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A Schiff Base Sensor Selective to Anions, Biological Activity, and Spectral Studies

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Abstract: In this study, synthesis, characterization, anion sensor properties, and DNA binding of a benzothiazole-based Schiff base 4-bromo-2-((6-methoxybenzo[d]thiazol-2-ylimino)methyl)phenol have been investigated. The structure of the Schiff base was revealed with elemental analysis and spectroscopic methods. The colorimetric and fluorescent anion sensor properties of the Schiff base in DMSO were investigated by adding an equivalent amount of anions. In this context, the solution containing Schiff base had a color change after the addition of F^-, CN^-, AcO^-, $H_2PO_4^-$ and OH^- anions, while the color change was not observed with the addition of Br^-, I^-, SCN^-, ClO_4^- and HSO_4^- anions. The anion-binding power of the compound was found to be $F^- > OH^- > AcO^- > CN^- > H_2PO_4^-$ using UV-Vis spectrophotometry, respectively. The antimicrobial activity of the compound was investigated against some microorganisms. The compound showed activity against bacteria and yeast. Schiff base showed a similar effect against both bacteria and yeast. Interactions between the compound and CT-DNA were studied with UV-Vis spectra. The UV-Vis experiment results confirm that the Schiff base binds to CT-DNA in an intercalative mode. The compound did not show any cleavage effect on SC DNA as hydrolytic and oxidative.

Keywords: Schiff base, Spectroscopy, Anion Sensors, DNA-Binding.

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INTRODUCTION

Some characteristic properties of Schiff bases, such as antimicrobial, antioxidant, anticancer, anti-helmintic, anti-inflammatory, anticonvulsant, antitubercular and analgesic activities (1-5) as well as chemosensory and DNA binding properties (6-19) have been reported. The technique used in chemosensors is concerned with the quenching of the colorimetric chemosensor of fluorescence by interaction with various anions. Chemosensors are very useful because they provide fast and visible color changes in the presence of ions. In the last decade, many chemosensors have been developed and studied to recognize and select ions with precise selectivity and sensitivity (4-20).

2-Hydroxy imines exhibit interesting properties both in solution and solid state. Tautomerism occurs in ortho-hydroxy imines. It was studied in ortho-hydroxy imines using various methods (21,22). If the H proton in the O-H is taken by the imine nitrogen, the keto-amine form occurs. This form is always observed if the imine is formed by 2-hydroxy-1-naphthaldehyde and aromatic amine. It is not observed in solution and solid state in Schiff bases composed of 2-hydroxy benzaldehyde and aromatic amines. But it has been observed in compounds derived from substituted salicylaldehydes and aromatic amines. Many properties of Schiff bases, especially sensor properties, are associated with tautomerism. Depending on the tautomerism of the ortho-hydroxy imines, hydrogen bonds such as O-H...N and N-H...O are formed (21,23).

Colorimetric sensors are used for ion detection because the signaling phenomenon is easily detected by the naked eye (22,24). A visible color change occurs after the formation of the hydrogen bond between the anion and the sensor. 2-Hydroxy Schiff bases can produce hydrogen bonds that can be used in chromogenic and fluorogenic sensors (11, 25). Benzothiazole-based Schiff bases are nitrogen and sulfur-heterocyclic bicyclic ring systems (26). Benzothiazoles have many applications. Anticancer, antimicrobial and antidiabetic, anticonvulsant, anti-inflammatory, antiviral, and antitubercular activities of benzothiazoles are known (27,28). Furthermore, biological activities of bicyclic Schiff bases have been investigated and reported as DNA repair agents (29).

In the present work, a receptor was prepared by the reaction of 2-amino-6-methoxybenzothiazole with 5-bromosalicylaldehyde with the ability to detect some anions by colorimetry and spectrophotometry (UV-Vis) in dimethyl sulfoxide (DMSO). In addition, the antimicrobial activity of the synthesized compound and its interaction with DNA was investigated.

