Vis Dual Beam-Labomed, Inc.).

The free radical scavenging activity was calculated using the following formula, the results being determined as % inhibition.

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

Where A_0 is absorbance of control and A_1 is the absorbance of compound or standard.

3. RESULTS AND DISCUSSION

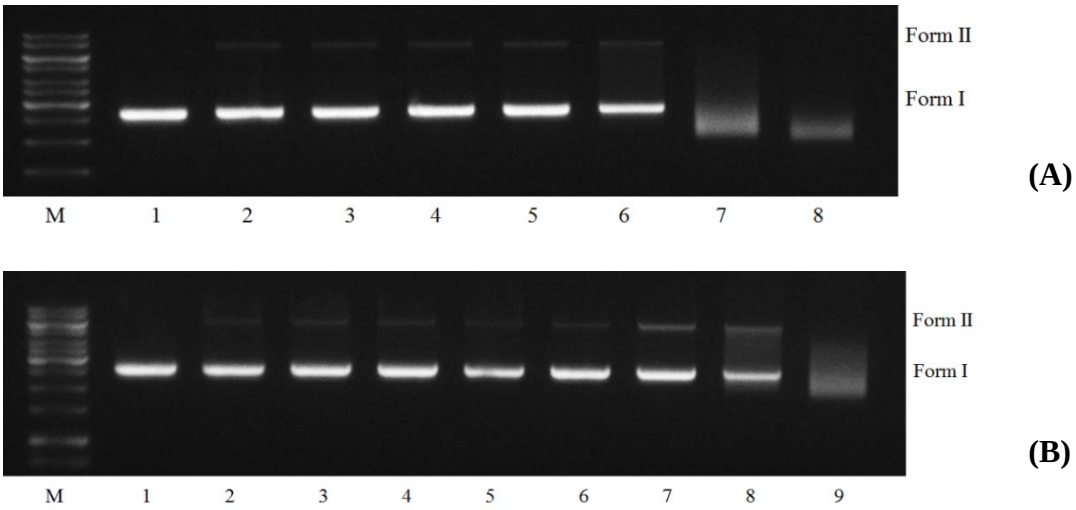
3.1. Antimicrobial activity

MIC values were measured against microorganisms with the microdilution method. The compound was found to be effective on yeast and bacteria. The values were read as the smallest concentration of the compound in the series that prevents visible growth of the test organism. Ampicillin, gentamicin and fluconazole antibiotics were used as positive controls. The antimicrobial activities of the compound varied between the concentrations of 64-256 µg/mL. The compound was found to be especially effective on *S. aureus* (ATCC 25923) and *E. faecalis* (ATCC 29212) bacteria (64 µg/mL). The compound showed the same antifungal activities (128 µg/mL) for both *C. albicans* (ATCC 60193) and *C. tropicalis* (ATCC 13803) yeasts, while it had a low effect on *P. aerouginosa* (ATCC 27853) bacteria (256 µg/mL).

3.2. DNA cleavage activity

Seven different concentrations (6.25, 12.5, 25, 50, 100, 200 and 400 µM) were prepared to determine the DNA cleavage activity of the compound used in the study. As a result of hydrolytic cleavage, it was observed that pBR322 plasmid DNA was cleaved by the compound at increasing concentrations and DNA was completely denatured at concentrations of 200 and 400 µM (Figure 2A). In the presence of an oxidizing agent (H_2O_2), the compound cleaved DNA at all concentrations and completely denatured DNA at a concentration of 400 µM (Figure 2B).

Figure 2. M: Marker, 1. Plasmid DNA, (A): Hydorlytic: 2. DNA+6,25 µM compound, 3. DNA+12,5 µM compound, 4. DNA+25 µM compound, 5. DNA+50 µM compound, 6. DNA+100 µM compound, 7. DNA+200 µM compound, 8. DNA+400 µM compound, (B): Oxidative: 2. DNA+6,25 µM compound+H_2O_2, 3. DNA+12,5 µM compound+H_2O_2, 4. DNA+25 µM compound+H_2O_2, 5. DNA+50 µM compound+H_2O_2, 6. DNA+100 µM compound+H_2O_2, 7. DNA+200 µM compound+H_2O_2, 8. DNA+400 µM compound+H_2O_2.

