# Some Novel Transition Metal Complexes of Polydentate Ligands: Synthesis, Physico-Chemical Characterization and DNA Interaction Study

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**JULY, 2021**

***Dedicated***
***to***
***My Beloved Parents***

## DECLARATION

I declare that the thesis entitled **Some Novel Transition Metal Complexes of Polydentate Ligands: Synthesis, Physico-Chemical Characterization and DNA Interaction Study** has been prepared by me under the supervision of Prof. Biswajit Sinha, Department of Chemistry, University of North Bengal. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

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# ABSTRACT

Molecules that interact with DNA have been studied extensively with the aim of designing new types of chemotherapy agents and probes for nucleic acid structures. Studying the interaction of transition metal complexes with DNA have been the main focus of recent investigations. Development of new molecules that can obstruct cancerous cells by interacting with DNA in a non-classical mechanism is an example of new anticancer approach. Transition metal complexes are ideal for these tasks, as their unique characteristics allow specific interactions with DNA and other important bio-molecules. Transition metals which are used largely in this area of research include platinum, ruthenium, rhodium, palladium, Vanadium and Nickel. Among these transition metals, Platinum(II) based compounds and related drugs fascinated the inorganic chemists during a long period because of its anti-cancer activity, especially cis-platin. But, these drugs have so many disadvantages too such as lesser solubility, dose-limiting side effects *etc*. which necessitates the upgradation of anticancer approach. To explore this area of research here we have dissertated interaction of some newly synthesized transition metal complexes with DNA.

Chapter I is an introductory one that describes different types of polydentate ligands and their transition metal complexes and their applications in biological fields. This chapter also contains a brief literature review on related works as well as the objective and application of the research works.

Chapter II contains the sources of the different chemicals and materials used in this research work. This chapter also contains details of the physico-chemical and spectroscopic techniques, *viz.*, Elemental analysis, Magnetic susceptibility measurement, IR, UV-Visible, NMR- spectroscopies, ESI-MS, *etc.*, used for the physicochemical characterization of the synthesized complexes. This chapter also describes briefly the procedure to study different applications of the compounds.

Chapter III involves synthesis, characterization of a new Zn(II) complex and its interaction with DNA by diverse physical and spectroscopic techniques. The newly synthesized complex was further examined for antioxidant activity.

Chapter IV includes preparation, characterization of a new tetradentate Co(II) complex and its interaction with DNA using different advanced techniques. The synthesized compound was also used to study antibacterial activity against gram positive (*S. aureus*, *B. subtilis*) and gram negative (*E. coli*, *P. aeruginosa*) bacteria.

Chapter V encompasses synthesis, characterization and DNA cleavage activity of Cu(II) complex. *In vitro* antioxidant activity of the new Cu(II) complex was also investigated.

Chapter VI includes the synthesis and physico-chemical properties of ionic liquid tagged azo-azomethine ligand and its Zn(II) complex. The newly synthesized complex was further researched for DNA interaction study.

Finally Chapter VII contains the concluding remarks of the research works embodied in this dissertation.

# PREFACE

I started the research work presented in this thesis entitled **Some Novel Transition Metal Complexes of Polydentate Ligands: Synthesis, Physico-Chemical Characterization and DNA Interaction Study** in the year 2013 under the supervision of Prof. Biswajit Sinha in the Department of Chemistry, University of North Bengal, India. In recent times, research on the detection and fixing of DNA defects at initial phase has received much attention and to diagnose / fix such defects various probes have been examined. Researchers to solve this issue have visualized the idea of binding event of any foreign molecule with biomolecules just like the Guest-Host interaction and Lock and Key Concept. In early studies researchers primarily used various organic molecules to facilitate such interaction with DNA. But ever since the establishments of cis-platin as chemotherapeutic agent, transition metal complexes have accessed much attention as they have the superiority of showing different geometries: the reactivity of transition metal complexes can be modulated by simply altering the metal ions and their oxidation states. Despite the success of cisplatin as anticancer agent, its clinical usefulness is limited by its severe side effects. The necessity of alternatives to cisplatin has inspired the researcher to do further work towards the improvement of novel metal-based drugs with superior properties.

The present dissertation focused on the synthesis, Physico-Chemical characterization and DNA interaction ability of newly synthesized transition metal complexes.

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# CHAPTER I

## General Introduction

The application of science towards mystical issues continues until there is a problem. Over the past few decades, the world has witnessed amazing discoveries. The cell is one of such mysterious inventions in the living system. Cell, the fundamental unit of life plays a crucial role in the life process [1-3]. The complex internal structures of cell have attracted researchers for a huge time. DNA, the heart of the cell in collaboration with other cellular systems governs metabolic processes and protein synthesis [4-6]. DNA carries the genetic particulars from one generation to the other. The information allied with DNA is transcribed to RNA which is followed by translation into synthesis of proteins. Copying defect in the course of replication in the genetic material causes mutation. This mutated DNA triggers a chain of defects in the synthesis of protein and eventually overall cellular action leading to some disease. Cancer is an example of such a disease that originates from this somatic mutation on cellular level [7-8]. In recent times, research on the detection and fixing of this DNA defects at initial phase has received much attention and to diagnose / fix such defects various probes have been examined. Researchers to solve this issue have visualized the idea of binding event of any foreign molecule with biomolecules [9-11] just like the Guest-Host interaction and Lock and Key Concept [12-13]. In early studies researchers primarily used various organic molecules to facilitate such interaction with DNA. But ever since the establishments of cis-platin as chemotherapeutic agent [14-15], transition metal complexes have accessed much attention as they have the superiority of showing different geometries: the reactivity of transition metal complexes can be modulated by simply altering the metal ions and their oxidation states. Despite the success of cisplatin as anticancer agent, its clinical usefulness is limited by its severe side effects such as dose-dependent nephrotoxicity, nausea and vomiting, ototoxicity, neurotoxicity, and myelosuppression [16-20]. The necessity of alternatives to cisplatin has inspired the researcher to do further work towards the improvement of novel metal-based drugs with superior properties.

### 1.1. Coordination chemistry:

Coordination chemistry as the name may seem to imply provides a link between different areas of chemistry. Coordination chemistry was a difficult domain for inorganic

chemists before the 19th century. After the Werner's theory in 1893, inorganic chemistry has moved towards a nice flow on coordination compounds. Currently, coordination chemistry is widely evolved, especially for designing and developing new bioactive donor ligands with transition metal ions. In the coordination compounds, the central metal ion is swaddled by electron rich ligand molecules: here metal acts as a Lewis acid and ligand as a Lewis base. Ligands are classified on the basis of number of donor sites: Ligands having only one donor site is called monodentate ligand while having two or more than two donor sites then they are categorized as **polydentate ligand**.

In living systems, there are several metal ions are found which form various kinds of complexes with bioactive polydentate ligand systems (like Porphyrin in chlorophyll).

The transition metals conflict from the main group metals in multiple characteristic properties. Among them the most notable aspect is the ability of transition metals to exhibit variable oxidation states and that's why they form stable coordination compounds. Bioactive ligand systems with certain transition metal ions play a crucial role in the processes of human life [20-28]. The physiological role of transition metal ions in these biological processes is definitely a fundamental question, but the difficulties linked to clarifying their role should not be minimized. It has already been established that the transition metal ions regulate a variety of processes in biology. The alliance between biology and inorganic chemistry is one of the areas where fascinating developments are taking place. This exciting advancement in the biochemical world fascinates inorganic chemists to do more work in this new domain called bioinorganic chemistry.

### 1.2. Biological significance of transition metals and their coordination compounds:

Transition metals are the vital elements for living system [29]. The significance of transition metals in the domain of bio-science, pharmaceutical fields and in agriculture is enhancing expeditiously day by day. From the Dose-response curve, the minimum requirement value of transition metals for human body has been found: [Mn = 2.3 mg, Co = 5 mg, Ni = 1.5 mg, Cu = 120 mg and Zn = 2.3 mg]. Small change in this amount may lead to several side effects. The biological significance of some transition metals is discussed below elaborately:

### 1.2.1. Manganese (Mn):

Manganese and its coordination compounds are biologically very much significant as they show a number of applications in biological fields [30-34]. Mn activates metalloenzymes like pyruvate carboxylase, arginase, superoxide dismutase (SOD). The enzyme possessing Mn-cluster takes a pivotal role in photosynthesis process via catalyzing the oxygen evolution step. Manganese is mainly centred in mitochondria and because of this it is engaged in operating the respiratory enzymes. Manganese deficiency in humans induces skeletal malformations and reduces reproductive function, but in excess the central nervous system and the brain is affected very badly. Mn (II) in combination with Fe (II) / Fe (III), creates active sites which helps in nitrogen fixation [35] in plants.

### 1.2.2. Cobalt (Co):

Coblat is very essential for human health [29-30]. It is present in Vitamin $B_{12}$ (cobalamin) which is a tetraazamacrocyclic cobalt (III) complex. Its corrin compounds are associated with blood plasma protein receptor which is necessary for the synthesis of hemoglobin, formation of myelin to support the production of red blood cells. It takes an important role in protein synthesis and in metabolism of carbohydrates, fats. Vitamin $B_{12}$ inhibits nerve damage via forming protective sheath. An adult human need 5 mg / day of Co for their normal development while overdosing may leads to polycythemia, abnormal thyroid functions, erythropoiesis and excess production of a hormone called erythropoietin (EPO) from kidney which leads to blood clots, heart attacks, strokes and many other related complications.

### 1.2.3. Nickel (Ni):

Animals and humans need a very small amount of nickel (trace element) [36-37]. Generally, a healthy human requires 25-35 μg of Ni per day. Higher levels of it become toxic and can cause many problems such as digestive problems, sinuses, allergies, high red blood cell count, kidney failure etc. Nickel containing Vitamin C is somewhat beneficial for improving liver cirrhosis and insulin production. Each cell inside the human body has 10 mg of nickel in nucleic acids (especially in RNA). It penetrates directly into the cell and experience redox metabolism producing reactive ROS. Urease

which catalyzes the hydrolysis of urea contains Ni-compounds at its active sites. It has an important role in the creation of Prolactin hormone.

### 1.2.4. Copper (Cu)

Copper has been identified as a key trace element for many biological activities [38-44]. In mammals, it is involved in respiration, energy metabolism, DNA synthesis, cytochrome c oxidase, superoxide dismutase, tyrosinase, ascorbate oxidase, amine oxidase, dopamine hydroxylase, and many other oxidases. It plays a key role in connective tissue, nerve covering and bone development in humans. It acts as a co-factor in many electron transfer and oxygen transport proteins like plastocyanin, azurin, hemocyanin etc. A normal adult human contains 85-110 mg of copper in the central nervous system, muscles, liver, heart, skin and in brain. Copper deficiency may leads to neurodegenerative disorders, Wilson's disease, Parkinson's disease etc. Furthermore, copper is used as mouthwash, toothpaste and other dental amalgams. Its complexes are utilized as nutrients to sustain the growth of plants. Recently a very few copper complexes have shown excellent anticancer and antimutagenic activity both *in vivo* and *in vitro*.

### 1.2.5. Zinc (Zn)

Zinc has been identified as a trace element for animals, humans, plants [45-54]. Zn exists in numerous metalloenzymes and gene regulatory proteins. It has an important role to carry genetic data during DNA replication. Zn is used extensively to form tendons, bones, ligaments, nails, skin, teeth, hair etc. The exploration of "zinc fingers" has prompted researcher to do serious research on the protein interaction of zinc ions. High toxicity of zinc may cause nausea, epigastric pain, diarrhea, vomiting, lethargy, hair loss, damage of the reproductive organs. Deficiency of Zn results: growth retardation, poor wound healing, impaired immune action and deterioration of mental function and cancer.

**1.3. Schiff Bases and its complexes:**

Schiff base was first synthesized by Hugo Schiff in the year 1864. Schiff bases contain azomethine linkage (-HC=N-) which is formed via a condensation reaction of primary amines with carbonyl compounds [55-57]. The detail of Schiff base preparation is widely spread in the literature. The general route of Schiff base preparation is given in figure 1.1.

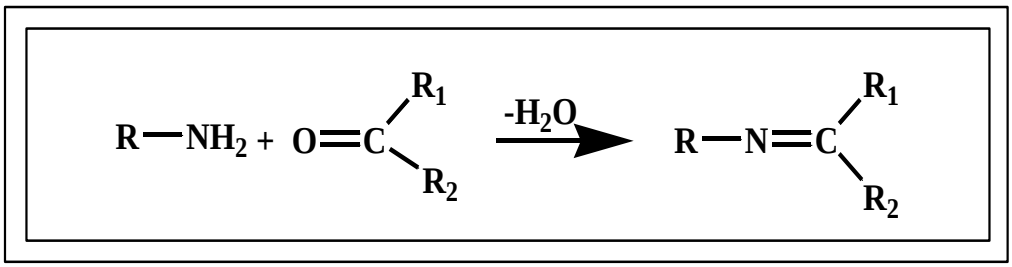


**Fig.1.1.** Condensation reaction for Schiff base formation.

Schiff bases obtained from aliphatic aldehydes are comparatively less stable and undergo polymerization [58] while which are obtained from aromatic aldehydes is more stable. The non-bonding electron pair on nitrogen of azomethine in Schiff bases is of ample biological and chemical importance [59] and provide remarkable chelating ability, particularly when it is in combination with other donor atoms around it. Depending on the nature of amines and aldehydes, Schiff bases can behave as polydentate ligands. Few examples of which are shown in figure 1.2:

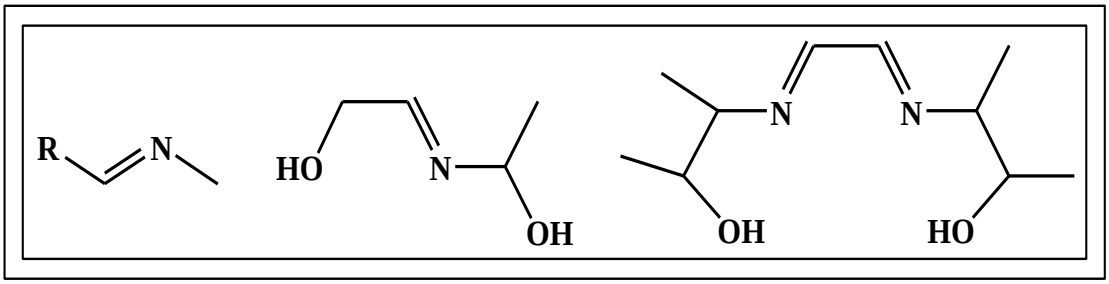


**Fig.1.2.** Schiff bases with different denticity.

Schiff base ligands are established as the most favorable and alluring ligands for the formation of transition metal complexes. They have the ability to stabilize metals under different oxidation states and thereby regulate the performance of metals in a variety of catalytic transformation. The Schiff base ligands can tune the steric and electronic effects around the metal core simply by incorporating a suitable sized electron donating or withdrawing substituent to the ligand [60]. Schiff base transition metal complexes have engrossed a key role in the advancement of coordination chemistry. In

the middle of 18th century (1840), first Schiff base transition metal complex bis(salicyldimino)Cu(II) was isolated by Ettling from the reaction of salicyladehyde, aqueous ammonia and cupric acetate.

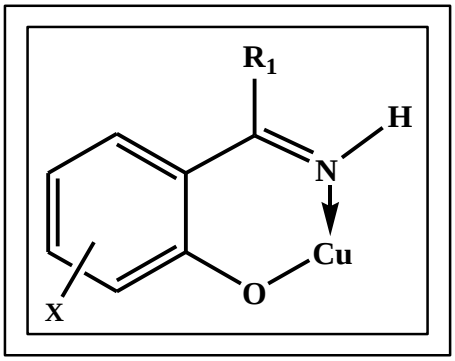


**Fig.1.3.** Structure of bis(salicyladimino)Cu(II).

But organized synthetic study of Schiff base metal complexes was initiated in 1931 by Pfeiffer and his co-workers. In the year 1966, Holm and Everett reviewed the properties, electronic structure and stereochemistry of the Schiff base complexes. A comprehensive review of Schiff base chemistry is mentioned in review [61-66].

### 1.4. Deoxyribonucleic Acid:

Deoxyribonucleic acid is the hereditary material which is present in all living organisms. In the year 1869, German biochemist Friedrich Miescher was the first to isolate "nuclein" which was later named as deoxyribonucleic Acid (DNA) [67]. But for several years, investigator did not figure out its significance. In 1953 James Watson, Maurice Wilkins, Francis Crick and Rosalind Franklin established the double helix structure of DNA which they recognized could transfer biological information [68-69]. Watson, Wilkins, Crick were awarded the Nobel Prize in 1962 for their research regarding the structure of DNA and its importance for transfer information in living materials.

The information in DNA is stored as a code made up of four chemical bases: adenine (A), guanine (G), cytosine (C), and thymine (T). Human DNA consists of about 3 billion bases, and more than 99 percent of those bases are the same in all people. The order, or sequence, of these bases determines the information available for building and

maintaining an organism, similar to the way in which letters of the alphabet appear in a certain order to form words and sentences [70-72].

DNA bases pair up with each other, A with T and C with G, to form units called base pairs. Each base is also attached to a sugar molecule and a phosphate molecule. Together, a base, sugar, and phosphate are called a nucleotide. Nucleotides are arranged in two long strands that form a spiral called a double helix [70]. The structure of the double helix is somewhat like a ladder, with the base pairs forming the ladder's rungs and the sugar and phosphate molecules forming the vertical sidepieces of the ladder.

Nucleic acids are the main chemical constituents present in the cell nucleus. DNA and RNA are the two major types of nucleic acids. DNA and RNA differ in their sugar unit only. When a cell divides, its DNA sequence was copied and transfer from one cell generation to the next cell [72]. DNA contains the "programmatic instructions" for cellular activities. When the organisms produce offspring, these programmatic instructions are copied in the form of DNA and passed down. In general DNA sequence must contain special features from organism through peculiar property to disease susceptibility. Nowadays well developed medicinal field, is able to replace the copied DNA sequence into RNA biomolcules, which are then used in protein synthesis to encode a specified protein sequence.

Many researchers struggled for the understanding of genetic information and gene expression in the development of new chemotherapeutic strategies. These effectively play a significant role in forensics, pharmaceutical applications, medical diagnosis, genetic screening, rational drug designing, diagnosis of drug resistance, food and agricultural analysis, environmental control and bioterrorism prevention [73-81].

DNA exists in many possible conformations that include A-DNA, B-DNA, and Z-DNA forms, although, only B-DNA and Z-DNA have been directly observed in functional organisms. On comparing B–DNA with A–DNA, the A–DNA is broad and has base pair bend out of the line to its helix axis instead of being perpendicular to it. Z–DNA has left– handed helix whose repeating units are dinucleotides and shows a "zigzag" backbone [82].

Deoxyribonucleic acid, DNA, is a molecule of great biological significance. The total DNA content of a cell is termed the *'Genome'*. The *'Genome'* is unique to an organism, and is the information bank governing all life processes of the organism, DNA being the form in which this information is stored [83-85]. DNA has two main functions,

1. *Transcription:* Information is retrieved from the DNA by ribonucleic acid, RNA, and utilized to synthesize proteins in the body. Proteins are involved in all body processes and play many roles. e.g. as hormones, enzymes, carriers, structural proteins, receptors, regulators etc.

2. *Replication:* DNA is responsible for its own regeneration, i.e., DNA self replicates. The two strands are held together primarily via Watson Crick hydrogen bonds where A forms two hydrogen bonds with T and C forms three hydrogen bonds with G.

**1.5. Interaction of transition metal complexes with Deoxyribonucleic Acid:**

Transition metals are considered attractive moieties for research in the fields of molecular biology, biotechnology and medicine. Dwyer recognised that the diversity of coordination metal complexes could be utilised to provide insight into the structure of biomolecules. His clear vision that "the size, charge distribution, stereochemistry, redox potential and other physical properties of the metal chelates can be varied readily during chemical synthesis, these substances would seem to be ideal pharmacological tools with which to investigate many functional systems in the living cell" is still evident. Metal complex-DNA interactions showcase the influence that the coordination geometry of the metal and the disposition of the ligands have on the binding activity. The early work in this area can be traced to Lippard and coworkers, concerning the binding of square planar platinum complexes to DNA, that eventually led to the discovery of the most popular anticancer drug, cis-platin [86] which covalently binds to DNA, cross-linking the double helix structure, preventing replication and even inducing cell death. With the success of cis-platin in cancer therapy, several research groups started to focus on various transition metal complexes as anti-cancer and anti-HIV agents [87-88]. Sigman's group working on copperphenanthroline complexes demonstrated the reversible binding of the complex in the DNA minor groove and its nuclease activity [89-90]. Barton and coworkers have shown that ruthenium phenanthroline and polypridyl complexes are excellent probes for the structure of nucleic acids [91-92]. Extensive work has been carried out with metal complexes of iron, cobalt, nickel and zinc with respect to either site specific DNA binding or DNA cleavage [93-95]. To understand the nature of interactions that can possibly occur between a metal complex and DNA, it is essential to start with the basic features of the DNA structure.

The nucleic acid monomers, guanine (G), adenine (A), thymine (T) and cytosine (C) have different metal ion affinities. The order of stability of 3d transition metal ion-nucleobase complexes are: G > A, C > T. At physiological pH the preferred binding sites on the nucleobases are: guanine N7, adenine N1 and/or N7, cytosine N3, thymine O4 [87]. Eichhorn and Shin studied the effect of various metal ions on the melting temperature of DNA. The authors suggest that magnesium ions increase $T_m$ by binding to phosphate and stabilizing the double helix, whereas copper ions decrease Tm by binding to the bases and destabilizing the double helix. Based on the metal-induced variation in $T_m$ they suggested that the relative metal affinity to the phosphate backbone of DNA follows the order $Mg^{2+} > Co^{2+} > Ni^{2+} > Mn^{2+} > Zn^{2+} > Cd^{2+} > Cu^{2+}$ [87].

### 1.5.1. Modes of DNA interactions:

Tansition metal complexes interact with DNA through various modes of binding. They are classified mainly into covalent and non-covalent modes of binding as represented in Fig.1.4.