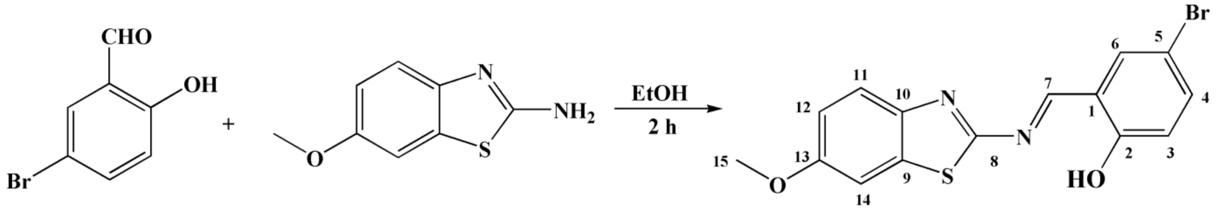
MATERIALS AND METHODS

Reagents and techniques

The ^{1}H- and ^{13}C-NMR spectra were recorded on a JOEL NMR- 400 spectrometer operating at 400 and 101,6 MHz, respectively. Infrared absorption spectra were obtained from a Perkin Elmer BX II spectrometer in KBr discs and were reported in cm^{-1} units. The UV-Vis spectra were measured using PG Instruments T+80 UV/Vis spectrophotometer. Elemental analyses were performed on a Vario EL III CHNS elemental analyzer. Melting points were measured with an Electro Thermal IA 9100 apparatus using a capillary tube. 2-Amino-6-methoxybenzothiazole, 5-bromosalicylaldehyde, EtOH, DMSO, Ethidium bromide (EB), calf thymus DNA (CT-DNA), $(Bu)_4NF$, $(Bu)_4NBr$, $(Bu)_4NI$, $(Bu)_4NCN$, $(Bu)_4NSCN$, $(Bu)_4NClO_4$, $(Bu)_4NHSO_4$, $(Bu)_4NCH_3COO$, $(Bu)_4NH_2PO_4$, $(Bu)_4NN_3$, $(Bu)_4NOH$ and DMSO were purchased from Aldrich. The Tris–HCl buffer solution was prepared with triple-distilled water. CT-DNA stock solution was prepared by diluting DNA to Tris–HCl/NaCl buffer (5 mM Tris–HCl, 50 mM NaCl, pH 7.2), and kept at 4 °C for no longer than two days.

Synthesis of 4-bromo-2-((6-methoxybenzo[d]thiazol-2-ylimino)methyl)phenol

2-Amino-6-methoxybenzothiazole (1.00 g; 5.55 mmol), ethanol (100 mL) and 5-bromo-2-hydroxybenzaldehyde (1.115 g; 5.55 mmol) were added to a 250 mL round-bottomed flask. The mixture was stirred and refluxed for 2 h. The compound was obtained from the evaporation of ethanol and was crystallized from $CHCl_3$:n-hexane (3:1) as a yellow crystal, m.p. 213-215 °C, 1.79 g (89%) yield (Scheme 1). Experimental: C, 49.05; H, 3.05; N, 7.71. Calculated: $C_{15}H_{11}BrN_2O_2S$; C, 49.58; H, 3.04; N, 7.71. FT-IR (KBr, cm^{-1}) νO-H; 3291 m-br, νAr-H; 3077 w, νC-H; 2974-2925 w, νC=N; 1598 s, νC=C; 1558 s, νC-N; 1497 s, νC-O; 1346 s, νC-O-C; 1262-1220-1169-1050 s. ^{1}H-NMR (DMSO); δ ppm, 9.30 (s, 1H, Ar-O<u>H</u>); 8.06 (s, 1H, Ar-C<u>H</u>=N-); 7.85-6.98 (m, 6H, Ar-<u>H</u>). ^{13}C-NMR (DMSO); δ ppm, 168.10 (s, 1C, <u>C8</u>); 163.08 (s, 1C, <u>C7</u>); 159.85 (s, 1C, <u>C2</u>); 157.91 (s, 1C, <u>C13</u>); 145.86 (s, 1C, <u>C10</u>); 137.78 (s, 1C, <u>C9</u>); 135.93 (s, 1C, <u>C6</u>); 132.28 (s, 1C, <u>C11</u>); 123.90 (s, 1C, <u>C4</u>); 122.25 (s, 1C, <u>C1</u>); 119.82 (s, 1C, <u>C3</u>); 116.48 (s, 1C, <u>C12</u>); 111.23 (s, 1C, <u>C5</u>); 105.56 (s, 1C, <u>C14</u>); 56.19 (s, 1C, <u>C15</u>). The carbons were numbered according to the order of Scheme 1.



