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Investigation of the Biological Activities of Sulfonamide-Based Imine Compounds

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ABSTRACT

In this study, three sulfonamide-based Schiff bases were prepared: 4-Fluoro-N-(2-hydroxy-5-methylbenzylidene) benzenesulfonamide (C**1**), 4-Fluoro-N-(2-hydroxy-5-nitrobenzylidene) benzenesulfonamide (C**2**) and 4-Fluoro-N-((2-hydroxynaphthalen-1-yl)methylene) benzenesulfonamide (C**3**). DNA cleavage and binding capabilities of the prepared compounds were investigated agarose gel electrophoresis and by UV-Vis spectroscopy, and their antioxidant capacities were investigated *in vitro* by DPPH, ABTS, FRAP, CUPRAC, superoxide and hydroxyl radical scavenging methods. In addition, the antimicrobial and antibiofilm activities of the compounds were examined. As a result of UV-Vis spectroscopy studies, compounds were observed to interact electrostatically with Calf Thymus DNA (CT-DNA). From the gel electrophoresis results, C2 and C3 cleaved pBR322 plasmid DNA (pDNA) hydrolytically and oxidatively at different concentrations, while C1 cleaved the DNA oxidatively. The antioxidant capacities of the compounds were compared with standard butylated hydroxytoluene (BHT) solution and ascorbic acid. C1 was the most active according to DPPH, ABTS radical scavenging, superoxide and hydroxyl anion scavenging activity results, while C3 was more active according to FRAP and CUPRAC tests. Compounds were found to be effective on the growth of both bacteria and yeasts by the Minimum Inhibitory Concentration (MIC) method. In the antifungal activity study using the disk diffusion method, C1 did not show antifungal activity, while C2 and C3 displayed antifungal activity. In addition, all compounds exhibited different levels of antibiofilm activity depending on the bacteria used.

Keywords: Sulfonamide-based Schiff bases, DNA binding, DNA cleavage, Antibacterial, Antifungal, Antioxidant.

1. INTRODUCTION

Imine compounds (Schiff bases) are organic compounds containing the azomethine group (C=N) that have common areas of use because of their easy synthesis, ability to interact by binding to DNA in various modes, strong metal-binding abilities and many important biological properties. Schiff bases, as a ligand family, are known for their strong coordination abilities and versatility. With antibacterial, antifungal and anti-tumor activity (Baseer and Mote, 2001), Schiff bases are great importance in many scientific fields (biological, industrial, pharmaceutical). In recent studies, different Schiff base derivative compounds that can act as anticancer and anti-HIV agents have attracted the attention of researchers (Uddin et al., 2020). It also has been reported in studies that a wide variety of Schiff bases show inhibitory activity against experimental tumor cells (Choiri et al., 2022). Schiff base complexes

have significant biological roles, such as oxygen transport in mammals and other organisms with respiratory systems (Szczepaniak and Bragiel, 1995).

Sulfonamides can easily be derived and are bacteriostatic agents with broad antimicrobial activity and free amino groups. They also are used in a wide variety of biomedical applications including antimicrobial (Genç et al., 2008), anticancer and anti-inflammatory (El-Sayed et al., 2011) treatment, and used as HIV protease inhibitors as well as antiviral agents (Clercq, 2001). Sulfonamides were the first clinical chemotherapeutic agents that could control and treat various bacterial infections in the human urinary tract and gastrointestinal system (Petri, 2006). They are of particular interest because they are used against pathogenic bacteria and tumor cells. Discovery or improving of new drugs for cancer is a global priority, as cancer remains a major health challenge. Many natural and synthetic compounds are being explored for their potential anticancer properties. Sulfonamide hybrids have been found to have a wide spectrum and very low side effects with multi-drug resistance activity (Li et al., 2022). At the same time, sulfonamide derivatives are recognized as good antimetabolites. Furthermore, many studies have revealed that sulfonamide-derived compounds have high antioxidant effects (Kausar et al., 2019; Lolak and Akocak, 2020).

DNA and RNA are considered the first intracellular target for drugs that are developed to treat disease because they play a critical role in the control of intracellular functions (Ganji et al., 2018). Sulfonamides have attracted attention for use in designing new drugs or restructuring old drugs for various targets in needed areas because of their wide range of bioactivity and multi-purpose structure.

Considering the biological importance of sulfonamides and Schiff bases, in this study, DNA binding, DNA cleavage, antioxidant activities (free radical removal DPPH and ABTS, metal reduction FRAP and CUPRAC, Superoxide and Hydroxyl anion radical scavenging methods), antimicrobial and antibiofilm features of 3 different sulfonamide-based Schiff base compounds were compared *in vitro*. These sulfonamide-based Schiff compounds were synthesized in order to search for new drug candidates with different effects and mechanisms that can lead to better activity.

2. MATERIAL AND METHOD

Reagents

All chemicals were purchased from Sigma-Aldrich and did not purify them further. They were commercially available.