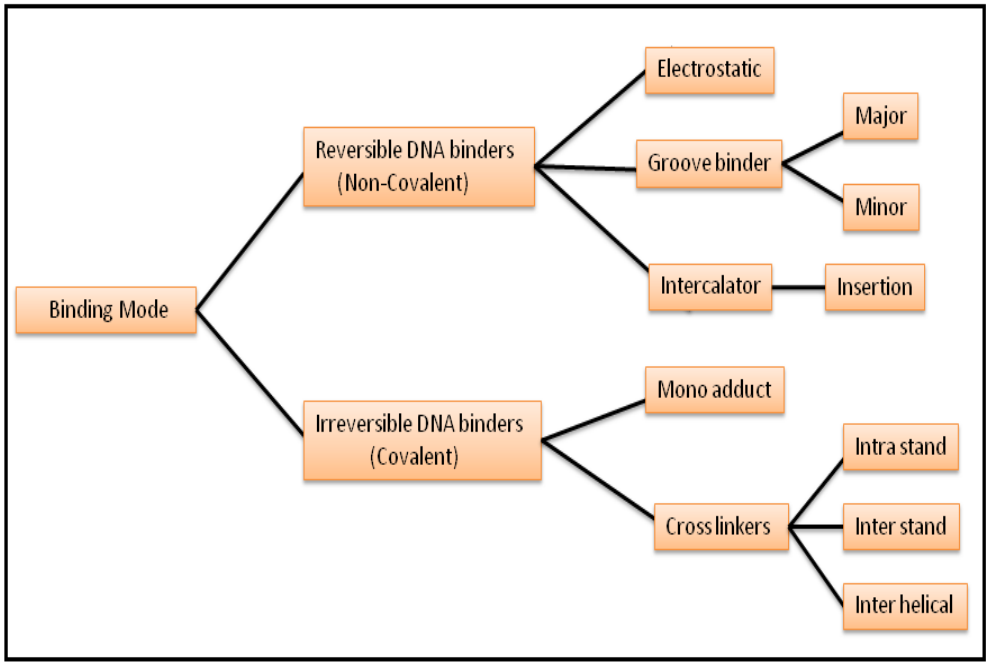


**Fig.1.4.** Modes of interaction of metal complex with DNA.

#### 1.5.1.1. Covalent interaction:

Highly electrophilic molecules can react with the nucleophilic sites of the DNA molecule. They bind DNA irreversibly and form permanent lesion in the DNA molecule.

Covalent binding in DNA is irreversible and invariably leads to complete inhibition of DNA processes and subsequent cell death. Cis-platin (cisdiamminedichloroplatinum) is a famous covalent binder used as an anticancer drug, and makes an intra/interstrand cross-link via the chloro groups with the nitrogens on the DNA bases [96]

**a. Mono adduct (base or phosphate binding)**

Among the different nucleophilic sites in DNA, either phosphate back bone or ring nitrogen bases are the favourable sites of covalent mode of interaction. The covalent mode of binding is often hindered by many factors like steric constraints and hydrogen bonding between the base pairs of helical DNA. Within the bases the covalent ability of ring nitrogen is in the order N7 guanine > N3 adenine > N7 adenine > N3 guanine. N7 position of guanine is the favoured site of much covalent interaction due to less hydrogen bonding and strain. Metal complexes with vacant coordination sites or labile site favour covalent mode of interaction with DNA [97]. Metal complexes can also bind covalently to the phosphate groups. This mode of binding usually occurs when the complex contains one or more coordinated aqua or halide ligands which are replaced by the phosphoryl oxygen atoms on interaction with DNA. The evidence for phosphate binding is sometimes confirmed by phosphodiester hydrolysis using bis (4-nitrophenyl) phosphate (BNPP) and ethyl 4-nitrophenyl phosphate (ENPP) [98].

**b. Cross linkers (Intra strand, Inter strand and Inter helical linker)**

Cross linking agents interact covalently with the nucleotide residues of the same DNA strand (intrastrand cross link) or from opposite strands [interstrand cross link]. Sometimes inter helical DNA cross linking may happen [99]. Cross linking agents are highly effective in killing rapidly dividing cells. It affects DNA replication and transcription by blocking the DNA strand separation [100]. Fig. 1.6 shows different class of cross linking agent with DNA. The widely used antitumor drug cisplatin reacts with DNA via cross-linking of two purine residues through the N7 atoms [101]. Among the

three type of cross linking, cis orientation of the ligands and its geometry favours formation of intra strand cross linked adduct between cisplatin and DNA.

#### 1.5.1.2. Non-covalent interaction:

Reversible DNA binders are chemical compounds that form non-covalent bonds with DNA. As DNA's backbone is negatively charged, reversible DNA binders tend to be cationic, but not electrophilic. In other words, the binder does not form electron-sharing relationship with any components of DNA. Compounds that bind to DNA by non-covalent interactions are generally kinetically inert.

**a. Electrostatic binding:**

This mode of binding is purely electrostatic attraction between the positively charged metal centre and negatively charged DNA backbone. Divalent cations like Mg(II) and Ca(II) are known to bind to DNA electrostatically and provide stability to the double helical structure. Transition metal complexes which bind to DNA by electrostatic mode are also known in literature [102-104]. Such an electrostatic association mode has been proposed for $[Ru(bpy)_3]^{2+}$ by Barton and co-workers [105].

**b. Groove binding:**

Groove-binding are the two most common modes by which small molecules bind directly and selectively to DNA. Groove-binding, which is predominantly entropy driven, involve covalent or non-covalent (electrostatic) interactions that do not perturb the duplex structure to any great extent [106]. Groove binders are reversible DNA binders which have additional intermolecular interaction other than electrostatic interaction. They include vander waals interaction and hydrogen bonding. Typically, the electrostatic interaction brings the binder into the proximity of the DNA. Additional intermolecular interaction occurs as a result of the close proximity. Though the DNA structure consists of distinctly different minor and major grooves, compounds binding to minor groove are more often encountered [107].

**c. Intercalation:**

Intercalation, which is an enthalpically driven process, results from the insertion of a planar aromatic ring system between DNA base pairs with concomitant unwinding and lengthening of the DNA helix. Intercalators as reversible DNA binders contain flat, aromatic molecules that insert between stacks of base pair. Based on Lerman proposal, intercalator sits perpendicular to the DNA axis allowing for π-π overlap with the base pairs thereby leading to simultaneous lengthening of the helix [108]. In essence, this destroys the existing hydrogen bonding between base pairs, thus, causing DNA to unwind [109]. Also, the steric hindrance of the intercalator blocks access of proteins required for DNA transcription and replication, and act as potent mutagens. Intercalators are valuable drugs many of which are currently used for the treatment of ovarian and breast cancers and acute leukaemia's [110]. Extensive work has been reported on metallo-intercalators containing platinum, ruthenium, rhodium, copper and so on which perform diverse functions from being structural probes of the nucleic acids to site specific targeting and identifying base mismatches [111-114].

**d. Insertion**

Insertion mode of binding of molecules is similar to the intercalative mode; however, in this case the molecules instead of stacking themselves between base pairs eject the bases of single base pairs and act as π-stacking replacement in the DNA base stack. Even though insertor concept was proposed by Lerman [115] when intercalator was proposed, until very recent work of Barton group on mismatch-specific DNA binding agents, the examples for this type of interaction were unknown. $[Rh(bpy)_2(chrysi)]^{3+}$and $[Rh(bpy)_2(phzi)]^{3+}$ are known metallo-insertors reported by Barton's research group [116].

**1.6. DNA Cleavage:**

Gel electrophoresis was used to study cleavage of supercoiled DNA by the different compounds. When circular plasmid DNA is put through electrophoresis, comparatively fast migration will be noticed for the intact supercoil form (Form I). If scission happens only on one strand (nicking), the supercoil will generate a slower moving open circular form (Form II). If both strands are cleaved, a linear form (Form III)

will be generated and that migrates between Form-I and Form-II [117]. Above said three forms of DNA are shown in **Fig.1.5.** [117].

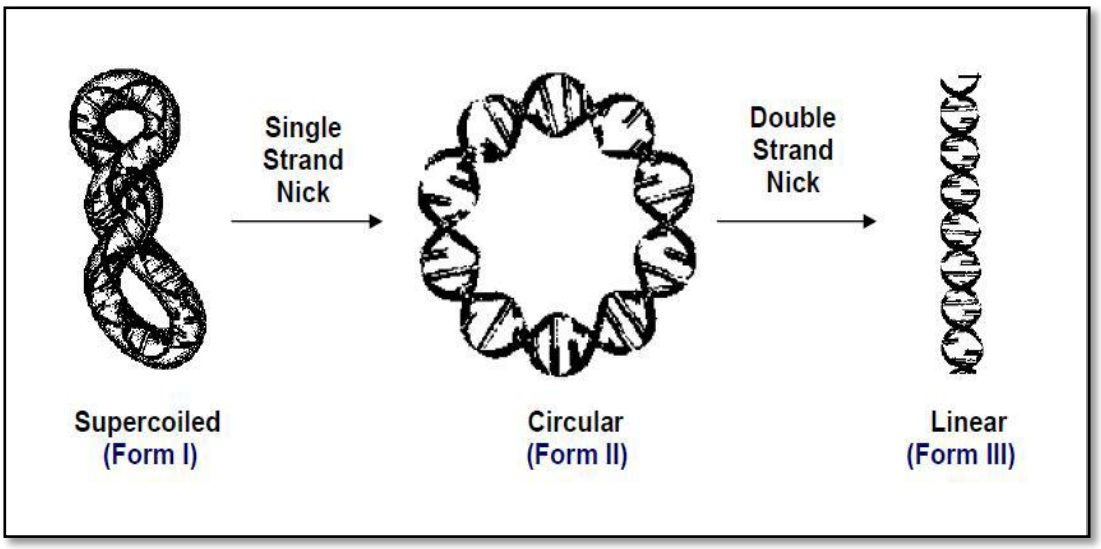


**Fig.1.5.** Representation of DNA cleavage process.

### 1.7. Antioxidant Activity:

It was found that generation of reactive oxygen and nitrogen species (ROS and RNS) play a crucial role in the pathogenesis and development of the disease. ROS are produced mainly in the biological system during the cellular metabolism and by the influence of environmental resources. Although ROS are produced during normal aerobic cell metabolism but its excessive production causes oxidative stress. Oxidative stress is a situation where in there is an imbalance between the generation of ROS and the capacity to neutralize them, leading to the oxidation of several intracellular components such as lipids, proteins and nucleic acids as well as extracellular matrix components like proteoglycans and collagens. This may result in the alteration of structure and biological functions of those molecules that are recognized as antigens by the immune system and autoantibodies formed against these modified molecules and create life limiting chronic diseases such as cardiac infection, hypertension, cancer, arteriosclerosis, rheumatism and cataracts, *etc* [118]. Antioxidant can control this autoxidation by interrupting the propagation or inhibiting the generation of free radicals and subsequently decrease the oxidative stress, improve immunity, and increase longevity. Most organisms are protected to some extent by free radical (peroxide, hydro-

peroxide or lipid peroxyl) damage by enzymes like super-oxide dismutase and catalase or compounds such as ascorbic acid, phenolic acids, tocopherols, polyphenols, glutathione and flavonoids. However, antioxidant supplements or dietary antioxidants may protect the body from the damaging effects created by free radicals. Antioxidants are considered as important nutraceuticals because of their many health benefits and are extensively used in the food industry [119-120]. The combination of certain foods, natural and synthetic rubbers, and gasolines with oxygen in air at room temperature cause to undesirable status such as rancidity in foods, loss of elasticity in rubbers and production of gums in gasolines. Currently, synthetic antioxidants are widely used because they are cheaper and effective than natural antioxidants. Antioxidant activity of a synthesized compound can be measured by using its scavenging potential for trapping of the free radicals. The finding for metal-derived antioxidants has received much attention. Recently, variety of metal complexes has been investigated as potential scavengers of ROS or as antioxidants [120].

**1.8. Antimicrobial studies:**

Antibacterial activity of a compound is associated with its preferential ability to provincially kill bacteria or slow down their rate of growth without being extensively toxic to nearby tissues [121]. Antibacterial agents are the most important in fighting infectious diseases. The antibacterial properties of metals have been recognized for centuries and have been represented by some of the most fundamental breakthroughs in medicinal history. Probably the first antibacterial experiment involved a metal compound in laboratory was done by Koch in 1890. The investigation involving the activity of mercuric chloride on anthrax spores and the introduction of the organoarsenical compound Salvarsan™ (in 1912) for the treatment of syphilis is considered to be the first synthetic therapeutic agent. The major hindrance associated with the chemical substances as antimicrobes is their toxicity to the host cell as well as microbial cells. Hence, the chemical substances used should have selective toxicity towards the harmful microbes but not much to the host tissues. Certain chemicals of synthetic and plant origin are toxic to the bacteria and fungi, but not to the host animal and their wide use as well as abuse, the appearance of bacterial resistance toward antibacterial agents has become a major problem for today's pharmaceutical industry [122-125]. The above problems motivate the scientists to synthesize and study the new agents for antimicrobial activities.

### 1.8.1. Antimicrobial activity of metal complexes:

Transition metal complexes with various ligands have been shown to exhibit antimicrobial activities against a spectrum of microbes and also they have been shown to possess toxicity against a number of cell lines of rodents and human in cell culture. Various organic ligands possess strong antibacterial, herbicidal, insecticidal and fungicidal properties [126]. Metal complexes or coordination compounds have been widely used in medicine [127] and pharmaceutical fields [128] because of their broad bioactivities against bacteria and fungi [129-130].

Christian Grams (Danish Physician) discovered the differential staining technique called as Gram staining. This technique differentiates the bacteria into two groups "Gram positive" and "Gram negative". Gram positive bacteria retain the crystal violet and resist decolorization with acetone or alcohol and hence appear deep violet in colour; while Gram negative bacteria, loose the crystal violet and counter-stained by saffranin and hence appear red in colour. The details regarding some bacteria are given below:

**(a) *Bacillus subtilis:***

*Bacillus subtilis* is a gram-positive, catalase-positive bacterium, generally found in the gastrointestinal tract of ruminants and humans and in soil. *B. subtilis* is rod-shaped, and can form a tough, protective endospore that allowing it to bear extreme environmental conditions [131].

**Fig. 1.6.** *Baciluss subtilis* bacteria.[131]

**(b) *Staphylococcus aureus:***

*Staphylococcus aureus* is a gram-positive, round-shaped, facultative anaerobic bacterium that is frequently found in the nose, respiratory tract, and on the skin. It is often positive for catalase and nitrate reduction. The cell wall of *S. aureus* is made up of peptidoglycan interspersed with teichoic acid and protein. Enzymes also contribute to the virulence of this organism. *S. aureus* is approximately 1μm diameter and it divides to form the cluster characteristic of the genes. In liquid media, it is present in different forms such as singles, pairs and short chains. It grows on nutrient agar medium, incubated in air for 18-24 h at the optimal growth temperature of 37 °C. Although *S. aureus* is not always pathogenic *S. aureus* causes skin infections like skin abscess, respiratory infections like sinusitis, and food poisoning. These bacteria are spread by direct contact with an infected person, by using a contaminated object, or by infected droplets dispersed by sneezing or coughing. The bacteria can also spread through the bloodstream and infect distant organs. Pathogenic strains often promote infections producing virulence factors such as potent protein toxins, and the expression of cell surface proteins that bind and inactivate antibodies [131].

**Fig 1.7.** *Staphylococcus aureus* bacteria. [131]

**(c) *Pseudomonas aeruginosa:***

*Pseudomonas aeruginosa* is a common gram-negative, rod-shaped bacterium that causes disease in plants and animals, including humans. It is a multidrug resistant pathogen, recognized for its ubiquity, its intrinsically advanced antibiotic resistance mechanisms, and its association with serious illnesses; especially hospital-

acquired infections such as ventilator-associatepneumonia and various sepsis syndromes. Treatment of *P. aeruginosa* infections is very difficult due to its natural resistance to antibiotics. When more advanced antibiotic drug regimens are needed adverse effects may result.

**Fig.1.8.** *Pseudomonas aeruginosa* bacteria [131]

**(d) *Escherichia coli***

*Escherichia coli* is a Gram-negative, facultative anaerobic, rod-shaped bacterium. This microorganism was first described by Theodor Escherich in 1885. Most *E. coli* strains harmlessly colonize the gastrointestinal tract of humans and animals as a normal flora but some strains causing diarrhea, intestinal and urinary tract infections. One strain can lead to kidney failure to mortality if not properly managed. Eating contaminated food and water is the most common way to get an E. coli infection. They grow best at 37 $^{\circ}$C [131].

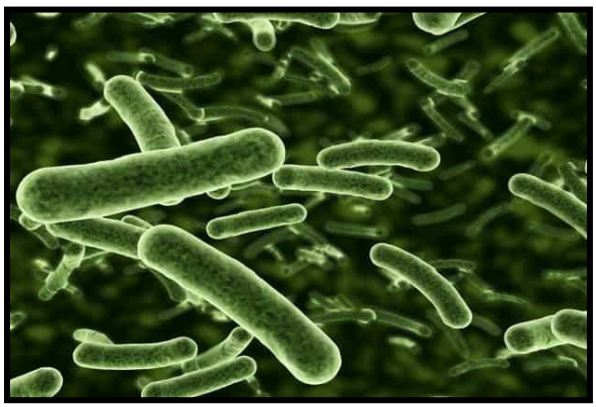

**Fig 1.9.** *Escherichia coli* bacteria.[131]

### 1.9. Molecular Docking:

Molecular docking is the study of how two or more molecular structures (e.g., drug and enzyme or protein) fit together [132]. In a simple definition, docking is a molecular modeling technique that is used to predict how a protein (enzyme) interacts with small molecules. The ability of a protein (enzyme) and nucleic acid to interact with small molecules to form a supramolecular complex plays a major role in the dynamics of the protein, which may enhance or inhibit its biological function. The behavior of small molecules in the binding pockets of target proteins can be described by molecular docking. The method aims to identify correct poses of ligands/complexes in the binding pocket of a protein and to predict the affinity between the ligand and the protein.

### 2.0. Literature Review:

Dwyer recognised that the diversity of coordination metal complexes could be utilised to provide insight into the structure of biomolecules [133-135]. His clear vision that "the size, charge distribution, stereochemistry, redox potential and other physical properties of the metal chelates can be varied readily during chemical synthesis, these substances would seem to be ideal pharmacological tools with which to investigate many functional systems in the living cell" is still evident [133-137]. Metal complex-DNA interactions showcase the influence that the coordination geometry of the metal and the disposition of the ligands have on the binding activity. For example, square planar complexes permit deeper insertion of an intercalator compared to octahedral or tetrahedral geometries [138]. Complexes such as [Pt(phen)(en)]2+ (where phen = 1,10-phenanthroline and en = 1,2-diaminoethane) can intercalate between the base pairs of DNA, [139-140] and depending on the choice of the ancillary ligand, may insert beyond the platinum (II) centre; effectively offsetting the size of small intercalating ligands such as phen. [141-143] However, when incorporated into octahedral complexes such as [Co(phen)3] 2+ or [Ru(phen)3] 2+, the geometric arrangement of the phen ligands can hinder full insertion [144]. For complexes such as [Ru(phen)2Cl2], they can inhibit covalent binding due to steric crowding by the DNA phosphate backbone [145]. Additionally, a study that compared zinc (tetrahedral) and cobalt (octahedral) complexes incorporating a porphyrin ligand showed that the cobalt complex bound to DNA via intercalation, however the zinc complex was inhibited by the presence of an axial water

ligand [146]. It is clear that different transition metal complexes can undergo vastly different binding interactions with DNA.

Cisplatin and its derivatives are capable of forming various DNA adducts including: monofunctional adducts in which one bond is formed with DNA and the other coordination site ligand remains aquated or protein-bound; 1,2-intrastrand adducts, the most common type, in which two bonds are formed upon the same strand between consecutive base pairs; 1,3-intrastrand adducts in which the bonds are formed with base pairs that are one base apart; and interstrand adducts in which bonds are formed on opposite strands of the double-helix. The cis geometry of cisplatin is vital to its in vivo activity; due to the trans effect, the trans isomer transplatin is much more rapidly degraded in vivo, is incapable of forming the most effective 1,2-intrastrand adducts, and its 1,3-intrastrand adducts are rapidly repaired relative to cisplatin [147]. The successes and limitations of cisplatin have inspired researchers to explore new designs for covalently binding platinum drugs. Initially, square-planar platinum(II) complexes with the general formula cis-$[PtX_2(NH_2R)_2]$, where NH2R is an inert amine and X is an anionic leaving group, were developed where the weak trans effect facilitated DNA binding and provided an overall neutral charge when administered [148-150]. Despite the large amount of research dedicated to creating complexes of this type, only five (Fig. 3) have gained approval for clinical use, and only some in all countries [151]; this is due to the problems of dose-limiting toxicity and intrinsic and acquired resistance that these agents experience [152-153]. Furthermore, each of these compounds are capable of binding to DNA via similar hydrolysis-mediated mechanisms as cisplatin [154-155]. The limitations of the above complexes have prompted medicinal chemists to study platinum complexes with structures that are far different from the typical cisplatin paradigm [156]. The use of metals with different coordination geometries to platinum(II) and a greater variety of ligands has resulted in a new library of complexes that exhibit different cellular behaviour and higher in vitro efficacy than current clinical compounds [157]. For example, platinum(IV) agents are currently being investigated as prodrugs that can preserve the active platinum(II) species until its release via intracellular reduction once the target cells are reached [158-159]. Oxidation can afford platinum(IV) complexes two additional ligands, which can be exploited for attaching tumour-targeting species [160], fluorescent ligands to allow tracking of the complex [161], hydrophobic groups to

increase lipophilicity [162], enzyme inhibitors to further increase survivability, and more [163].

Platinum anticancer complexes consisting of two or more centres that are tethered together have been in development since the late 1980s [164-166]. These complexes have attracted considerable interest as their multinuclear nature allows for a greater number of possible DNA binding adducts than cisplatin, [167-168] making it more difficult for cells to repair DNA damage and subsequently develop drug resistance [169]. Additionally, many multinuclear compounds are charged and therefore water soluble, allowing for ease of administration, faster DNA binding and higher cellular uptake than cisplatin due to electrostatic attractions [170-171]. The leading complexes of this type were initially based upon cisplatin motifs with aliphatic amine substituents. This triggered the development of multinuclear complexes with a large variety of tethers and active ligands [172-179]. In many cases, the resulting cytotoxicity was equal to or greater than that of cisplatin, cellular uptake was significantly higher, and interstrand cross-linking was confirmed [180-181]. Currently, the most well-known multinuclear Pt complex is [({trans-$PtCl(NH_3)_2$}2μ-(trans-$Pt(NH_3)_2$- ($H_2N$-$(CH_2)_6NH_2)_2$)]$^{4+}$ (BBR3464); this trinuclear complex entered Phase II trials in 2001 [182-183]. BBR3464 is more active than cisplatin in a wide variety of cell lines, including those that are cisplatin-resistant. This high cytotoxicity is attributed to a variety of factors, including the formation of interstrand crosslinks up to six bases apart, high DNA binding affinity due to a 4+ charge, [184] increased cellular uptake relative to cisplatin, [185] lower DNA repair protein expression [186] and lower reactivity with intracellular thiols [187-188]. Phase I clinical trials of BBR3464 revealed high systematic toxicity in participants [189]. This was mediated through alternate treatment plans and the complex proceeded to Phase II trials [190], however it has not progressed further due to a low rate of activity in patients.

