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A STUDIES ON DNA CLEAVAGE, BINDING, AND ANTIBACTERIAL ACTIVITY

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ABSTRACT

Due to the tendency of DNA-bound medications to interfere with transcription and replication, a crucial phase in cell growth and division, DNA, the storage and carrier molecule of genetic information, has been a key target for drug interaction. Three binding processes—external binding, intercalative binding, and groove binding—can interact with DNA to interact with tiny molecules like metal complexes. These DNA-complex interactions can harm cancer cells by preventing cell division and leading to cell death. The invention of new copper-based metallodrugs as well as research into the DNA binding and cleavage abilities of copper(II) complexes have increased significantly. Many intercalating substances that were effective against neoplastic cells and microbiological organisms do so by binding to DNA in an intercalative manner. Three techniques, including electronic absorption titration, fluorescence investigations, and viscosity tests, can be used to assess the degree of DNA binding to the metal complexes.

KEY WORDS: DNA Cleavage, DNA Binding, and Antibacterial Activity

1. INTRODUCTION

Since a few years ago, metal-based medications have been utilized to treat diseases, but the main obstacle was the lack of a differentiation between therapeutic and hazardous levels. Undoubtedly, one of our society's top health issues has been cancer, which is currently the main focus of medicinal chemistry. Cancer was mostly treated with platinum medicines including cisplatin, carboplatin, and oxaliplatin. One of the most popular and commonly used metal-based anticancer medicines for cancer therapy is cisplatin (Cis-diammino di chloroplatinum (II)), but it has intrinsic drawbacks such substantial side effects, general toxicity, and developed drug resistance. As a result, significant efforts to replace this medication with non-platinum-based medicines are currently underway. Many complexes have been created by reworking the existing structure by substituting ligands or by creating new molecules with improved cytotoxicity and safety characteristics. The focus of future research has been on creating therapeutic medications that selectively target cancer cells while sparing healthy ones. The most potential cisplatin

substitutes have been thought to be copper(II) complexes. Being a bioessential transition metal ion, copper can be used more effectively at the cellular level in complexes with tunable coordination geometries in a redox active environment. Based on the cytotoxic effects, copper(II) based complexes seem to be particularly promising for anticancer therapy. According to in vitro investigations on MCF-7 human breast cancer cells, certain nickel(II) complexes actively limit cell growth. They also have antibacterial action.

Due to their numerous uses as therapeutic agents, structural probes, DNA footprinting agents, sequence-specific binding, and more, transition metal complexes play a significant role in the chemistry of nucleic acids. It appears that selecting the coordinated ligands is just as crucial as selecting the metal ions. These organic molecules (ligands), in addition to being an integral component of physiologically active complexes, can also exert biological activity on their own. Pyridoxal semicarbazone (PLSC) based complexes' coordination chemistry has proven to be particularly intriguing because it can exist in neutral, mono-, and dianionic forms depending on pH. Regardless of the neutral (keto form) or monoanionic (enolic form) forms of coordinated ligands, the pyridoxalic fragment behaves as a zwitter ion. It is a great chelating agent because it binds through imine nitrogen, phenolic, and carbonyl oxygen atoms, which is the most common tridentate (ONO) coordination mode.

In industrial, agricultural, and pharmaceutical chemistry, metal complexes are crucial. Recent investigations have shown that the primary criterion for pharmaceutical research has been the identification of metal-based anticancer drugs.Under physiological conditions, metal complexes that effectively bind and cleave DNA may be taken into consideration as possible candidates for use in the creation of biotechnological instruments and therapeutic drugs. Biological experiments have been conducted to determine the viability of the synthesized metal complexes' uses in the current inquiry. This chapter has been broken into three sections for your convenience.

- DNA binding studies
- DNA cleavage studies
- Antibacterial activity

DNA binding studies

DNA binding studies were carried out by three methods listed below.

- Electronic absorption titration method.
- Competitive fluorescence studies.
- Viscosity measurements.

