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Synthesis, Structural Characterization and Investigation of DNA/BSA Binding Properties of a Homo-disulphide Schiff Base Compound Carrying Oxo Propargyl Group

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ABSTRACT

In this work, a new homo-disulphide Schiff base compound (HDSB) was prepared and its structure was characterised by common spectroscopic and analytical methods. The compound was obatined from the condensation reaction of 2-aminothiophenol and 2-hydroxy-4-(prop-2-yn-1-yloxy)benzaldehyde in benzene. In the reaction, both Schiff base condensation and oxidation of thiols into disulphide formed. The isolated compound was structurally characterized by single crystal X-ray diffraction experiment. The homo-disulphide Schiff base compound (HDSB) was screened for its DNA/BSA binding properties using UV-Vis absorption and emission spectral studies. The compound showed considerable binding affinity to double-stranded fish sperm DNA (FSds-DNA) with binding constant of 4.1×10^4 M^{-1}. Spectral measurements suggest that HDSB interacts with DNA in a minor groove binding mode. The compound also showed binding properties towards BSA (bovine serum albumin). The incremental addition of HDSB to the BSA solution resulted in a significant decrease in the characteristic emission band of BSA in the range of 320-500 nm (λ_{exc}: 280 nm) showing the binding interactions between HDSB and BSA.

Keywords: Homo-disulphide Schiff base, Structural characterization, DNA/BSA binding, Spectroscopy.

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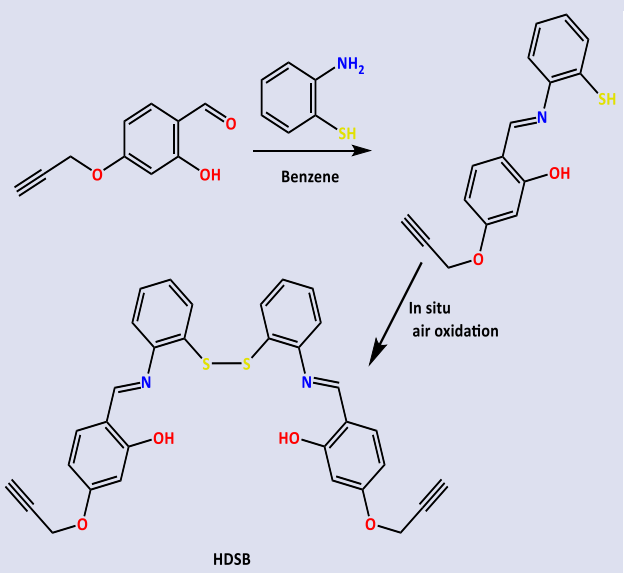
Introduction

In recent years, promising studies on drugs targeting tumors, arthritis, diabetes and neurological disorders have been conducted [1,2]. By studying the factors that affect the biological activities, molecules with specific properties as drugs are designed and their activities are examined [3]. The serious side effects of metal-based chemotherapy agents such as cisplatin and their limited use in some cancer types have led scientists to use organic compounds as anticancer agents [3]. Since cisplatin-based compounds show antitumor activity by binding to DNA, small organic or metal-organic molecules targeting DNA have been synthesized [4]. Examining the interactions of small molecules with DNA has created an important field of study. Molecules can bind to DNA by non-covalent interactions such as electrostatic, groove, intercalative, and partial intercalative bonding [5]. Schiff bases, also known as imine compounds, are compounds with a wide variety of biological activity such as antibacterial, antifungal, herbicide, anti-inflammatory, anticancer, anti-diabetic and antitumor activity [6, 7]. On the other hand, it has been reported that Schiff base compounds of the oxo-propargyl group increase biological activity [8, 9].

Synthesis and structural studies of Schiff bases containing disulphide groups attract attention due to their chelating properties, electron transfer abilities and biological properties [10,11]. Disulphides formed by oxidative dimerization of thiols attract attention in organic chemistry and biochemistry, and various oxidizers are used for this conversion [12–15]. It has been reported that the thiol to disulphide conversion can be done under different experimental conditions [16]. Various Schiff bases containing disulphide groups were synthesized and their biological properties and chemosensor properties were investigated [11,17–20]. In this study, a new homo-disulphide compound HDSB (Scheme 1) was synthesized from the reaction of the salicylaldehyde compound carrying the oxo propargyl group and 2-aminothiophenol.



Scheme 1. Synthesis reaction of homo-disulphide Schiff base compound (HDSB)

The structure of the synthesized compound HDSB was characterized by FTIR, 1H NMR and elemental analysis methods. In addition, the crystal structure of the compound was elucidated by single crystal X-ray diffraction study. DNA and BSA binding properties of the synthesized homo-disulphide compound were investigated by spectrophotometric methods.

Materials and Methods

All reagents and solvents were obtained from commercial sources (Aldrich or Merck). The starting 2-hydroxy-4-(prop-2-yn-1-yloxy)benzaldehyde was prepared according to the reported procedure [8, 21]. The structural characterization data are provided in the supplementary documents. Elemental analyses (C, H and N) were performed using a LECO CHNS 932. Infrared spectrum was obtained using KBr disc (4000-400 cm^{-1}) on a Perkin Elmer Spectrum 400 FT-IR. The electronic spectra in the 200-900 nm range were obtained on a Perkin Elmer Lambda 45 spectrophotometer. Mass spectra of the ligands were recorded on a LC/MS APCI AGILENT 1100 MSD spectrophotometer. ^{1}H NMR spectrum in $CDCl_3$ was recorded on a Bruker 400 MHz instrument. TMS was used as internal standard.

Synthesis of Homo-disulphide Schiff Base Compound (HDSB)

2-Hydroxy-4-(prop-2-yn-1-yloxy)benzaldehyde (0.88 g, 5 mmol) was dissolved in benzene. 2-amino benzenethiol (0.625 g 5mmol) was added to this solution. The colour turned to yellow with the addition of aldehyde. The mixture was refluxed at 80 ºC for 8 hours. The progress of the reaction was checked by TLC. Upon consumption of starting compounds, the reaction solution was allowed to cool to the room temperature. Yellow needle-like crystals formed were filtered and dried in air.