Physical Measurement

In UV-Vis measurements of the compounds, methanol was used as a solvent and recorded with UV-4000 (ORI, Germany). A UV-Vis absorption was used to determine DNA binding properties, Perkin Elmer BX II spectrometer was used for Infrared absorption spectra, Electro-Thermal IA 9100 instruments were used for melting points.

Chemistry

The investigated compounds were synthesized by Tekin et al. (2022) and prepared according to the literature. By matching the FT-IR, UV-Vis and melting point data with the literature values, confirmed the chemical composition of the compounds (Table 1). The schematic representation of the molecular structures of the compounds is given in Figure 1.

Table 1. Analytical results of C1, C2 and C3

Compounds	Molecular Formula	M (Calc.) g/mol*	M (Found) g/mol*	M.p (°C)*	M.p (°C) †	Yield (%)*	Yield (%)†	Color*,†
C1	$C_{14}H_{12}FNO_3S$	293.31	294.05	115	115-117	82	80	Pale pink
C2	$C_{13}H_9FN_2O_5S$	324.28	325.25	132-134	131-134	81	78	Yellow
C3	$C_{17}H_{12}FNO_3S$	329.35	331.25	192-194	192-194	78	75	Pale brown

* (Tekin et al., 2022)

†This study

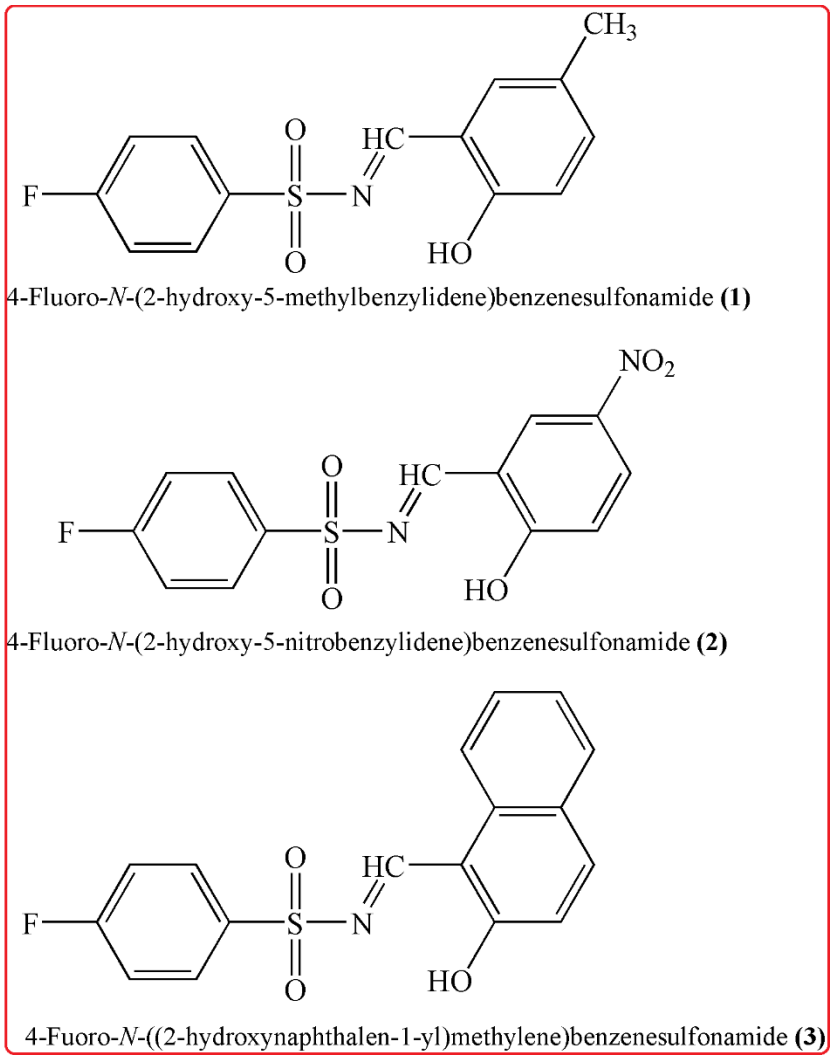


Figure 1. Schematic representation of the molecular structures of Schiff bases (**1-3**)

UV-Vis spectrum

Calf Thymus-DNA (CT-DNA) was used to determine the DNA binding effects of the compounds. Increasing concentrations of DNA were added to 8 mM Tris-HCl, 50 mM NaCl, and 1 mM EDTA (TNE) buffer (pH: 7.4) that were used for binding compounds to DNA, and measurements were made in a spectrophotometer (T+80 UV/Vis) between 200-600 nanometer wavelengths. The experiment continued until the observed changes in the absorption of the peaks formed at the end of the

measurements were stabilized. The binding constant (kb) of compounds with DNA was calculated according to Sairaj et al. (2022).