The biological effects of ruthenium(II) and (III) complexes are increasingly being recognised, due in part to the stable, well characterised and predictable structures that can be produced through judicious choice of ligands [191]. After the discovery of the antitumor potential of ruthenium red [192] research revealed that ruthenium(III) complexes such as fac-$[RuCl_3(NH_3)_3]$ and cis-$[RuCl_2(NH_3)_4]Cl$ also demonstrated anticancer activity [193]. Further development produced the first and only ruthenium(III) complexes to reach clinical trials: NAMI-A (imidazolium trans-

[tetrachlorido(imidazole)(dimethylsulfoxide) ruthenate(III), and KP1019 (indazolium trans-[tetrachloridobis(1H-indazole)ruthenate(III)], each of which were reported to prevent metastasis formation and inhibit already advanced tumours with relatively low toxicity [194–196]. These ruthenium(III) complexes are theorised to be inert until activation by reduction within hyperoxic cancerous cells. Each complex is capable of covalent binding to DNA [197]; however, their overall mechanisms differ in that NAMI-A interferes with the regulation of the cell cycle and the extracellular matrix, preventing further tumour metathesis [198], while KP1019 causes direct cell apoptosis via the intrinsic mitochondrial pathway and the formation of reactive oxygen species. NAMI-A and KP1019 have each completed a phase I clinical trial in 2004 and 2008, respectively, while further trials are being planned for KP1019 [198]. Ruthenium complexes such as $[Ru(\eta6\text{ -arene})(A_L)X]+$ , where $A_L$ is a bidentate ligand and X is a halide, have also been developed that are generally water soluble, relatively inert toward degradation under physiological conditions [199] and have shown potent cytotoxicity in a range of cancerous cell lines [200-201]. Similarly to platinum intercalators, a range of properties can be achieved through modulation of the η6 -arene and $A_L$ [202]. For example, complexes of the type [Ru(η6 -arene)- (en)(Cl)]+ form monofunctional adducts with the guanine bases of DNA [203-204] and anticancer activity increases with the size of the arene (benzene < p-cymene < biphenyl < dihydroantracene < tetrahydroanthracene) [205]. NMR studies have shown that these complexes can covalently bind to DNA, although the arene can also intercalate from the minor groove [206-207].

The first non-platinum(II) covalently binding metal complex to undergo clinical trials for cancer treatment was the titanium complex budotitane ($[Ti(bzac)_2(OEt)_2]$, where bzac = 1-phenylbutane-1,3-dionate [208]. Metallocene dihalides, such as the metal dichloride (M(CP)2(Cl)2) (where M = Ti or V, CP = cyclopentadienyl anions, were also reported to have effective anticancer activity and offered a different spectrum of activity [209-210]. Specifically, titanocene dichloride has also been tested in some phase I and II clinical trials, [211] and has been found to localise within the nucleus of xenografted cells. The DNA binding of these titanium complexes is attributed to the hydrolysis of the ethoxy or halide ligands; however, this occurs extracellularly unlike cisplatin [212-213] and can result in the formation of multinuclear complexes that are also and studies that suggest that are also active [214]. Aside from the proven covalent DNA interactions, and studies that suggest that transferrin may play a role in the tumour penetration of these

compounds [215], not much else is known regarding the cytotoxic mechanisms of titanium complexes. Cobalt has been used to deliver coordinative DNA binders to cancerous cells such as nitrogen mustard ligands or 8-hydroxyquinoline [216-217]. Similarly to the prodrug approach for platinum(IV) complexes, the release of the active species is mediated via reduction from Co(III) to Co(II); this can be artificially induced with ionising radiation, or it can occur without stimuli within the hypoxic regions of cancerous cells [218]. Rhodium complexes have been extensively studied due to their selectivity toward DNA sequences and their nuclease-cleaving ability [219]. For example, the N4-tetradentate complex $[Rh(Me_2trien)(phi)]^{3+}$ (where Me2trien = 2R,9R-diamino-4,7- diazadecane and phi = phenanthrene-9,10-diimine,) was specifically designed to intercalate, from the major groove, into the 5′-TGCA-3′ sequences of DNA [220]. It was found that both π-stacking forces and water-mediated hydrogen bonds each contributed to the interaction. Other intercalating metal complexes that incorporate an N4-tetradentate ligand such as $[M(N,N'$-bis-5-(triethylammoniummethyl)-salicylidene-2,3-naphthalendiiminato)$]^{n+}$ (where M = copper, nickel or zinc) have been synthesised and their DNA binding affinities determined by spectroscopic and computational methods [221]. It was reported that each complex bound to DNA via intercalation, although large differences in binding affinity between each metal were observed. It was hypothesised that the Ni complex bound with the highest affinity due to its square planar coordination geometry which would allow it to insert deeply between the DNA base pairs, relative to the octahedral geometry of the Cu and Zn complexes [221].

**2.1. Objectives and scope of the present work:**

An elaborated investigation of the literature exposed that numerous metal complexes were considered for DNA binding and cleaving capabilities, but out of those N, O supported frameworks have been accepted most. Thus in the present research work, we have chosen to work with new type of N, O framework based metal complexes. The research work conferred in this thesis was accomplished with the following objectives:

1. Synthesis of new type of N, O based polydentate ligand systems and their transition metal complexes.
2. Characterization of synthesized compounds by UV-Visible spectroscopy, FTIR, NMR, EPR, PXRD and single crystal X-ray diffraction analysis.

3. Studies on interaction of synthesized complexes with DNA by using different biophysical methods like UV-Visible absorption titration, thermal denaturation, competitive binding assay and gel electrophoresis.
4. Validation of interactions of synthesised complexes with DNA using molecular modelling studies.
5. Studies on antimicrobial and antioxidant activity of synthesized complexes.

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# CHAPTER II

# EXPERIMENTAL SECTION

## 2.1. Chemicals and Reagents

All the analytical grade (A.R.) chemicals were used without further purification for the syntheses of various compounds and metal complexes. However, for spectroscopic techniques and conductivity measurements only proper spectroscopic grade solvent were used. The list of various chemicals and reagents are given in Table 2.1.

**Table.2.1.** List of chemicals and reagents.

| Chemicals | Molecular Weight (g/mol) | Source | CAS No. |
|---|---|---|---|
| **3, 3' Methylenedianiline** | 198.26 | Sigma-Aldrich, Germany | 19471-12-6 |
| **2-hydroxy-5-nitro benzaldehyde** | 167.12 | Sigma-Aldrich, Germany | 97-51-8 |
| **5- bromo salicylaldehyde** | 201.02 | Sigma-Aldrich, Germany | 1761-61-1 |
| **1-methyl imidazole** | 82.10 | Sigma-Aldrich, Germany | 616-47-7 |
| **2-bromoethylamine hydrobromide** | 204.89 | Sigma-Aldrich, Germany | 2576-47-8 |
| **Potassium hexa fluorophosphate** | 184.06 | Sigma-Aldrich, Germany | 17084-13-8 |
| **Salicylaldehyde** | 122.12 | Sigma-Aldrich, Germany | 90-02-8 |
| **4-chloroaniline** | 127.57 | Sigma-Aldrich, Germany | 95-51-2 |

| | | | |
|---|---|---|---|
| **4-hydroxy benzoic acid** | 138.12 | Sigma-Aldrich, Germany | 99-96-7 |
| **Ethidium bromide** | 394.31 | Sigma-Aldrich, Germany | 1239-45-8 |
| **Deoxyribonucleic acid sodium salt from calf thymus** | | Sigma-Aldrich, Germany | 73049-39-5 |
| **Trizma hydrochloride** | 157.60 | Sigma-Aldrich, Germany | 1185-53-1 |
| **Cobalt acetate** | 177.021 | Sigma-Aldrich, Germany | 71-48-7 |
| **Zinc acetate. $2H_2O$** | 219.50 | Thomas Baker | 5970-45-6 |
| **Copper(II) nitrate trihydrate** | 10031-43-3 | Sigma-Aldrich, Germany | 241.60 |
| **Acetone (AR)** | 58.08 | S. D. Fine Chemicals, India | 67-64-1 |
| **Methanol** | 32.04 | Sigma-Aldrich, Germany | 67-56-1 |
| **Ethanol** | 46.07 | S. D. Fine Chemicals, India | 64-17-5 |
| **Dimethylformamide** | 73.09 | S. D. Fine Chemicals, India | 68-12-2 |
| **Diethylether** | 74.12 | S. D. Fine Chemicals, India | 60-29-7 |
| **Sodium nitrite** | 68.99 | Merck, India | 7632-00-0 |
| **Sodium Hydroxide** | 39.99 | Merck, India | 1310-73-2 |
| **Sodium carbonate** | 105.98 | Merck, India | 497-19-8 |
| **Spectroscopic grade** | 18.02 | S. D. Fine | 7732-18-5 |

| | | | |
|---|---|---|---|
| **water (For Spectroscopy)** | | Chemicals, India | |
| **Dimethylsulfoxide (For spectroscopy)** | 78.13 | S. D. Fine Chemicals, India | 67-68-5 |

**2.2. Physico-chemical methods used to characterize synthesized compounds:**

To characterize the structure of synthesized ligands and their metal complexes, Different Physico-chemical techniques have been used. The descriptions of these methods are given below:

**2.2.1. Elemental analysis:**

Euro VECTOR EA 3000 analyzer was used for the elemental micro-analyses (C, H and N) of all the synthesized compounds. The metal contents of the complexes were determined with Atomic Absorption Spectrophotometer (Varian SpectrAA 50B) by using suitable standard metal solutions procured from Sigma-Aldrich, Germany.

**Fig.2.1.** Atomic Absorption Spectrophotometer (Varian SpectrAA 50B).

**2.2.2. Thin layer chromatography:**

The thin layer chromatography (TLC) on silica gel plates were used to confirm the purity of the prepared ligands and the complexes. UV-light was used to view the TLC plate and iodine is used as and when required.

**2.2.3. Magnetic susceptibility measurement:**

Magnetic susceptibilities were measured at room temperature with a Sherwood Scientific Ltd magnetic susceptibility balance (Magway MSB Mk1). The MSB works on the basis of a stationary sample and moving magnets. The pairs of magnets are placed at opposite ends of a beam so placing the system in balance. Introduction of the sample between the poles of one pair of magnets produces a deflection of the beam that is registered by means of phototransistors. A current is made to pass through a coil mounted between the poles of the other pair of magnets, producing a force restoring the system to balance. At the position of equilibrium, the current through the coil is proportional to the force exerted by the sample and can be measured as a voltage drop.

The solid sample is tightly packed into weighed sample tube with a suitable length (*l*) and noted the sample weight (*m*). Then the packed sample tube was placed into tube guide of the balance and the reading (*R*) was noted. The mass susceptibility, $\chi_g$ is calculated using by equation 1:

$$\chi_g = C_{Bal} \times l \times \frac{(R - R_0)}{m \times 10^9} \qquad (1)$$

where $l$ = the sample length (in cm), $m$ = the sample mass (in g), $R$ = the reading for the tube plus sample, $R_0$ = the empty tube reading and $C_{Bal}$ = the balance calibration constant. Thus molar susceptibility is $\chi_M = \chi_g \times \text{M.Wt of the sample}$. The molar susceptibility is the corrected with diamagnetic contribution. The effective magnetic moment $\mu_{eff}$ is calculated by using equation 2:

$$\mu_{eff} = 2.83\sqrt{T \times \chi_A} \quad \text{B.M} \qquad (2)$$

where $\chi_A$ is the corrected molar susceptibility. The MSB was calibrated with $Hg[Co(SCN)_4]$ before the actual measurements.

**Fig 2.2.** The magnetic susceptibility balance (Magway MSB Mk1).

### 2.2.4. Infrared spectroscopy:

FTIR spectra were recorded in KBr pellets with a Perkin-Elmer Spectrum FTIR spectrometer (RX-1) working in the range 4000-400 $cm^{-1}$ at ambient temperature. KBr used for preparing the pellets was dryed in a hot air oven for 24 hours and then kept in vacuum desiccators over anhydrous $CaCl_2$ before use.

**Fig 2.3.** Perkin-Elmer Spectrum FT-IR spectrometer (RX-1).

### 2.2.5. Electronic spectroscopy

UV-Visible spectra of the ligands and their complexes were recorded in a quartz cell (1 cm path length) on a Jasco (V-530) double beam spectrophotometer equipped with a thermostated bath (maintained at 25±0.1 °C) using either water or DMSO as the reference solvent in accordance with the solubility of the compounds.

**Fig 2.4.** Jasco V-530 double beam UV-VIS Spectrophotometer.

### 2.2.6. Nuclear Magnetic Resonance (NMR) spectroscopy ($^{1}$H NMR and $^{13}$C NMR):

$^{1}$H and $^{13}$C NMR spectra were recorded on a FT-NMR (Bruker Avance-II 400 MHz) spectrometer using $D_2O$, $CDCl_3$ and DMSO-$d_6$ as the solvent. Chemical shifts **($\delta$)** are quoted in ppm downfield of internal standard tetramethylsilane (TMS).

**Fig 2.5.** FT-NMR (Bruker Avance-II 400 MHz).

### 2.2.7. ESI-MS:

Mass spectra were recorded on a Waters ZQ-4000 spectrometer using water and chloroform as the solvent. Chemical shifts **(*δ*)** are quoted in ppm downfield of internal standard tetramethylsilane (TMS).

**Fig 2.6.** ESI-MS **(**Waters ZQ-4000 spectrometer).

### 2.2.8. Conductivity

Specific conductances were measured at (298.15 ± 0.01) K with a Systronic conductivity TDS-308 meter. The conductance measurements were carried out by using a dip-type immersion conductivity cell, CD-10 with a cell constant of 1.0 ± 10% $cm^{-1}$. The instrument was standardized by using 0.1 (M) aqueous KCl solution. Measurements were made in a thermostatic water bath maintained at the experimental temperature with an accuracy of ± 0.01 K. The molar conductance of the complexes ($\Lambda_m$) was determined by using the relation $\Lambda_m = 1000\times\kappa/c$, where $c$ and $\kappa$ stands for the molar concentration of the metal complexes and specific conductance, respectively. The complexes ($1 \times 10^{-3}$ M) were dissolved in respective solvent and their specific conductance was measured at (298.15±0.01) K.

**Fig 2.8.** Systronic conductivity TDS-308 meter.

### 2.2.9. Mass measurements:

Mass measurements were carried out on digital electronic analytical balance (Mettler Toledo, AG 285, Switzerland) as shown in Fig 2.9. This Digital balance can measure mass to a very high precision and accuracy. The mass measurements were accurate to ± 0.01 mg.

**Fig 2.9.** Digital electronic analytical balance (Mettler Toledo, AG 285).

## 2.3. DNA interaction study:

### 2.3.1 Absorption study:

The DNA binding experiment of synthesized metal complexes with CT-DNA were performed in Tris-HCl buffer (50 mM, pH 7.2). The ratio of UV absorbance at 260 and 280 nm was used to acutain protein contamination of DNA. If this ratio >1.86 for CT-DNA solution, DNA is considered to be was free from protein contamination [1]. The CT-DNA concentration is also determined by absorption spectroscopy at 260 nm ($\varepsilon_{260}$= 6600 $mol^{-1}$ $cm^2$). The absorbances measurements were recorded for solution with a constant/preferred concentration of the synthesized complex (10 µM) but with varied concentration of the CT-DNA. The stock aqueous solutions of the complexes were prepared in the range of $1\times10^{-4}$ M and such solution were then diluted with Tris-HCl buffer to get the desired experimental concentrations. During the titration equal amount of CT-DNA stock solutions were added to both the complex solutions and the reference solutions to eliminate the effect of CT-DNA absorbance. Using the absorption data, the intrinsic binding constant ($K_b$) can be obtained as usual [2] by plotting [DNA] / ($\varepsilon_a$ - $\varepsilon_f$) *versus* [DNA] using Wolfe-Shimer equation [3] as shown below:[3]

$$\frac{[\mathrm{DNA}]}{\varepsilon_a - \varepsilon_f} = \frac{[\mathrm{DNA}]}{\varepsilon_b - \varepsilon_f} + \frac{1}{K_b(\varepsilon_a - \varepsilon_f)} \qquad (3)$$

where, $\varepsilon_a$, $\varepsilon_b$ and $\varepsilon_f$ correspond to apparent extinction coefficient of the complex, $A_{obs}$/[complex], extinction coefficient for the complex in fully bound form, and in free form, respectively. The intrinsic binding constant ($K_b$) can be obtained from the ratio of slope to intercept.

### 2.3.2. Ethidium Bromide (EB) competitive study with fluorescence emission spectroscopy:

To investigate the effectiveness of binding modes of the complexes to DNA, a competitive binding study was performed by following EB displacement strategy through fluorescence emission spectroscopy. The competitive binding experiment was carried out

in Tris-HCl buffer by keeping a constant ratio of concentration of [DNA]/[EB] =1.13 constant and varying the complex concentrations from 0 to 60 μM. During this experiment the emission spectra were recorded in the wavelength range 550-700 nm by exciting the EB-DNA conjugate at the wavelength 540 nm (i.e., $\lambda_{ex}$=540 nm). The obtained spectra were analyzed by using the classical Stern–Volmer equation[4] which is given below as equation [4]:

$$\frac{I_0}{I} = 1 + K_{SV}[Q] \qquad (4)$$

where $I_0$ and I are the fluorescence intensities in the absence and presence of the quencher, respectively, $K_{sv}$ is the linear Stern–Volmer quenching constant, Q is the concentration of the quencher. The relative binding affinities of the complexes to CT-DNA was determined from the ($K_{sv}$) values.

**2.3.3. Thermal denaturation study:**

Thermal denaturation studies of DNA were carried out by Jasco UV-Visible V-530 model spectrophotometer equipped with a temperature controlling programme assembly that helps to raise the temperature of the experimental solutions by 1 $^{\circ}$C min $^{-1}$. The absorbance of the CT-DNA solutions (100 μM) both in absence and presence of soluted metal complexes (10 μM) were monitored continuously at 265 nm [4].

**2.3.4. Viscosity measurements:**

Viscosities of the experimental solutions determined through Ostwald capillary type viscometer thermostated at 25 ± 1 $^{\circ}$C. The concentration of CT-DNA was kept constant (100 μM) during the measurement and concentration of ligand/complex solution was gradually increased (10 -100 μM). With a digital stopwatch the flow times were measured with an accuracy of ± 0.20 s. The relation [5] used to calculate the viscosities:

$$\eta = (t - t^o)/ t^o \qquad (5)$$

where, t is the flow time of CT-DNA containing ligand or complex solution and $t^o$ is the flow time of buffer alone in seconds.[2] The obtained data were presented as $(\eta/\eta_o)^{1/3}$ versus 1/ R (R = [DNA]/[Complex]), where $\eta_o$ and η is the viscosity of DNA in the absence and that in the presence of the ligand or complex in experimental solutions, respectively.

**2.4. DNA cleavage study:**

The DNA cleavage reaction was investigated by gel electrophoresis technique. The DNA cleavage activity was adjudged by examining the transformation of supercoiled form of pBR322 DNA (Form I) to circular (Form II) and linear (Form III) form. To perform cleavage reaction, solution of pBR322 DNA was prepared in Tris-HCl buffer and each reaction mixture contains 20μM DNA, 200μM $H_2O_2$ and 5-15μM synthesized complex. After incubation for 2 hours, 2 μL of the loading buffer and 1% agarose gel (containing 1 μg/cm$^3$ ethidium bromide) was mixed to each reaction mixture. The electrophoresis was performed in Tris-acetic acid-EDTA buffer for 2h at 50V. After the completion of electrophoresis, the gel was captured photographically under UV light.

**2.5. Antioxidant and free radical scavenging activity study:**

Several biochemical experiments were performed to determine the antioxidant or free radical scavenging activities of the synthesized complexes.

**2.5.1. DPPH radical scavenging assay:**

DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging activity was monitored by using a literature method given by Saha *et al* [5]. Various concentrations of complexes were prepared and mixed with freshly prepared DPPH solution (1mM; diluted in 95% methanol) and kept in dark. After 30 minutes of reaction absorption was measured at 517 nm to determine the percent of inhibition and compared with the standard ascorbic acid. The percent of inhibition was calculated using the following relation [6]:

$$\text{Percentage of scavenging} = \frac{A_0 - A_1}{A_0} \times 100 \qquad (6)$$

where, $A_0$= absorbance of the control and $A_1$= absorbance in the presence of samples and standard.

**2.5.2. Hydroxyl radical scavenging assay:**

Hydroxyl radical scavenging activity was studied according to a standard protocol with slight modification.[6] Hydroxyl radical ($OH^{\bullet}$) was generated through Fenton reaction and inhibition was measured at 532 nm. Mannitol was used as a standard for hydroxyl radical scavenging activity. Equation [7] was used for calculation of percent of inhibition.

**2.5.3. Superoxide radical scavenging assay:**

Superoxide radical scavenging assay was studied by following a literature method[7] with slight modifications. Non-enzymatic combination of phenazine methosulfate (PMS) and reduced nicotinamide adenine dinucleotide (NADH), superoxide radical ($O_2^{\bullet-}$) was produced and measured by the reduction of nitro blue tetrazolium (NBT) to purple-colored formazan. Quercetin was used as standard for the scavenging of superoxide radical.

**2.5.4. Nitric oxide (NO) radical scavenging assay:**

Nitric oxide radical (NO) quenching activity was studied as per GriessI-llosvoy reaction [8]. NO was generated through the reaction between aqueous sodium nitroprusside (SNP) and oxygen. Diazotization of nitrite ions with sulphanilamide and subsequent coupling with N-(1-naphthyl) ethylenediamine dihydrochloride (NED), pink azo dye was generated. Curcumin was used as standard for the above experiment. The percentage inhibition was calculated using equation [6].

**2.5.5. Hydrogen peroxide scavenging assay:**

Hydrogen peroxide ($H_2O_2$) scavenging activity was studied by following FOX reagent method [9]. Sodium pyruvate was used as standard.

**2.5.6. Peroxynitrite scavenging activity:**

Peroxynitrite ($ONOO^-$) scavenging activities of β-cyclodextrin derived Schiff base Zn(II) complexes were compared against the standard gallic acid following a previously standardized method [10].

**2.5.7. Singlet oxygen scavenging assay:**

Singlet oxygen ($_1O^2$) generated through the reaction of sodium hypochloride (NaOCl) with $H_2O_2$ and scavenging activity of singlet oxygen was determined by monitoring the bleaching of N, N-dimethyl-4-nitrosoaniline (RNO) using the method of Pedraza-Chaverrí *et al*[11]. with minor modifications. Lipoic acid was used as standard for the above experiment.

**2.5.8. Hypochlorous acid scavenging assay:**

HOCl scavenging activity was studied by monitoring the decrease in absorbance of catalase following a standard method with minor modification [12]. Ascorbic acid was used as standard for this experiment.

**2.5.9. Measurement of reducing power:**

The ferric reducing power was determined by the method of Oyaizu *et al* [13]. and Butylated hydroxytoluene (BHT) was used as standard.

**2.5.10. Iron chelation assay:**

Iron chelation activity was studied by the method of Haro-Vicente *et al* [14]. by measuring the decrease of intensity of violet complex (generated on coupling of $Fe^{2+}$ and ferrozine) on addition of various concentrations of compounds. The absorbance was measured at 562 nm. Etylenediaminetetraacetate (EDTA) was used as standard.

**2.5.11. Total antioxidant activity (TAA):**

Total antioxidant activity (TAA) was measured following the method of Prietto *et al* [15]. by the reduction of $Mo^{6+}$ to $Mo^{5+}$. Various concentrations of complex solution

were mixed with 1 mL of reaction mixture containing sulfuric acid, sodium phosphate and ammonium molybdate. The absorbance was measured at 695 nm against a suitable blank. Ascorbic acid was used as standard for this experiment. The antioxidant activity was measured by the capacity of the complex to reduce molybdenum (VI) to molybdenum (V) using equation [7]:

$$\text{Total antioxidant activity} = \frac{A_0 - A_1}{A_0} \times 100 \quad (7)$$

where, $A_0$ = the absorbance of the blank and $A_1$ = absorbance in the presence of the samples and standard (ascorbic acid).