Molecular Formula: $C_{32}H_{24}N_2O_4S_2$. Molecular weight: 564.65 g/mol. Yield: 85%. Colour: Yellow. E.N.: 119 °C. FTIR (ATR, cm^{-1}): 3274, 3241, 3060, 2857, 2692, 2115, 1606, 1506, 1377, 1339, 1282, 1232, 1188, 1115, 1020, 963, 883, 784, 752, 638, 558. Elemental analyses found (calculated for $C_{32}H_{24}N_2O_4S_2$) %: C, 67.79(68.06); H, 4.13(4.28); N, 4.83(4.96). ^{1}H NMR ($CDCl_3$, ppm) d H: 13.45 (*b*, OH, 2H), 9.05 (*s*, CH=N, 2H), 7.80-6.55 (*m*, $CH_{aromatic}$ 14 H), 4.63 (*s*, OCH_2, 4H), 2.58 (*s*, C≡CH, 2H).

DNA Binding Studies

Absorption spectral measurements

The DNA binding properties of the homo-disulphide compound (HDSB) was studies by UV-Vis spectroscopic measurements. The absorption spectra of HDSB in DMSO (2.0 ×10-5 M) containing Tris-HCl buffer solution (pH = 7.0) were taken in the presence of increasing amount of double-stranded fish sperm DNA (FSds-DNA) at 230-730 nm range. The spectral changes of HDSB in the presence of DNA were taken into account to determine the binding properties.

The absorbance was measured for calculating the percentage of DNA binding using equation given below.

$[DNA]/(\varepsilon a–\varepsilon f) = [DNA]/(\varepsilon b–\varepsilon f) + 1/Kb(\varepsilon b–\varepsilon f)$

In the equation given above, where εa is the apparent extinction coefficient obtained by the calculation of A_{obds}/[Ligands or complexes], εf is the extinction coefficient of the compounds in its free form, εb=extinction coefficient for the compounds in the fully bound form, and [FsdsDNA] is the concentration of dsDNA in terms of base-pairs: K_b indicates the binding constant of the compound with DNA and is calculated from the slope of the line drawn between [DNA]/(εa – εf) and [DNA].

Competitive Binding Studies

Ethidium bromide (EB) is a DNA intercalating agent and it gives a characteristic emission band at 500-700 nm range (λexc = 526) when it binds to the DNA. The replacement of EB in the DNA-EB complex by another molecule results in quenching in the emission band. The quenching of the emission band of the DNA-EB are often referred to the competitive binding of the molecule via intercalation or groove binding. In the emission spectral measurements, to the constant concentration of FSds-DNA (75 µM) solution pre-treated with 5 µM EB in Tris-HCl, increasing amount of the compound (HDSB, 0-100 µM in DMSO) were added. The emission spectra of the solutions were recorded in the wavelength range of 570-750 nm upon irradiation at 526 nm. The quenching of the emission band was followed and the quenching constants (Ksv) was calculated from the Stern-Volmer equation given below:

$$F0=F = 1 + KSV[Q]$$

Where; F0: emission intensity of DNA-EB in the absence of HDSB, F: emission intensity of DNA-EB in the presence of HDSB and [Q]: the total concentration of HDSB.

Bsa Binding Studies

The bovine serum albumin (BSA) properties of the homo-disulphide Schiff base compound (HDSB) was investigated by florescence spectral measurements [22]. The emission spectra of BSA (2.5 µM) solution in Tris-HCl buffer (pH = 7.4) were recorded in the range of 320-500 nm (λexc: 280 nm) upon incremental addition of HDSB (0-100 µM in DMSO). For each measurement, the mixtures were shaken and stands for 20 min at three different temperatures (288, 300 and 310 K). Concentration of BSA was determined by using the molar absorption coefficient of BSA at 279 nm (43824 mol−1Lcm−1). The quenching constant of the emission band of BSA in the presence of HDSB was calculated using Stern-Volmer equation (F0/F) versus log [Q].

X-ray crystallography

Single crystal X-ray crystallographic data for the homo-disulphide Schiff base compounds (HDSB) were recorded at 293(2) K on a Bruker APEX 2 CCD diffractometer using Mo- Kα radiation (λ= 0.71073 Å). Data reduction was performed using Bruker SAINT [23]. SHELXT was used to solve and SHELXL to refine the structure [24, 25]. The structure of the compound was solved by direct method and refined on F^2 using all the reflections. The hydrogen atoms bonded to carbon and oxygen atoms were inserted at calculated positions using a riding model.

Results and Discussion

Chemistry

The reactions between the 2-aminothiophenol and salicylaldehyde derivatives results in thiol Schiff base compounds. However, thiol Schiff base compounds are often susceptible to air oxidation that usually gave homo-disulphide Schiff base derivatives [26]. Moreover, it was reported that solvents can induce air oxidation of thiophenols into and homo-disulphides [27]. A speculated mechanism of conversion of the thiol Schiff bases to their homo-disulphide derivatives through reaction with oxygen have been proposed [26]. In this study, a homo-disulphide Schiff base compound (HDSB) was directly prepared by the condensation reaction of 2-aminothiophenol and 2-hydroxy-4-(prop-2-yn-1-yloxy)benzaldehyde in benzene. In the reaction, both Schiff base condensation and oxidation of thiols into disulphide occurred. The yellow-coloured homo-disulphide Schiff base compound (HDSB) is soluble in MeOH, EtOH, CHCl3, diethyl ether, THF, DMF and DMSO. The structure of the compound was characterized by common spectroscopic and analytical methods (FTIR, 1H NMR and elemental analysis). Furthermore, the crystal structure of the compound was also examined by single crystal X-ray diffraction study. The FTIR spectrum of the compound was carried out and spectral data are given in the experimental section. The spectrum of the compound is shown in Figure 1. The FTIR spectrum of the compound showed peaks at 3274, 3060 and 2857 cm-1 due to the n(C-H) stretching frequencies. In the spectrum, a relatively weak band at 3400-3500 cm-1 range is due to the phenolic group stretching's n(O-H). The thiol Schiff base compounds show the n(S-H) stretching frequency at 2500-2600 cm-1 range[28]. In the spectrum of the synthesized compound, no peak due to the n(S-H) group stretching was observed and this is indicative of a stoichiometric formation of homo-disulphide Schiff base compound in the reaction [29]. Moreover, a weaker peak at 553 cm^{-1} due to the ν(S-S) stretching frequency confirms the homo-disulphide structure. In the spectrum of the compound, a relatively weaker peak at 2115 cm^{-1} can be assigned to the alkyne group ν(C≡N) on the phenol ring [30]. The IR spectrum of the compound displayed a strong peak at 1606 cm^{-1} and this peak is assigned to the which could be due to ν(C=N) stretching frequency. The FTIR spectral data of the homo-disulphide compound are similar to those of similar homo-disulphide Schiff base compounds reported in literature [12].

