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DNA Cleavage Mechanism by Metal complexes of Cu(II), Zn(II), Mn(II) and Co(II) with a Schiff-base Ligand

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Abstract

Metal ions and metal complexes are important components of nucleic acid biochemistry, participating both in regulation of gene expression and as therapeutic agents. Four new transition metal complexes of Cu(II), Ni(II), Co(II) and Zn(II) have been synthesized from a new Schiff base ligand derived from 4-bromo aniline, 2-hydroxy-1-naphthaldehyde. The ligand and its complexes have been characterized by spectral analysis. The *in vitro* antimicrobial activity of the investigated compounds was tested against the bacteria such as *Bacillus subtilis, Escherichia coli* and fungi *Aspergillus flavus* and *Aspergillus niger.* The data indicate that most of the metal complexes have higher antibacterial activity than the free ligand. DNA cleavage experiments performed on pBR322 DNA using metal complexes in the presence of H_2O_2 showed that all the complexes afford a pronounced DNA cleavage.
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Keywords: 2-hydroxy-1-naphthaldehyde , DNA cleavage, antimicrobial activity, metal complex

INTRODUCTION

Schiff bases are versatile organic compounds which are widely used and synthesized by condensation reaction of different amino compound with aldehydes or ketones known as imine. Schiff base ligands are considered as privileged ligands as they are simply synthesized by condensation. They show broad range of application in medicine, pharmacy, coordination chemistry, biological activities, industries, food packages, dyes, and polymer and also used as an O_2 detector [1]. The study of transition metal complexes is of current interest because of their potential applications as metallo-drugs, new compounds having a wide range of pharmacological effects, such as antiviral, antimicrobial and cytotoxic agents, among others [2]. In particular, antitumor activity has devoted great attention. Cancer is a group of diseases due of an uncontrolled cell proliferation [3]. DNA replication, transcription and protein synthesis are the primarily steps in cell growth and division so, DNA is an important target molecule for many anticancer therapies. The use of metal

complexes as antitumor agents began with the discovery of the properties of cisplatin, over 50 years ago [4]. Ever since, a lot of research has been developed to design and synthesize new metal complexes that can cause structural damage to DNA as well as being able to block the access of essential proteins to DNA to fulfill the basic cellular functions of replication and transcription [5]. Cleavage of one or both strands of DNA is a normal and necessary process to maintain cell viability: topoisomerase enzymes resolve topological problems during DNA replication and transcription, different nucleases participate in repair mechanisms and DNA degradation, that is one of the hallmarks of apoptotic programmed cell death. So, the activity of many antitumor drugs is based on their ability to interfere with these physiological processes, introducing extended damage to the DNA which can trigger apoptosis, and leading to the cell death. On the other hand, groove-binding molecules can bind to both the major and the minor groove of DNA, either impairing the access to proteins or disrupting the double helix of DNA [6,7].

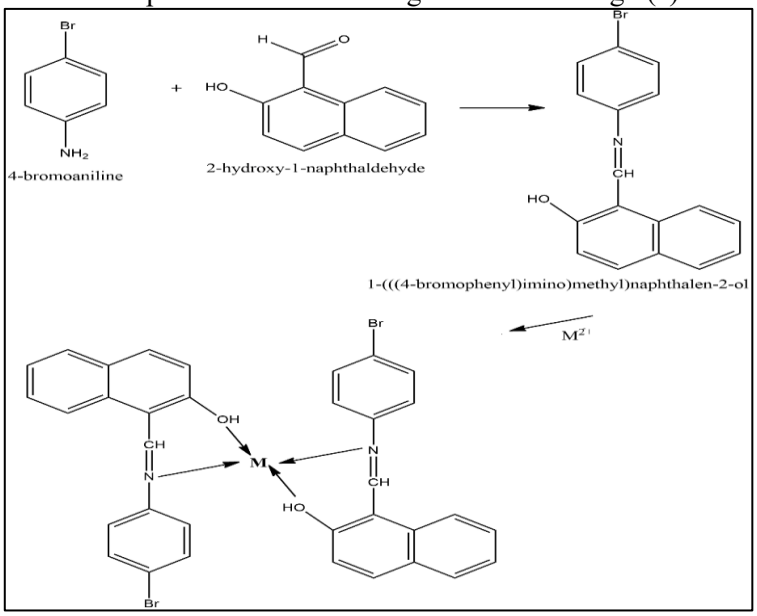
Here, four new transition metal complexes of Cu(II), Ni(II), Co(II) and Zn(II) have been synthesized from a new schiff base ligand derived from 4-bromo aniline, 2-hydroxy-1-naphthaldehyde, cleavage experiments of these complexes with DNA, with the aim to get information about the mechanisms of action involved. The DNA cleavage properties of the complexes have been investigated by gel electrophoresis. Experimental results indicate that all complexes cleave.