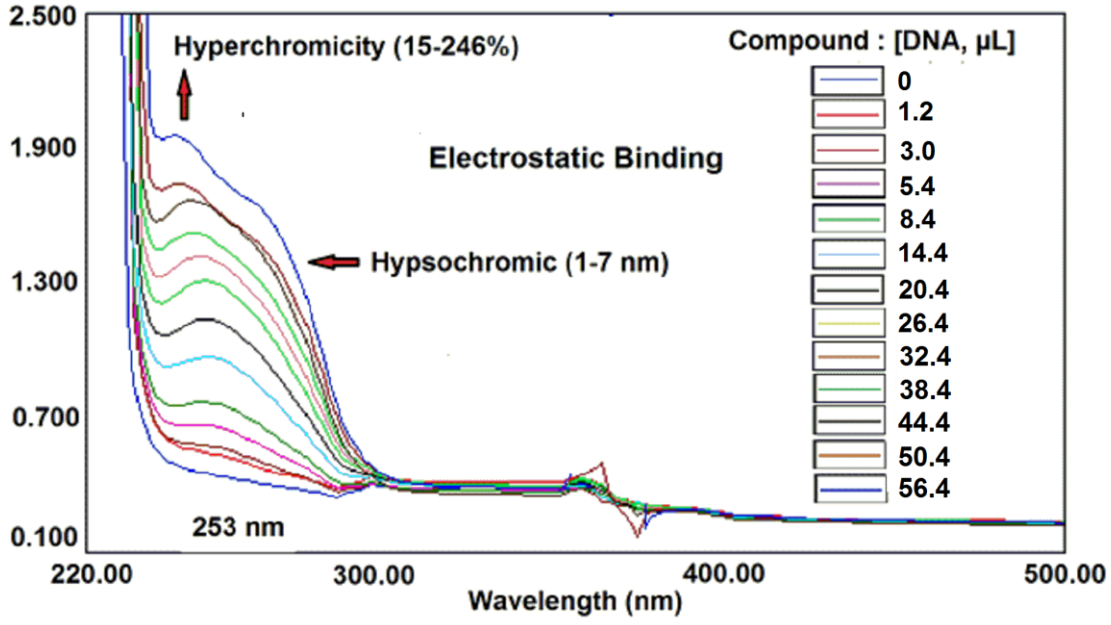


3.3. UV-Visible field absorption spectroscopy

The reactions of the compound with CT-DNA were examined by UV-Vis absorption spectroscopy. Differences in maximum absorbance of the free molecule and the molecule bound to DNA were compared to determine changes after interactions between the compound and DNA.

When the UV-Vis spectra of the compound are examined, it was observed that the change in absorbance intensity was increased in the direction of hyperchromism by gradually increasing the concentration of CT-DNA applied. 15-246% hyperchromism in the compound and 1-7 nm hypsochromism was observed with absorption at 253 nm (Figure 3).

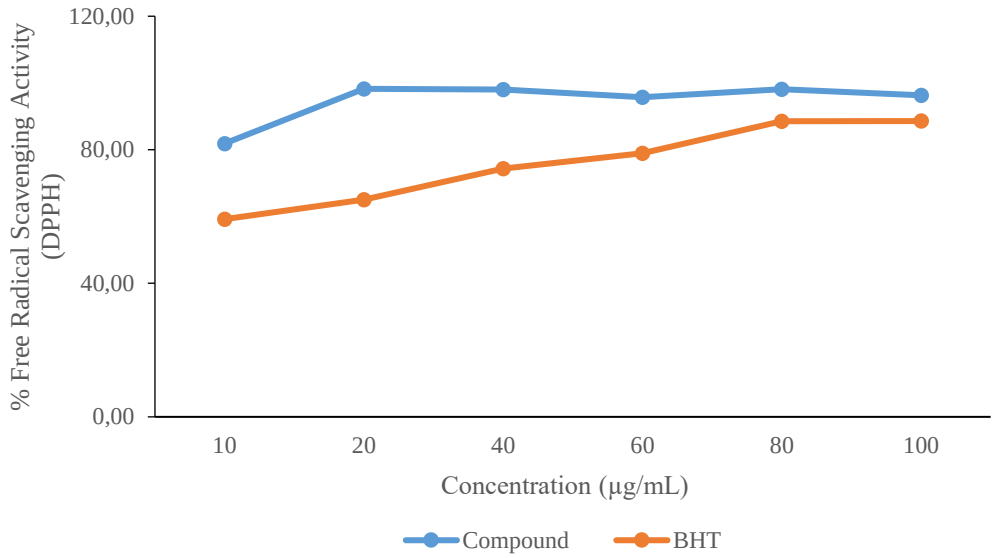
Figure 3. Absorption spectra trace of the compound with CT-DNA.