The ^{1}H NMR spectrum of the compound was recorded in $CDCl_3$ and the obtained data are presented in the experimental section. The spectrum of the compound displayed a signal at 13.45 ppm due to the phenolic OH protons. The presence of the signal due to the phenolic OH is indicative of enolic structure in solution. The azomethine proton (HC=N) resonance appears as a singlet at 9.05 ppm. The aromatic protons of the compounds were observed as multiplets at 7.80-6.55 ppm range. In addition, sharp signals at 4.63 and 2.54 ppm are assigned to the O-CH_2 and terminal acetylenic C≡CH protons. Integration values in the spectrum is in well agreement with the proposed structure.

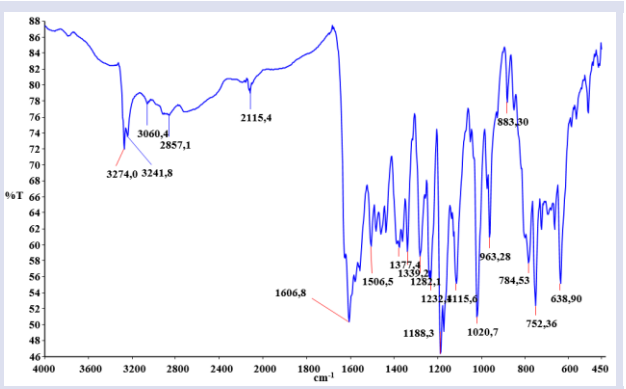


Figure 1. FTIR spectrum of the homo-disulphide Schiff base compound (HDSB)

Molecular Structure of Homo-Disulphide Schiff Base Compound (Hdsb)

Single crystals suitable for X-ray diffraction studies were obtained by recrystallization of the compound from chloroform solution. The definite structure of the compound was obtained from X-ray diffraction study. The X-ray refinement values and other crystallographic data obtained from X-ray diffraction studies of the compound are presented in Table 1. The structure of the compound was solved in the triclinic unit cell and P-1 space group. Molecular structure of the compound obtained from X-ray study is given in Figure 2. The disulphide bond (S-S) in the molecule form by the dimerization of two identical thiol units. The S1-S2 disulphide bond in dimeric molecule has a distance of 2.0215(10) Å (Table 2), which is very close to the S-S single bond distance observed in reported similar structures [12]. The N1-C10 and N2-C23 imine bond distances are 1.271(3) and 1.282(3) Å, respectively, and these distances are within the expected C=N double bond distance [31]. In addition, O2-C6 and O3-C25 distances have characteristic C-O single bonds. The propargyl groups (C1-C2-C3 and C30-C31-C32) in the phenolic rings in the compound have an approximate linear geometry. The bond lengths of the C1-C2 and C31-C32 alkyne groups in the propargyl group are 1.160(4) and 1.153(4) Å, respectively, showing a C≡C triple bond character. The phenolic groups (O2H and O3H) in the compound made intramolecular hydrogen bonds (O2-H···· N1 and O3-H····

N2) with the imine bond nitrogen atoms (N1 and N2). In addition, phenolic groups interacted weakly with the sulphur atoms (O2-H·····S1 and O3-H·····S2) in the disulphide bond.

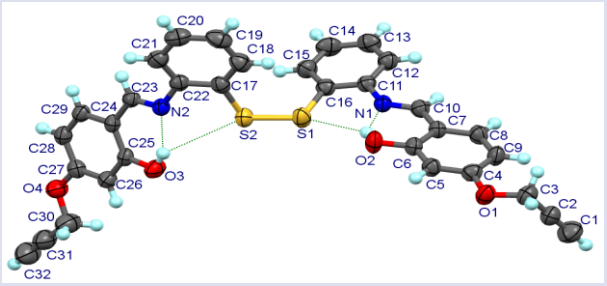


Figure 2. Molecular structure of the compound with atom numbering (thermal ellipsoid 50% probability). Hydrogen bonds are shown as dashed lines.

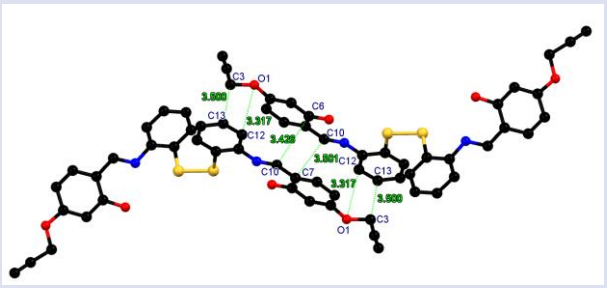


Figure 3. π-π interactions in the structure of the compound

When the data obtained from the X-ray diffraction studies of the compound were examined, it was determined that there were repeated π-π interactions between the molecules. One edge of the phenol and benzene rings in the compound formed head-tail type π-π interactions with the same edge in the neighbouring molecule (Figure 3). In addition, weak intermolecular C-H····O, C-H····S and C-H····π interactions ensured the stability of the crystal lattice. The packing diagram of the molecule showing the π-π stacking interactions is given in Figure 4.