Cleavage Activity on DNA

The cleavage activities of the compounds on DNA were examined by gel electrophoresis (GE) technique and pBR322 pDNA was used. The hydrolytic and oxidative effects of the concentrations of the studied compounds prepared with dimethyl sulfoxide (DMSO) on plasmid DNA (pDNA) were determined. Test compounds were treated with 10 mM Tris-HCl buffer (pH: 7.4). In the oxidative cleavage activity, in addition to DNA and buffer, hydrogen peroxide (H_2O_2), was used which is an inducing agent. The incubated (37°C, 3h) mixture was run in an electrophoresis tank (60V, 1h) using 1% agarose gel. Finally, the bands that formed in the gel were visualized (Hangan et al., 2022).

Antioxidant Assay

The free radical scavenging capability of the compounds were carried out using the 2,2-diphenyl-1-picrylhydrazil (DPPH) radical (Blois, 1958) and standard butylated hydroxytoluene (BHT) solution was used for comparison. Determination of ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation scavenging activity was measured at 734 nm (Re et al., 1999). It is an electron-based antioxidant method in which a radical cation is formed from the ABTS compound and this cation is removed by antioxidants (Kurt, 2018).

Different concentrations of compounds (10-100 µg/mL) were used in the Ferric Reduction/Antioxidant Potency (FRAP) Test (Abu Bakar et al., 2014). The FRAP reagent was made; sodium acetate buffer (0.3 M), 2,3,5-triphenyltetrazolium chloride (TPTZ) solution (10 mM), and $FeCl_3$ (20 mM) solution were added at a ratio of 10:1:1, respectively and mixed (37°C, 15min) and then incubated in the dark (30min). Measurements were made at 593 nm absorbance values.

Determination of Cu(II) Ion Reducing Antioxidant Capacity reducing (CUPRAC) of compounds was carried out using the Apak et al. (2005) method. For the procedure, 250 µL of $CuCl_2$ (0.01 M), neocuproin (0.0075 M) and ammonium acetate solution (1 M) were put into each spectrophotometer cuvette to which 1000 µL of distilled water was distributed equally among the cuvettes. The cuvettes were then incubated in the dark (RT, 30 min). Measurements were made against a blank and using the BHT (0.001 g/mL) standard as a positive control. After incubation, absorbance values at 450 nm were compared with the BHT standard.

The hydroxyl radical scavenging capability of the compounds were carried out at 510 nm according to the method of Zhang et al. (2011) and ascorbic acid was used for the positive control. The Superoxide anion radical scavenging activity of the compounds (Hangan et al., 2022) was measured at 560 nm. All of the spectroscopic values in the antioxidant tests were formulated and used in terms of % activity.

Antibacterial, Anti-yeast and Anti-mould Activity

Broth microdilution method was used to determine the minimum inhibition concentration (MIC) values (mg/mL) in antibacterial and anti-yeast studies. Gentamicin and Ampicillin for bacteria and Fluconazole for yeasts were used as control antibiotics. MIC and disk diffusion tests were conducted according to Clinical and Laboratory Standards Institute (CLSI) guidelines interpretive criteria. Mold spores of *Aspergillus fumigatus* NRRL 163 and *Penicillium notatum* NRRL 807 species were obtained by modifying the Azgın (2013) method. Miconazole 10 (MCZ10) and Amphotericin B 20 (AMB20) were used as control antibiotics. Zone diameters formed in anti-mold activity were measured and recorded (mm) (Chohan et al., 2004).

Antibiofilm Activity

Absorbance values of "F" plates were recorded at 620 nm with microplate reader (Merritt et al., 2005). The percentages of biofilm inhibition of compounds against seven different microorganisms were found.

3. RESULTS AND DISCUSSION

UV-Vis spectrum

Determining the type of interaction DNA has with chemical substances is very important for the development of new drugs. One of the methods to understand the effect drugs can have on DNA is UV-Vis absorption titration, which is evaluated by the change of DNA or the absorption of the drug. In this method, the changes in the maximum absorbance of the free molecule and the molecule bound to DNA are compared to comprehend the efficacy of the drug on DNA. Although this type of binding is weaker than other types of interaction, it plays a considerable role in the biological activities of drugs. The electronic absorption spectra of some sulfonamide-based Schiff bases were investigated, and the π→π* and n→π* assignments in aromatic rings and azomethine groups were observed in the range of 230–280 nm and 340–350 nm, respectively (Salehi et al., 2018).

UV-Vis spectra of C1-2-3, prepared for this study, were taken in DMSO. Two bands were observed in C1 and C2. In C1, π–π* and n–π* transitions for aromatic C=C and imine C=N were observed at 260 and 338 nm, respectively, and in C2, 259 and 315 nm, respectively. For C3, three bands of π–π*, n–π* and n–π* transitions were observed at 259, 316 and 356 nm, and assigned to the C=C, C=N, S=O groups, respectively. The observed UV-Vis results and the data are similar to those in the literature (Tekin et al., 2022).