Scheme 1. Synthesis of the Schiff base.

Screening for antimicrobial activities

Staphylococcus aureus ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 254992, *Candida albicans* ATCC 60193, and *Candida tropicalis* ATCC 13803 were used as microorganisms. Ampicillin and fluconazole were used as controls in this study as they are well-known broad-spectrum antibiotics that have different mechanisms of activity, such as interruption of cell wall synthesis (ampicillin) (30). The compounds were dissolved in DMSO (dimethyl sulfoxide) to a final concentration of 500 µg/mL. The concentration of the compounds on different plates was 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL, 15.6 µg/mL, 8 µg/mL, 4 µg/mL, 2 µg/mL, and 1 µg/mL.

DNA-Binding experiments

The UV-Visible spectral titrations were carried out in Tris–HCl/NaCl buffer at room temperature to investigate the binding affinity between CT-DNA and the Schiff base. The UV-Vis absorbance at 260 and 280 nm of CT-DNA solution in Tris buffer gives a ratio of 1.8–1.9, indicating that the DNA was sufficiently free of the protein (31). Tris–HCl/NaCl buffer (1.8 mL) and the solutions of Schiff base (1.8 mL, 1×10^{-5} M) were respectively placed into two cuvettes. Then one aliquot (5.4 µL, 0.01 M) of buffered CT-DNA solution was added to each cuvette in order to eliminate the absorbance of DNA itself. Before the absorption spectra were recorded, the Schiff base-DNA solutions were incubated at room temperature for 5 min.

DNA Cleavage experiments

The DNA cleavage activity of the Schiff base was studied by agarose gel electrophoresis method. pBR322 DNA (0.1 µg µL^{-1}) in Tris-HCl buffer (10 µM, pH:7,2) treated with the compound at 37 °C for 3 h. To determine the mechanism of cleavage activity H_2O_2 was added to one of the group of mixture as an oxidizing agent. After incubation loading buffer was added and samples were subjected to electrophoresis for 1 hour at 60 V on 1% agarose gel in TBE (Tris-Boric acid-EDTA, pH:8.0) buffer. Then, the bands were visualized by UV light and photographed.

Anion Sensors Measurements

The Schiff base (0.05 µmol) was dissolved in DMSO (50 mL). Tetrabutylammonium salts(F$^-$, Br$^-$, I$^-$, CN$^-$, SCN$^-$, ClO_4^-, HSO_4^-, CH_3COO^-, $H_2PO_4^-$, OH$^-$) (0.05 µmol) were dissolved in DMSO (50 mL). Each solution of tetrabutylammonium salts was added to the Schiff base solution (1:1) in the tube. After mixing them, UV absorption spectra and the photos were taken at room temperature under daylight and UV light (λ= 365 nm).

RESULTS AND DISCUSSION

Spectroscopic Studies

FT-IR studies

The IR spectrum of the Schiff base is given in Figure 1. The vibration bands with the wavenumbers (ν, cm^{-1}) of 3291 (Ar-OH), 3077 (Ar-H), 2925 (aliph-H), 1598 (C=N + C=C), 1558 (C=C), 1497 (C-N), 1346 (C-O) and 1262-1220-1169-1050 cm^{-1} (Ar-O-CH_3) were observed in the spectrum. Also, the C=N and C=C peaks overlap at 1598 cm^{-1} in the spectrum (Figure 1). Due to the intramolecular hydrogen bond O-H...N, a peak at 2828 cm^{-1} was observed in the spectrum of the compound. The presence of a strong band at 1346 cm^{-1} in the Schiff base indicates that the compound is in phenol-imine form due to the stabilization of the phenolic C-O bond. Very strong etheric ArC-O-C vibrations were observed between 1262 cm^{-1} and 1050 cm^{-1} for the compound.