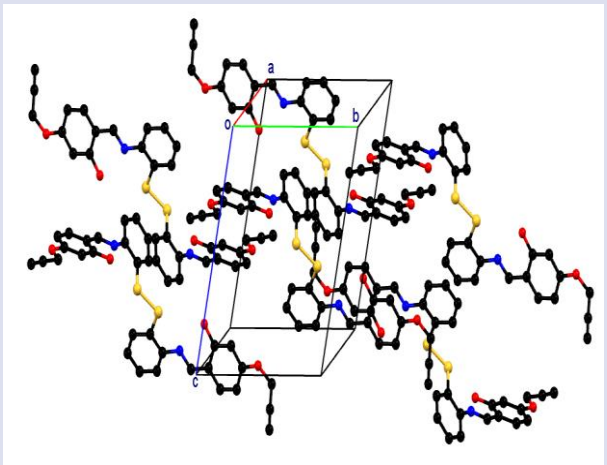


Figure 4. Packing diagram of HDSB

Table 1. Single crystal X-ray crystallographic data for the compound

Molecular formula	**$C_{32}H_{24}N_2O_4S_2$**
Molecular weight (g/mol)	564.65
Temperature/K	298.0
Crystal system	Triclinic
Space group	P-1
a/Å	10.8703(11)
b/Å	12.2621(11)
c/Å	13.2290(9)
α/°	107.894(7)
β/°	91.944(7)
γ/°	115.300(9)
Volume/Å^3	1488.8(3)
Z	2
Crystal size/mm^3	0.15 × 0.13 × 0.11
Irradiation	Mo-Kα (λ = 0.71073)
Refl. collected	12448
Independent refl.	6550 [R_{int} = 0.0317, R_{sigma} = 0.0644]
Final R indexes [I>=2σ (I)]	R_1 = 0.0521, wR_2 = 0.1191
Final R indexes [all data]	R_1 = 0.0953, wR_2 = 0.1426
CCDC	2171343

Table 2. Bond distances for the Schiff base compound (Å)

S(1)-S(2)	**2.0215(10)**	**C(4)-C(5)**	**1.382(3)**	**C(18)-C(19)**	**1.373(4)**
S(1)-C(16)	1.778(2)	C(4)-C(9)	1.392(3)	C(19)-C(20)	1.383(4)
S(2)-C(17)	1.780(3)	C(5)-C(6)	1.370(3)	C(20)-C(21)	1.386(4)
O(1)-C(3)	1.426(3)	C(6)-C(7)	1.417(3)	C(21)-C(22)	1.385(3)
O(1)-C(4)	1.367(3)	C(7)-C(8)	1.389(3)	C(23)-C(24)	1.450(3)
O(2)-C(6)	1.343(3)	C(7)-C(10)	1.446(3)	C(24)-C(25)	1.398(3)
O(3)-C(25)	1.342(3)	C(8)-C(9)	1.370(3)	C(24)-C(29)	1.399(3)
O(4)-C(27)	1.369(3)	C(11)-C(12)	1.391(3)	C(25)-C(26)	1.383(3)
O(4)-C(30)	1.405(3)	C(11)-C(16)	1.391(3)	C(26)-C(27)	1.379(3)
N(1)-C(10)	1.271(3)	C(12)-C(13)	1.374(4)	C(27)-C(28)	1.388(3)
N(1)-C(11)	1.413(3)	C(13)-C(14)	1.376(4)	C(28)-C(29)	1.369(3)
N(2)-C(22)	1.412(3)	C(14)-C(15)	1.379(3)	C(30)-C(31)	1.466(4)
N(2)-C(23)	1.282(3)	C(15)-C(16)	1.385(3)	C(31)-C(32)	1.153(4)
C(1)-C(2)	1.160(4)	C(17)-C(18)	1.378(3)		
C(2)-C(3)	1.463(4)	C(17)-C(22)	1.402(3)		

UV-Vis Absorption and Photoluminescence Properties

The UV-Vis absorption and emission properties of the compounds were investigated in solution medium. The effect of the solvent on the absorption and emission properties were examined in different solvents (diethyl ether, chloroform, methanol and dimethyl sulfoxide). The absorption spectra and emission spectra of the compound are given in **Figure 5**. In methanol, the compound shows two well separated absorption bands in the range of 260-420 nm. The first band at 260-300 nm range (λ_{max}: 287 nm) can assigned to the π-π* electronic transition due to the π-electrons in the structure of the compound. The latter band with higher absorbance values was seen at 306-420 nm range (λ_{max}: 341 nm) and this electronic absorption were assigned to the π-π* and n-π* transitions. Two separated absorption bands were preserved when the solvent was changed. However, depending on solvents,

the absorption values and position of the bands showed some shifts. The absorption bands of the compound showed bathochromic shifts in chloroform and diethyl ether. Moreover, the absorption values also increased (hyperchromic effect). The bathochromic shifts in low polar solvents (chloroform and diethyl ether) showed the interactions of the solvent with apolar groups of the compound. In DMSO, the absorption bands were also shifted longer wavelengths. The photoluminescence properties of the compound in the solutions were also studied. The solution of the compound was excited with the maximum absorption wavelength. The compound exhibited emission band at 350-550 nm range. In dimethyl sulfoxide and methanol, the compound showed an emission band at 350-500 nm range (λ_{exc}: 341 nm for dimethyl sulfoxide and 335 nm for methanol). In chloroform and diethyl ether, the compound exhibited dramatically different emission properties. In diethyl ether, the compound showed dual emission when irradiated at 345 nm, two emission bands were observed at 350-600 nm range. The first band with lower emission intensity at 350-380 nm range (λ_{max}: 365 nm) is narrow. The second band was broad and observed at 380-600 nm range. In chloroform solution, the compound showed similar emission characteristic to that of diethyl ether solution.

