

Biological activity and fluorescent property of Benzimidazole derivative of N,N,O-donor Schiff base with BSA protein

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ABSTRACT

Benzimidazole compounds are a class of heterocyclic, aromatic compounds which contribute to an elemental structural characteristic of six-membered benzene merged to five-membered imidazole moiety. Nowadays it is an important organic moiety because which possesses potent inhibitor of various enzymes involved in a wide range of therapeutic uses which are anticancer, antimicrobial, analgesics, antiviral, and antihistamine, as well as used in cardiovascular disease, neurology, ophthalmology, and more. For this purpose we synthesized of N,N,O-donor Schiff base (**HL**) by the refluxing of 2-(1H-benzimidazol-2-yl) aniline and 5-nitro salicyldehyde in ethanol medium. The interaction of Ligand (**HL**) towards Bovine serum albumin (BSA) was examined with the help of absorption spectroscopic tools. From this study which suggested that Ligand was strongly interaction with Bovine serum albumin (BSA) protein. The antibacterial activity of Ligand (**HL**) was studied by diffusion method with some pathogenic bacteria namely *Escherichia coli*, *Vibrio cholerae*, *Klebsiella pneumonia* and *Bacillus cereus*.

Key words: Benzimidazole Schiff base; BSA binding, Optical property; Antibacterial Activity.

1. INTRODUCTION

Benzimidazole compounds are important in medicinal chemistry and agrochemistry because of their high efficiency and low toxicity. Benzimidazoles have been and are still widely used to control varieties of fungal diseases, but their resistance problems may result in failure to control disease [1]. Therefore, it is necessary to discover and develop novel agrochemicals that could overcome or minimize the side effects of agrochemicals that are currently used [2]. Schiff bases contain N, O, N- organic moiety has played an important role in the development of coordination chemistry as they readily formed stable Ligands with most of the transition metals [3]. Schiff bases and their coordinating compounds are well known to be biologically important of interest for their antibacterial, antifungal and anti-inflammatory agents [4]. Many of them exhibit ability to bind toxic and heavy metals, catalytic reduction and photochromism. Benzimidazoles are outstandingly efficient compounds, extensive biochemical [5] and pharmacological studies have established that these molecules are effective against various strains of microorganisms [6]. Due to their enormous medicinal value, the research and development of benzimidazole-containing drugs is an increasingly active and attractive topic of medicinal chemistry.

Based on these facts, we decided to synthesize Schiff base of 2-(2-aminophenyl)-1-H-benzimidazole and 5-nitro salicylaldehyde in ethanol medium using acid catalyst and the products were isolated simply by filtration. In the present work, we report the interaction between ligand (**HL**) and BSA under physiological conditions by spectroscopic methods. Meanwhile the optical waveguide properties of HL-BSA microstructures measured by optical and fluorescence microscopy study. The compound is strongly bound with protein, and then we investigated the antibacterial activity by agar disk diffusion method against some pathogenic bacteria. From this study, the ligand behaves moderate activity with pathogenic bacteria.

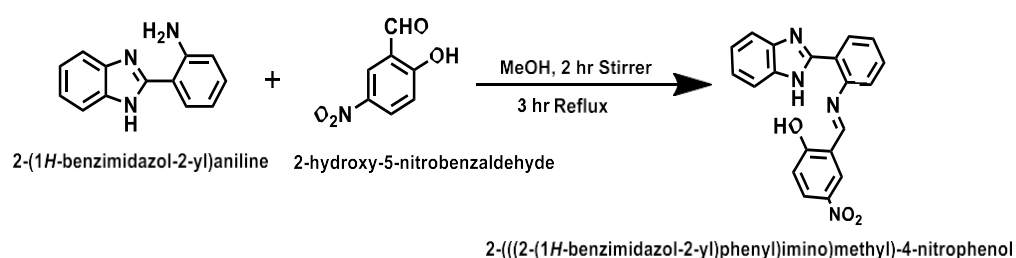
2. MATERIALS AND PHYSICAL MEASUREMENTS

All chemicals and reagents were obtained from commercial sources and used as received, unless otherwise stated. Solvent (MeOH, DMF) were distilled from an appropriate drying agent. The Ligand was synthesized by using the following procedure. Electronic absorption spectrum was recorded on a SHIMADZU UV-1800 spectrometer. Optical microscopy images

were taken using an NIKON ECLIPSE LV100POL upright microscope equipped with a 12V-50W halogen lamp.