The binding affinity between CT-DNA and C**1-2-3** was studied by UV-Vis spectroscopy in Tris-HCl/NaCl buffer at room temperature according to the literature (Yapar et al., 2022) and was presented in Figures 2, 3 and 4. In absorption spectroscopy, if there is an effect contrary to DNA, a hypochromic effect is observed, and if there is a DNA interaction (electrostatic or intercalative), a hyperchromic effect is observed. In addition, the redshift of the maximum absorptions indicates that the distinction between HOMO and LUMO energy levels is reduced and that the compounds are mutually related to DNA.

In the presence of CT-DNA, an increase in peak intensities in the absorption spectra of Schiff bases **1-3** was observed, and their absorption was also redshifted (bathochromic; 1-2 nm). The degree of redshift and hyperchromism in absorption was generally related to the electrostatic bond strength. As seen in Figure 2, the change in absorbance intensity with the concentration of CT-DNA added to C1 appears to be in the direction of hyperchromism (14-625%), and a 1-2 nm bathochromic (red) shift was observed at 258 nm absorption. In C2, the change in absorbance intensity with added CT-DNA concentration was in the direction of hyperchromism (24-740) and a 258 1-2 nm bathochromic (red) shift was observed. When the UV-Vis spectrum of C3 is examined, the change in absorbance intensity with the added CT-DNA concentration is hyperchromism (20 -600%) and a bathochromic (red) shift of 1-2 nm at 258 nm occurred. These results show the effect of hyperchromicity in the absorption spectra of the compounds and indicate that the Schiff bases (1-3) bind to DNA in an electrostatic mode.