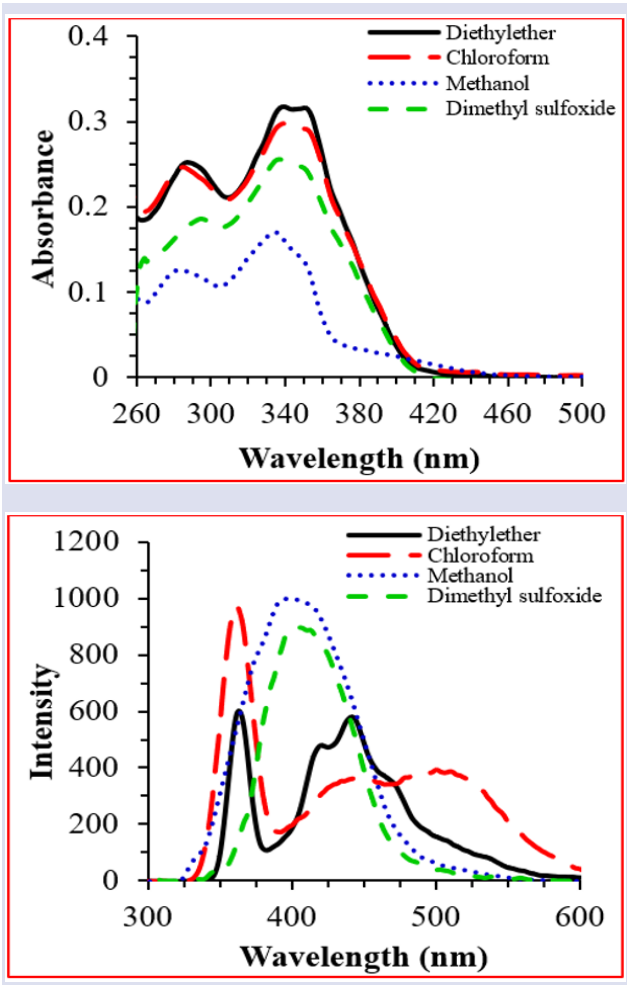


Figure 5. Absorption and emission spectra of the HDSB in different solvents (10^{-5} M)

Dna Binding Properties

DNA-targeting drugs are of great interest because the cause of many types of cancer is associated with DNA damage. There are many metal-organic and organic structures interacting with DNA in the literature [7]. DNA-targeted molecules interact with DNA in three modes (intercalation between base pairs, groove bonding, and electrostatic interactions). In order to examine the interactions between homo-disulphide compound and dsDNA synthesized within the scope of this study, UV-Vis spectra were obtained by adding increasing concentrations of FS-dsDNA to the compound solution. The UV-Vis absorption spectra obtained at increasing DNA concentrations of the compound are given in Figure 6. The interaction of small molecules with DNA usually results in a change in the UV-Vis absorption spectrum (hyperchromic or hypochromic effect and red or blue shift). The synthesized homo-disulphide compound showed two absorption bands of π-π* and n-π* electronic transitions in the 230-550 nm range. Addition of DNA at increasing concentrations (constant compound concentration) caused shifts in the absorption bands of the compound and decreased absorbance values. While no significant change was observed in the maximum absorption wavelength in the 230-300 nm range in the spectrum of the compound, the absorbance values gradually decreased. On the other hand, with increasing DNA addition, a noticeable red shift was observed in the absorption band observed in the 330-530 nm range. In addition, the addition of DNA caused a gradual decrease in the absorption values of this band. The DNA binding constant (K_b) of the HDSB compound was calculated taking into account the change in the absorption band (with the addition of DNA) observed in the 330-530 nm range [32]. The compound had a DNA binding constant of 4.1×10^4 M^{-1} and showed lower DNA binding affinity than ethidium bromide, a DNA intercalating molecule ($K_b = 1.4 \times 10^6$ M^{-1}). It is thought that HDSB compound synthesized according to this obtained binding constant value interacts with DNA in minor groove binding mode. Also, the DNA binding constant of HDSB compound is within the range of minor groove binding agents reported in the literature [33].

In order to further investigate the DNA binding mode of the synthesized homo-disulphide Schiff base compound, competitive DNA binding studies were carried out with ethidium bromide (EB). As it is known, EB is a DNA binding agent and interacts with DNA by intercalating. The EB molecule is inserted between the DNA base pairs. The DNA-EB complex formed by the interaction of EB with DNA creates a characteristic emission in the range of 550-800 nm when excited at 526 nm [34]. In the presence of a second molecule that can intercalate with DNA close to or better than EB, a competition for DNA binding is expected. The decrease in the intensity of the emission band formed by the DNA-EB complex is generally attributed to the release of DNA-bound EB from the DNA-EtBr complex, the excited state energy transfer, or the conformational change of DNA [35]. The emission spectrum obtained by

adding increasing concentrations of the synthesized compound (HDSB) to the solution containing DNA-EB is shown in Figure 7.

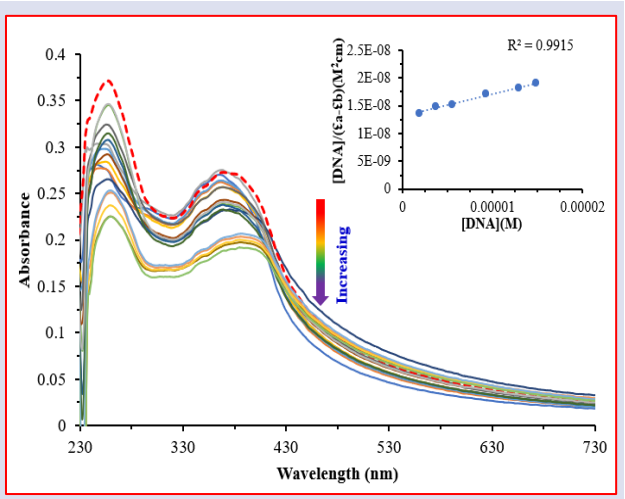


Figure 6 Absorption spectra of HDSB, in 2 mM Tris–HCl/2 mM NaCl buffer at pH 7.1 upon the addition of FSdsDNA. Inset: plot of [DNA]/Ɛa–Ɛf) vs. [DNA] in for the titration of FSdsDNA with HDSB (0-100 µM)