MATERIALS AND METHODS

4-bromo aniline, 2-hydroxy-1-naphthaldehyde, were obtained from Sigma Aldrich . The metal chlorides utilized in the synthesis of the complexes were obtained from Merck. Common solvents like ethanol, acetone and Dimethyl sulphoxide (DMSO) utilized at various steps of this work were purified according to the standard procedure explained in qualitative analysis by Vogel.

Schiff base ligand is synthesised by condensation of 4-bromo aniline (0.01 mol) with 2-hydroxy-1-naphthaldehyde (0.01 mol) in alcoholic medium and the mixture is refluxed for 4-5 hours at 40-50°C.After completion of the reaction, yellow solid product obtained is washed with ethanol and dried at room temperature.

The schiff base ligand(0.01mol) and metal chloride salt (0.005 mol) are dissolved in ethanol and mixed in 1:2 (Metal:Ligand) molar ratio and refluxed for 4-5 hours at 50-60°C. The resultant complex is filtered and washed well with ethanol and dried in room temperature. All the metal complexes are coloured , stable and stored in desiccator over anhydrous $CaCl_2$. Synthesis of Schiff base ligand and its metal complex sown in scheme I. The ^{13}C and ^{1}H spectra of schiff base ligand shown in Fig.1(a) and Fig.1(b). respectively



Scheme 1: Synthesis of Schiff base metal complexes (M= Cu(II), Ni(II), Co(II) and Zn(II))