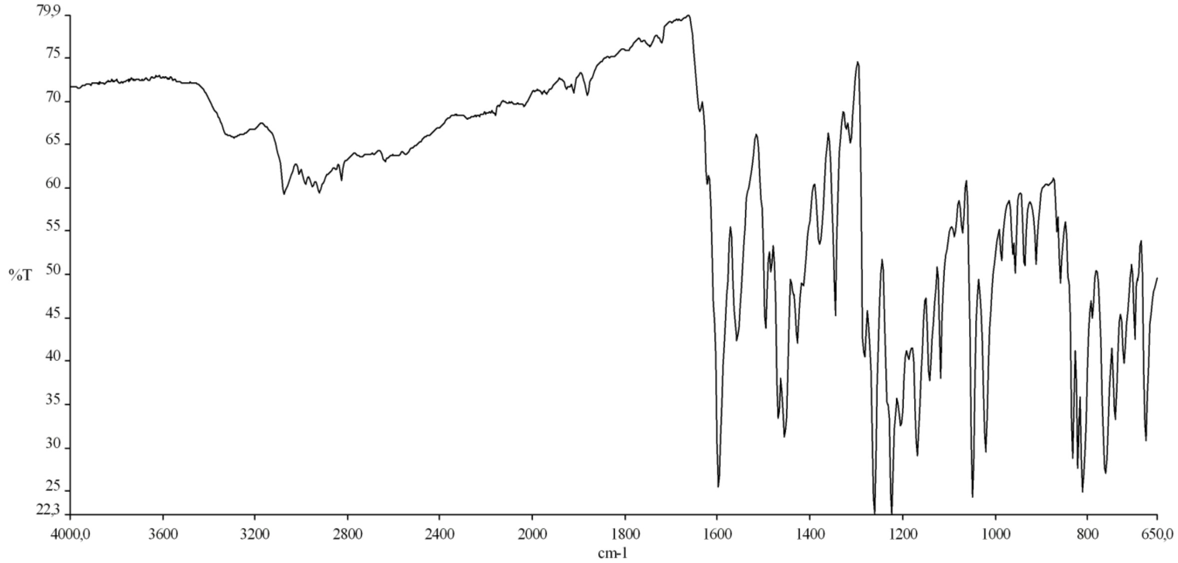


Figure 1. FTIR spectrum of the Schiff base.

^{1}H- and ^{13}-CNMR spectroscopy

The NMR spectrum of the compound is given in Figures 2 and 3. According to the ^{1}H NMR results, the Schiff base has phenol-imine form in DMSO solution (Fig. 2). In the NMR spectrum of the compound, a singlet for the $OC\underline{H_3}$ protons, and multiplets for the Ar-H protons are observed. In the compound, the $O\underline{H}$ and $-C\underline{H}=N-$ protons are observed as a singlet. It can be said that the compound is in phenol-imine form in DMSO since the imine ($-C\underline{H}=N-$) and hydroxyl ($O\underline{H}$) protons do not split. From the ^{13}C NMR spectrum, it appears that the compound has 15 signals (Figure 3). When the chemical shift values are ordered from highest to lowest, they are N-$\underline{C}$(S)-, -$\underline{C}$H=N, Ar$\underline{C}$-OH, Ar$\underline{C}$-OCH_3, Ar-$\underline{C}$, and $O\underline{C}H_3$, respectively, for the compound. According to the NMR results, the compound is in the phenol-imine form in DMSO solution.