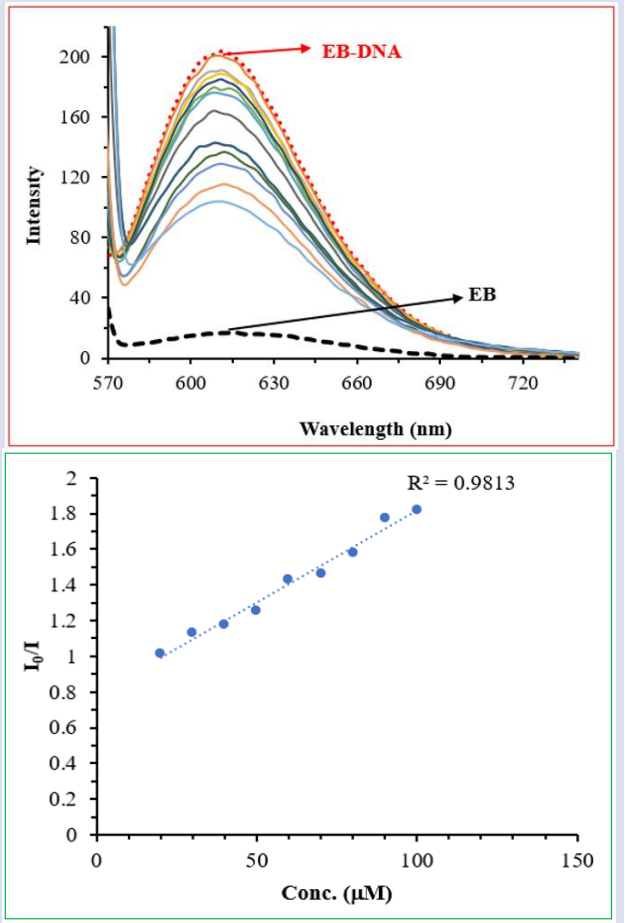


Figure 7. The emission spectra of the FSdsDNA-EB complex (75 µM) in the presence of various concentrations (0-100 µM) of HDSB in 2 mM Tris-HCl buffer (pH 7.1). Stern-Volmer plot of fluorescence titrations of HDSB with FSdsDNA. (λ_{exc}: 526 nm).

As seen in Figure 7, DNA-EB emission intensity decreased with HDSB addition and showed fluorescence quenching effect. This decrease observed in the fluorescence spectrum does not clearly show the intercalation of the compound with DNA, but confirms that it exhibits significant interaction with DNA. The slope of the *Io*/*I* versus [concentration] graph obtained from the Stern-Volmer equation gives the quenching constant K_{SV}. The nonlinearity of the plot of *Io*/*I* versus [concentration] suggests that emission quantification occurs through both dynamic and static damping mechanisms [36]. The fact that HDSB compound showed almost linear damping effect in the range of 20-100 µM indicates that the damping mechanism is static [36]. The K_{SV} value for the compound calculated from the Stern-Volmer equation is 1.02×10^4 M^{-1}, which is close to some compounds that competitively bind to DNA with ethidium bromide.

Bsa Binding Properties

In addition to many important physiological functions of biomolecules such as serum albumins, they have very important roles in the transport and metabolism of many endogenous and exogenous compounds in metabolism. Due to its structural similarity to human serum albumin, bovine serum albumin (BSA) is the most studied protein for the investigation drug-protein interactions. Fluorescence spectroscopy is one of the most commonly used methods to investigate the interaction of small molecules with proteins. BSA is a fluorescent due to the presence of the amino acid residues such as phenylalanine, tyrosine and tryptophan. The interaction of BSA with small molecules generally results in a reduction in emission intensity (fluorescence quenching) [37]. BSA solution shows an emission band in the range of 320-400 nm when irradiated at 280 nm. The gradual addition of synthesized homo-disulphide Schiff base (HDSB) to the BSA solution causes an obvious decline in the emission band (Figure 8) showing the interactions of HDSB with BSA. With the increase of HDSB, a linear decrease in the emission intensity of BSA was observed. On the other hand, in the presence of HDSB, a new emission band appeared at 420-500 nm range (λ_{exc}: 280 nm). The formation of the new band is due to the emission characteristic of HDSB and the emission characteristic of the HDSB was discussed in section 3.3. The quenching constant (K_{SV}) was calculated using the Stern–Volmer equation [38]. The quenching constant (K_{SV}) was found to be 1.88×10^5 M^{-1}, showing significant BSA-HDSB interaction.