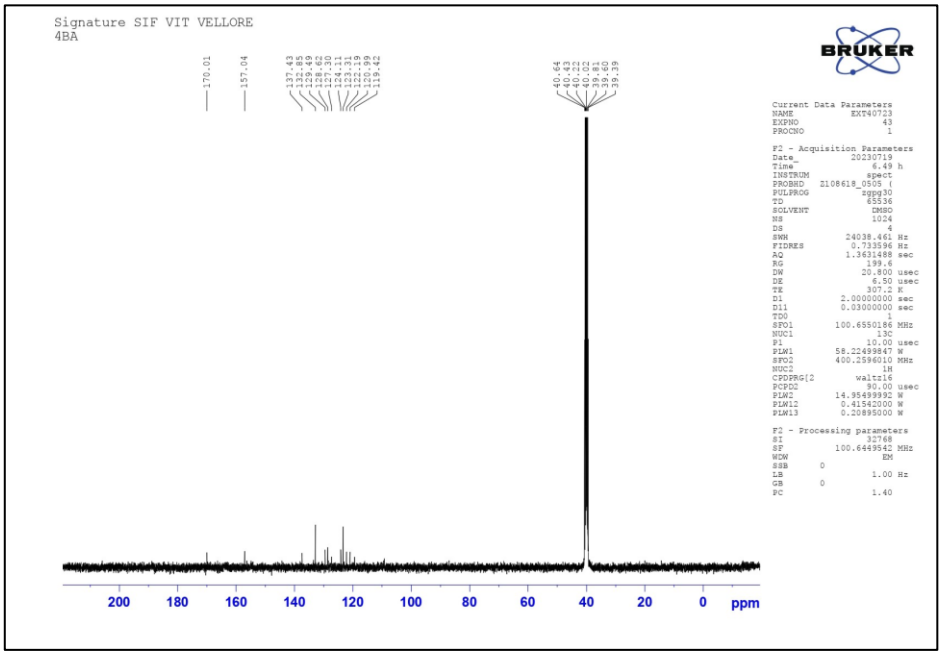


Fig.1 (a): ^{13}C spectrum of schiff base ligand

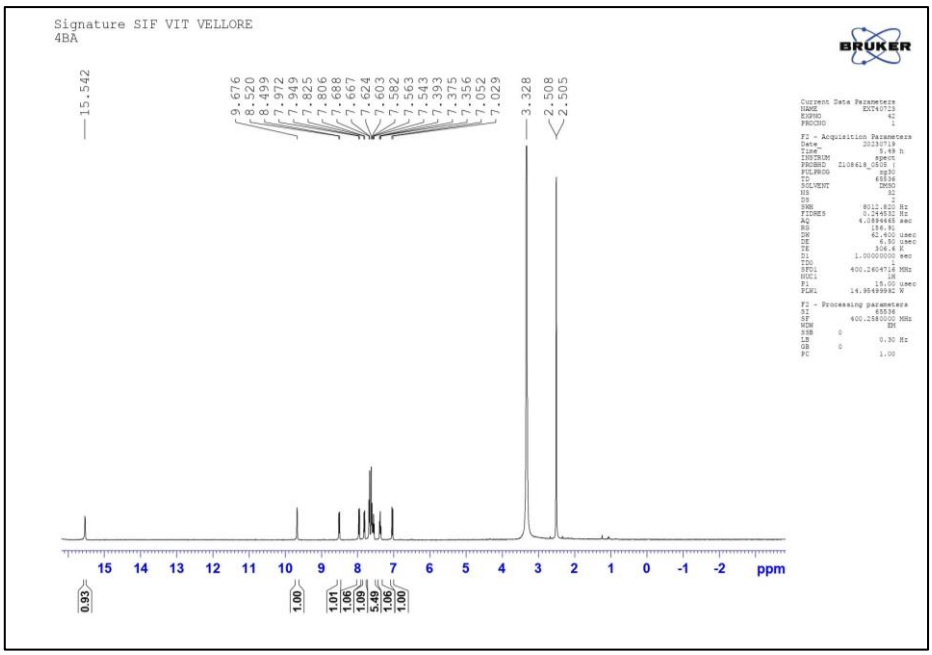


Fig.1(b): ^{1}H spectrum of Schiff base ligand

DNA Cleavage

Cleavage of DNA is a vital process in all living systems. For example, topoisomerase enzymes resolve topological problems of DNA in replication, transcription and other cellular transactions by cleaving one or both strands of the DNA . Another example are restriction enzymes (or restriction endonucleases), which protect the cell against virus infection by cleavage of the foreign DNA , or by degrading cellular DNA during apoptosis of the affected cell . Finally, the activity of many anticancer drugs rely on their ability to introduce extended damage to the DNA in the (affected) cells (e.g. bleomycin), which can trigger apoptosis, leading to the cell death. The DNA cleavage of super coiled pBR322 DNA promoted by metal complex was proceeded by addition of reaction mixture (20µl). The reaction mixture 20µl containing pBR322 DNA, 50 mM Tris–HCl, pH 7.4, 50mm NaCl ,10mM H_2O_2 added in a different volume , followed by adding Millipore water for final volume .Then the mixed solutions were incubated at 37º C for 1 hr. They checked by agarose electrophoresis method. Gel electrophoresis experiments were performed using pBR322-DNA with ligand, complex in presence and absence of H_2O_2.

Antimicrobial activity

Disc Preparation: The 6mm (diameter) discs were prepared from Whatmann No. 1 filter paper. The discs were sterilized by autoclave at 121°C.After the sterilization the moisture discs were dried on hot air oven at 50°C.Then various solvent extract discs and control discs were prepared.

Collection of test microorganism : The Bacterial strains of *Escherichia coli (gram negative)* and *Bacillus subtilis (gram positive)* were obtained from Microbial Type culture Collection Centre (MTCC), Chandigarh. The fungal strains of *Aspergillus flavus*, and *Aspergillus niger* were used.

Assay of Antibacterial Activity: Antibacterial activity was determined by disc diffusion method as described by Bauer *et al*., 1966 [8]. By inoculating a loopful of strain in Mueller Hinton broth separately and incubated at 37°C on a rotary shaker for 12 hrs. Then 0.1 ml of fresh inoculum (containing around 1 - 2 × 10^6 CFU/ml as per McFarland standards) was spread onto the surface of sterile Mueller Hinton agar plates using a sterilized spreader. Then discs impregnated with compounds (1mg/disc) and solvent controls were placed on plates with the help of a sterilized forceps and the plates were incubated aerobically at 37°C. Similarly a negative solvent control (DMSO) disc were placed on each pathogen inoculated plates. The entire microbial assay was carried out under strict aseptic conditions. The zone of inhibition (mm) of the different metals was examined after 12-18 hrs.