### 2.5.12. Lipid peroxidation inhibition assay:

Lipid peroxidation inhibitory activity was determined by studying the inhibition of $OH^-$ catalyzed malondialdehyde (MDA) production from the polyunsaturated fatty acid (PUFA) as per the method of Kizil *et al* [16-18]. Mice brain homogenate was used for the lipid peroxidation inhibition assay. Brain homogenate was prepared by centrifuging of mice brain with phosphate buffer (and potassium chloride. Various concentrations of complexes were mixed with the homogenate (100 µl) followed by addition of ferrous sulfate and ascorbic acid and incubated for 1 h at 37°C. Following incubation, TCA (Trichloroacetic acid) and TBA (thiobarbituric acid) were added in the reaction mixture and then heated at 95°C in water bath for 30 min. Then the mixtures were cooled to room temperature and centrifuged for 2 min. The absorbance of the supernatant liquid was measured at 532 nm. The resultant data was compared with the standard Trolox.

## 2.6. Antibacterial activity of the synthetic compounds using well diffusion method:

### 2.6.1. Preparation of synthetic compounds for microbiological assay:

The test solution of synthetic compound was prepared in DMSO at a concentration of 5, 7.5 and 10 mg/ml.

### 2.6.2. Test Organisms:

Two pathogenic gram positive bacteria *Staphylococcus aureus*, *Bacillus subtilis* and two pathogenic gram negative bacteria *Escherichia coli*, *Klebsiella pneumonia* were used for assessing the antibacterial activities using well diffusion method. All bacterial strains were cultivated in nutrient broth medium (NB) and incubated at 37 °C for 24 hr. This was used for the antibacterial activity in the well diffusion assay.

### 2.6.3. Preparation of media:

The nutrient agar (NA) medium was obtained from Hi-media laboratory Pvt Ltd, Mumbai, India. NA (2.8g) was suspended in 100 ml of distilled water, boiled and dissolved completely. It was sterilized by autoclaving at 15 lbs. pressure (121 ºC) for 15 min and poured into sterile Petri dish and allowed to set. Sterility check was done after the agar was solidified [19].

### 2.6.4. Preparation of culture media and inoculation method:

The pure cultures of each bacterium were prepared in Nutrient broth and incubating over night at 37 ºC. After incubation, all the required plates were inoculated by dipping a non-toxic sterile cotton swab into the microbial growth. Excess of inoculate was removed by pressing and rotating the swab firmly against the side of the tube, above the level of the liquid. The medium was inoculated by evenly streaking the swab over the entire surface of the plate in three directions.

### 2.6.5. Agar well diffusion method:

The assay for antibacterial activities was conducted by agar well diffusion method [20]. Sterile nutrient agar (NA) was poured into sterile Petri dishes, which was inoculated with the test organisms. Sterile cork borer was used to prepare well (9 mm) on each Petri plates. About 200 µl of synthetic compound (10mg/mL) was poured into each well (each microorganism inoculated plates) using sterile pipette and incubated at 37 ºC for 24h. After incubation all the tested plates were examined and the diameters of the inhibition zones were measured. All the experiments were carried out in triplicates. DMSO was

used as a control. The zone of inhibitions is expressed as mean values with standard deviation of mean (SD).

### 2.7. Molecular modeling analysis:

#### 2.7.1. Swiss target prediction:

The bioactive small molecules, drugs and metabolites have tendency to bind with proteins and other macromolecule targets to modulate their activities which has visible expression in organisms [2]. The synthesized complexes were checked for possible bioactivity with Swiss target prediction web server (http://www.swisstargetprediction.ch).

#### 2.7.2. Molecular Docking:

The target proteins identified by Swiss target prediction were chosen as receptor protein. The X-ray crystallographic structures of these receptor proteins were obtained from RCSB PDB (*https://www.rcsb.org).* They were used for molecular docking analysis against the synthesized chemicals. Molecular docking was performed using Auto Dock Vina. The receptor structures were defined as rigid and the grid dimensions varied from being 80, 80 and 80 for X, Y and Z dimensions for proteins having PDB ID's 1D3G, 1JY1, 1R2D, 2NL9 and 4LXD while 100, 100 and 100 for 3D2Q and 4CIM. Whereas for the protein with PDB ID 2ON9 it is 60, 60 and 60 respectively. The Exhaustiveness of the simulation was set to 8. Every protein of interest was made ready by removing the water molecules and by adding polar hydrogen to them. Gasteiger charges were added to the proteins on the basis of electronegativity equilibration and all non-polar hydrogens were merged [2]. This was performed because Auto Dock uses the united atom model to represent molecules. Gasteiger charges were also calculated for the respective complexes and all the torsions were allowed to rotate.

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## CHAPTER - III

## DNA BINDING, AMELIORATION OF OXIDATIVE STRESS AND MOLECULAR DOCKING STUDY OF Zn(II) METAL COMPLEX OF A NEW SCHIFF BASE LIGAND

## 3.1. Introduction:

Molecules that interact with DNA have been studied extensively with the aim of designing new types of chemotherapy agents and probes for nucleic acid structures [1-2]. Studying the interaction of transition metal complexes with DNA have been the main focus of recent investigations. Cisplatin (cis-diamminedichloridoplatinum(II)) is the first clinically approved anticancer drug; its various analogues are found to be capable to bind to DNA, halting its replication and induce apoptosis [3]. However, these complexes have so many disadvantages: poor solubility [4], dose-limiting side effects like nausea, nephrotoxicity and neurotoxicity [5] and acquired resistance in many cancer types [6]. These basic problems associate with chemotherapy necessitate the evolvement of new anticancer approaches.

Development of new molecules that can obstruct cancerous cells by interacting with DNA in a non-classical mechanism is an example of new anticancer approach. Transition metal complexes are ideal for these tasks, as their unique characteristics allowing specific interactions with DNA and other important bio-molecules [7].Transition metals that are utilized extensively in these field include platinum [8], ruthenium [9], Zinc [10], titanium [11], rhodium [12], copper[13], palladium [14], gold [15], Vanadium [16] and iron [17]. Schiff base metal complexes have also gained recognition for their utilities in these biological processes [18]. In recent years, many studies associated with Schiff base metal complexes show promising anticancer activity and are of great interest in both chemistry and biology [19].

Zinc, which is known as “essential trace element” plays an important part in many biological processes. It is essential for the functionality of more than 300 enzymes and

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vital for the stabilization of DNA, gene expression [20]. It is involved in immune responses, in homeostasis, in apoptosis and in ageing. Zn-binding proteins and enzymes are protective in stress, infections [21]. To explore the chemistry of Zn(II) complexes and DNA interaction, here we have synthesized and characterised a new Zn(II) complex and studied its interaction with Calf Thymus DNA(CT DNA) by different physical and spectroscopic techniques.

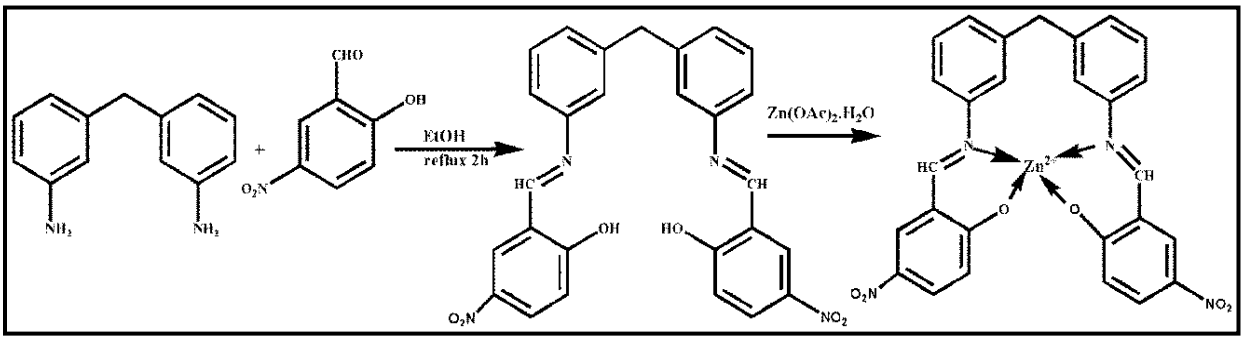
### 3.2. Experimental Section:

#### 3.2.1. Synthesis of Schiff Base Ligand:

A hot ethanolic solution of 3, 3' Methylenedianiline (0.1mol) was refluxed with 2-hydroxy 5- nitro Benzaldehyde (0.2 mol) in a round bottom flask for 2h with constant stirring. The resulting yellow colored solid product was separated by filtration, washed with ethanol followed by diethyl ether and dried in vacuum desiccator over anhydrous calcium chloride.

#### 3.2.2. Synthesis of Metal Complex:

Hot ethanolic solution of Zn(II) acetate was added dropwise to the equimolar ethanolic solution of synthesized ligand. The resulting mixture was then stirred and refluxed (at 40-50 °C) for 2 h whereupon the Zn(II) metal complex was precipitated out. The dark yellow colored solid complex was separated by filtration, washed with ethanol and dried in vacuum desiccator.




**Scheme.3.1.** Synthesis of schiff base ligand and its Zn(II) complex.

### 3.3. Results and Discussion:

The formation of the investigated Schiff base ligand and its metal complex is represented in Scheme.3.1.

### 3.3.1. Characterization of the ligand and its Zn(II) complex:

The synthesized ligand and its corresponding Zn(II) complex have been characterized by different analytical and spectroscopic techniques (IR, $^{1}H$ and $^{13}C$ NMR, UV-Visible, molar conductance *etc.*). The analytical data of the synthesized ligand and its Zn(II) complex along with physical properties is listed in Table 1. The ligand and the metal complex are moisture insensitive, air stable and soluble in DMSO and DMF. The analytical data are in good agreement with calculated values and are consistent with formation of mononuclear Zn(II) complex having metal to ligand ratio 1:1.

#### 3.3.1.1. Molar Conductance:

The synthesized Zn(II) complex was dissolved in DMSO solvent and molar conductance of $10^{-3}$ mol $dm^{-3}$ complex solution was measured at room temperature (Table 3.1.). The molar conductance value indicates that the metal complex is a non-electrolyte [28].

**Table. 3.1.** Analytical and Physical data of Schiff base ligand and its Zn(II) complex:

| Compound | m.p. (◦C) | Colour (% yield) | Mol. Wt. (gm) | % Found (calcd.) | | | $\Lambda_m\ \Omega^{-1}\ mol^{-1}\ cm^2$ |
|---|---|---|---|---|---|---|---|
| | | | | C | H | N | |
| $H_2L$ ($C_{27}H_{20}N_4O_6$) | 140 | Yellow (84) | 496 | 65.71 (65.32) | 4.16 (4.03) | 11.13 (11.29) | _ |
| [Zn(L)] ($C_{27}H_{18}N_4O_6Zn$) | >250 | Dark Yellow (78) | 559 | 58.06 (57.96) | 3.41 (3.22) | 9.94 (10.02) | 14.11 |

### 3.3.1.2. Infrared Spectra:

The coordination of the ligand to the metal ion have been studied by comparing the IR data of the ligand with its Zn(II) complex. The IR spectra of synthesized Schiff base and its corresponding Zn(II) complex is shown in Fig.3.1. There are some appreciable shifts in the stretching frequencies of Zn(II) complex compared to free ligand. The Schiff base shows a characteristic strong band at 1618 $cm^{-1}$due to the azomethine ν(C=N) [28]. This band gets shifted to 1599 $cm^{-1}$ in the synthesized complex indicating the participation of the azomethine nitrogen in complexation [28]. A broad band at 3447 $cm^{-1}$ due to phenolic -OH group of ligand is disappeared in the corresponding Zn(II) complex suggesting the involvement of oxygen atom of -OH group in coordination to Zn(II) ion [28]. The coordination of O atom of phenolic -OH group to metal ion is further proved by the shift in ν(C-O) band of ligand at 1099 $cm^{-1}$ to higher frequency at 1105 $cm^{-1}$ in Zn(II) complex [29]. The participation of phenolic O and azomethine N in complexation are confirmed by the development of two non-ligand bands in the spectra of complex at 535 $cm^{-1}$ and 466 $cm^{-1}$ due to ν(Zn-O) and ν(Zn-N), respectively [28]. Therefore, the above arguments together with the elemental analyses indicated that Schiff base ligand behaves as a dibasic tetradentate ligand coordinated to the metal ion via the azomethine N and deprotonated phenolic O and the complex possibly have a tetrahedral geometry because of $d^{10}$ configuration of Zn(II).

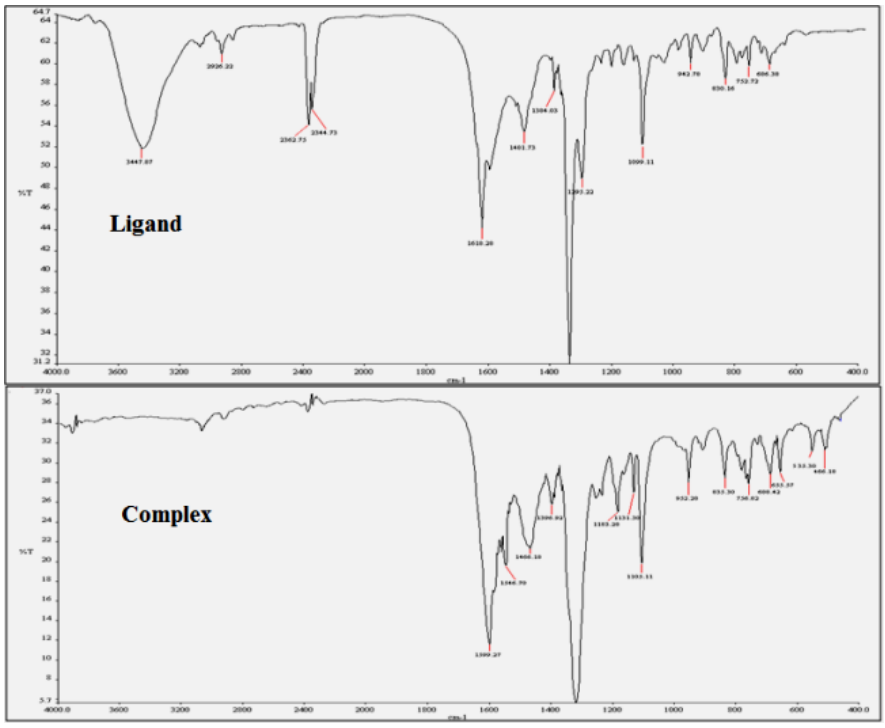



**Fig.3.1.** FTIR Spectra of the synthesized ligand and its Zn(II) complex.

### 3.3.1.3. NMR Spectra:

The $^{1}H$ NMR spectra of Schiff base ligand and its Zn(II) complex is shown in Fig.3.2. The synthesized Schiff base showed a singlet at δ 9.17 ppm because of azomethine proton (-CH=N-) [24]. However, in case of Zn(II) complex this -CH=N- peak is shifted to 8.70 ppm suggesting the involvement of azomethine nitrogen in coordination with Zn(II). Moreover, the ligand showed a signal at 10.29 ppm due to hydroxyl (-OH) proton which disappears in the synthesized complex revealing the deprotonation of –OH group during complexation [30].

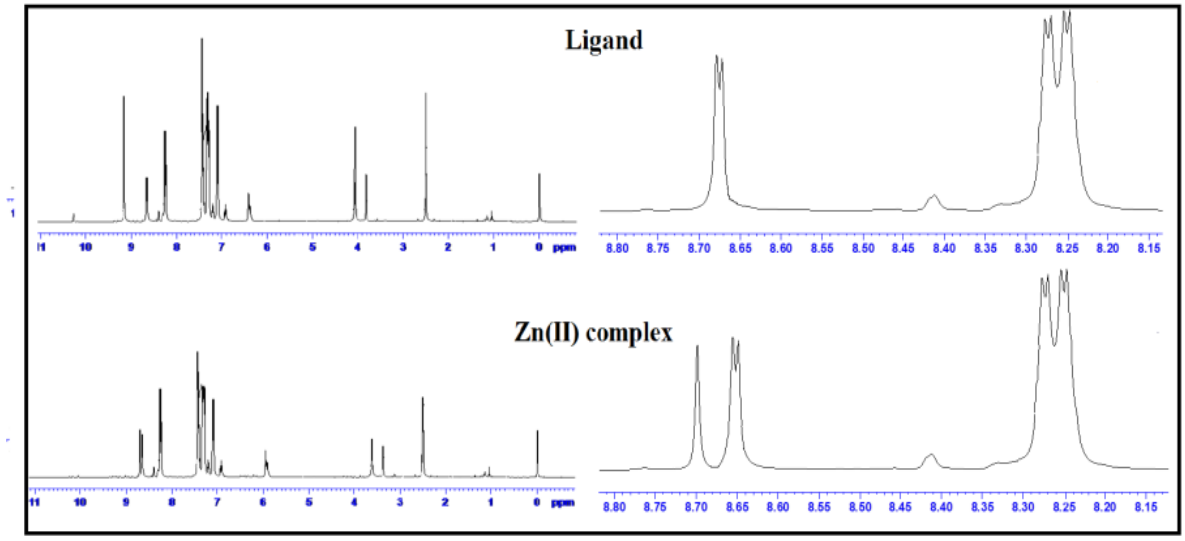



**Fig.3.2.** $^{1}$H NMR spectrum of Schiff base ligand and its Zn(II) complex.

The $^{13}$C NMR spectrum of both ligand and complex show peaks at 116.19-153.12 ppm for aromatic carbons and at 42.09 for -$CH_2$ (Fig.3.3.). The ligand signals at 167.71 ppm and 169.01 ppm for -CH=N and -C-OH group are shifted upfield to 160.03 ppm and 162.12 ppm for the Zn(II) complex. There are no appreciable changes in other peaks. So the changes in peak position for -CH=N and -C-OH carbons in the synthesized complex with respect to ligand confirms the complexation.

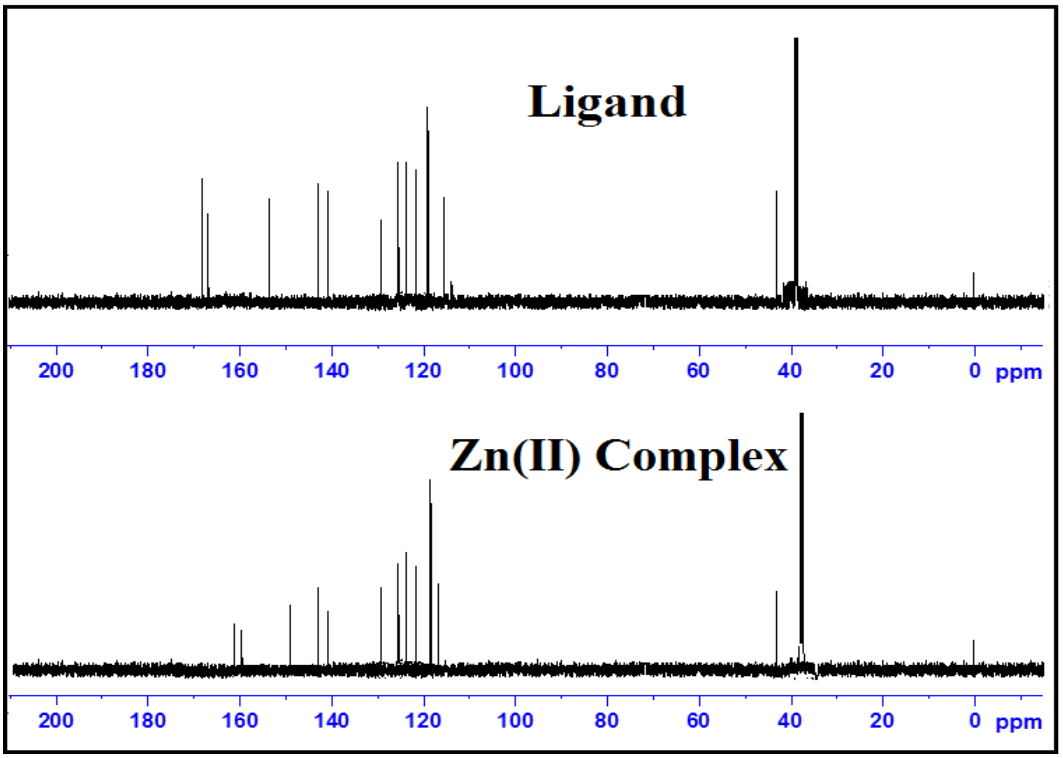



**Fig.3.3.** $^{13}$C NMR spectrum of Schiff base ligand and its Zn(II) complex.

### 3.3.1.4. Electronic Spectra:

The UV-visible absorption spectra of the Schiff base and its Zn(II) complex were recorded in DMSO ($1\times10^{-5}$ M) at room temperature, depicted in Fig.3.4. The electronic absorption spectrum of Schiff base contains two bands at 380 and 430 nm. The band at higher energy (380 nm) is attributed to π-π* transitions of phenyl ring while the lower energy band at 430 nm is assigned to n-π* transition of azomethine (-CH=N-) group [30]. These absorption bands also exist in the spectrum of Zn(II) complex but undergoes blue shift. This shifts in the spectra of the synthesized complex supports the coordination of the ligand to Zn(II) ion. The Zn(II) complex because of its $d^{10}$ configuration do not show any d-d transition and hence its diamagnetic and possibly having tetrahedral geometry.

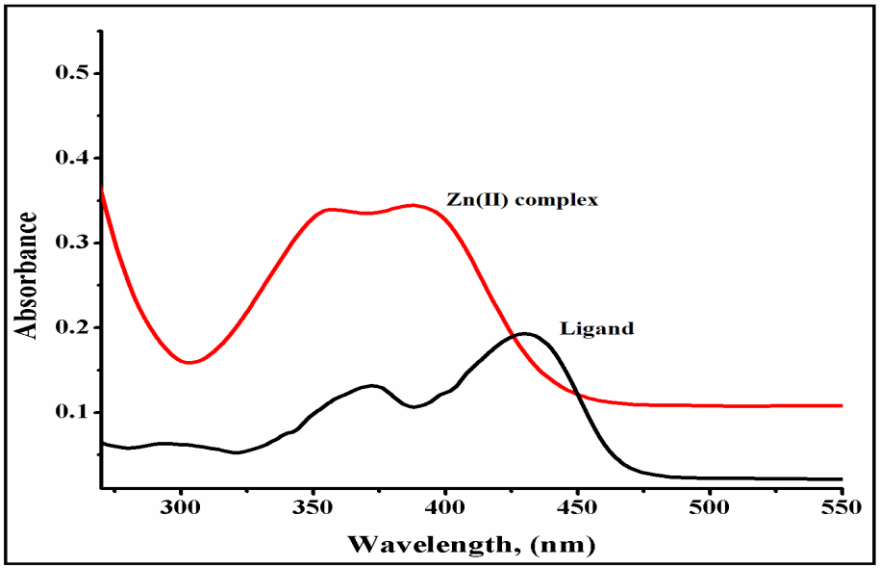



**Fig. 3.4.** UV-visible spectra of Synthesized ligand and its Zn(II) complex in DMSO.

### 3.3.2. DNA binding study:

#### 3.3.2.1. Absorption study:

Electronic absorption spectroscopy serves as an effective tool to study the binding mode of DNA with metal complexes. The binding behavior of DNA to metal complex is often investigated by the changes in the wavelength and in the absorbance. A metal complex binds to DNA via intercalation usually results in bathochromism (red shift in wavelength) and hypochromism shift because of strong stacking interaction of DNA base pairs with aromatic chromophore [22]. Here on increasing the amount of DNA in the complex solution resulted in red shift (1.8 nm for n-π* band, Fig.3.5.) and noticeable hypochromicity too, indicating intercalative mode of binding. The intrinsic binding constant ($K_b$) was evaluated using Wolfe-Shimer equation and the value of $K_b$ was found to be $(2.30 \pm 0.06) \times 10^5 M^{-1}$. Though this value is slightly lower than standard EB ($1.4\times 10^6 M^{-1}$) but mostly higher than other reported Zn(II) complexes [31-33].