3.4. Free radical scavenging activity (DPPH method)

The antioxidant activity of the compound was investigated with the DPPH method which is commonly used. Results were compared against BHT control. The antioxidant activity data revealed that the compound exhibited good antioxidant activity as concentrations increased in the DPPH method (Figure 4).

Figure 4. Free radical scavenging activity of the compound.



DNA is considered to be the primary intracellular target of drugs developed for the treatment of many diseases due to its role in regulating cell viability and function. Therefore, there is increasing interest in the search for new molecules that can interact with DNA (Erkkila *et al*., 1999; Chouai *et al*., 2005; Liu *et al*., 2010). It is known that compounds capable of interacting with DNA have potential biological and pharmacological activities, and that this activity is closely related to the binding affinity and mode of binding of the compound to DNA.

Schiff bases and metal complexes are an important classes of compounds that attract attention with their diverse biological and pharmaceutical effects. Many Schiff base ligands are reported to have antibacterial, antifungal and antitumor activity (Wang *et al*., 2006; Quiao *et al*., 2011; Tabassum *et al*., 2013; Alizadeh *et al*., 2015). Considering all these properties, it is thought that newly synthesized Schiff bases and complexes may have similar properties. Therefore, the investigation of the interactions and biological activities of Schiff bases and complexes with DNA is very important for disease prevention and design of new chemotherapeutic drugs. DNA binding is particularly important for development of new chemotherapy drugs. Basically, the complex binds to DNA through three non-covalent modes: electrostatic, groove and intercalation binding (Rambabu *et al*., 2019).

Mermer *et al*. (2019) investigated the antimicrobial and antioxidant activities of synthesized Schiff base derivatives. They studied antimicrobial activity by using *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 13883, and *Acinetobacter haemolyticus* ATCC 1900. Antioxidant activity was investigated using DPPH (2,2-diphenyl-1-picrilhydrazil) radical. As a result, they found that all synthesized compounds had antioxidant and antimicrobial activities against tested microorganisms.

Alizadeh *et al*. (2014) in their study examined the in vitro DNA binding and pBR322 plasmid DNA cleavage activities of benzothiazole Schiff-base complexes. They found that the DNA binds electrostatically or to grooves and breaks down oxidatively.

Tadavi *et al*. (2018) studied the biological activities of a new Schiff base and complexes derived from the condensation of 2-hydroxy-6-isopropyl-3-methyl benzaldehyde and 1,2-diaminopropane. They investigated the antioxidant, antimicrobial and DNA cleavage activities of all compounds. Antibacterial activities were determined against *E. coli*, *P. aeruginosa*, *B. subtilis* and *S. aureus*. Antifungal activities were examined against *C. albicans, A. flavus, A. niger* and *C. neoformans* yeasts. Antioxidant activities were tested with 2,2-diphenyl-1-picrilhydrazil using the free radical scavenging method (DPPH). DNA cleavage activity was examined using plasmid DNA pBR322 in the presence of H_2O_2. As a result, they found an increase in antibacterial activity of complex compounds and showed low antifungal activity. They also indicated that all compounds exhibited antioxidant activity and the DNA cleavage occurred only with the Co complex.

4. CONCLUSIONS

In this study, the newly synthesized and characterized sulfonic acid-based imine compound was investigated for antimicrobial activity, DNA binding, DNA cleavage and antioxidant activity. The sulfonic acid-based imine compound was shown to have high antibacterial activity against *S. aureus* and *E. faecalis* bacteria. The interaction of the compound with CT-DNA was studied with UV-Vis absorption spectroscopy. It was revealed that the compound bind to CT-DNA through the electrostatic mode. When DNA cleavage activity results are analyzed, it was determined that the compound cleaved pBR322 plasmid DNA both hydrolytically and oxidatively depending on concentration. The free radical scavenging activity against DPPH was found to have good antioxidant properties. Also, the compound exhibited higher activity than the standard BHT.

Due to specific binding properties of Schiff bases and their various applications in cancer therapy, these compounds were reported as suitable candidates for antimicrobial, artificial nuclease, DNA probe, and antitumor drugs (Kiran *et al*., 2015). Research and design of new molecules that can interact with DNA is one of the most promising ways to discover new DNA- targeting anticancer drugs to be used in chemotherapy (Qiao *et al*., 2011). Therefore, it is very important to investigate the interaction of molecules with DNA in order to develop effective chemotherapeutic agents and better anticancer and antibacterial drugs.

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