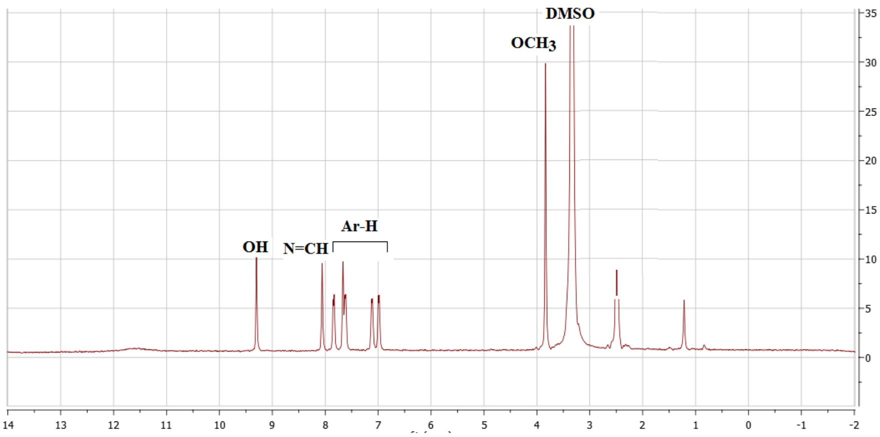


Figure 2. ^{1}H-NMR spectra of the Schiff base.

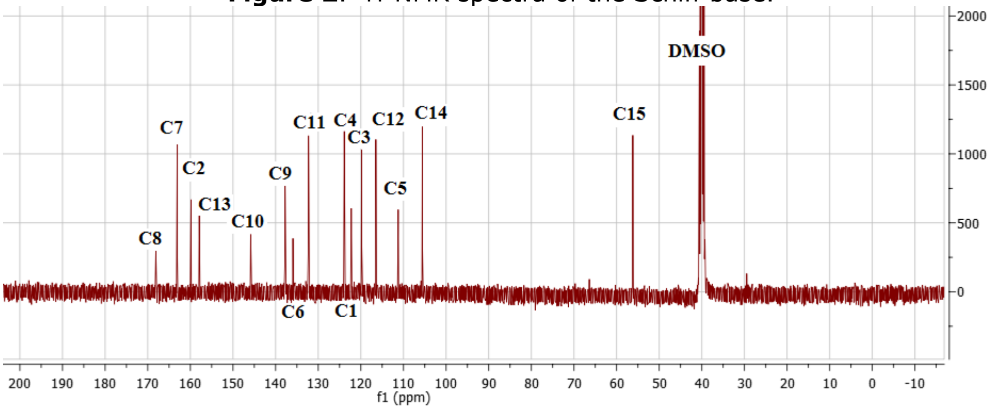


Figure 3. ^{13}C-NMR spectra of the Schiff base.

UV-Vis spectroscopy

In this study, the electronic spectrum of the compound was also investigated in dimethyl sulfoxide (Figure 4). Some imines give absorption bands in both polar and non-polar solvents greater than 400 nm. It was reported that the new band in 2-hydroxy imines belongs to the keto-amine form in polar and non-polar solvents in both acidic and basic solutions. A new peak was not observed after 400 nm in DMSO for the Schiff base. Hence, the compound has phenol-imine form in DMSO. According to the UV-Vis results of the compound, only a shoulder and a band appear at 276 nm and 399 nm, which are assigned to the $\pi-\pi^*$ and $n-\pi^*$ transition of the C=C and C=N.

As a result, the compound was in the phenol-imine form according to the FTIR, UV-Vis, ^{13}C-NMR and ^{1}H-NMR data.

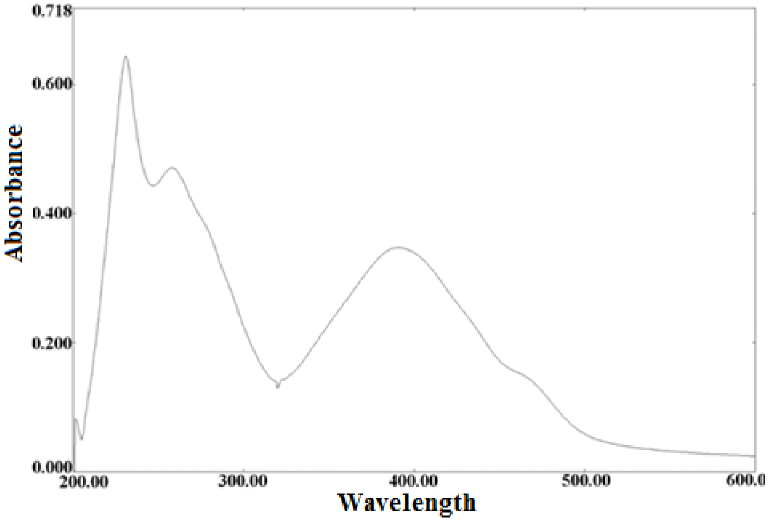


Figure 4. UV-Vis spectrum of the Schiff base.