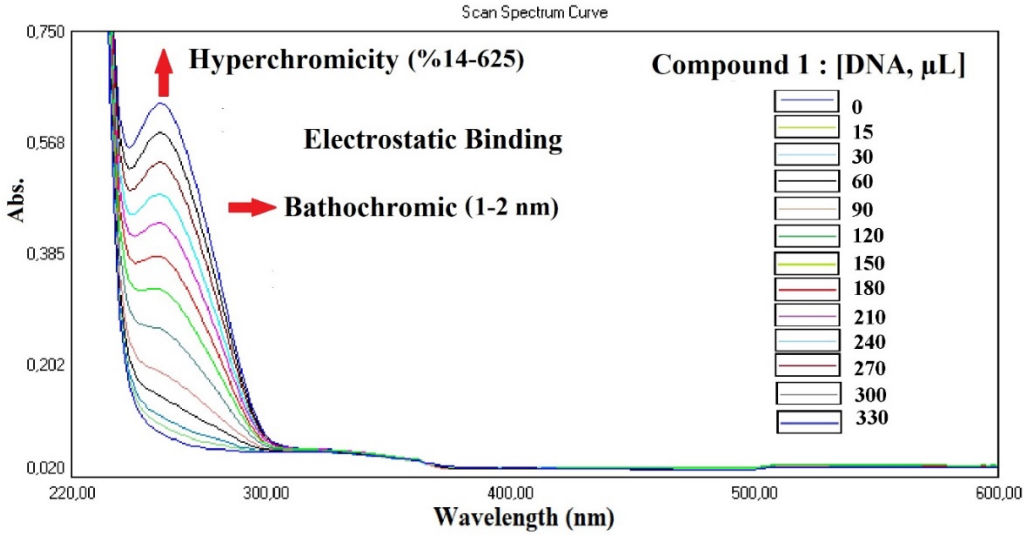


Figure 2. UV-Vis spectrum of C1

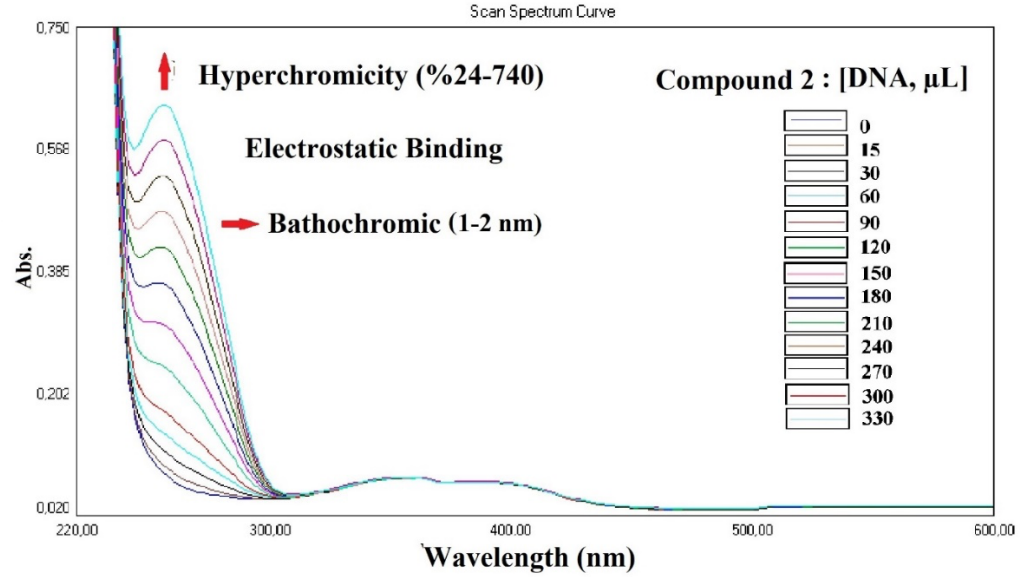


Figure 3. UV-Vis spectrum of C2

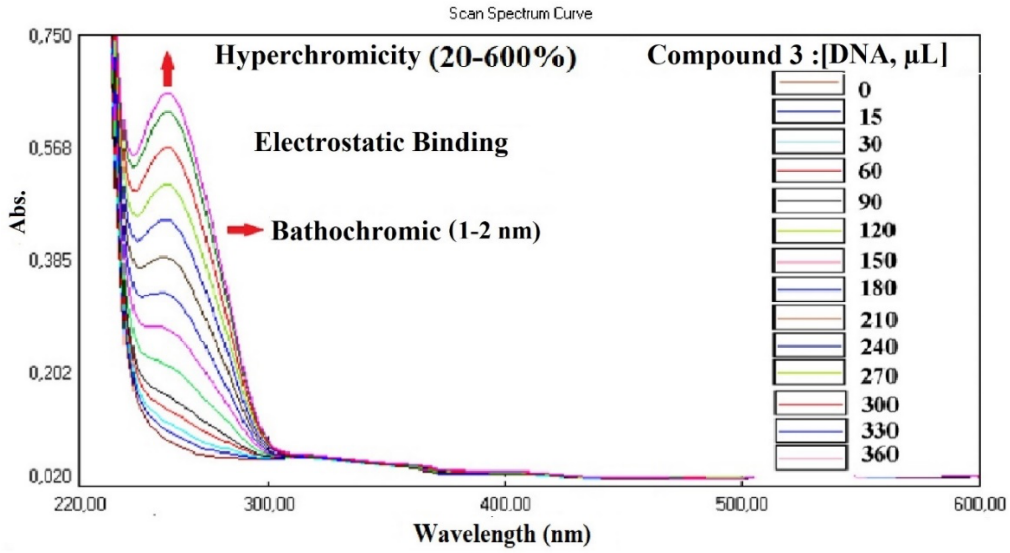


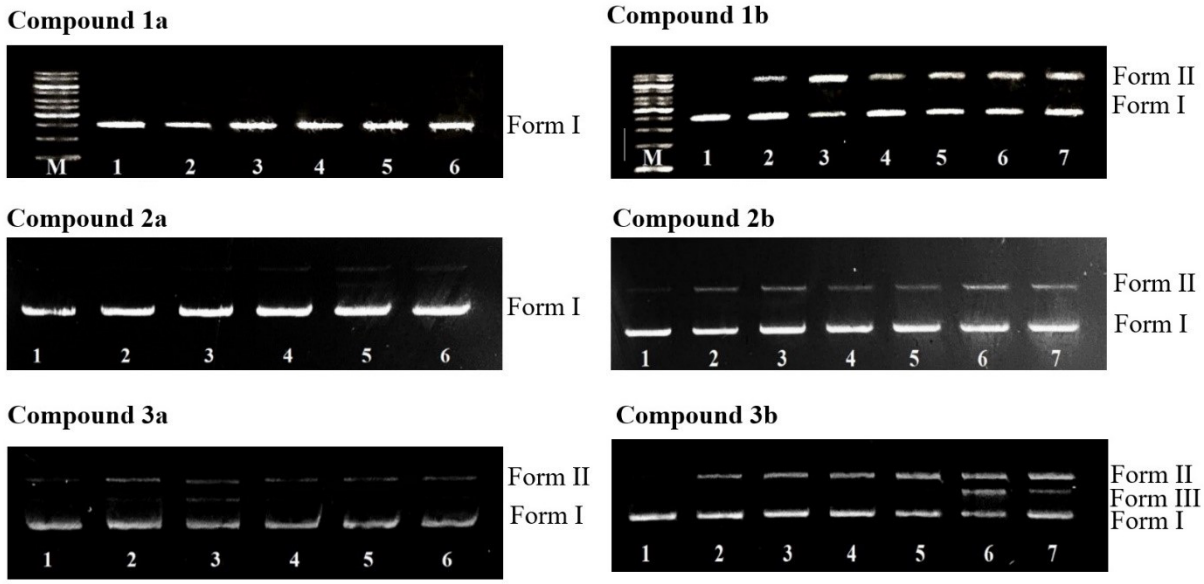
Figure 4. UV-Vis spectrum of C3

DNA Cleavage

The coaction of C1, C2 and C3 with pDNA were examined by GE. Three forms of pDNA are usually observed in the gel image: Supercoiled (Form I), nicked (Form II) and linear (Form III). Form I is the uncleaved form of pDNA (pBR322) and migrates faster on the gel. If cleavage occurs in a single strand of pDNA, the supercoiled form of pDNA unwinds into Form II, which migrates slower on the gel. When the two strands are cleaved together, pDNA differentiates into Form III, which has a rate of movement between Form II and Form I.