The above studies have been carried out for Ni(II), Cu(II) and Zn(II) complexes of PLHBH, PLNBH, PLDCBH, PLFBH and PLTMBH.

2. RESEARCH METHODOLOGY

2.1 ELECTRONIC ABSORPTION TITRATION METHOD

The technique of electronic absorption spectroscopy has been extensively employed to investigate the degree and mechanism of DNA binding to the metal complex. The DNA double helix structure's spectrum characteristics include hypochromic and hyperchromic effects. DNA helical axis concentration and conformational changes cause the hypochromic shift, while structural DNA damage causes the hyperchromic effect. The degree of hypochromism indicates the strength of intercalative binding, whereas hyperchromism is caused by non-intercalative or electrostatic binding to metal complexes.

In both covalent and non-covalent ways, transition metal complexes can bind to DNA. Due to the strong - interaction between the DNA base pairs and the aromatic chromophore of the ligand, hypochromism with appreciable bathochromic (red) shift or hypsochromic (blue) shift can be seen in cases where the complex binds to DNA in an intercalative manner. The strength of intercalation is indicated by the degree of hypochromism.

PLHBH, PLNBH, PLDCBH, PLFBH, and PLTMBH Ni(II), Cu(II), and Zn(II) complex absorption titration studies were carried out using various concentrations of CT DNA (10-100l), while maintaining a constant concentration of the complex. After DNA was added to the metal complex, the resultant solution was given 10 minutes to equilibrate before each spectra was recorded. The intrinsic binding constant (Kb), which was calculated using the equation, was used to determine the quantitative extent of the metalcomplex's DNA binding strength.

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

The slope of the [DNA]/(a - f) vs. [DNA] plot and the intercept of the plot were both equal to 1/(a - f).

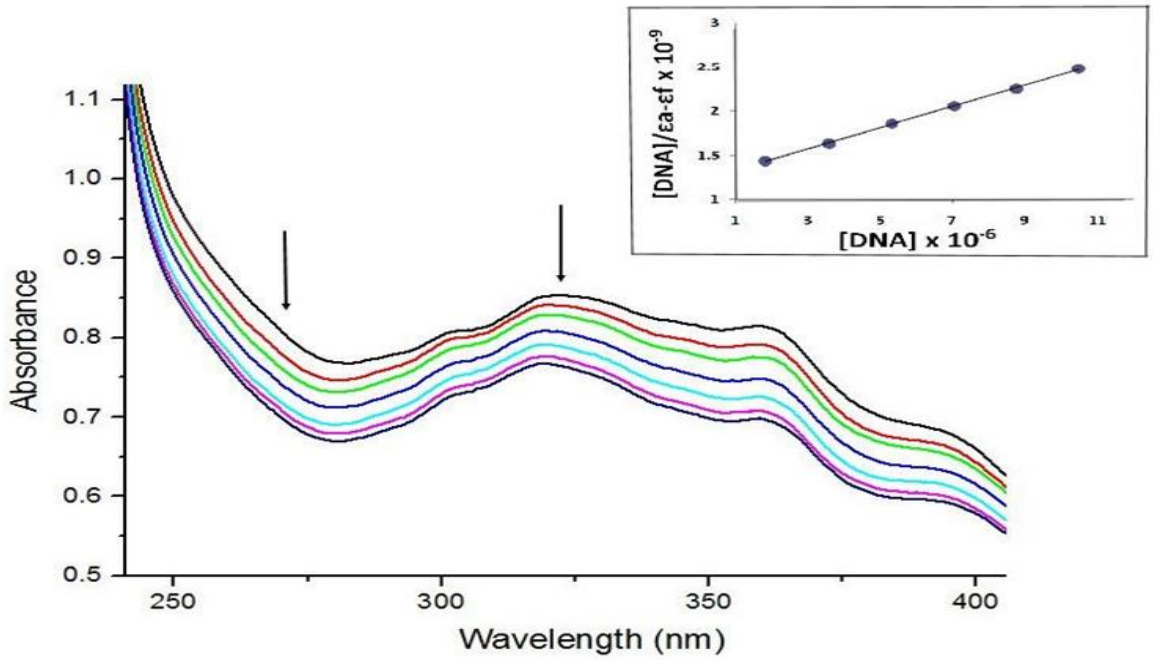

Fig. 1: Absorption spectrum of Ni(II)-PLHBH complex (20μM) in absence (-black) and presence (-colours) of increasing amounts of DNA (10-100μM). Arrow indicates hypochromism with increase in concentration of DNA. Inset: Plot of [DNA]/(ε_a - ε_f) versus [DNA]