2.1. Synthesis of Organic ligand (HL):

The ligand **HL** was Synthesize by adding 1.045 g (5.0 mmol) of 2-(1H-benzimidazol-2-yl)aniline and then 0.835 g (5.0 mmol) of 5-nitro salicylaldehyde with 10 mL of ethanol in a round bottom flask and then reflux up to 3 hour shown in **Scheme 1**. The precipitate was filtered and washed with ethanol, finally crude product was collected by recrystallization in ethanol, Yields >80%.



Scheme 1: synthetic strategy of ligand HL

2.2. Antimicrobial Screening:

The synthesized organic compound was used for antibacterial activities in vitro against two gram negative (*Vibrio cholera*, *E. coli*) and two gram positive (*Bacillus cereus*, *Staphylococcus aureus*) pathogenic bacteria. The test compound and standard antibiotics are dissolved in DMF solvent. The compound and standard were added to the agar plates and incubation of the plates was done at 37°C for 24 hours [7]. The percentage of inhibition was measured, and then compare to the standard antibiotics [8].

3. RESULT AND DISCUSSION

3.1. Spectral Study:

The electronic spectrum of the ligand was recorded in methanol at room temperature. The spectra of the Schiff base **HL** exhibit two main peaks: at 398.0 nm and 280.0 nm. The first and second peaks were attributed to benzene $n \rightarrow \pi^*$ and imino $\pi \rightarrow \pi^*$ transitions. All the

spectra lower bands than 400 nm of ligand shows due to intramolecular $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions for the aromatic ring.

3.2. Protein (Bovine Serum Albumin) binding with Ligand (HL):

The absorption spectra of BSA in the absence and presence of ligand at different concentrations were studied (**Fig.1**). From this study, upon increasing the concentration of the ligand the absorption of BSA increases regularly. This is due to the adsorption of BSA on the surface of the Ligand. From these data the apparent association constant (K_{app}) of the Ligandes with BSA has been determined using the Benesi-Hilderbrand equation [9]

$$1/(A_{obs} - A_0) = 1/(A_c - A_0) + 1/K_{app} (A_c - A_0) [comp]$$

Where, A_{obs} is the observed absorbance of the solution containing different concentrations of the Ligand at 278 nm, A_0 and A_c are the absorbance of BSA and the Ligand at 280 nm respectively, with a concentration of Ligand, and K_{app} represents the apparent association constant. The enhancement of absorbance at 278 nm was due to adsorption of the surface Ligand, based on the linear relationship between $1/(A_{obs} - A_0)$ vs. reciprocal concentration of the Ligand with a slope equal to $1/K_{app}(A_c - A_0)$ and an intercept equal to $1/(A_c - A_0)$. The value of the apparent association constant (K_{app}) determined from this plot is $3.68 \times 10^4 \text{ M}^{-1}$ ($R = 0.9863$ for five points).

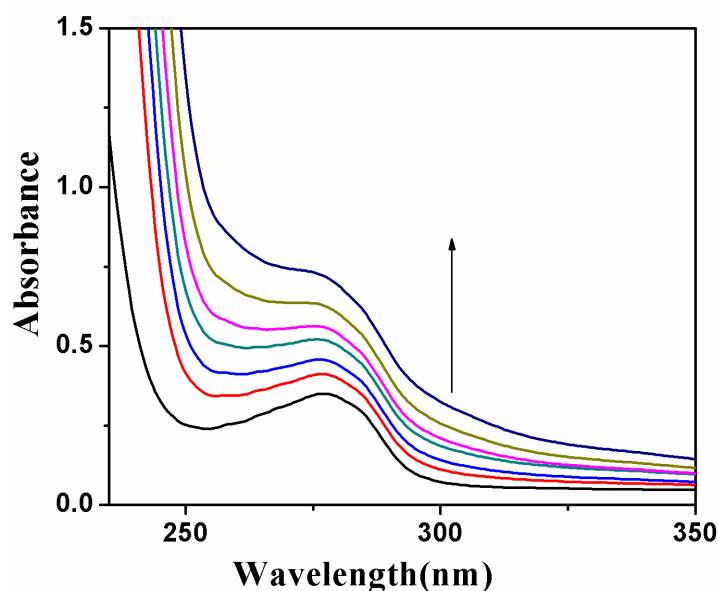


Fig. 1: Electronic spectral titration of Ligand (HL) with BSA in the presence of phosphate buffer, pH 7.4. Arrow indicates an increase in the concentration of Ligand.