In a previous study, it was revealed that Cu(II) complexes cleave DNA more efficiently than ligands and other complexes in the presence of an oxidant when a N,O-chelating Schiff base ligand containing azo and sulfonamide fragments and some transition metal complexes are used (Alaghaz et al., 2015). In a comparative study with Benzene Fused Monocyclic Enediynyl Amides and Sulfonamides, it was determined that sulfonamides do not show any DNA cleavage activity (Basak et al., 2002).

Based on this information, C1 was not hydrolytically active but had an effect on pDNA in the presence of an oxidizing agent (H_2O_2). C2 hydrolytically formed a single cleave (Form II) in DNA starting from the lowest concentration of 100 µM and was more effective in the presence of a H_2O_2. C3 hydrolytically formed a single cleave (Form II) in DNA starting from a lowest concentration of 25 µM and a double cleave (Form III) in pDNA starting from a concentration of 400 µM in the presence of an oxidizing agent (H_2O_2) (Figure 5).



a: Hydrolytic cleavage (M marker, 1 pDNA, 2-6. DNA+ 25, 50, 100, 200, 400 µM, respectively), b: Oxidative cleavage (M marker (1 kb), 1 pDNA, 2-6. DNA+ 25, 50, 100,200, 400 µM+H_2O_2, respectively).

Figure 5. C1, C2 and C3 DNA cleavage activity

Antioxidant Assay

Oxidative stress is a condition where the balance between the production and elimination of reactive oxygen species (ROS) is disturbed. ROS are molecules that contain oxygen and have high reactivity, such as hydroxyl radicals, peroxy radicals and superoxides. ROS can cause damage to cellular components and contribute to the development of various diseases, such as cancer, atherosclerosis, diabetes mellitus, Alzheimer's diseases, cardiovascular events, aging and inflammation (Akhtar et al., 2016).

The antioxidant potential of a compound is the ability of the compound to slow down oxidative stress. Three main mechanisms of action are used to evaluate the antioxidant potential, including the free radical scavenging mechanism, the pro-oxidant reduction mechanism and the pro-oxidant chelation mechanism (Kanwal et al., 2016).

We evaluated the antioxidant activity in vitro of different samples using six methods: DPPH radical, ABTS radical, FRAP, CUPRAC, Superoxide anion and Hydroxyl anion radical scavenging assays. These methods are based on the ability of antioxidants to scavenge free radicals or reduce metal ions by transferring hydrogen atoms or electrons. Each method involves generating a different radical that acts through various mechanisms and measuring it spectrophotometrically at a fixed time point or an interval. In one study that determined the DPPH activity of sulfonamide mixed Schiff base compounds of anthranilic acid, it was found that the compounds with good antioxidant activities enabled the hydrogen atoms to show antioxidant potency due to the presence of -NH_2 and -OH groups in their structures (Kausar et al., 2019). The antioxidant potential of sulfonamide compounds synthesized from different concentrations was investigated in another study. The compounds exhibited dose-dependent radical scavenging activities against ABTS, DPPH, lipid peroxidation, superoxide and nitric oxide anion (Zhong et al., 2007). Furthermore, comparative IC_{50} (nM/mL) inhibitor concentrations against different free radicals were statistically significant (Table 2).

In the current study, different concentrations of Schiff bases (**1 - 3**) showed dose-dependent antioxidant activity. The IC_{50} inhibitory concentrations of **C1-2-3** against various free radicals were statistically significant and are given in Table 2. The DPPH and ABTS radical scavenging and Superoxide and Hydroxyl anion scavenging activity of C1 was higher than that of C2 and C3 but similar to the controls (Table 2). The FRAP and CUPRAC-reducing activity of C3 was higher than that of C1 and C2 (Table 3-4).

Table 2. The radical scavenging capacity of synthesized compounds (IC_{50}, µg/mL)

Compounds	DPPH	ABTS	Superoxide Anion	Hydroxyl Anion
1	4.25 ± 0.05	7.79 ± 0.11	3.54 ± 0.09	5.01 ± 0.13
2	4.68 ± 0.08	9.62 ± 0.12	3.08 ± 0.08	5.32 ± 0.10
3	4.95 ± 0.10	8.44 ± 0.04	8.61 ± 0.31	5.07 ± 0.08
BHT	2.86 ± 0.04	5.73 ± 0.17	-	-
Ascorbic Acid	-	-	4.18 ± 0.07	4.00 ± 0.13

Data are mean ± standard deviation (n=3)
- = not calculated.