3. RESULTS AND DISCUSSION

3.1 COMPETITIVE DNA BINDING FLUORESCENCE STUDIES

Due of the strong fluorescence intensity of ethidium bromide (3,8-diamino-5-ethyl-6-phenylanthridium bromide) (EB) when bound to the nucleic acid, it has been utilized as a probe. By observing the variations in the emission intensity of EB bound to CT DNA when a metal complex is introduced, the competitive DNA binding of complexes has been examined [9]. Due to its intercalation between the neighboring DNA base pairs in the presence of CT-DNA, EB exhibits strong fluorescence at a wavelength of between 580 and 600 nm. The metal complex has an attraction for DNA, which causes the emission intensity of the EB-DNA adduct to decrease. The competitive binding of the metal complex to CT DNA, which displaces EB, or alterations in the secondary structure of DNA can be responsible for the degree of fluorescence quenching of EB coupled to CT DNA. When Ni(II), Cu(II), and Zn(II) complexes are added to DNA that has already been pretreated with EB, the intensity of the emission is quenched, indicating that EB has been removed from the EB-DNA adduct. The Stern-Volmer equation(2) was used to calculate the fluorescence quenching.

$$I0/I = 1 + Ksv\ r \qquad (2)$$

where I0 and I represent the DNA-EB adduct's fluorescence intensity in the absence and presence of the complex, respectively. Ksv is the Stern-Volmer quenching constant, and 'r' is the proportion of the complex's concentration to DNA's. The extent of the complex's binding to CT DNA is shown by the Ksv value, which is determined by the slope-to-intercept ratio in a plot of I0/I versus [complex]/[DNA]. The emission spectra of EB bound to DNA in the absence and presence of metal complexes. The arrow in the figures denotes a reduction in emission intensity with an increase in complex concentration. The plot of I0/I versus [Complex]/[DNA] is shown in the insets of figures-2. The values of the Stern-Volmer constant for the complexes of Ni(II), Cu(II), and Zn(II) are provided.