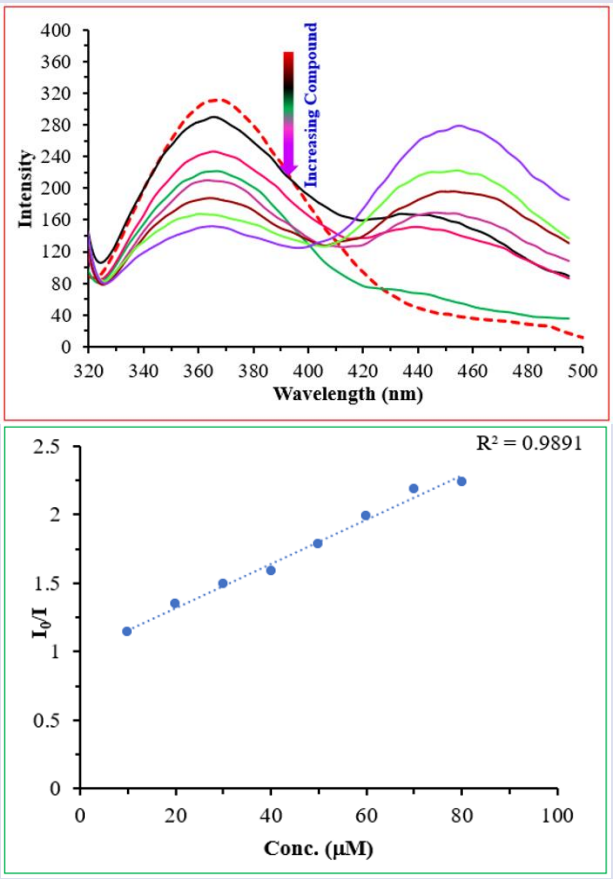


Figure 8 Emission spectra of BSA (λ_{exc}: 280 nm, λ_{em}: 362 nm) in the presence of increasing amounts of HDSB (0-100 µM in DMSO). Stern-Volmer plot for HDSB with BSA protein

Conclusion

A homo-disulphide Schiff base compound (HDSB) was prepared and its DNA/BSA binding properties were investigated. The crystal structure of the compound was determined by single crystal X-ray diffraction experiment. The synthesised compound showed binding affinities towards both DNA and BSA. The spectral measurements suggested that the compound can be considered as a new DNA minor groove binding agent.

Conflicts of interest

There are no conflicts of interest in this work.

References

[1] Abdel-Mohsen H. T., Sudheendran K., Conrad J., Beifuss U., Synthesis of disulfides by laccase-catalyzed oxidative coupling of heterocyclic thiols, *Green Chem.,* 15 (2013) 1490–1495.

[2] Ali A. Q., Teoh S. G., Salhin A., Eltayeb N. E., Khadeer Ahamed, M. B., Majid A. M. S. A., Synthesis of isatin thiosemicarbazones derivatives: In vitro anti-cancer, DNA binding and cleavage activities, *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.,* 125 (2014) 440–448.

[3] Amirnasr M., Bagheri M., Farrokhpour H., Schenk K. J., Mereiter K., Ford P. C., New Zn(II) complexes with N2S2 Schiff base ligands. Experimental and theoretical studies of the role of Zn(II) in disulfide thiolate-exchange, *Polyhedron,* 71 (2014) 1–7.

[4] Annaraj B., Balakrishnan C., Neelakantan M. A., Synthesis, structure information, DNA/BSA binding affinity and in vitro cytotoxic studies of mixed ligand copper(II) complexes containing a phenylalanine derivative and diimine co-ligands, *J. Photochem. Photobiol. B Biol.,* 160 (2016) 278–291.

[5] Annaraj B., Neelakantan M. A., Synthesis, crystal structure, spectral characterization and biological exploration of water soluble Cu(II) complexes of vitamin B6 derivative, *Eur. J. Med. Chem.,* 102 (2015) 1–8.

[6] Behpour M., Ghoreishi S. M., Mohammadi N., Soltani N., Salavati-Niasari M., Investigation of some Schiff base compounds containing disulfide bond as HCl corrosion inhibitors for mild steel, *Corros. Sci.,* 52 (2010) 4046–4057.

[7] Bharti S., Choudhary M., Mohan B., Rawat S. P., Sharma S. R., Ahmad K., Syntheses, spectroscopic characterization, SOD-like properties and antibacterial activities of dimer copper (II) and nickel (II) complexes based on imine ligands containing 2-aminothiophenol moiety: X-ray crystal structure determination of disulfide Schif, *J. Mol. Struct.,* 1164 (2018) 137–154.

[8] Bhowon M. G., Jhaumeer Laulloo S., Hosten E. C., Khodabaccus M. M., Rhyman L., Ramasami P., Synthesis, spectroscopic, biological and DFT studies of new t-butyl substituted salicylaldimines having disulfide moiety, *J. Mol. Struct.,* 1175 (2019) 13–23.

[9] Chen Y., Ren J. Q., Zhang X. G., Wu D. Y., Shen A. G., Hu J. M., Alkyne-Modulated Surface-Enhanced Raman Scattering-Palette for Optical Interference-Free and Multiplex Cellular Imaging, *Anal. Chem.,* 88 (2016) 6115–6119.

[10] Demircioğlu Z., Synthesis, crystal structure, spectroscopic characterization, chemical activity and molecular docking studies of (E) – 2 - (((3 - chloro – 4 -methylphenyl) imino) methyl) – 6 - ethoxyphenol , *J. Mol. Struct.,* 1246 (2021) 131114.

[11] Gandhimathi S., Theetharappan M., Bhuvanesh N. S. P., Neelakantan M. A., Crystal structure, theoretical and experimental electronic structure and DNA/BSA protein interactions of nickel(II) N2O2 tetradentate Schiff base complexes, *Polyhedron,* 138 (2017) 88–102.

[12] Geethanjali H. S., Nagaraja D., Melavanki R. M., Exploring the mechanism of fluorescence quenching in two biologically active boronic acid derivatives using Stern-Volmer kinetics, *J. Mol. Liq.,* 209 (2015) 669–675.

[13] Gu J., Codd R., Copper(II)-based metal affinity chromatography for the isolation of the anticancer agent bleomycin from Streptomyces verticillus culture, *J. Inorg. Biochem.,* 115 (2012) 198–203.

[14] Gungor O., Kocer F., Kose M., Cu(II) complexes of biguanidine ligands: Structural characterisation, DNA binding and antimicrobial properties, *J. Mol. Struct.,* 1204 (2020) 127533.

[15] Güngör S. A., Tümer M., Köse M., Erkan S., Benzaldehyde derivatives with functional propargyl groups as α-glucosidase inhibitors, *J. Mol. Struct.,* 1206 (2020).

[16] Güngör S. A., Tümer M., Köse M., Erkan S., N-substituted benzenesulfonamide compounds: DNA binding properties and molecular docking studies, *J. Biomol. Struct. Dyn.,* (2021) 1–15.