Table 3. Measurement of CUPRAC metal reduction capacity of compounds (1-3) at λ=450 nm

	Amount of compound (µg/mL)	Standard (BHT)	C1	C2	C3
1	10	0.18 ± 0.05	0.10 ± 0.06	0.14 ± 0.03	0.11 ± 0.05
2	20	0.41 ± 0.08	0.10 ± 0.03	0.15 ± 0.06	0.19 ± 0.04
3	40	0.82 ± 0.06	0.13 ± 0.07	0.16 ± 0.05	0.20 ± 0.06
4	60	1.05 ± 0.07	0.14 ± 0.05	0.21 ± 0.05	0.24 ± 0.03
5	80	1.48 ± 0.09	0.15 ± 0.08	0.24 ± 0.04	0.25 ± 0.06
6	100	1.49 ± 0.07	0,16 ± 0.07	0.28 ± 0.08	0.30 ± 0.06

Table 4. Measurement of FRAP metal reduction capacity of compounds (1-3) at λ=593 nm (Mean absorbance ± SEM)

	Amount of compound (µg/mL)	Standard (BHT)	C1	C2	C3
1	10	0.25 ± 0.04	0.11 ± 0.03	0.12 ± 0.04	0.32 ± 0.04
2	20	0.37 ± 0.06	0.12 ± 0.04	0.21 ± 0.06	0.32 ± 0.05

3	40	0.52 ± 0,05	0.14 ± 0.03	0.22 ± 0.05	0.33 ± 0.06
4	60	0.59 ± 0.06	0.14 ± 0.04	0.23 ± 0.07	0.33 ± 0.04
5	80	0.97 ± 0.07	0.15 ± 0.05	0.30 ± 0.06	0.39 ± 0.06
6	100	0.99 ± 0.04	0.29 ± 0.07	0.42 ± 0.08	0.44 ± 0.07

Antimicrobial Activities

Antibiotics show their effects by stopping the growth of microorganisms or by killing them. They have various mechanisms of action such as inhibition of cell wall synthesis, DNA replication, RNA transcription and protein synthesis. However, these effects have also been shown to promote the development of resistant strains (Sharma et al., 2009). As a result, studies are underway to develop antimicrobial treatments (bacteriostatic or bactericidal) to prevent the threat of antibiotic-resistant pathogens (More et al., 2014).

Some sulfonamide Schiff bases have performed well against resistant pathogens. While MIC data were 32-128 mg/mL, sulfonamide alone was 32-128 mg/mL inactive and MIC was >512 mg/mL (Mondal et al., 2017). In another study, the antibacterial and antifungal activities of Sulfonamide Schiff bases and metal complexes were evaluated, and it was determined that Schiff base metal complexes had stronger antibacterial and antifungal activity compared to Schiff bases (Chohan et al., 2004). In a study with turmeric-derived sulfonamide Schiff bases, turmeric derivatives showed activity against all bacterial strains used in the study, which may be due to the synergistic effect of sulfonamide and turmeric components (Ahmed et al., 2016).

To determine the antibiotic potential of Schiff bases 1-3 in the current study, their antimicrobial activities were determined via MIC values for Gram-positive *B. subtilis* ATCC 6633 (Bs), *S. aureus* ATCC 25923 (Sa), *E. faecalis* ATCC 29212 (Ef) and *L. monocytogenes* ATCC 7644 (Lm); Gram-negative *P. aeruginosa* ATCC 27853 (Pa), *E. coli* NRRL B-3704 (EcN), *E. coli* ATCC 25922 (EcA) *and P. vulgaris* ATCC 13315 (Pv); and yeast *C. albicans* 60193 (Ca) (Table 5). In addition, the antifungal activities of the compounds against *P. notatum* NRRL 807 (Pn) and *A. fumigatus* NRRL163 (Af) were examined by the disk diffusion method, and inhibition zone diameters were measured and evaluated (Table 6).

As seen in Table 5, all synthesized compounds 1-3 inhibited the growth of bacteria at certain concentrations. When the MIC values of the compounds were examined, C1 and C2 were most effective against Pv and Ef *bacteria,* while C3 was more effective against EcN bacteria and Ca yeast. When comparing C1 and C2, C1 appears to be more effective against Pv and Ef bacteria.

As given in Table 6, C1 did not show antifungal activity against test molds, C2 showed antifungal activity against Pn strain and C3 showed activity against Pn and Af strains.