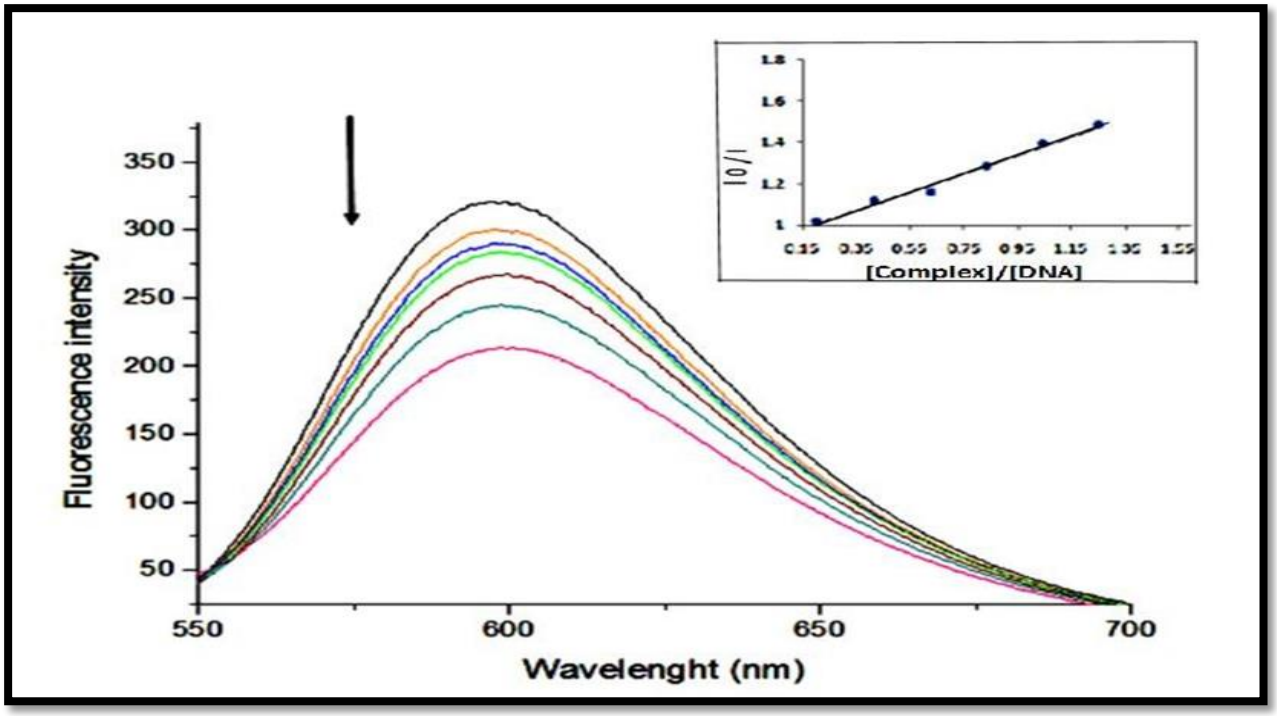

Fig.2.: Emission spectrum of EB bound to CT-DNA in the absence (-black) and presence (-colours) of Ni(II)-PLHBH complex (10-100μM). Arrow indicates quenching with increase in concentration of complex. Inset: Stern- Volmer

According to the values of the Stern-Volmer constants, the Cu(II) complexes of all the ligands demonstrated superior binding ability than Ni(II) and Zn(II) complexes. The intercalative mechanism of binding for the Ni(II), Cu(II), and Zn(II) complexes is suggested by competitive DNA binding fluorescence experiments. The metal complexes' planar ligands displace EB from intercalative binding sites, which results in a dampening of DNA-

EB's emission intensity. The linear Stern-Volmer equation was in good agreement with the graphs of I0/I versus [complex]/[DNA]. The complexes' Ksvvalues are listed below in the order they appear.

Ni(II) complexes: PLFBH >PLDCBH > PLHBH > PLTMBH > PLNBH

Cu(II) complexes: PLFBH > PLDCBH > PLTMBH ≈ PLHBH > PLNBH

Zn(II) complexes: PLDCBH≈ PLFBH > PLTMBH > PLHBH > PLNBH

3.2 VISCOSITY MEASUREMENTS

At 25°C, viscosity tests were done to determine how the metal complexes bond to the CT-DNA. The most important test of DNA binding in solution was hydrodynamic tests, which were thought to be sensitive to length change. The DNA helix lengthens when base pairs separate to make room for the bound ligand, according to a traditional intercalation model, which increases CT-DNA viscosity. The DNA helix might be bent (or kinked), but its length and consequent viscosity could be reduced by non-classical intercalation of ligand. By extending the DNA helix by intercalation, EB has been identified as a possible intercalator that exhibits a notable increase in viscosity. The complexes increased in relative viscosity as the concentration was raised, similar to EB, showing ligand insertion between DNA base pairs. The resulting information has been plotted as [Complex]/[DNA] vs ('sp/sp)1/3.

According to all systems, EB > Cu(II) > Ni(II) > Zn(II) has been followed by the binding affinity to DNA, which may be what causes the viscosity to increase. The viscosity of CT DNA changes in the presence of EB and complexes are depicted.

It has been noted that as the concentrations of Ni(II), Cu(II), and Zn(II) complexes rise, the viscosity of the CT DNA also rises steadily. Such behavior is consistent with intercalators like EB, which extend the DNA double helix and ultimately cause intercalation to increase the relative specific viscosity.