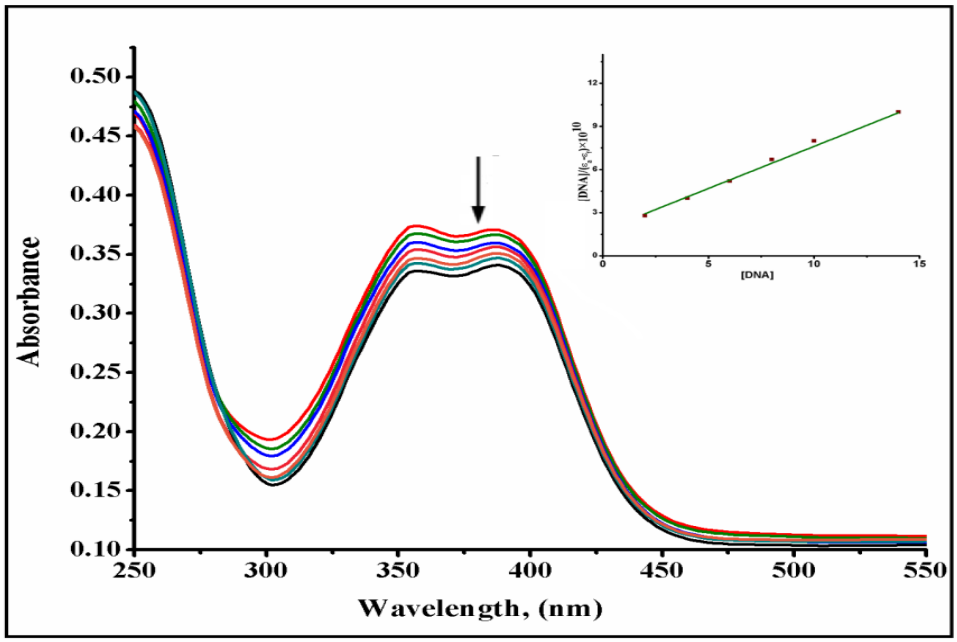



**Fig.3.5.** Absorption spectra of Zn (II) complex (orange line) in absence and presence of increasing amount of CT DNA (0–15 µM), Inset: plot for binding constant ($K_b$).

### 3.3.2.2. Ethidium Bromide (EB) competitive study with fluorescence emission spectroscopy:

Ethidium Bromide (EB)-DNA conjugate exhibit an intense emission band at 592 nm because of intercalation of the planar phenanthridine ring of EB between adjacent DNA base pairs [35]. A significant quenching of the EB-DNA emission band may be observed if a foreign molecule (which can intercalate to DNA equally or stronger than EB) is added into EB-DNA solution [35]. The synthesized Zn(II) complex do not show any emission band in solution or in the presence of CT- DNA or EB when excited at 540 nm. So the observed changes in the emission spectra of EB-DNA solution, when Zn(II)complex is added, are vital to examine the Ethidium Bromide displacement ability of the synthesized complex. The addition of Zn(II) complex in EB-DNA solution causes a significant quenching of the intense emission band at 592nm shown in Fig.3.6. The calculated quenching constant ($K_{sv}$) value for the synthesized complex is (3.0 ± 0.02) $\times 10^5$ $M^{-1}$ which is mostly higher than other reported Zn(II) complexes [31,34]. Therefore, the observed quenching suggest remarkable ability of Zn(II) complex to displace

intercalator EB from EB-DNA conjugate and divulging indirectly the intercalative mode of binding to DNA [22].

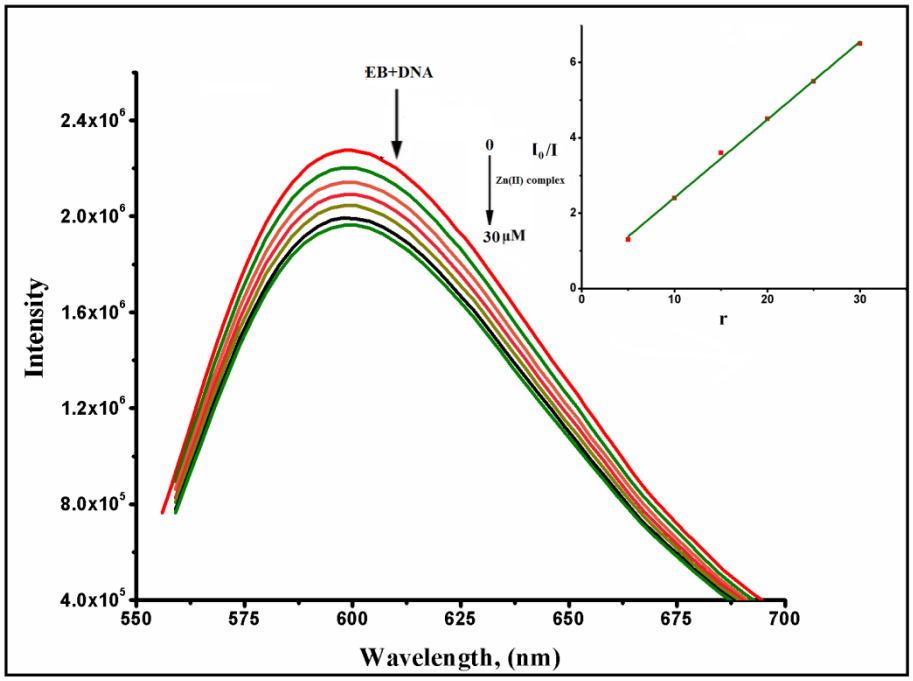



**Fig.3.6.** Emission spectra of EB bound to the DNA in absence and presence of increasing amount of Zn(II) complex (0–30 µM), Inset: plot for quenching constant (Ksv).

### 3.3.2.3. Thermal denaturation study:

Thermal denaturation study provides information regarding the extent of intercalation [36]. The intercalation of complex into DNA base pairs generally increases the melting temperature ($T_m$) of DNA [22]. Fig.3.7. shows thermal denaturization profile of CT-DNA solution both in absence and in presence of Zn(II) complex. The recorded value of $T_m$ for CT-DNA solution was $79.0^0$C. But in presence of Zn(II) complex, $T_m$ value increases dramatically to $85.0^0$C. These increase in melting temperature of CT-DNA in presence of complex ($\Delta T_m$= $6.0^o$C) indicates intercalative binding of Zn(II) complex into DNA [36].

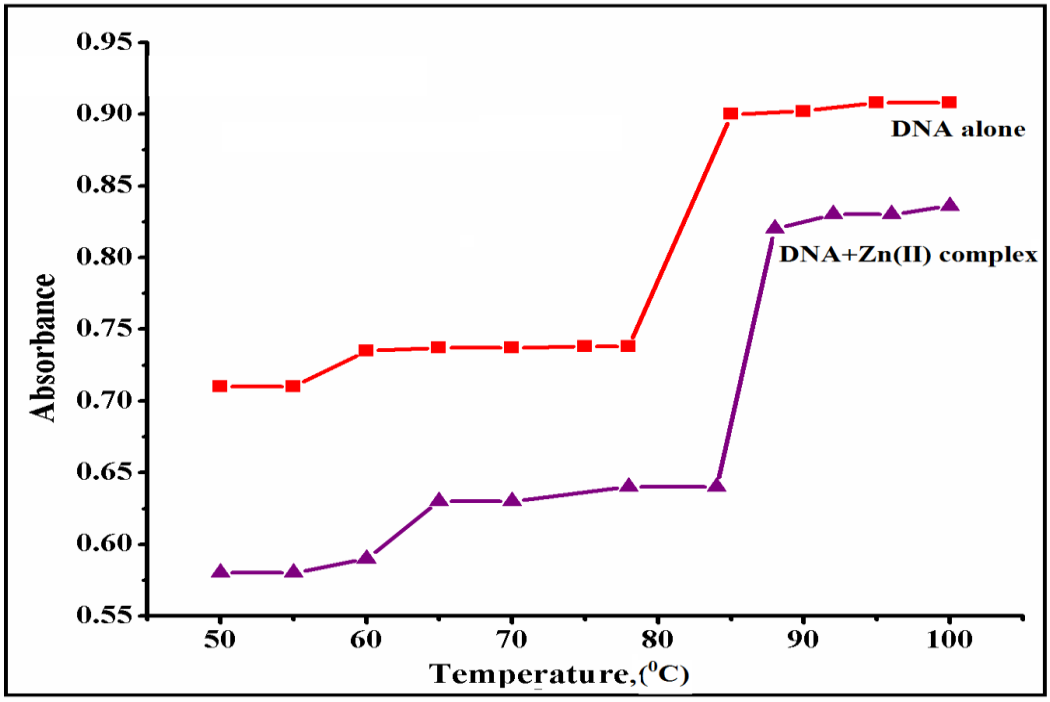



**Fig.3.7.** Plot of absorbance versus temperature ($^0$C) for the melting of 1) CT DNA alone (red line), 2) CT DNA + Zn(II) complex (purple line).

### 3.3.2.4. Viscosity measurement:

Viscosity measurement is a substantial tool to study the binding mode of compounds to DNA. The interaction of DNA with metal complexes via intercalation generally results in an increase of the separating distance of the DNA base pairs present at the intercalation site in order to host the binding compound. As a result the length of the DNA helix increases that leads to an increase in DNA viscosity [37]. While in case of binding to DNA via non-classic intercalation (like external groove-binding or electrostatic interaction) results in a kink or bends in the DNA helix. This kink or bends in DNA helix causes slight shortening of the effective length of DNA helix; in such cases the changes in DNA viscosity is less prominent or no change at all [37]. Ethidium bromide is a well known DNA intercalator that increases the relative viscosity of DNA by lengthening the DNA helix. Upon increasing the concentration of Zn(II) complex in the CT-DNA solution, the relative viscosity of DNA

solution increases steadily almost similar to the nature as in case of Ethidium Bromide (shown in Fig.3.8). This increase in viscosity in case of complex solution suggests that the synthesized Zn(II) complex could bind to DNA via intercalation mode which is consistent with the UV-visible and fluorescence spectral data.

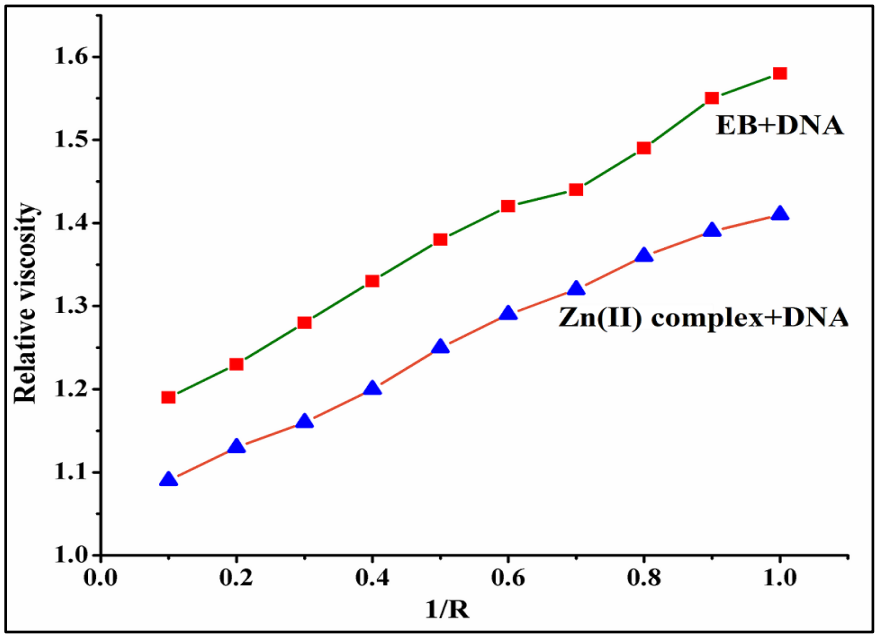



**Fig.3.8.** Effect of increasing amounts of (a) EB (b) Zn(II) complex on the relative viscosity of CT-DNA.

### 3.3.2.5. DNA cleavage study:

The DNA cleavage reaction was monitored by gel electrophoresis. To perform cleavage reactions, solutions of pUC19 DNA were prepared and then diluted with loading dye using 1% agarosegel. To each solution obtained, 3 µl of EB (0.5 µg ml−1) was added and mixed well. In the next step, warm agarose was poured and instantly held tightly with a comb to develop sample wells. The gel was placed in an electrophoretic tank and adequate electrophoretic buffers were added to enclose the gel to 1 mm depth. DNA sample (20 µM), 30 µM complex and 10 µMH2O2 in the aforesaid buffer (pH = 7.2) were mixed with a loading dye and filled into the well of the submerged gel using a micropipette. A 50 mA electric current was passed and the gel was taken out from the buffer. The gel was photographically captured under UV light after the completion of

electrophoresis.method with different concentration of Zn(II) complex in presence of $H_2O_2$. The ability of the complex to cleave DNA is determined by transformation of DNA from supercoiled form (Form 1) to nicked form (Form 2) and linear form (Form 3). For Form I, the fastest migration is observed. If only one strand is cleaved, then Form 1 relaxes to convert into a slower-moving Form II. If both the strands are cleaved, Form 3 is then generated which moves in between Form I and Form II [38]. The result of DNA cleavage is shown in Fig.3.9.

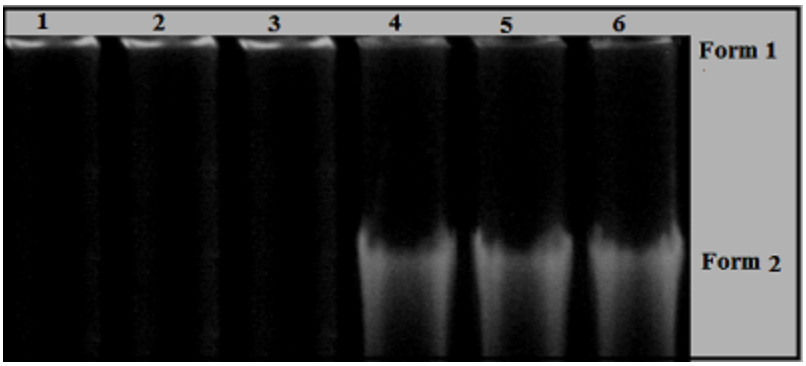



**Fig.3.9.** Changes in the agarose gel electrophoretic pattern of pBR322 plasmid DNA induced by $H_2O_2$ for ligand and Zn(II) complex. Lane (1): DNA control, Lane (2): DNA + $H_2O_2$, Lane (3): DNA+ 10 μM ligand + $H_2O_2$, Lane (4-6): DNA+ Zn(II) complex + $H_2O_2$, [complex] = 5, 10, 15μM respectively.

Here for control experiments (lane 1, 2) and in presence of ligand (lane 3), no DNA cleavage is found as there is no metal complex in role while with increasing the concentration of the synthesized complex (lane 4-6), the percentage of Form 1 starts to diminish gradually where as Form II increased. These indicates that the synthesized Zn(II) complex have acted on supercoiled plasmid DNA as there was remarkable difference in bands of the complex compared to band of the control DNA. But in case of ligand there is no appreciable change in the bands with respect to control DNA. The experiment is modulated by hydroxyl radical or peroxo species originated from $H_2O_2$. The existence of smears in the gel photograph suggesting radical cleavage [39]. The detail mechanism of DNA cleavage by metal complex in presence of $H_2O_2$ has been reported by various research groups [39,40]. As the synthesized complex is efficient to

cleave the supercoiled plasmid DNA into open circular form, it can be inferred that the complex may inhibit the growth of pathogens by cleaving the genome [24].

### 3.3.3. Molecular modeling analysis:

#### 3.3.3.1. Swiss target prediction:

The Swiss prediction report for the synthesized Zn(II) complex showed probable association with biological network. It is seen that among the probable targets 20% are enzymes and 40% are cytosolic proteins and there is a huge chunk of another 40% which are unclassified proteins (Fig.2). Among the list of predicted targets there are important compounds such as BCl-2 like protein 1 which are linked to various cellular activities especially pro apoptotic functions. Then again there is Bcl-2-like protein 2 which is also linked to similar function [41]. Apoptosis regulator Bcl-2 is another similar protein which shows interaction with our Zn(II) complex. Induced myeloid leukemia cell differentiation protein Mcl-1 is another protein of BCl-2 family which is essential for apoptosis regulation. All these proteins are essentially related to carcinogenesis pathway [42]. So it is quite likely that the synthesized Zn(II) complex might play a role in general oncology. Moreover, there are other important proteins in this aspect. Microtubule-associated protein Tau promotes microtubule assembly and stability, and might be involved in the establishment and maintenance of neuronal polarity which suggest probable implications in neurodegenerative diseases. They are vital proteins in Alzheimer's disease [43]. These apart important DNA repair enzymes like Tyrosyl-DNA phosphodiesterase 1 and Muscleblind-like protein groups also seems to have some affinity towards the synthesized complex. These multivaried activities suggest importance of the synthesized complex in biological systems.

#### 3.3.3.2. Molecular Docking:

The synthesized Schiff base Zn(II) complex has interesting target partners. To better understand their interaction with the complex and to understand which compound has better interaction we have conducted the molecular docking experiments. Tyrosyl-DNA phosphodiesterase 1 has the best binding affinity with the complex (Table.3.2).

**Table. 3.2.** Binding affinity of the Zn(II) complex with different proteins:

| Protein name | Gene name | PDB ID | Binding affinity(kcal/mol) |
|---|---|---|---|
| Dihydroorotate dehydrogenase (quinone), mitochondrial | DHODH | 1d3g | -9.2 |
| Tyrosyl-DNA phosphodiesterase 1 | TDP1 | 1jy1 | -10.5 |
| Bcl-2-like protein 1 | BCL2L1 | 1r2d | -9.6 |
| Induced myeloid leukemia cell differentiation protein Mcl-1 | MCL1 | 2nl9 | -10 |
| Microtubule-associated protein tau | MAPT | 2on9 | -7.1 |
| Muscleblind-like protein 1 | MBNL1 | 3d2q | -8.7 |
| Bcl-2-like protein 2 (by homology) | BCL2L2 | 4cim | -9.4 |
| Apoptosis regulator Bcl-2 (by homology) | BCL2 | 4lxd | -8.5 |

These enzymes have DNA repair capacity and catalyze different hydrolysis reactions in nucleotides. Moreover, it is seen earlier from DNA interaction study that the Zn(II) complex has different DNA binding and cleaving activity. So, a high affinity of magnitude -10.5 kcal/mol in autodock enviroment confirms those previous claims. Then again induced myeloid leukemia cell differentiation protein Mcl-1 has the second best binding affinity. This protein plays a major role in apoptotic regulations. It is already seen that apart from Mcl-1 protein different Bcl-2 family proteins has major activity with the synthesized Zn(II) complex. Proteins like Bcl-2-like protein 1, Apoptosis regulator Bcl-2, Bcl-2-like protein 2 are all members of Bcl-2 family of proteins and all have good binding affinity with the synthesized complex (Fig.3.10). The proteins of Bcl-2 family

are all linked to carcinogenesis pathway. So it is confirmed that along from nucleotide interaction the synthesized Zn(II) complex has possible implications in carcinogenesis.

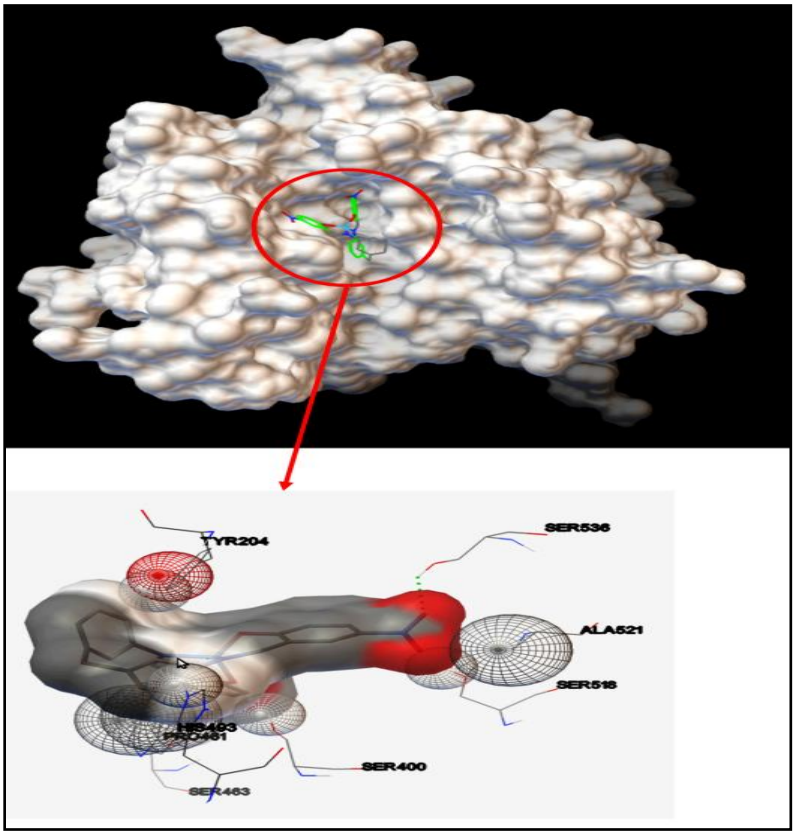



**Fig.3.10.** Top- The molecular surface view of the protein molecule and the docked complex molecule at its binding site.

### 3.3.4. In vitro antioxidant activity:

The free radical scavenging activity of Zn(II) complex in dose dependent manner and the differences in the activities compared with standard compound is seen. Half maximal inhibitory concentration ($IC_{50}$) of Zn(II) complex are shown in the Table.3.3. The synthesized Zn(II) complex showed lower $IC_{50}$ value than ascorbic acid, mannitol, sodium pyruvate, curcumin, quercetin in DPPH, hydroxyl radical, $H_2O_2$, nitric oxide, superoxide anion, hypochlorous acid scavenging assay and found comparable with peroxinitrate shown by gallic acid and lipid peroxidation shown by trolox [Fig.3.11-Fig.3.15.].