Colorimetric anion sensing of the compound

For the colorimetric anion sensor properties of the Schiff base, the color of the solution formed by the addition of anions to the DMSO solution containing the Schiff base was photographed and is shown in Figure 5.

As shown in the photographs taken under two different lights, the color of the compound changes with the addition of F^-, CN^-, AcO^-, $H_2PO_4^-$ and OH^- anions to the sensor compound (Figures 5a; under day light, 5b and 5c; under UV lamp). In daylight, the observed color changes were from yellow to red for F^- and CN^-, dark orange for AcO^- and OH^-, and to light orange for $H_2PO_4^-$ (Fig. 5a). Significant fluorescence changes were observed under long-wave UV lamp for anions (Figure 5b). The color of the anion solutions is light-red for F^- and CN^-, and it is light-orange for AcO^-, $H_2PO_4^-$ and OH^-. There were more pronounced fluorescence changes under the shortwave UV lamp. In the solutions, turquoise and light-red were observed for F^-, AcO^-, $H_2PO_4^-$ and OH^-, and CN^-, respectively. Intensive color change is formed by the addition of anions such as F^-, CN^-, AcO^-, $H_2PO_4^-$ and OH^-. This shows that there is strong binding between these selective ions and the Schiff base. For the above anions, the sensor compound gave a visible color change under both the short wave and the long wave UV lamp. There is no visible color or fluorescence change for all other less basic anions (Br^-, I^-, SCN^-, ClO_4^- and HSO_4^-) studied under both daylight and UV lamp. Accordingly, we can say that there is very weak binding or no binding between these anions and the Schiff base.

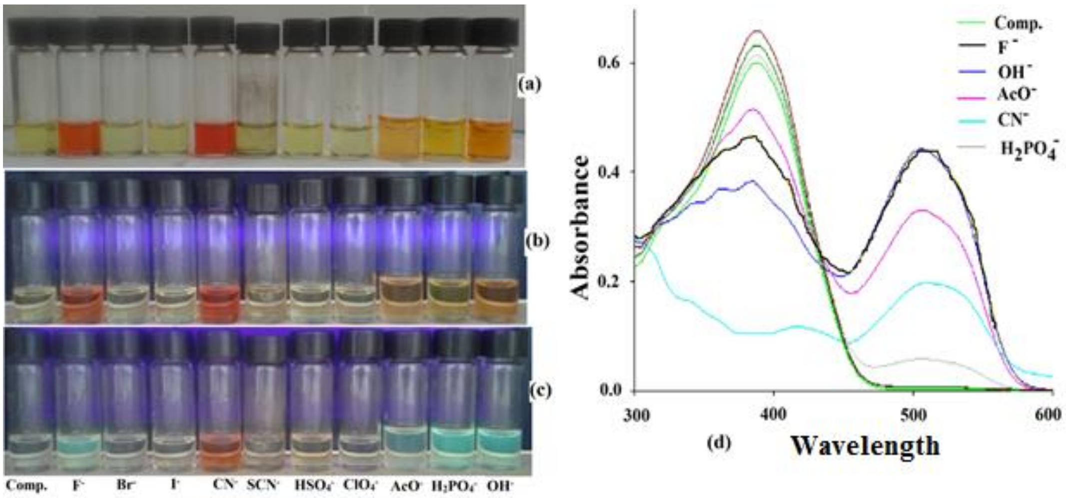


Figure 5. a) Color changes of compound (50 μM) under normal light, **b**) hand held UV lamp (λ=365 nm), **c**) hand held UV lamp (λ=254 nm), d) UV-Vis absorption spectra upon the addition of 1.0 equivalence of various anions in DMSO.