Assay of Antifungal Activity: Antifungal activity test was carried out following the modification of the method originally described by Bauer *et al.,* (1966) [8]. Potato Dextrose Agar (PDA) was prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled to 45ºC. The cooled media was added 10ml/L tartaric acid (10%) act as antibacterial agents and poured on to sterile petriplates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swab. The various solvents extract prepared discs individually were placed on the each petriplates and also placed control and standard (Amphotericin B (100 Units/ Discs)) discs. The plates were incubated at 28ºC for 72 hrs. After incubation period, the diameter of the zone formed around the paper disc were measured and expressed in mm.

RESULTS AND DISCUSSION

The interactions of metal complexes with DNA are now well documented. In recent years a considerable amount of work has been done on the coordination chemistry of copper(II) complexes with Schiff base ligands to model the physical and chemical behaviour of biological copper systems. Cupric ions have been shown to bind to the DNA bases adenine, guanine and cytosine. These ions can be reduced and then oxidized by dioxygen, leading to hydroxyl radical production close to the metal binding site, which can damage DNA in site-specific reactions [1]. DNA is the primary pharmacological target of many antitumoral drugs. Although eukaryotic DNA has a nucleosomal conformation different from plasmidic DNA, this one has been widely used to correlate in vitro DNA damage with eukaryotic cell functionality [6]. When circular plasmidic DNA is run in gel electrophoresis, the SC form is observed as a fastest migration band (Form I). If one strand is cleaved, the SC will relax to produce a slower-moving open circular form (Form II) [7].

Gel electrophoresis experiments were performed using pBR322-DNA with ligand, complex in presence and absence of H_2O_2. All complexes exhibit cleavage ability at low concentration (40µM). The activity was much higher for the complex in presence of H_2O_2. When pBR322DNA is subjected to electrophoresis, relatively fast migration will be observed for the intact super coil form (Form I). If scission occurs on one strand (nicking), the super coil will relax to generate a slower moving open circular form (Form II). If both strands are cleaved, a linear form (Form III) that migrates between Forms I and II will be generated. From Fig. 2-Fig.6, the schiff base and complexes shows more activity in the presence of oxidant which may be due to the reaction of hydroxyl radical with DNA. These hydroxyl free radicals participate in the oxidation of the deoxyribose moiety followed by hydroxyl cleavage of sugar phosphate backbone. The results of DNA cleavage studies have been shown in Fig.2-6. Metal complexes is able to convert DNA (Form I) into open circular (Form II) [9]. All the complexes was found to be highly active in cleaving DNA in the presence of hydrogen peroxide. The H_2O_2 is coordinated to the metal ions of the complexes. This coordinated peroxide ion attacks phosphate DNA [10].