On the other hand, Zn(II) complex showed higher $IC_{50}$ value than lipoic acid and EDTA in singlet oxygen and iron chelation scavenging assay. In case of total antioxidant activity, Zn(II) complex showed better scavenging activity than the standard ascorbic acid. The reducing power of Zn(II) complex was also determined and found that the

**Table.3.3.** Percentage of inhibition at highest concentration and $IC_{50}$ values (in parentheses) of Zn(II)-Complex and standard for different antioxidant and free radical scavenging assays.

| Parameter | Zn(II)-Complex | Standard |
|---|---|---|
| DPPH | 43.52±0.04** | 58.5±0.02 |
| | (272.25±1.06**) | (202.06±0.12) |
| Nitric oxide | 57.43±0.48** | 100±0 |
| | (145.42±2.18*) | (60.93±0.04) |
| Hydroxyl radical | 56.9±0.42* | 31.31±0.84 |
| | (129.97±1.05*) | (591.48±9.50) |
| Hydrogen peroxide | 25.14±0.6** | 7.64±0.68 |
| | (744.25±70.57$^{NS}$) | (2147.45±248.44) |
| Hypochlorous acid | 47.52±0.58* | 62.36±0.36 |
| | (214.22±2.13*) | (132.46±4.37) |
| Peroxynitrite | 16.87±0.86$^{NS}$ | 17.68±0.21 |
| | (1034.4±65.65*) | (799.39±36.99) |
| Singlet oxygen | 24.81±1.07*** | 77.97±0.34 |
| | (637.85±36.65**) | (48.43±3.65) |
| Hemolytic | 28.19±0.24** | 73.93±1.01 |
| | (440.12±4.85**) | (78.31±2.35) |
| EMSA | 43.49±0.36* | 78.67±0.67 |
| | (266.02±0.55***) | (67.03±0.64) |

Units in µg/ml. Data expressed as mean±S.D. *-p<=0.05(two sided), **-p<=0.01(two sided), **-p<=0.001(two sided), $^{NS}$-p>0.05(two sided)

reducing capacity of Zn(II) complex extract was increased in a dose dependent manner comparable to the reference compound ascorbic acid and ligand.

In the present antioxidant profiling, Zn(II) complex showed potential free radical scavenging activities. The molecule DPPH is a free radical that can accept an electron or hydrogen radical to become stable and reacts with reducing agent to form new bond, changing the color of the solution. The colored DPPH solution mixed with natural

antioxidants. DPPH gives rise to the reduced form with the loss of violet color by the effect of natural antioxidants. Thus, DPPH scavenging activity by Zn(II) complex proves the presence of significant antioxidant properties. But in case of ligand the results are not satisfactory.

Human beings are exposed to $H_2O_2$ indirectly. Inhibition of $H_2O_2$ indirectly from environment is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals ($OH^{\cdot}$) that can cause lipid peroxidation and DNA damage in the body. Therefore, the ability of Zn(II) complex to scavenge $H_2O_2$ proves beneficial for our health.

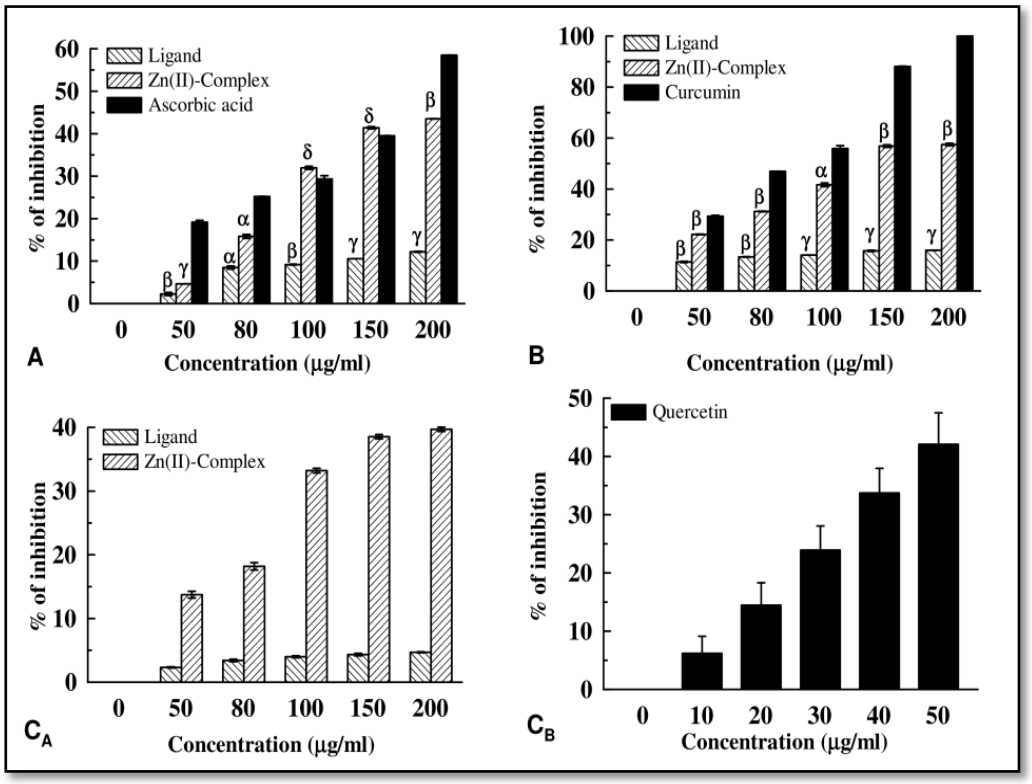



**Fig.3.11.** Antioxidant activity of Zn(II) metal complex. **(A)** DPPH activity. **(B)** Nitric oxide scavenging activity. **(C)** Super oxide radical scavenging activity. Data expressed as mean ± S.D. $^{\alpha}$ $p<0.05$; $^{\beta}$ $p<0.01$; $^{\gamma}$ $p<0.001$; $^{\delta}$ Non significant when compared with standard.

Nitric oxide plays an important role as pro-inflammatory mediators. Nitric oxide ($NO^{\cdot}$) is synthesized from the amino acid L-arginine by the activation of nitric oxide synthase (NOS). During chronic inflammation iNOS (Calcium independent isoform of NOS) is activated by LPS (Lipopolysaccharide) and produces huge amount of nitric oxide. The active $NO^{\cdot}$ translocate NF-κβ and leads to the formation of cancer. In mitochondria excess amount of nitric oxide reacts with superoxide radical to produce reactive peroxynitrite radical which further causes oxidative stress related disorder. In the present study, it is demonstrated that nitric oxide is down regulated by Zn(II) complex when compared to standard curcumin. Thus, Zn(II) complex might inhibit the inflammation related disorders.

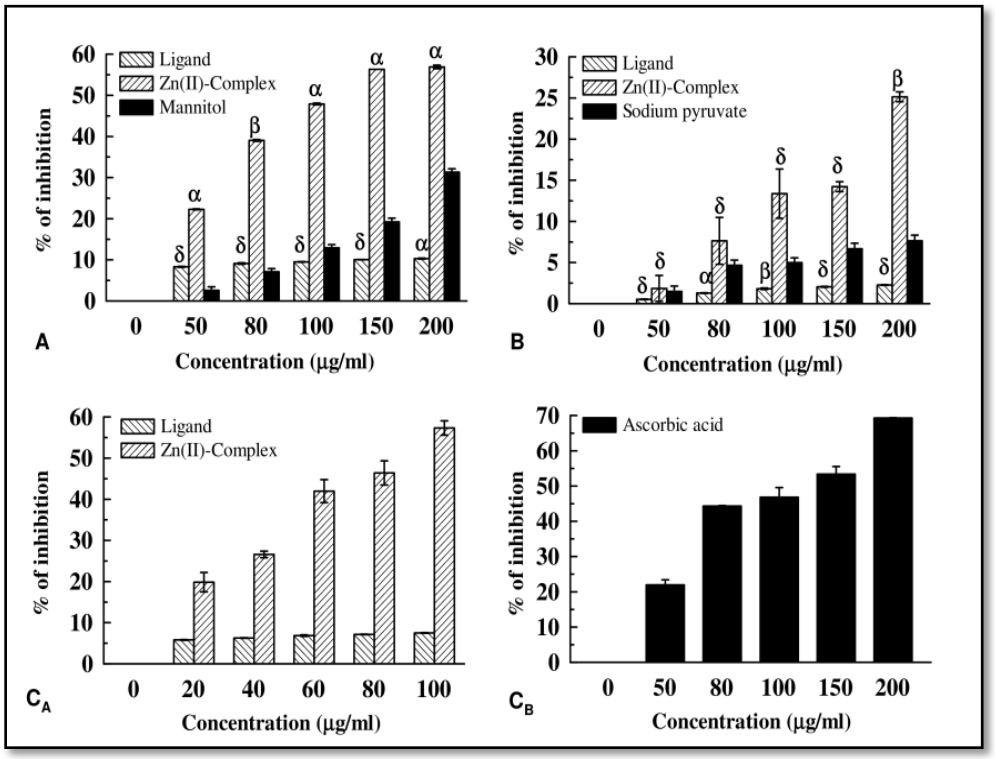



**Fig.3.12.** Antioxidant activity of Zn(II) metal complex. **(A)** Hydroxyl radical scavenging assay. **(B)** Hydrogen peroxide scavenging activity. **(C)** Total antioxidant scavenging assay. Data expressed as mean ± S.D. $^{*}$ $p<0.05$; $^{**}$ $p<0.01$; $^{***}$ $p<0.001$; $^{NS}$ Non significant when compared with standard.

Hydroxyl radical generated from hydrogen peroxide by Fenton reaction is one of the potent reactive oxygen species in the biological system that react with phospholipids containing polyunsaturated fatty acid moieties of cell membrane and cause damage of cell [44].

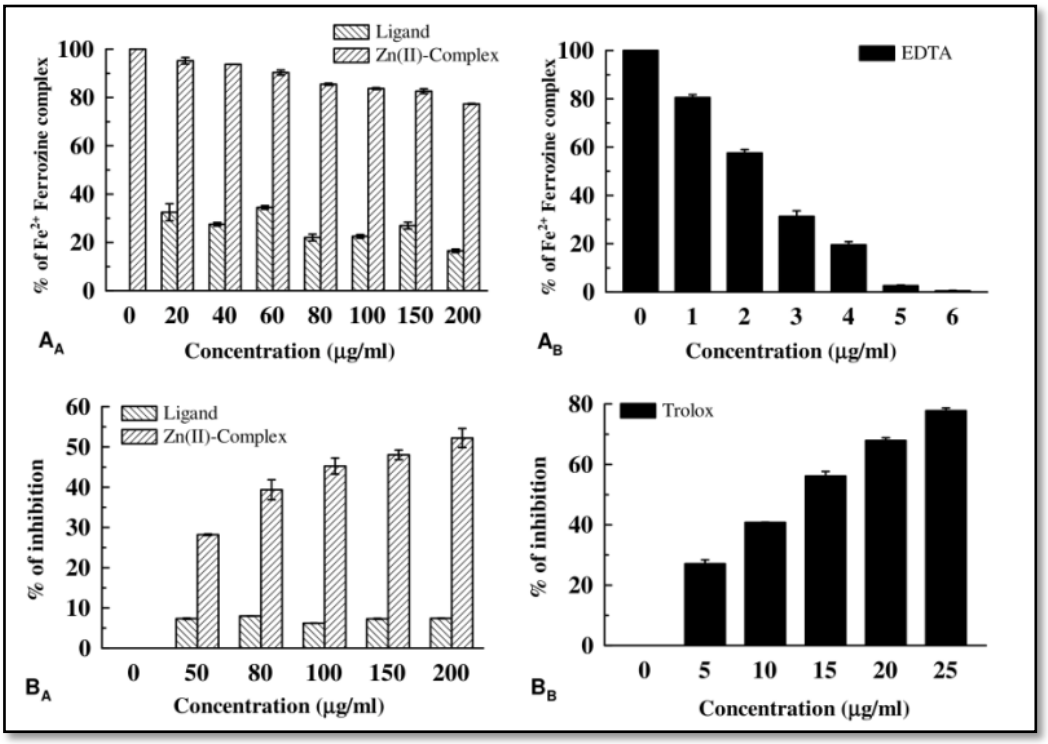



**Fig.3.13.** Antioxidant activity of Zn(II) metal complex. **(A)** Iron chelation assay. **(B)** Lipid peroxidation activity. Data expressed as mean ± S.D. $^{*}$ $p<0.05$; $^{**}$ $p<0.01$; $^{***}$ $p<0.001$; $^{NS}$ Non significant when compared with standard.

Hypochlorous acid produced from the site of chronic inflammation resulting from the oxidation of $Cl^-$ ion by the neutrophil enzyme, myelo-peroxidase. Hypochlorous acid degrades heme prosthetic group and inactivates the antioxidant enzyme catalase.

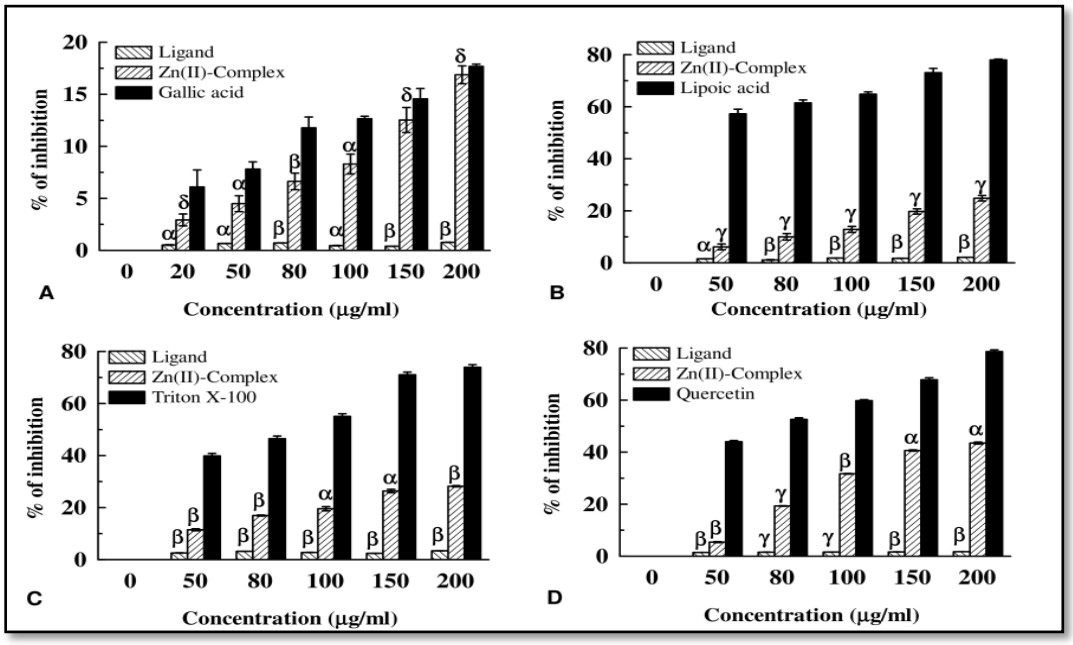



**Fig.3.14.** Antioxidant activity of Zn(II) metal complex. **(A)** Peroxynitrite scavenging assay. **(B)** Singlet oxygen scavenging assay. **(C)** Hemolytic assay **(D)** Erythrocyte membrane stabilizing activity. Data expressed as mean ± S.D. $^{\alpha}$ $p<0.05$; $^{\beta}$ $p<0.01$; $^{\gamma}$ $p<0.001$; $^{\delta}$ Non significant when compared with standard.

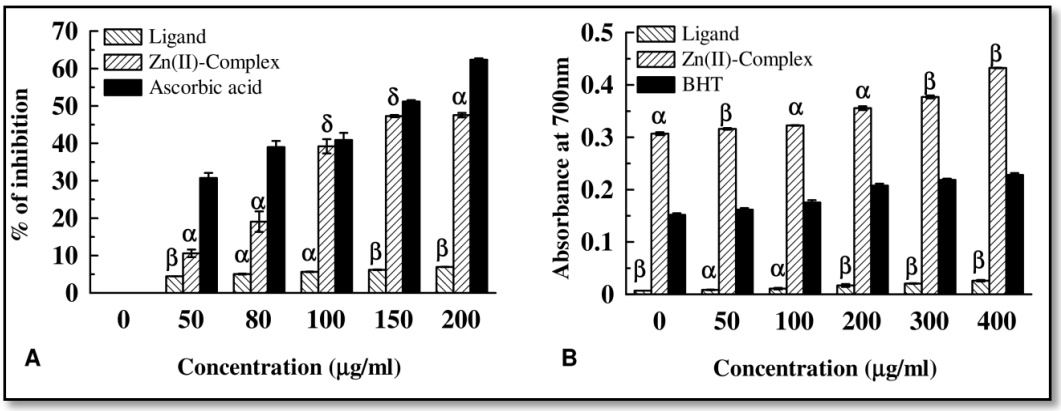



**Fig.3.15.** Antioxidant activity of Ligand and its Zn(II) metal complex. (A) Hypochlorous acid scavenging assay. (B) Reducing power assay. Data expressed as mean ± S.D. $^{\alpha}$ $p<0.05$; $^{\beta}$ $p<0.01$; $^{\gamma}$ $p<0.001$; $^{\delta}$ Non significant when compared with standard.

Zn(II) complex prove that it has the potentiality to scavenge proxynitrate, hydroxyl radical, superoxide, singlet oxygen and other free radicals too that cause the harmful effect in our biological system. In comparision to the ligand the Zn(II) complex shows far better free radical scavenging activity (Fig. 11 to Fig. 15). Thus, Zn(II) complex might prove to be a key component in prevention of various diseases related to oxidative stress and free radical generation.

### 3.4. Conclusions:

The synthesized Schiff base ligand and its Zn(II) complex have been characterized by different spectral and analytical techniques. Result of the physical measurements exhibit that the Zn(II) metal ion is coordinated by two azomethine nitrogen and two phenolic oxygen atoms and hence it possibly adopts a tetrahedral geometry. The synthesized complex is stable and non electrolyte in nature. The ability of the synthesized complex to bind to DNA was monitored by different techniques. The experimental results indicate that the Zn(II) complex interacts remarkably with CT-DNA and confirms an intercalative mode of binding to CT-DNA. Apart from high binding affinity with DNA at stable energetics it is also seen that the complex has interaction potential with other biologically important pathways like apoptosis regulation which hints towards possible involvement with Carcinogenesis. In the present study it is also evident that the synthesized complex aided in the recovery of oxidative stress and inhibited lipid peroxidation. On the basis of these facts, antioxidant therapy by synthesized Zn(II) complex alone or in combination with other pharmacological strategies appear as the most reasonable treatment of oxidative stress induced several mental disorders like mental stress, trauma, anxiety etc.

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# CHAPTER-IV

# DNA INTERACTION AND MOLECULAR DOCKING STUDY OF A NOVEL SCHIFF BASE Co(II) COMPLEX

## 4.1. Introduction:

Schiff base metal complexes, recognised by azomethine or imine group (>C=N-), are the most comprehensively studied coordination compounds. They have been extensively involved as ligands in the preparation of transition metal complexes [1,2]. In neotric scientific studies an ample amount of research connected with Schiff base complexes showed remarkable biological activity and are of great significance in both chemical and biological field [2-4]. The most vital step for such investigation is the identification of target. For cancer, DNA has been marked as the principal target thus, the study of DNA binding and cleavage activity by metal complexes is the key for developing new metal-based drugs. Development of such molecules that can interact with DNA of cancerous cell and obstruct them is an example of new anticancer approach. This has paved way for an increase in anticancer therapeutics in search of metallo-nucleases [4-7]. Transition metals which are used largely in this area of research include platinum [8], ruthenium [9-10], titanium [11], rhodium [12], palladium [13], gold [14], Vanadium [15] and Nickel [16]. Among these transition metals, Platinum(II) based compounds and related drugs fascinated the inorganic chemists during a long period because of its anti-cancer activity, especially cis-platin [5,6]. But, these drugs have so many disadvantages too such as lesser solubility, dose-limiting side effects like nephrotoxicity and neurotoxicity and acquired resistance in most type of cancers [6] which necessitates the upgradation of anticancer approach [17].

Cobalt which is an integral constituent of cyanocobalamin (vitamin B12), is very essential in our circulatory system [18,19]. Cobalt also functions as an activating agent for diverse enzyme systems. But interaction of Cobalt complexes with DNA is rarely available in the literature. To explore the chemistry of Co(II) complex and assess its

biological activity, here we have prepared a new Co(II) complex and investigated its interaction with DNA using different physico-chemical and spectroscopic techniques.

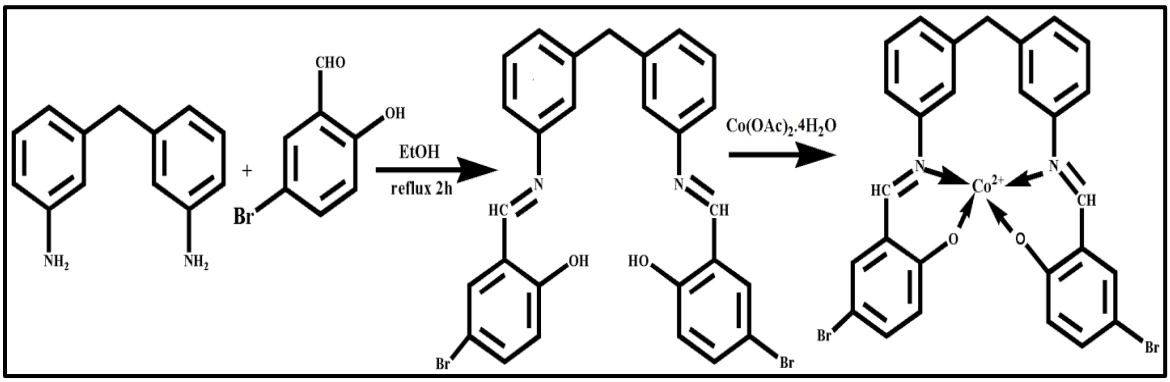
## 4.2. Experimental Section:

### 4.2.1. Synthesis of Schiff Base Ligand:

To a hot ethanolic solution of 3, 3' Methylenedianiline (0.1 mol), an ethanolic solution of 5- bromo salicylaldehyde (0.2 mol) was added in dropwise manner and refluxed it for 1h. The resultant orange colored ligand was separated by filtration, washed several times with ethanol and ether and dried in vacuum desiccator.

### 4.2.2. Synthesis of Metal Complex:

The metal complex was prepared by adding hot ethanolic solution of Co(II) acetate to the equimolar amount of hot ethanolic solution of synthesized ligand (Scheme.4.1.). The resulting reaction mixture was then refluxed for 2 h at 50°C whereupon the Co(II) metal complex was precipitated out. The blue colored precipitate was filtered off, washed with ethanol and dried in vacuum desiccator.




**Scheme.4.1.** Synthesis of schiff base ligand and its Co(II) complex.

### 4.3. Results and Discussion:

#### 4.3.1. Characterization of the ligand and its Co(II) complex:

The synthesized compounds have been characterized by using different analytical and spectroscopic tools like IR, Proton NMR, Magnetic moment, UV-Visible, molar conductance *etc*. The analytical data of the synthesized compounds is listed in Table.4.1.

#### 4.3.1.1. Molar Conductance:

The room temperature molar conductance of synthesized Co(II) complex ($10^{-3}$ mol $dm^{-3}$ ) was measured in DMSO (Table1). The molar conductance value indicates that the metal complex is a non-electrolyte [20].