3.3 DNA CLEAVAGE STUDIES

Studies on DNA cleavage were conducted to assess the title compounds' Ni(II), Cu(II), and Zn(II) complexes' capacity to cut DNA.Super coiled (SC) plasmid pBR322 DNA (100ng/L) was incubated with various complex concentrations (20–60 M) in DMSO in Tris HCl buffer (pH 7.2) for 1 hour at 37°C in the gel electrophoresis experiment. Under the influence of electric potential, plasmid DNA migrates. Being a charged species, DNA will move toward the anode in an electric field. When the circular plasmid DNA is electrophoresed, the super-coiled DNA (Form I) migrates quite quickly. The supercoiled shape will relax to create a slower-moving nicked circular

form (shape II) if scission takes place on one strand. A linear form (Form III) that transitions between the super coiled form and the nicked circular form is created if both strands cleave. By using a hydrolysis mechanism, hydrolytic cleavage agents cut phosphodiester bonds in nucleic acids. Figures 3 to 7 show the gel electrophoresis patterns for plasmid DNA breakage by the metal complexes.

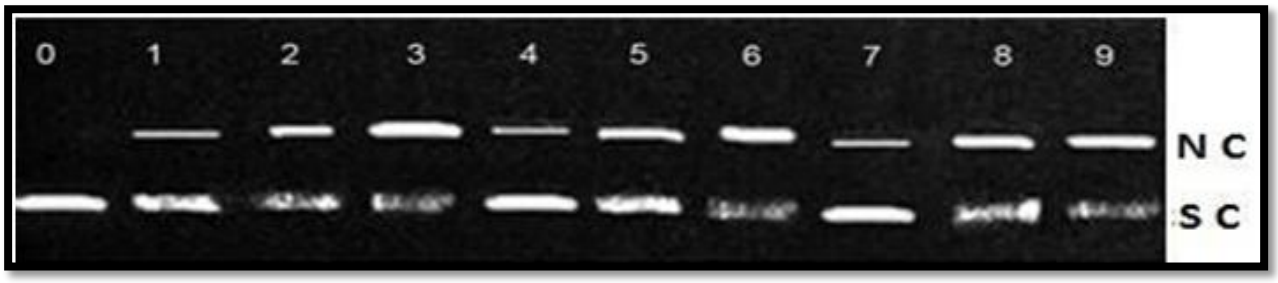

Fig. 3: Agarose gel electrophoresis pattern for the cleavage of supercoiled pBR 322 DNA by complexes. Lane 0, DNA control, Lane 1-3 DNA + Ni(II)-PLHBH (20,40,60 µM), Lane 4-6 DNA + Cu(II)- PLHBH(20,40,60 µM), Lane 7-9 DNA + Zn(II)-PLHBH (20,40,60 µM).

Fig. 4: Agarose gel electrophoresis pattern for the cleavage of supercoiled pBR 322 DNA by complexes. Lane 0 DNA control, Lane 1-3 DNA + Ni(II)-PLNBH (20,40,60 µM), Lane 4-6 DNA + Cu(II)- PLNBH(20,40,60 µM), Lane 7-9 DNA + Zn(II)-PLNBH (20,40,60 µM).

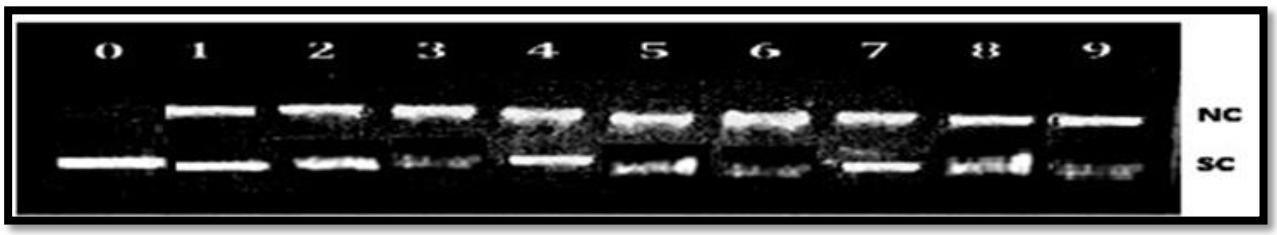

Fig. 5.: Agarose gel electrophoresis pattern for the cleavage of supercoiled pBR 322 DNA by complexes. Lane-0 DNA control, Lane 1-3 DNA + Ni(II)-PLDCBH (20,40,60 µM), Lane 4-6 DNA + Cu(II)- PLDCBH(20,40,60 µM), Lane 7-9 DNA + Zn(II)-PLDCBH (20,40,60 µM).