[17] Jamshidvand A., Sahihi M., Mirkhani V., Moghadam M.,

Mohammadpoor-Baltork I., Tangestaninejad S., Studies on DNA binding properties of new Schiff base ligands using spectroscopic, electrochemical and computational methods: Influence of substitutions on DNA-binding, *J. Mol. Liq.,* 253 (2018) 61–71.

[18] Kumar S., Pandya P., Pandav K., Gupta S. P., Chopra A., Structural studies on ligand–DNA systems: A robust approach in drug design, *J. Biosci.,* 37 (2012) 553–561.

[19] Lehrer S., Corrections - Solute Perturbation of Protein Fluorescence. The Quenching of the Tryptophyl Fluorescence of Model Compounds and Lysozyme by Iodide Ion, *Biochemistry,* 10 (1971) 4995–4995.

[20] Liu S., Chen B., Yang Y., Yang Y., Chen Q., Zeng X., Electrochemical oxidations of thioethers: Modulation of oxidation potential using a hydrogen bonding network, *Electrochem. Commun.,* 109 (2019) 106583.

[21] Manivel J., Sangeetha S., Murali M., DNA and BSA Interaction, DNA Cleavage and *In Vitro* Cytotoxicity of Copper(II) Complexes: [Cu(bba)(phen)]$(ClO_4)_2$ is Promising Chemotherapeutic Scaffold, *J. Sci. Res.,* 12 (2020) 111–133.

[22] Moosun S. B., Bhowon M. G., Hosten E. C., Jhaumeer-Laulloo S., Crystal structures, antibacterial, antioxidant and nucleic acid interactions of mononuclear, and tetranuclear palladium(II) complexes containing Schiff base ligands, *J. Coord. Chem.,* 69 (2016) 2736–2753.

[23] Moosun S. B., Jhaumeer-Laulloo S., Hosten E. C., Gerber T. I. A., Bhowon M. G., Antioxidant and DNA binding studies of Cu(II) complexes of N,N′-(1,1′-dithio-bis(phenylene))-bis(salicylideneimine): synthesis and characterization, *Transit. Met. Chem.,* 40 (2015) 445–458.

[24] Moubeen S. A. M., El-Shahat M. F., Aziz A. A. A., Attia A. S., Synthesis, characterization and biological evaluation of novel octahedral Ru(III) complexes containing pentadentate Schiff base ligands, *Curr. Chem. Lett.,* 10 (2021) 17–32.

[25] Neelakantan M. A., Balakrishnan C., Balamurugan K., Mariappan S. S., Zinc(II)-N2O2 ligation complex-based DNA/protein binder and cleaver having enhanced cytotoxic and phosphatase activity, *Appl. Organomet. Chem.,* 32 (2018) 1–18.

[26] Neelakantan M. A., Balakrishnan C., Selvarani V., Theetharappan M., DNA/BSA binding interactions and VHPO mimicking potential of vanadium(IV) complexes: Synthesis, structural characterization and DFT studies, *Appl. Organomet. Chem.,* 32 (2018) 1–16.

[27] Ramadan R. M., Elantabli F. M., El-Medani S. M., Conversion of thiol to homodisulfide-Schiff base derivative: Synthesis, molecular structure, crystal structure and DFT studies, *J. Mol. Struct.,* 1196 (2019) 547–554.

[28] Raman N., Sobha S., Thamaraichelvan A., A novel bioactive tyramine derived Schiff base and its transition metal complexes as selective DNA binding agents, *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.,* 78 (2011) 888–898.

[29] S. Bruker, APEX2 and SAINT Bruker, (1998).

[30] Shafaatian B., Mousavi S. S., Afshari S., Synthesis, characterization, spectroscopic and theoretical studies of new zinc(II), copper(II) and nickel(II) complexes based on imine ligand containing 2-aminothiophenol moiety, *J. Mol. Struct.,* 1123 (2016) 191–198.

[31] Shafaatian B., Ozbakzaei Z., Notash B., Rezvani, S. A., Synthesis, characterization, single crystal X-ray determination, fluorescence and electrochemical studies of new dinuclear nickel(II) and oxovanadium(IV) complexes containing double Schiff base ligands, *Spectrochim. Acta-Part A Mol. Biomol. Spectrosc.,* 140 (2015) 248–255.

[32] Sharma A. K., Chandra S., Complexation of nitrogen and sulphur donor Schiff's base ligand to Cr(III) and Ni(II) metal ions: Synthesis, spectroscopic and antipathogenic studies, *Spectrochim. Acta-Part A Mol. Biomol. Spectrosc.,* 78 (2011) 337–342.

[33] Sheldrick G. M., Crystal structure refinement with SHELXL, *Acta Crystallogr., Sect. C Struct. Chem.* 71 (2015) 3–8.

[34] Sheldrick G. M., SHELXT - Integrated space-group and crystal-structure determination, *Acta Crystallogr. Sect. A Found. Crystallogr.,* 71 (2015) 3–8.

[35] Shi J. H., Chen J., Wang J., Zhu Y. Y., Binding interaction between sorafenib and calf thymus DNA: Spectroscopic methodology, viscosity measurement and molecular docking, *Spectrochim. Acta-Part A Mol. Biomol. Spectrosc.,* 136 (2015) 443–450.

[36] Vardhan H., Yusubov M., Verpoort F., Self-assembled metal-organic polyhedra: An overview of various applications, *Coord. Chem. Rev.,* 306 (2016) 171–194.

[37] Wu W. Bin, Wong Y. C., Tan Z. K., Wu J., Photo-induced thiol coupling and C-H activation using nanocrystalline lead-halide perovskite catalysts, *Catal. Sci. Technol.,* 8 (2018) 4257–4263.

[38] Zhang Q., Ni Y., Kokot S., Combined voltammetric and spectroscopic analysis of small molecule-biopolymer interactions: The levodopa and serum albumin system, *Talanta,* 88 (2012) 524–532.