**Table 4.1.** Analytical and Physical data of Schiff base ligand and its Co(II) complex:

| Compound | m.p. (◦C) | Colour (% yield) | Mol. Wt. (gm) | % Found (calcd.) | | | $\Lambda_m$ ($\Omega^{-1}$ $mol^{-1}$ $cm^2$) |
|---|---|---|---|---|---|---|---|
| | | | | C | H | N | |
| $H_2L$ ($C_{27}H_{20}N_2O_2Br_2$) | 154 | Orange (76) | 564 | 57.31 (57.47) | 3.62 (3.57) | 4.43 (4.96) | _ |
| [Co(L)] ($C_{27}H_{18}N_2O_2Br_2Co$) | >250 | Blue (69) | 621 | 51.06 (52.21) | 2.98 (2.92) | 4.26 (4.51) | 13.83 |

#### 4.3.1.2. Electronic Spectra and magnetic moment measurement:

The absorption spectra of the newly synthesized molecules were recorded in DMSO depicted in Fig.4.1. The ligand displayed bands at 375 nm and 432 nm, attributed to π-π* transition of phenyl ring and n-π* transition of azomethine (-CH=N-), respectively [22]. However, these absorption bands were undergo blue shift in the spectra of Co(II) complex, confirming the coordination of ligand to Co(II) ion. In addition the Co(II) complex showed a band at 598 nm due to $^4A_2 \rightarrow {}^4T_1$ (P) transition which is a typical one

for tetrahedral Co(II) complex [23]. The value of magnetic moment 4.56 BM also supports the tetrahedral geometry of the synthesized complex [23].

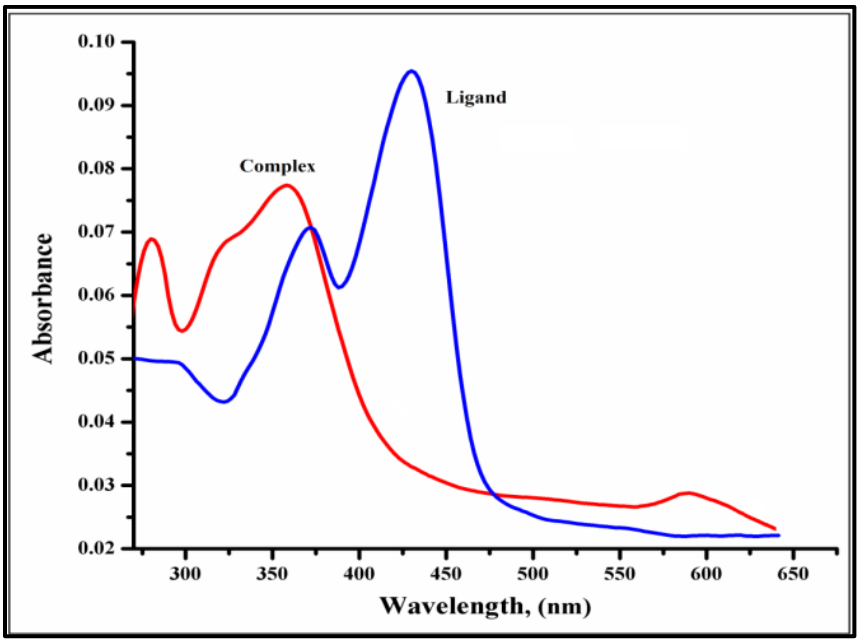



**Fig.4.1.** UV-visible absorption spectra of ligand and its Co(II) complex in DMSO.

### 4.3.1.3. Infrared Spectra:

IR spectra afford relevant clue regarding the coordination pattern of ligand to the metal ion. The IR spectrum of the Cobalt(II) complex was matched with the spectrum of ligand to identify the changes come about during complexation. The synthesized Schiff base ligand exhibits a characteristic band at 1594 $cm^{-1}$ due to azomethine group ν(C=N) shown in Fig.4.2. This stretching vibration band is shifted to 1600 $cm^{-1}$ in the Co(II) complex indicating the involvement of the nitrogen atom of (>C=N-) group in complexation [20]. In the spectra of synthesized ligand, a broad band was noticed at 3447 $cm^{-1}$due to phenolic -OH group of ligand which is found to disappear in the spectra of synthesized complex suggesting the participation of phenolic oxygen in bonding with Co(II) ion through deprotonation [21]. The coordination of phenolic oxygen to metal ion is further confirmed by the shift in ν(C-O) stretching band of ligand at 1199 $cm^{-1}$ to 1169 $cm^{-1}$ upon complexation. The appearance of two non-ligand bands in the IR spectra of synthesized complex at 541 $cm^{-1}$ and 461 $cm^{-1}$ due to ν(Co-O) and ν(Co-N) confirms the

participation of phenolic O and azomethine N in complexation. In conclusion, the above arguments suggest the dibasic tetradentate behavior of the synthesized compound.

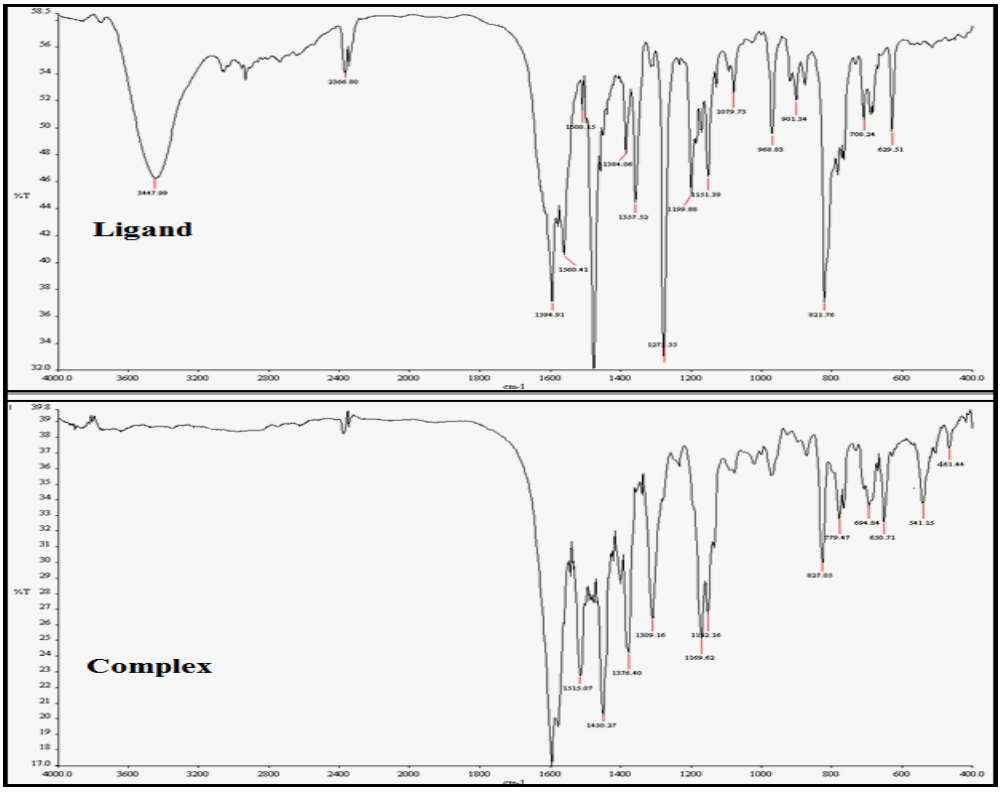



**Fig.4.2.** FTIR Spectra of the synthesized ligand and its Co(II) complex.

### 4.3.1.4. NMR Spectra:

The proton NMR spectra of synthesized ligand and was recorded in DMSO-$d_6$ solution (Shown in Fig.4.3.). The aromatic protons showed signals in the region 6.8-7.8 ppm. The formation of Schiff base is affirmed by the occurrence of azomethine (HC═N) proton signal at 9.20 ppm. Moreover, the ligand showed a hydroxyl (-OH) proton signal at 10.40 ppm [22].

The $^{13}C$ spectra of the ligand (Fig. 4.3.) showed peaks at 111.13-147.11 ppm for aromatic carbons and at 42.09 for -CH2. Peaks at 165.21 ppm and 168.23 ppm for –CH=N and –C–OH group also indicates the formation of Schiff base ligand.

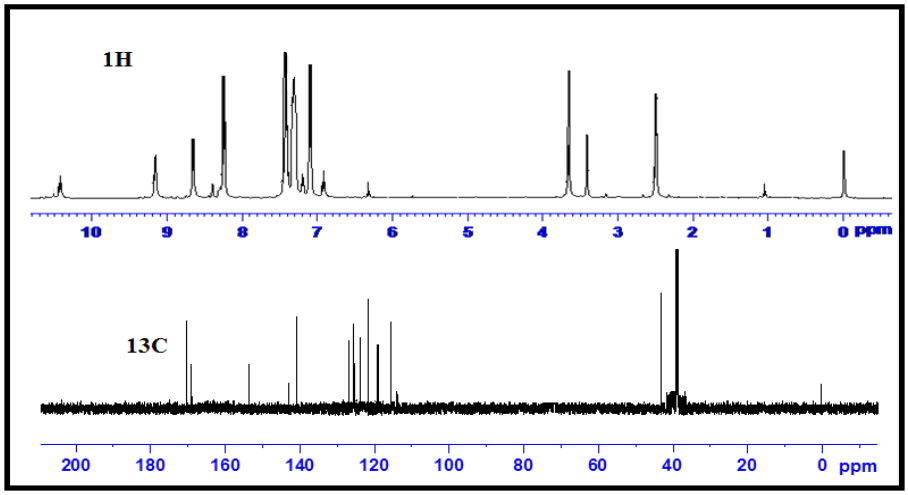



**Fig.4.3.** $^{1}H$ and $^{13}C$ NMR spectrum of Schiff base ligand.

### 4.3.1.5. Thermogravimetric analysis:

Thermogravimetric analysis (TGA) delivers a quantitative measurement of change in weight with temperature, and is commonly used to explore the thermal stability of synthetic molecules along with compositional analysis. Thermal stability of the Cobalt(II) complex was studied in the temperature range 50-1000 $^{\circ}C$ at the rate of 10 $^{\circ}C$/min. The gradual degradation of synthesized complex with temperature and formation of metal oxides is shown in Fig.4.4. The synthesized complex is quite stable up to 200 $^{\circ}C$ which indicates the absence of any counter ions in the complex molecule. The sharp weight loss of 26.25% (calcd. 26.76%) at the first decomposition stage at 281 $^{\circ}C$ was because of liberation of two bromine atoms. Thereafter, the Co(II) complex showed a gradual decomposition up to 850 $^{\circ}C$ due to the loss of the remaining ligand moiety and ultimately the sample decomposes to corresponding metal oxide. The weight of the residue corresponds to cobalt oxide.

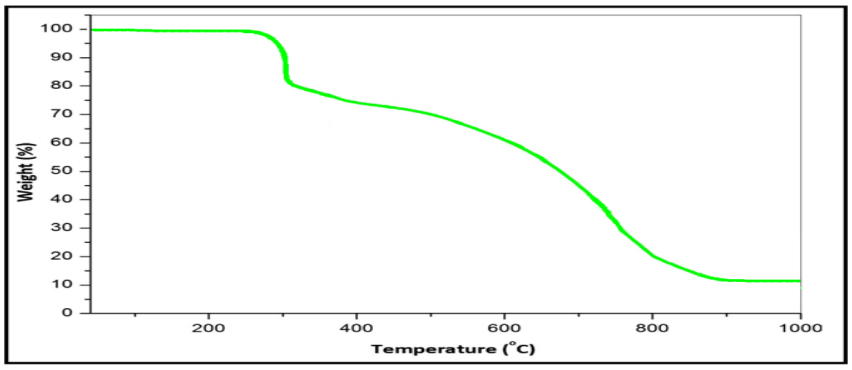



**Fig.4.4.** TGA curve of the Co(II) complex in the temperature range 50-1000 $^{o}$C.

### 4.3.1.6. Powdered XRD spectra:

The powdered XRD investigation of ligand exhibited sharp peaks, showing its crystalline nature. But upon complexation the peak intensity was reduced and line broadening wanoticed (Fig.4.5). This suggests the change in crystalline nature of ligand upon complexation and showing amorphous nature of the synthesized complex. The crystalline sizes were computed using Debye Scherer's equation: $D= 0.9\ \lambda/\beta\ \cos\theta$, where constant 0.9 is the shape factor while $\lambda$, $\beta$, $\theta$ are the X-ray wavelengths (1.5406 Å), FWHM i.e., full width at half maximum, Bragg diffraction angle respectively. The experimental average grain size of the ligand molecule and its complex were found to be 25.71 nm and 11.76 nm respectively.

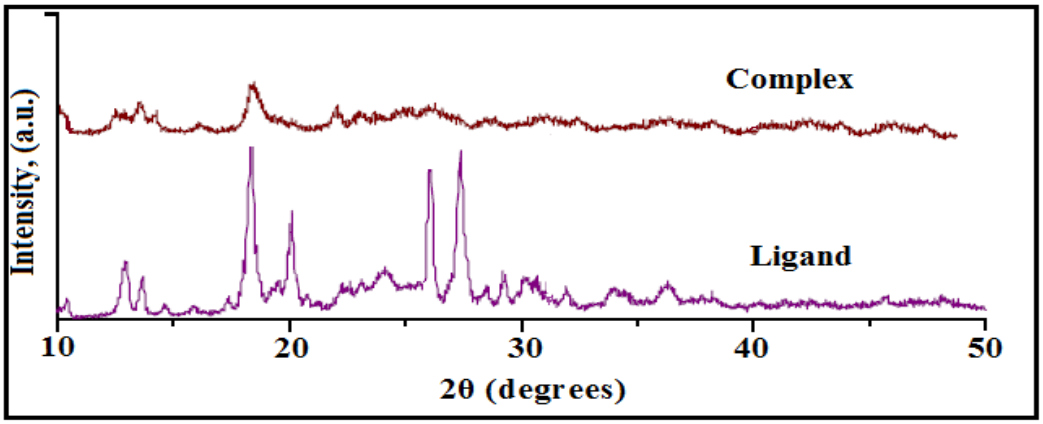



**Fig.4.5.** PXRD patterns of the ligand and its Co(II) complex.

#### 4.3.1.7. Stability constant and stoichiometry of complex:

Stoichiometry of the synthesized complex has been determined by spectrophotometric titration using mole-ratio method. The changes in the electronic spectra of ligand (initially $1\times 10^{-5}$ mol · $L^{-1}$) were recorded by stepwise addition of 2.5 µL of $Co(OAc)_2$ ($1 \times 10^{-3}$ mol · $L^{-1}$) metal solution at each step until the complexation was completed. During the titration, a gradual rise in the intensity at 432 nm, stipulates the formation of complex as presented in Fig. 4.6. The spectrophotometric data was analyzed with the absorbance values at $\lambda$ =432 nm as shown in Fig.4.6. (inset), which suggests the stoichiometry to be 1:1. Also using the ($c_M c_L$/A *versus* $c_M$) data, the stability constant of the complex was computed to be $\log K_f = 5.90 \pm 0.02$ at 25 °C.

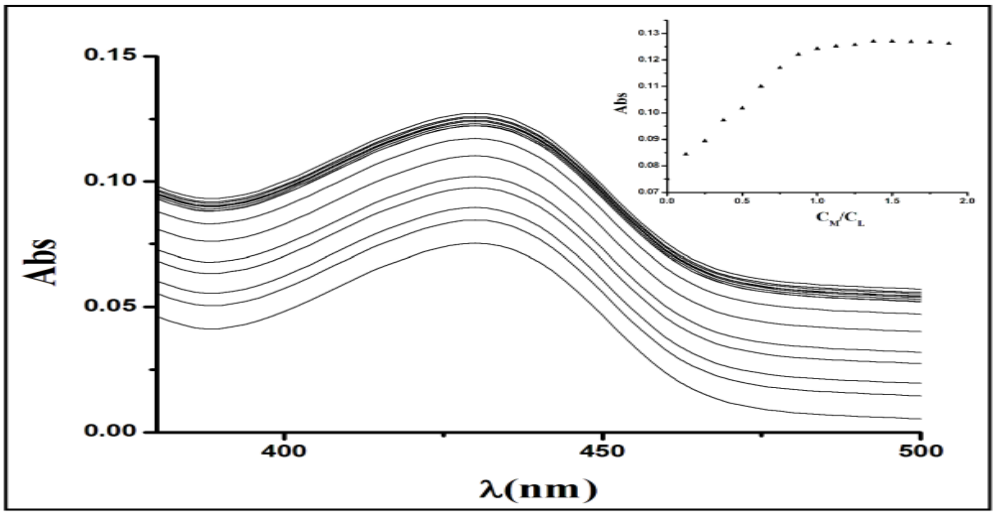



**Fig.4.6.** UV–Vis spectra of the ligand in presence of increasing concentrations of Co(II) ions. Inset: Absorbance plot for the ligand with Co(II) ion against *c*M:*c*L.

### 4.3.2. DNA interaction studies:

#### 4.3.2.1. Electronic absorption titration:

Absorption spectroscopic titration functions as a vital tool to study the interaction of DNA with small molecules. If a metal complex binds to DNA through intercalative mode, the obtained results show changes in absorbance and wavelength. The binding affinity of the synthesized metal complex with CT-DNA was studied in a solvent mixture

of 1% DMSO and 99% Tris‑HCl buffer employing a fixed concentration of complex to which increasing amount of CT-DNA solution was added. While recording the absorption spectra, equal increment of CT-DNA was added to both the complex solution chamber and the reference chamber to exclude the absorbance of free CT-DNA.

On successive addition of DNA in the complex solution chamber, change in both absorbance and wavelength (2.2 nm for n-π* band) were observed (shown in Fig.4.7.) because of strong stacking interaction between DNA base pairs and aromatic chromophore suggesting an intecalative mode of binding [24,25]. In order to calculate the binding ability of metal complex to CT-DNA, the intrinsic binding constant ($K_b$) was determined using Wolfe-Shimer equation:

$$[\mathrm{DNA}]/(\varepsilon_a - \varepsilon_f) = [\mathrm{DNA}]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f)$$

and the value of $K_b$ is $(2.5\pm0.2) \times 10^4\ M^{-1}$.

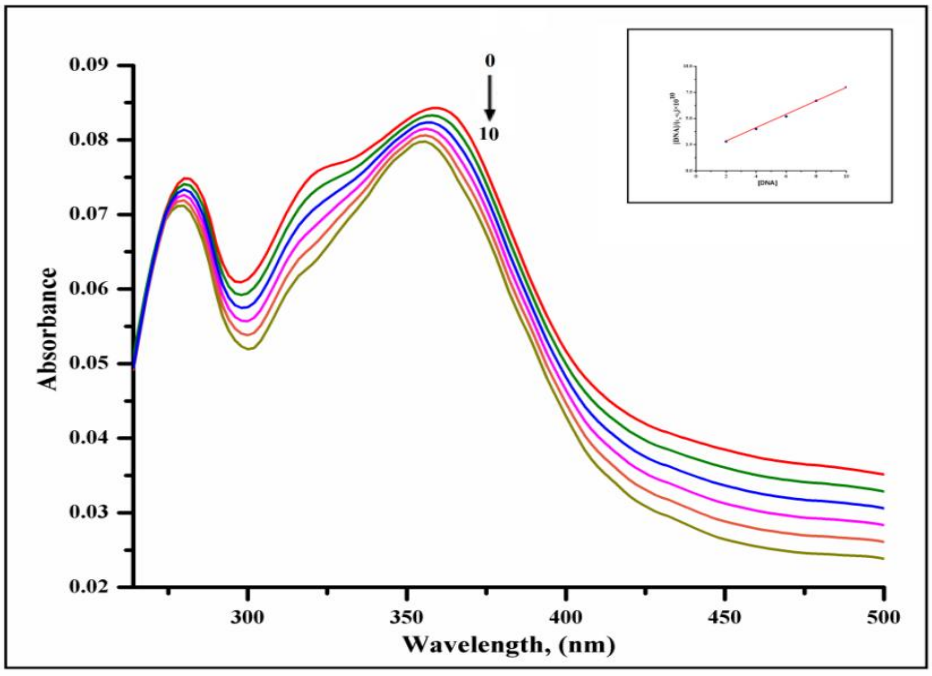



**Fig.4.7.** Absorption spectra of Co(II) complex (red line) in the absence and presence of increasing amount of CT DNA (0–10 µM), Inset: plot for binding constant ($K_b$).

### 4.3.2.2. Ethidium Bromide (EB) competitive study with fluorescence emission spectroscopy:

To affirm the mode of binding of synthesized Co(II) complex to CT-DNA, a competitive binding experiment was performed using Ethidium Bromide displacement strategy. EB-DNA couple shows an intense emission band at 592 nm because of intercalation of the planar phenanthridine ring of EB between adjacent DNA base pairs [26]. A noticeable quenching of the EB-DNA emission band may be seen if a foreign molecule having the ability to intercalate to DNA equally or stronger than EB, is added into EB-DNA solution [27].

The competitive binding experiment was performed in Tris-HCl buffer, keeping [DNA]/ [EB] =1.13 with increasing the concentration of the synthesized complex. The addition of Co(II) complex solution in EB-DNA solution resulted in significant quenching in the emission intensity (shown in Fig.4.8). This lowering in emission intensity of EB-DNA couple upon addition of Co(II) complex suggests displacement of EB which can be delineated as intercalative mode of binding. The quenching constant or quenching strength ($K_{sv}$) of synthesized complex toward EB-DNA conjugate was further calculated using the classical Stern‑Volmer equation [28]:

$I_0/I = 1 + K_{SV}r$, Where I and $I_0$ are the fluorescence intensities in the presence and absence of the quencher and r is the ratio of total concentration of complex to that of DNA. The calculated value of $K_{sv}$ for the synthesized complex is $(1.1\pm0.3)\times10^4$ $M^{-1}$.

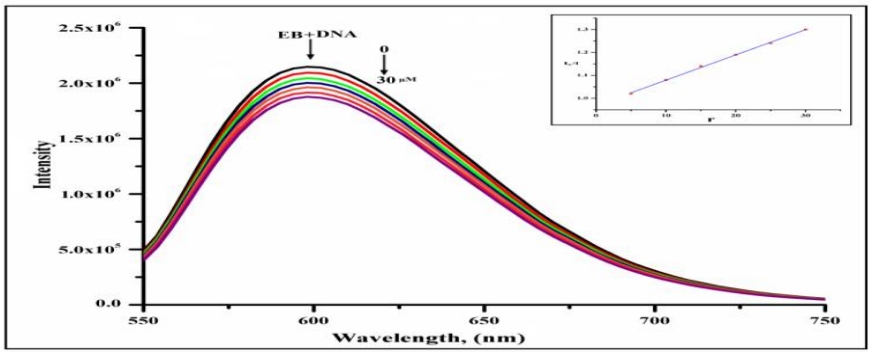



**Fig.4.8.** Emission spectra from EB bound to the DNA in the absence and presence of increasing amount of Co(II) complex (0–30 μM), Inset: plot for quenching constant (Ksv).