Table 5. MIC values of C1, C2 and C3

			MIC values (µg/mL)					
Microorganisms			C1	C2	C3	Gentamicin	Ampicillin	Fluconazole
Bacteria	Gr-	Pa	512	512	512	0.08	2	-
		EcN	512	128	64	0.125	32	-
		EcA	256	128	128	0.125	32	-
		Pv	16	64	128	0.125	0.06	-
	Gr+	Bs	128	256	256	0.008	0.06	-
		Sa	512	128	256	1	0.016	-
		Ef	32	32	128	1	0.016	-
		Lm	256	128	256	1	0.016	-
Yeast		Ca	256	256	128	-	-	0.063

Table 6. Antifungal activity values of test compounds

	Disc zone diameter (mm)				
Microorganisms	C1	C2	C3	Miconazole 10	Amphotericin B 20
Pn	-	8	15	31	32
Af	-	-	12	30	32

Ligand: > 14 mm significant activity; 7-13= mm moderate activity; < 7mm weak activity

Antibiofilm Activity

The resistance of bacteria to antibiotics is mostly the production of biofilms, which is a quorum sensing (QS) feature (More et al., 2014). Serious infections in medicine and more than 50% of infections in humans are caused by biofilm (Spoering and Lewis, 2001). Industrial sectors suffer significant economic losses due to bacterial biofilm formation (Van Houdt and Michiels, 2010). It is very important to produce new-generation drugs that are effective against biofilm formation for the prevention of these infections. Various methods have been developed to prevent biofilm formation and very successful results have been obtained with the application of compounds with antimicrobial properties (Artini et al., 2017). In one study that examined the antibiofilm activity of silver complex sulfonamides, they found that it destroyed a *Pseudomonas aeruginosa* biofilm and showed a significant difference in biofilm formation when compared to the positive control (Siqueira et al., 2021). In another study investigating the anti-biofilm activity of gold-complex Sulfonamides, they found that the test compounds effectively inhibited methicillin-resistant *Staphylococcus aureus* biofilm formation (Mizdal et al., 2018).

In order to determine the biofilm inhibition effects of Schiff bases **1-3** used in the study, the MIC values of C1-2-3 were found and showed biofilm inhibition percentages corresponding to seven different microorganisms (Figure 6). The highest and lowest antibiofilm values of the compounds were calculated. The highest antibiofilm value of C1 was with Ef (77%) and the lowest antibiofilm value was with Bs (8%), while C2 showed the highest with Pv (84.7%) and the lowest with Pa (8%). For C3**,** the highest was with Pv (75.4%) with Ca and the lowest was with Sa (11%). All three compounds showed better antibiofilm activity on Enteric bacteria species (Ea, Pv, Ef).

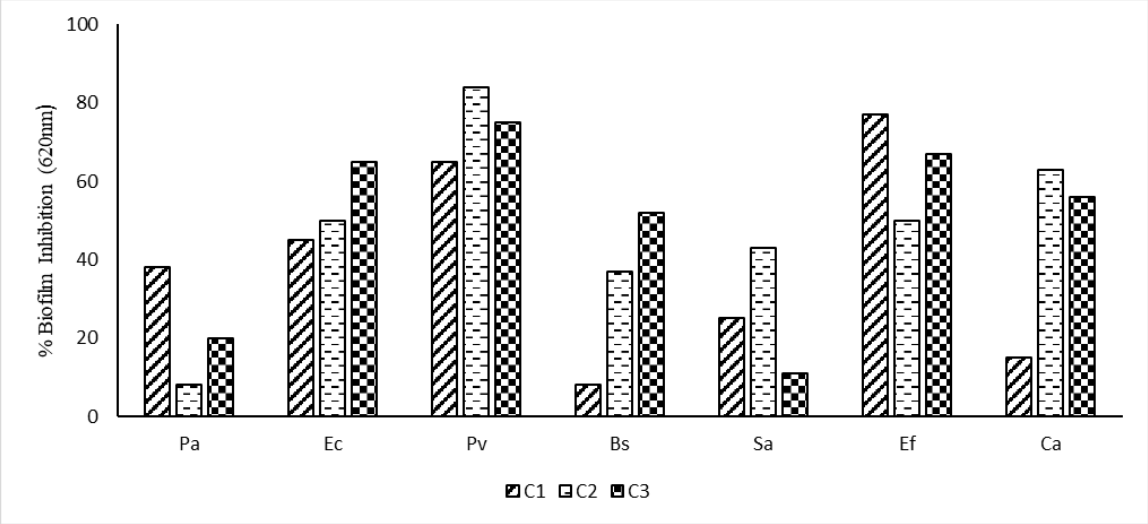


Figure 6. Biofilm inhibition plot of compounds

4. CONCLUSION

The process of drug development involves the discovery and optimization of novel molecules with therapeutic potential. It aims to create effective and safe drugs that can treat various diseases and improve human health. Many different antibiotics have been put on the market in the past, taking into account all the parameters necessary for drug design. Research is still ongoing with the modification of parent compounds to improve efficacy with minimal side effects. The sulfonamide-based Schiff bases synthesized in this study were found to have DNA binding, DNA cleavage, and antibacterial and antioxidant properties. These results suggest a need for clinical trials of these compounds. However, it can be said that more *in vivo* and *in vitro* studies are needed to synthesize similar compounds as those used in the study and to assess their biological activities more comprehensively.

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