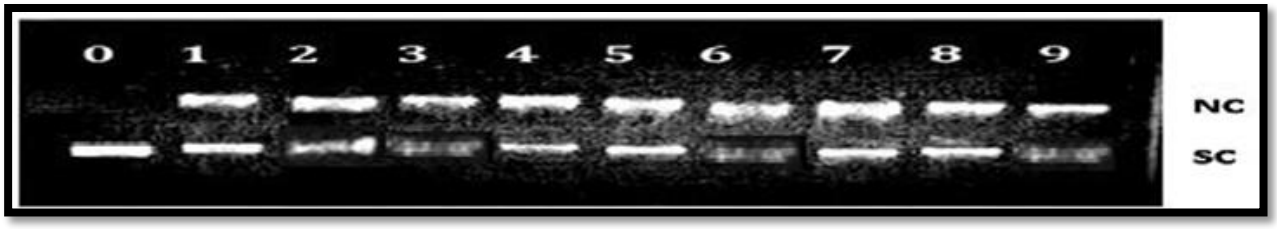

Fig. 6: Agarose gel electrophoresis pattern for the cleavage of supercoiled pBR 322 DNA by complexes. Lane 0 DNA control, Lane 1- 3 DNA + Ni(II)-PLFBH (20,40,60 µM), Lane 4-6 DNA + Cu(II)-PLFBH(20,40,60 µM), Lane 7-9 DNA + Zn(II)-PLFBH (20,40,60 µM).

Fig. 7: Agarose gel electrophoresis pattern for the cleavage of supercoiled pBR 322 DNA by complexes. Lane 0, DNA control, Lane 1- 3, DNA + Ni(II)-PLTMBH (20,40,60 µM), Lane 4-6, DNA + Cu(II)- PLTMBH(20,40,60 µM), Lane 7-9, DNA + Zn(II)-PLTMBH (20,40,60 µM).

In the current experiment, it was found that all of the complexes were capable of cleaving DNA without the need for any additional reagents, indicating that DNA was being broken down hydrolytically. This may be because the ligands contained nucleophiles such hydroxyl groups.

Figures 3 to 7 show that all the complexes are capable of converting DNA from its super coiled (SC) form to its nicked circular (NC) form, indicating that the complexes have nicking activity. The cleavage activity also increased with an increase in the concentration of the metal complexes.

3.4 ANTIBACTERIAL ACTIVITY

Based on Tweedy's chelation theory and the Overtones idea, the increased activity of the metal complex when compared to ligand can be explained.According to Overtones' idea of cell permeability, the lipid membrane that surrounds the cell facilitates the passage of only lipid-soluble molecules. According to Tweedy's chelation theory, chelation entails a reduction in the polarity of the metal ion because the donor groups of the ligand partially share the metal ion's positive charge. This strengthens the metal complex's lipophilic nature and facilitates its entry into

the bacterial cell's lipid membranes. The metal complexes also interfere with cellular respiration, which in turn prevents protein synthesis and limits organism growth.

The ligands were tested for antibacterial activity against gram negative bacteria (Escherichia coli and Pseudomonas aeruginosa) and gram positive bacteria (Staphylococcus aureus and Bacillus cereus) using their complexes of Fe(III), Co(II), Ni(II), Cu(II), Zn(II), and Cd(II). The information in tables 1–5 shows that metal complexes had more antibacterial action than free ligand.