The ability of Schiff base (50 μM) to detect F^-, CN^-, AcO^-, $H_2PO_4^-$ and OH^- anions was studied using UV-Visible spectrophotometry in DMSO as shown in Figure 5c. A band at 385 nm in the UV-Vis spectrum for the Schiff base was observed before the addition of the selective anions. When the F^-, CN^-, AcO^-, $H_2PO_4^-$ and OH^- anions were gradually added, the intensity of the band decreased at 385 nm and a new band was formed at 505 nm. In 2-hydroxy Schiff bases, the absorption band at > 400 nm was reported to belong to the ketamine form (32). In this case, the keto-amine form occurs because the H atom from the O-H group is transferred to the N atom of the CH=N group. This resulted in a change of color from yellow to red and orange. This band was probably due to the intramolecular charge transfer of electrons, which are resultant phenol protons and the anion interactions (16-20). All the other anions (Br^-, HSO_4^-, Cl^- and ClO_4^-) did not exhibit any discernible changes (15-20). UV-Vis results show that the Schiff base exhibits excellent selectivity for F^-, CN^-, AcO^-, $H_2PO_4^-$ and OH^- anions in the presence of other anions and can be useful in practical applications. Furthermore, the anion-binding capacity of the compound was found to be $F^- > OH^- > AcO^- > CN^- > H_2PO_4^-$ using a UV-Visible spectrophotometer, respectively.

In our previous study, while the salicylaldehyde-based sensor did not show selectivity against phosphate anion (17), the 5-bromosalicylaldehyde-based sensor showed selectivity against phosphate in this study. In this study, addition of phosphate anion to the DMSO solution of the 5-bromosalicylaldehyde-based sensor resulted in a rapid color change from yellow to light orange with an accompanying new band appearing at 505 nm in the absorption profile. The title compound showed chromogenic and fluorogenic sensor properties against phosphate anion. In addition, for the same anions under both daylight and UV lamp, each sensor gave a different solution color. Consequently, it can be said that the substituted bromine in the title compound affects the anion selectivity.

These results demonstrate that the receptor title Schiff base exhibits good selectivity for F^-, OH^-, AcO^-, CN^- and $H_2PO_4^-$ anions in the presence of other anions and is useful in practical applications.

Minimum Inhibitory Concentration (MIC)

Minimum inhibition concentration (MIC) was determined with broth microdilution test. The average of three experimental results is given in Table 1. The antimicrobial activity spectrum of the

Schiff base showed great diversity. As can be seen from Table 1, it can be said that Schiff base antimicrobial activity results have a similar effect against bacteria and yeast. This compound showed activity against the tested microorganisms. Surprisingly, the compound exhibits similar activity, although Gram-positive, Gram-negative and yeast cell walls are very different.

Table 1. MIC (μg/mL) of the compound.

Microorganisms	Compound	Antibiotic	
		Ampicillin	Fluconazole
***S. aureus* ATCC 25923**	256	5	-
***E. faecalis* ATCC 29212**	128	8	-
***E. coli* ATCC 25922**	128	-	-
***P. aeruginosa* ATCC 254992**	256	5	-
***C. albicans* ATCC 60193**	128	-	128
***C. tropicalis* ATCC 13803**	128	-	64

Interaction of Schiff base and DNA

The effect of Schiff base on CT-DNA was investigated using electronic spectroscopy. With the increase in the concentration of CT-DNA, decreases of 18.89-56.26% and 13.50-20.00%, and higher wavelength of 1-37 nm and 1-3 nm are observed at 258 nm and 390 nm (Figure 6). Furthermore, the red shift of maximum absorption shows the energy decrease in energy between the HOMO and LUMO. This indicates that DNA interacts with the Schiff base (14-20). If the DNA interacts with the Schiff base, п-п* transition energy decreases, and the absorption shifts to red. The decrease, increase and shift long wave of absorption in the UV absorption spectra show that the Schiff base interacts with CT-DNA, presumably because of stacking of the interaction between an aromatic ring and the base pairs of DNA (14-20). According to the UV-Vis results, the compound binds to CT-DNA intercalatively.