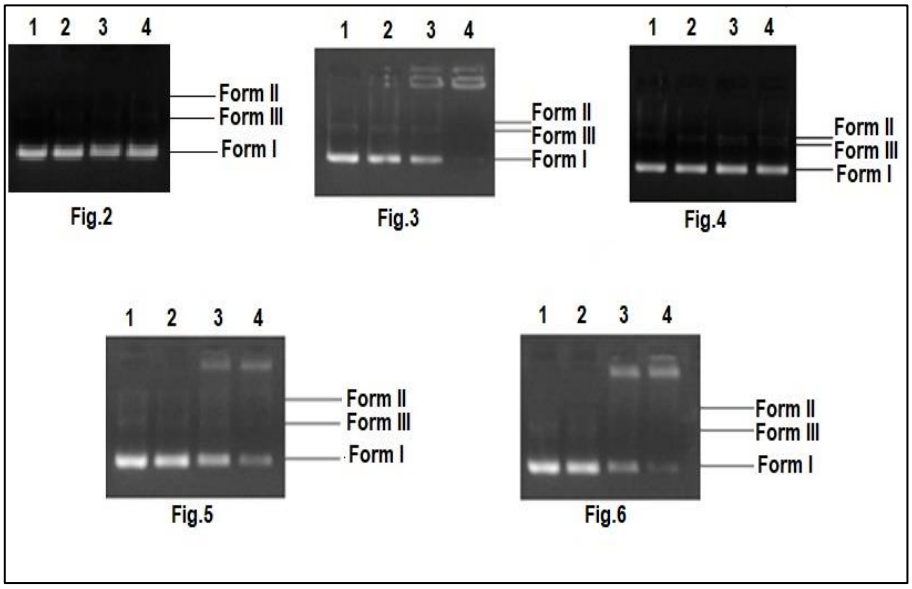


Fig. 2: DNA cleavage of Schiff base [Lane 1: DNA(Control); Lane 2: DNA+H_2O_2 (1mM); Lane 3:DNA+H_2O_2 (1mM)+ Schiff base (30µM) ; Lane 4:DNA+H_2O_2 (1mM)+ Schiff base (40µM)]; **Fig. 3: DNA cleavage of Cu complex** [Lane 1: DNA(Control); Lane 2: DNA+H_2O_2 (1mM); Lane 3:DNA+H_2O_2 (1mM)+ Cu complex (30µM) ; Lane 4:DNA+H_2O_2 (1mM)+ Cu complex (40µM)]**; Fig. 4: DNA cleavage of Ni complex [**Lane 1: DNA(Control); Lane 2: DNA+H_2O_2 (1mM); Lane 3:DNA+H_2O_2 (1mM)+ Ni complex (30µM) ; Lane 4:DNA+H_2O_2 (1mM)+ Ni complex (40µM)**]; Fig. 5: DNA cleavage of Co complex** [Lane 1: DNA(Control); Lane 2: DNA+H_2O_2 (1mM); Lane 3:DNA+H_2O_2 (1mM)+ Co complex (30µM) ; Lane 4:DNA+H_2O_2 (1mM)+ Co complex (40µM)]**; Fig. 6: DNA cleavage of Zn complex** [Lane 1: DNA(Control); Lane 2: DNA+H_2O_2 (1mM); Lane 3:DNA+H_2O_2 (1mM)+ Zn complex (30µM) ; Lane 4:DNA+H_2O_2 (1mM)+ Zn complex (40µM)]

The bacterial activity of synthesized compounds was tested against gram (+), gram (-) bacteria and fungal species . The results are presented in Table 1. These results were also compared with a standard antibacterial drug (Ciprofloxacin) and standard antifungal drug (Amphotericin-B). From the obtained results, complexes showed good antibacterial activity.

Table 1: Assay of antimicrobial activity

S. No.	Microorganism	Zone of Inhibition (mm in diameter)					
		Standard*	Schiff base	Cu complex	Ni complex	Co complex	Zn complex
1	*Bacillus subtilis*	23	12	19	15	15	16
2	*Escherichia coli*	25	17	22	21	19	18
3	*Aspergillus flavus*	13	-	8	-	-	-
4	*Aspergillus niger*	12	-	7	-	7	-

* Ciprofloxacin (5mcg), AP-Amphotericin-B

The present study showed high antibacterial activity against the gram-positive *B. subtilis* and gram-negative *E.coli*, which are pathogens widely associated with urinary tract infections. Antifungal activity was obtained against *Aspergillus niger* and *Aspergillus flavus.* Compounds showing minimum / nil activity against *Aspergillus niger , Aspergillus flavus* (Table 1).

Metal complexes have seen wide application as molecular tools for *in vitro* studies of a variety of biochemical problems [2]. *In vivo* applications are less evident and represent an area for further exploration. Application of metal complexes that target disease-associated DNA/RNA as potential therapeutic agents should enjoy a promising future.

CONCLUSION

A new Schiff base ligand is synthesized using 4-bromo aniline, 2-hydroxy-1-naphthaldehyde. It acts as a tetradendate ligand and forms stable complexes with transition metal(II) ions such as copper(II), nickel(II),

cobalt(II) and zinc(II) in ethanol. The ligand and its complexes were characterized by spectral data. Results of all the characterizations indicated that, four co-ordinated geometry is allocated to these metal complexes and it is implied that ligand acts as bidentate and coordinate to metal through N and O atoms . A comparative study of the ligand and its complexes indicates that the complexes exhibit slightly higher antimicrobial activity than the free ligand. The DNA cleavage activity of all the complexes was examined using gel electrophoresis experiment. DNA cleavage studies revealed that all the complexes were cleaved into Form I & II nuclease activity in the presence of oxidant.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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