3.3. Fluorescence microscopy study of Ligand with BSA molecules

The optical wave properties are investigated by fluorescence microscopic tools. The bluish green micro-rods were observed by using fluorescence microscopy shown in **Fig. 2**. Upon excitation with UV and blue light sample shows rod shaped particles with bluish green and yellowish-green luminescence respectively [10]. In solution phase the Ligand are enter into the BSA microparticles and possibly to be distributed randomly confirmed by luminescence property. It is thought that for the period of the development of the doped microparticles, hydrophobic and π - π interactions induces the aggregation process of BSA and Ligand molecules into microrods [11]. The Fluorescence intensity also increases upon high dilution on BSA molecules. This optical property also allied to the single-crystalline character and the strong fluorescence emission activities of the Ligand-BSA microrods.

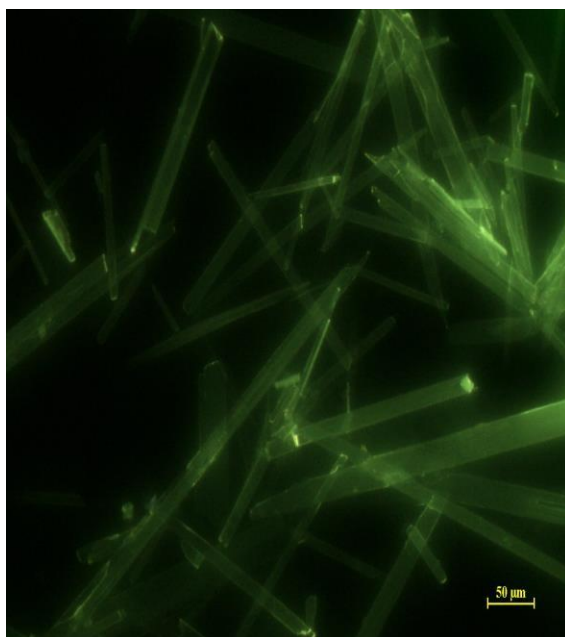


Fig. 2: Fluorescence microscopy image of Ligand with BSA microrods.

3.4. Antibacterial activity:

The antimicrobial activity of the ligand was investigated by the agar disc diffusion method [12]. The standard drug used was Levofloxacin. From this assay we found that compound is moderate inhibitory activity than standard drug shown in **Fig.3**. The activities of the ligand

can be explained by lipophilicity character [13]. The lipid membrane of the bacterial cell prefers only lipid soluble materials and controls the antimicrobial activity [14]. Thus the Ligand penetrates the bacterial cell membranes and decreases the growth of the microorganisms. From this plot; we conclude that, ligand is more active towards *E. coli* and *V. Cholera* higher than *B. Cereus*.

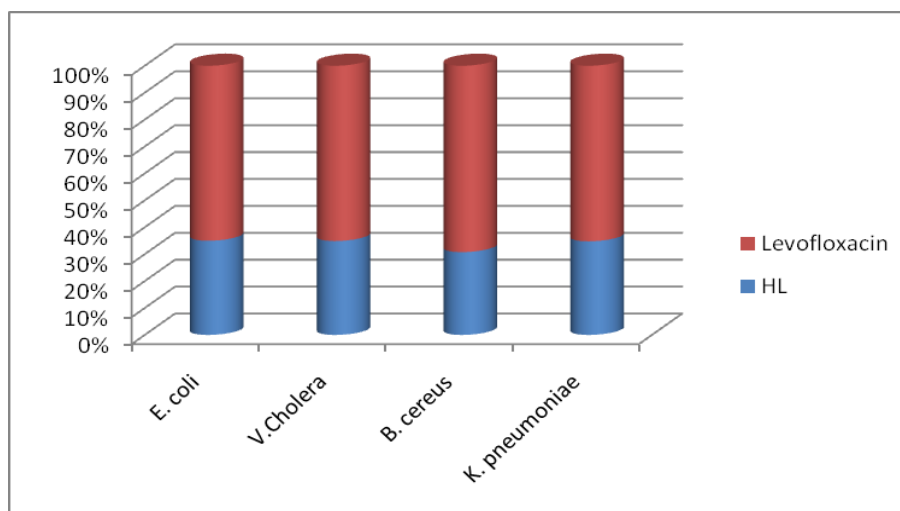


Fig. 3. Comparison the antibacterial activities of **HL** and standard Levofloxacin drug.

4. Conclusion

The synthesis and characterization of N,O,N- donor set have been performed. On the basis of the physical and spectral data of the Schiff base has phenolic oxygen, benzimidazol nitrogen and the imine nitrogen donor sites. The binding mechanisms study suggested that the ligand strongly binds with BSA through a static quenching mechanism. Ligand binds with BSA to form microrods, which might be introduced as the building block for novel optoelectronic devices. The antibacterial activity confirmed that ligand is good biologically active.

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