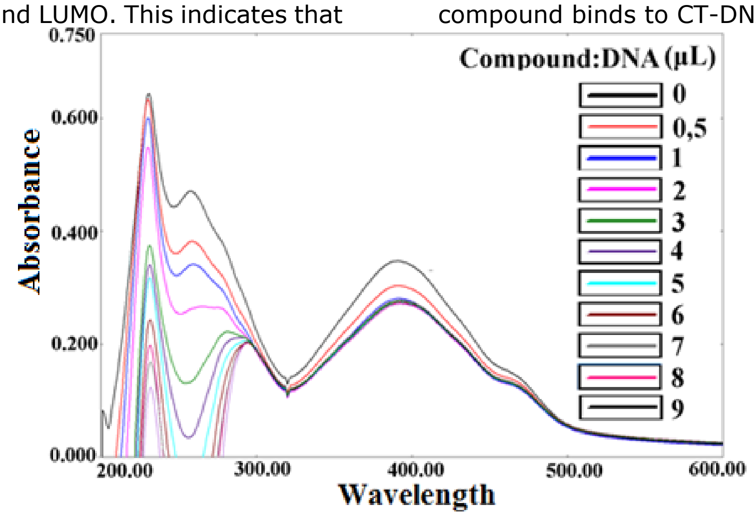


Figure 6. Absorption spectra of compound in the absence and presence of increasing amounts of CT-DNA at room temperature in Tris–HCl/NaCl buffer (pH 7.2).

DNA-Cleavage activity

The cleavage of DNA is determined with the agarose gel electrophoresis method. Supercoiled (SC) plasmid pBR322 DNA was used in the study. Cleavage experiments were performed by adding Schiff base at concentrations ranging from 5 μM to 400 μM in TAE buffer.

If the DNA interacts with the Schiff base, SC DNA form is degraded to nicked circular (NC) form. According to the result of the cleavage assay, it is seen that the SC form of DNA is preserved (Fig.7). Consequently, the Schiff base which is both hydrolytic and oxidative does not show any cleavage of SC DNA (Figure 7 a: hydrolytic b: oxidative).

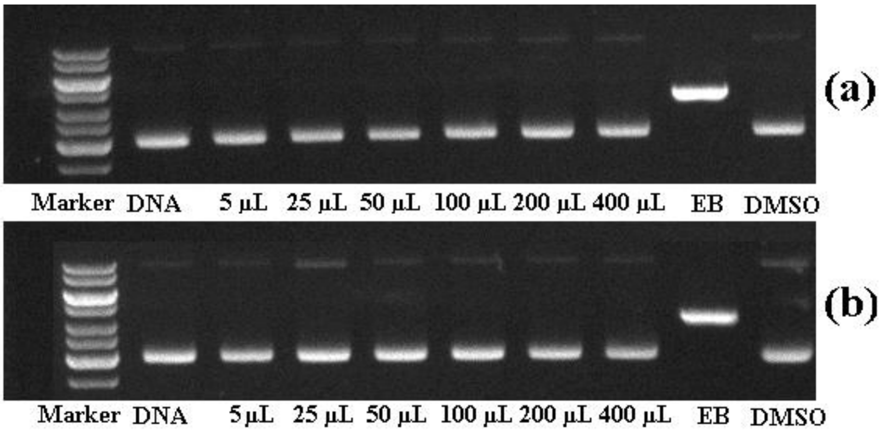


Figure 7. Agarose gel electrophoresis patterns for the hydrolytic cleavage of pBR322 DNA by Schiff base. DNA: Deoxyribonucleic acid; µL: Microliters; EB: Ethidium bromide; DMSO: Dimethyl sulfoxide.

CONCLUSIONS

In this work, a benzothiazole-based Schiff base was synthesized and its structure was elucidated. In addition, biological activity and chemical sensor applications were investigated. Minimum inhibitory concentration (MIC) against yeast and bacteria was determined. Interactions with DNA were studied. It was found that it binds interactively to DNA, but it does not cleave DNA. The sensor properties were tested against anions. The Schiff base was found to be selective for fluoride, cyanide, acetate, dihydrogen phosphate and hydroxyl anions in DMSO. A color change was observed for these anions, but not for the other anions. As a result of all these studies, it was found that it could be used as an agent for DNA and anions.

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