TABLE 1.- ANTIBACTERIAL ACTIVITY OF PLHBH AND ITS COMPOUNDS (MM)

Compound	**Gram negative bacteria**		**Gram positive bacteria**	
	E.coli	***P. aeruginosa***	***S.aureus***	***B.cereus***
PLHBH	11	15	14	12
Fe(II)-PLHBH	19	16	16	13
Co(II)-PLHBH	18	15	17	15
Ni(II)-PLHBH	20	17	21	19
Cu(II)-PLHBH	26	25	22	21
Zn(II)-PLHBH	20	21	20	18

TABLE-2.ANTIBACTERIAL ACTIVITY OF PLNBH AND ITS COMPLEXES (MM)

Compound	Gram negative bacteria		Gram positive bacteria	
	E.coli	*P. aeruginosa*	*S.aureus*	*B.cereus*
PLNBH	09	11	10	10
Fe(III)-PLNBH	16	13	15	12
Co(II)-PLNBH	17	16	13	15
Ni(II)-PLNBH	20	19	21	18
Cu(II)-PLNBH	24	22	23	20
Zn(II)-PLNBH	19	17	16	16

TABLE-3.ANTIBACTERIAL ACTIVITY OF PLDCBH AND ITS COMPLEXES (MM)

Compound	Gram negative bacteria		Gram positive bacteria	
	E.coli	*P. aeruginosa*	*S.aureus*	*B.cereus*
PLDCBH	17	15	12	16
Fe(III)-PLDCBH	20	18	19	18
Co(II)-PLDCBH	21	19	20	17
Ni(II)-PLDCBH	27	25	24	21
Cu(II)-PLDCBH	32	30	29	27
Zn(II)-PLDCBH	24	21	23	20
Cd(II)-PLDCBH	22	20	17	19

TABLE-4.ANTIBACTERIAL ACTIVITY OF PLFBH AND ITS COMPLEXES (MM)

Compound	Gram negative bacteria		Gram positive bacteria	
	E.coli	*P. aeruginosa*	*S.aureus*	*B.cereus*
PLFBH	19	16	14	17
Fe(III)-PLFBH	20	19	21	19
Co(II)-PLFBH	21	18	23	20
Ni(II)-PLFBH	28	26	24	22
Cu(II)-PLFBH	34	31	30	29
Zn(II)-PLFBH	25	24	22	21

TABLE-5.ANTIBACTERIAL ACTIVITY OF PLTMBH AND ITS COMPLEXES (MM)

Compound	Gram negative bacteria		Gram positive bacteria	
	E.coli	*P. aeruginosa*	*S.aureus*	*B.cereus*
PLTMBH	15	10	13	11
Fe(III)-PLTMBH	17	13	15	19
Co(II)-PLTMBH	20	18	14	20
Ni(II)-PLTMBH	25	23	20	22
Cu(II)-PLTMBH	29	25	25	24

Zn(II)-PLTMBH	22	19	21	18
Cd(II)-PLTMBH	19	17	16	19

All of the ligands' Cu(II) complexes have demonstrated excellent antibacterial activity. Due to the presence of ring-activating fluoride and chloride groups in the ligands, the metal complexes of PLFBH and PLDCBH have demonstrated good antibacterial activity, which can be attributed to mesomeric effect and -I effect.

4. CONCLUSION

All of the complexes interact with CT-DNA by intercalative mode through nitrogenous bases of DNA, according to evaluations of DNA binding studies using the UV-visible absorption titration method, competitive fluorescence studies, and viscosity measurements of the Ni(II), Cu(II), and Zn(II) complexes of all the ligands. The fluorescence quenching experiments and viscosity measurements were in agreement with the binding constant values (Kb) of the metal complexes. Compared to Ni(II) and Zn(II) complexes, the Cu(II) complexes demonstrated greater DNA binding capabilities. All of the complexes of Ni(II), Cu(II), and Zn(II) were able to break the DNA of the plasmid pBR322 and change the highly coiled form of DNA into a circular form with nicks, indicating that the complexes exhibit nicking activity. According to the investigations on antibacterial activity, metal complexes were more effective than free ligands.

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