### 4.3.2.3. DNA melting study:

DNA melting study is an essential tool to measure the extent of intercalation [29]. Intercalation of foreign molecules into DNA base pairs increases the value of melting temperature ($T_m$). For performing this experiment, solutions of CT-DNA and complex was prepared in 1% DMSO and 99% Tris-HCl buffer solvent mixture. Fig.4.9. presents the melting curve of CT-DNA solution both in absence and in presence of Co(II) complex. The measured value of $T_m$ for CT-DNA solution was $79.0^0$C which increased noticeably ($86.0^0$C) in presence of Co(II) complex. These increase in $T_m$ value supports intercalative binding of Co(II) complex [29].

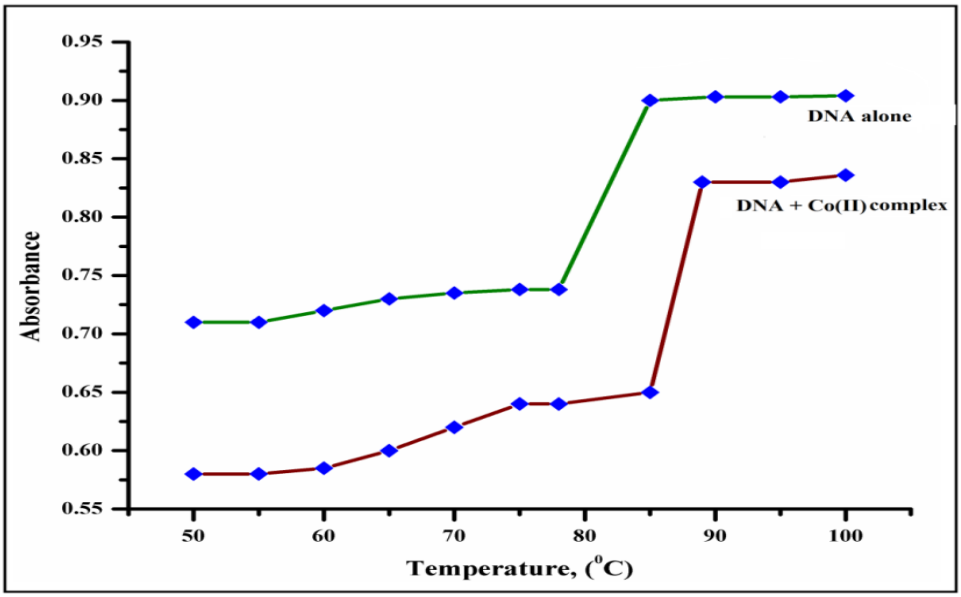



**Fig.4.9.** Plot of absorbance versus temperature ($^0$C) for the melting of 1) CT DNA alone 2) CT DNA + Co(II) complex.

### 4.3.2.4. Viscosity measurement:

Hydrodynamic tactics (like viscosity measurement), which are sensitive to length of DNA, are substantial tool to investigate the binding mode of metal complexes to DNA. An intercalative mode of interaction results in increase in viscosity values because of lengthening of DNA helix. Viscosity measurements were carried out using a capillary type viscometer (Ostwald viscometer) thermostated at 25±1 $^\circ$C keeping the concentration

of CT-DNA (100 μM) constant while varying the concentration of complex (10-100 μM). The relative viscosity of DNA in absence ($\eta_o$) and presence ($\eta$) of Co(II) complex was measured using the relation: $\eta = (t - t^o)/ t^o$, where t is the flow time of CT-DNA containing complex solution and $t^o$ is the flow time of buffer alone in seconds [30]. The obtained results were represented as $(\eta/\eta_o)^{1/3}$ versus 1/ R (R = [DNA]/[Complex]).

Here with increasing the concentration of Co(II) complex, the relative viscosity of DNA increases sharply similar to the nature of well known intercalator EB (depicted in Fig.4.10.) suggesting intercalative binding.

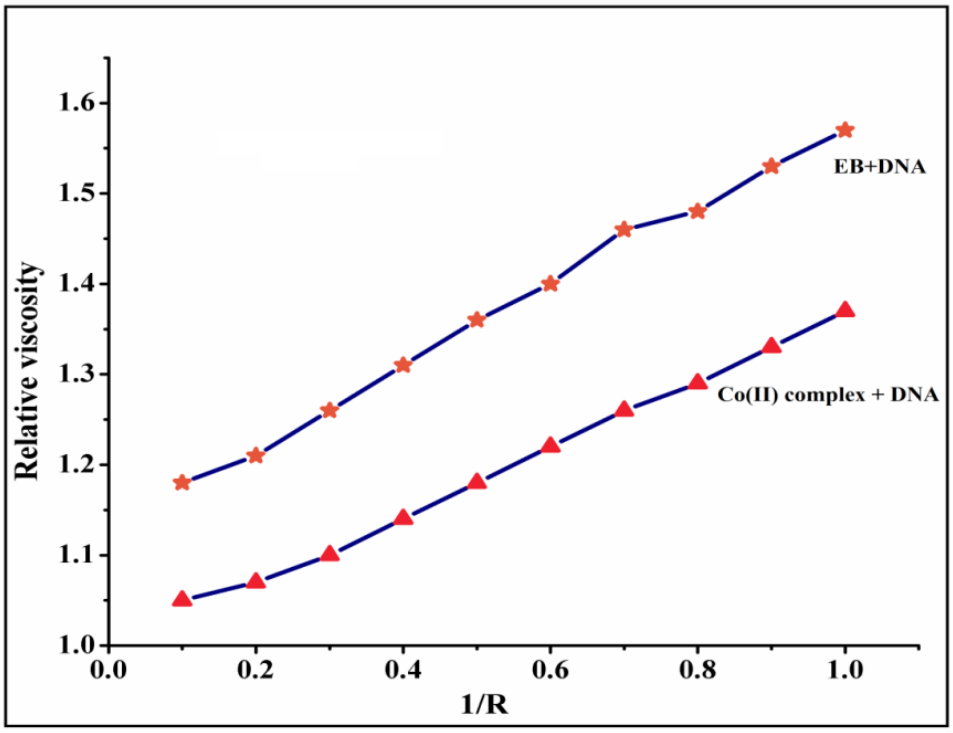



**Fig.4.10.** Effect of increasing amounts of (a) EB (b) Co(II) complex on the relative viscosity of CT-DNA.

### 4.3.3. Molecular Docking study:

The bioactive molecules and drugs have a tendency to bind with proteins and other important macromolecules to modulate their biological functions. The synthesized Co(II) complex is examined for possible bioactivity using Swiss targets prediction web server (http://www.swisstargetprediction.ch). The Swiss prediction report shows the probable

association of synthesized complex with biological network. The repot proposed that among the probable targets, 20% are enzymes, 27% are cytosolic proteins and there is a huge chunk of unclassified proteins of 27%. Among the series of predicted targets, some are very important compounds such as BCl-2 like protein 1, microtubule-associated protein tau, and several receptor proteins which are linked to various cellular activities [34]. The proteins like progesterone receptor and Mcl-1 has showed high binding affinity with the Co(II) complex (Table.4.2; Fig.4.11). The progesterone receptor has the maximum interaction with the complex having binding affinity -9.7 kcal/mol. So, it might have some implications in inhibition or expression of progesterone hormones in biological systems.

**Table.4.2.** Binding affinity of the Co(II) complex with different proteins:

| Protein Name | Gene Name | PDB ID | Binding affinity (kcal/mol) |
|---|---|---|---|
| Progesterone receptor | PGR | 1a28 | -9.7 |
| Dihydroorotate dehydrogenase (quinone), mitochondrial | DHODH | 1d3g | -9.0 |
| Tyrosyl-DNA phosphodiesterase 1 | TDP1 | 1jy1 | -9.4 |
| Bcl-2-like protein 1 | BCL2L1 | 1r2d | -8.6 |
| Induced myeloid leukemia cell differentiation protein Mcl-1 | MCL1 | 2nl9 | -9.4 |
| Microtubule-associated protein tau | MAPT | 2on9 | -6.7 |
| Muscleblind-like protein 1 | MBNL1 | 3d2q | -8.7 |
| Bcl-2-like protein 2 (by homology) | BCL2L2 | 4cim | -9.4 |
| Apoptosis regulator Bcl-2 (by homology) | BCL2 | 4lxd | -9.5 |

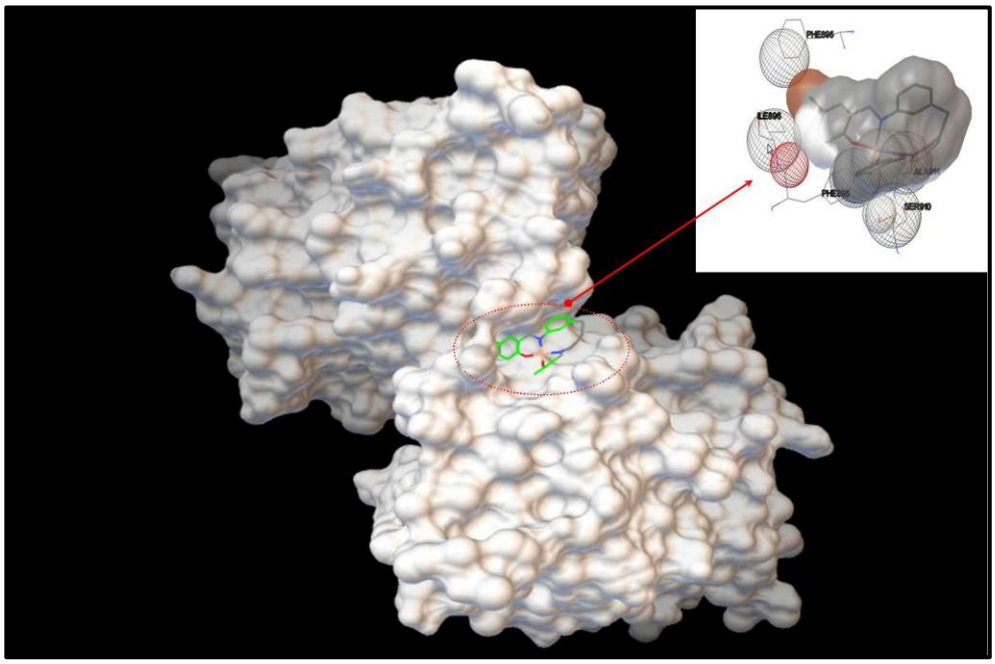


**Fig.4.11.** The molecular interaction of the proteins with Co(II) complex.

### 4.3.4. Antibacterial activity:

The synthesized compounds along with Cobalt acetate salt were examined for antibacterial activity following standard procedure of well diffusion method [35]**.** Two pathogenic gram positive bacteria *Bacillus subtilis* (MTCC-121), *Staphylococcus aureus* (MTCC-3160) and two pathogenic gram negative bacteria *Escherichia coli* (MTCC-1698), *Pseudomonas aeruginosa* (MTCC-1035) were used for assessing the antibacterial activities. The test solution of the compounds was prepared in DMSO at a concentration of 5, 7.5 and 10 mg/ml. The zone of inhibitions is expressed as mean values with standard deviation of mean (SD).

The Synthesized Co(II) complex shows significant antimicrobial activities (zone of inhibition, mm) against the *E. coli* and *P. aeruginosa* (Table.4.3; Fig.4.12) and moderate activity against *B. subtilis* and *S. aureus*. But such zone of inhibition is very low in case of ligand and Cobalt acetate salt. This implies that both gram positive and gram negative bacterial were susceptible to such metal complex and may provide a support to protect against bacterial disorder. So this Co(II) complex might prove beneficial as a novel drug candidate against bacterial infection in future.

**Table 4. 3.** Antibacterial activity of Co(II) complex (zone of inhibition in mm):

| Sample | Concentration (mg/ml) | Diameter of zone of inhibition (mm.) | | | |
|---|---|---|---|---|---|
| | | ***B. subtilis*** | ***S. aureus*** | ***E. coli*** | ***P. aeruginosa*** |
| Co(II) complex | 5 | 11.5±0.7 | 12.5±1.41 | 13±1.41 | 13.5±0.7 |
| | 7.5 | 10.5±0.7 | 10.5±0.7 | 15±1.41 | 18.5±0.7 |
| | 10 | 14±1.41 | 14±1.41 | 16±2.82 | 12.5±0.7 |
| DMSO | | 8.5±0.7 | 8.5±0.7 | 8.5±0.7 | 8.5±0.7 |

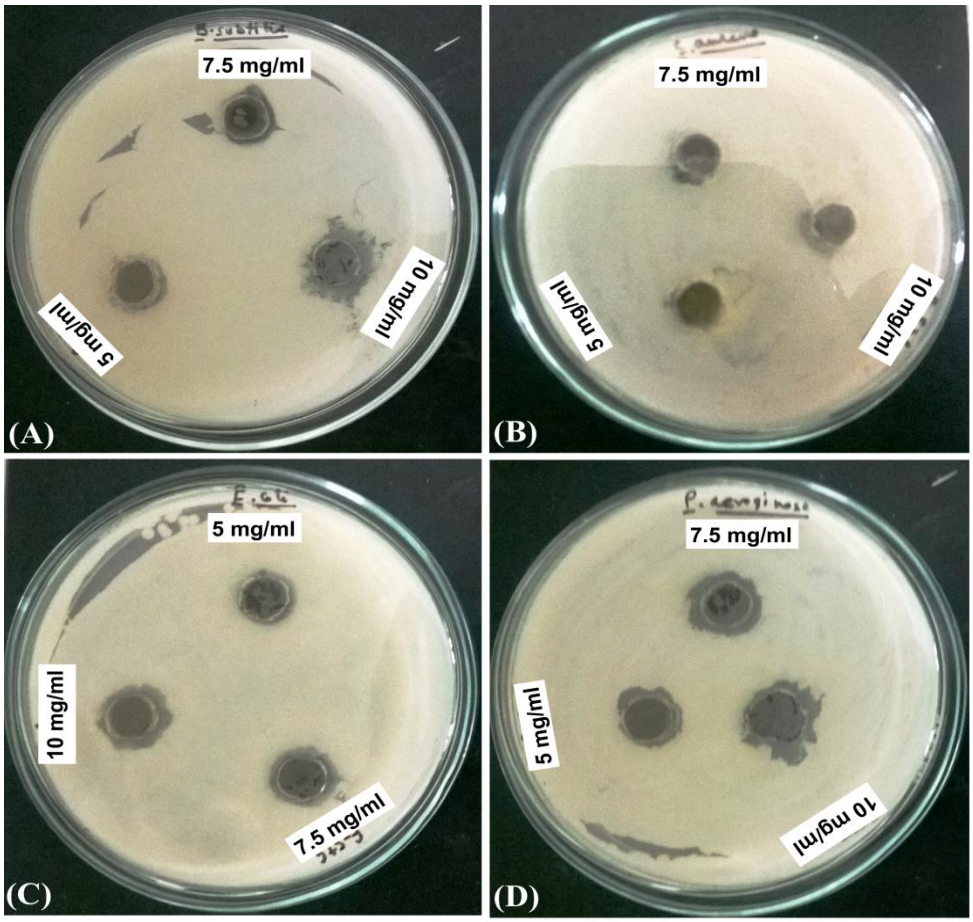



**Fig.4.12.** Anti- bacterial activity of Co(II) complex on (A) *B. subtilis*, (B) *S. aureus*, (C) *E. coli* and (D) *P. aeruginosa*

## 4.4. Conclusions

Here in this chapter, a biologically important ligand and its Co(II) complex have been prepared and characterized. The physico-chemical and spectroscopic results reveal that

the synthesized complex is mononuclear and posses tetrahedral geometry around the Co(II) ion. The results of DNA binding study suggest that the synthesized complex act as an efficient metallointercalators. The result of antibacterial activity showed that both gram positive and gram negative bacterial were susceptible to such metal complex and may provide a support to protect against bacterial disorder. According to molecular docking analysis, proteins like progesterone receptor and Induced myeloid leukemia cell differentiation protein Mcl-1 has high binding affinity with the Co(II) complex. Because of such benevolent biological activities, the synthesized complex may be productive for the design of new metal-based drugs.

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# CHAPTER-V

## SYNTHESIS, CHARACTERIZATION, ANTIOXIDANT ACTIVITY AND DNA CLEAVAGE STUDY OF NOVEL COPPER (II) COMPLEX

## 5.1. Introduction

Design and synthesis of metal–organic hybrid complexes have drawn much attention over the years due to their fascinating architectures and topologies, including their varied potential applications as catalysts, and chemical sensors [1-4]. In modern research, functional hybrids have been inquired enormously for the remedy of several diseases like malaria, HIV, cancer, fungal and bacterial affliction etc [5]. One of the merits of employing this type of metal organic frameworks is because of the carriage of tunable inorganic-organic components. Thereby simply altering the components in these materials, the disparity in the attributes can be attuned for their prospective utilization. Amongst the d-block elements, copper is certainly the most preferred transition metal for the synthesis of novel metal-organic hybrid compounds; as it's both Cu (I) and Cu (II) ions in association with several organic joiners can shape interesting network configurations and packing motifs [6-9].

Oxygen is very essential for our living system but when it is present in excess amount in our body its turn deleterious for immune cells by the formation of reactive oxygen species (ROS). Increase amount of ROS in our body may be the reason of the development of several diseases like cancer, diabetes, obesity, rheumatoid arthritis, cognitive disorders etc as it damages normal biochemical activities in the living organism [10-11]. So to combat this foul species various antioxidants must be developed in the organism.

Therefore, herein this chapter copper (II) metal based hybrid material has been synthesized and their potential effects in alleviating oxidative disorder were studied through various biochemical methods.

**CCDC NO: 1823459**

## 5.2. Experimental Section:

### 5.2.1. Synthesis of [Diaquo {bis (*p*-hydroxybenzoato-$\kappa^1O^1$)} (1-methylimidazole-$\kappa^1N^1$)} copper (II)]:

To a methanolic solution of 4-hydroxy benzoic acid (0.277g, 2mmol) (20 ml), copper nitrate (0.2417g, 1mmol) is included and the resulting blue colored mixture is stirred at room temperature for 30 minutes. To this blue coloured solution, 1-methyl imidazole (0.082g, 1 mmol) is included in a drop wise fashion. As the color of the solution changes from light blue to dark blue, a few drops of triethyl amine was mixed and after that it was refluxed for 4 hrs at 160 °C. The mother liquor was then moved into a 50 ml beaker and permitted to remain at room temperature for a couple of days. After a few days the compound was acquired as a blue crystal. Single crystals suitable for X-ray diffraction were collected by hand picking under a microscope (40X). The synthesized copper complex was soluble in most of the polar solvent except water and it did not melt up to 280 °C. Yield: 0.383 g, 78% (based on Cu). Elemental analysis calcd (%) for [Cu($C_{18}H_{20}N_2O_8$).$2H_2O$]: C 43.94, H 4.92, N 5.69, Cu 19.91; found: C 43.77, H 4.69, N 5.55, Cu 12.96.

## 5.3. Results and Discussion:

### 5.3.1. Characterization of the synthesized compound:

#### 5.3.1.1. X-ray crystallographic Studies:

The synthesized compound crystallizes as orthorhombic system having the space group $P2_12_12_1$ with 4 formula units in the unit cell (Z = 4). Table5.1. presents the crystal data along with structure refinement variables for the synthesized Cu (II) complex.

**Table 5.1.** Crystal data collection and structure refinement for the complex

| **Crystal data** | |
|---|---|
| **Chemical formula** | [Cu($C_{18}$ $H_{20}$ $N_2$ $O_8$).2$H_2O$] |
| $M_r$ | 491.93 |
| **Crystal system, space group** | Orthorhombic, $P2_12_12_1$ |
| **Temperature (K)** | 298.8 (7) |
| **Colour, habit** | Blue, Parallelepiped |
| **Size, mm** | 0.50 × 0.40 × 0.20 |
| ***a* , *b*, *c* (Å)** | 7.0603 (3), 12.1658 (5), 24.2757 (8) |
| ***α*, *β*, *γ* (°)** | 90 |
| ***V* (Å$^3$)** | 2085.14 (14) |
| ***Z*** | 4 |
| **Radiation type** | Mo $K\alpha$ |
| **Density (calculated), Mg/m$^3$** | 1.567 |
| **Absorption coefficient, mm$^{-1}$** | 1.106 |
| **Data collection** | |
| **Diffractometer** | xcalibur, sapphire3 |
| $T_{min}$, $T_{max}$ | 0.608, 0.809 |
| $\theta_{max}$,$\theta_{min}$ | 29.271, 3.453 |
| ***h*** | -9→7 |
| ***k*** | -16 →15 |
| ***l*** | -25 →32 |
| **Reflections collected** | 6703 |
| **Unique reflections** | 4513 |
| **Observed reflections (>2σ(*I*))** | 4110 |
| $R_{int}$ | 0.0363 |
| **Refinement** | |
| **Refinement method** | Full-matrix least-squares on $F^2$ |
| **Data/restraints/parameters** | 451 /12/308 |
| **Final *R* indices [I>2σ(I)]** | $R_1$ = 0.0363, w$R_2$ = 0.0859 |

**CCDC NO: 1823459**

X-ray analysis report revealed that the asymmetric unit contains one Cu (II) ion, one 1- methyl imidazole, two benzoic acid moiety and two coordinated $H_2O$ molecules. In addition two guest $H_2O$ molecules are also present in the asymmetric unit (Fig.5.1.).

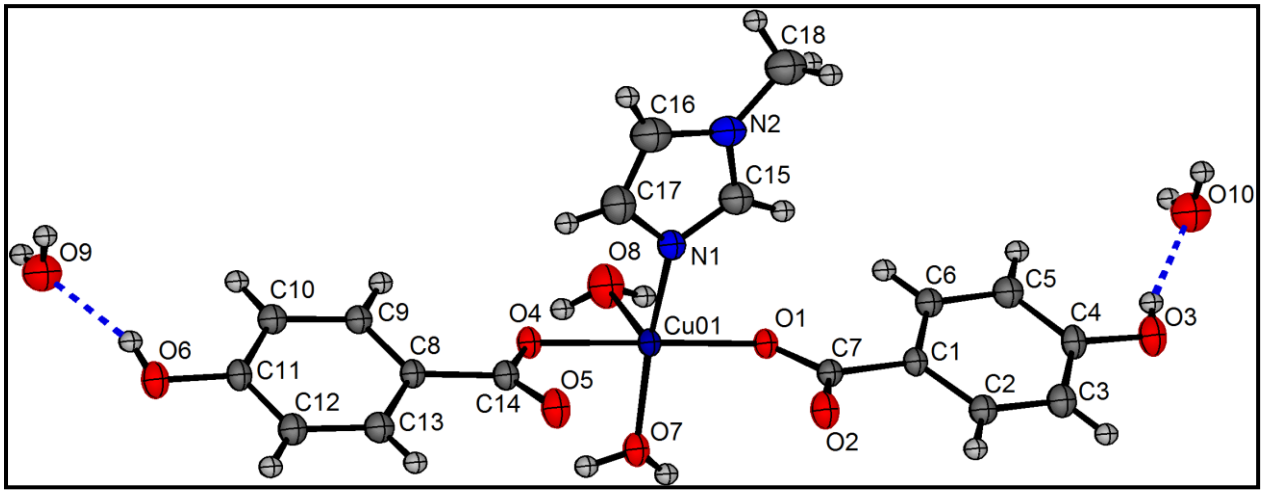


**Fig.5.1.** Coordination geometry of Cu(II) ion in the complex.

The synthesized complex molecule adopts a square pyramidal geometry having the molecular formula $[Cu(C_{18}H_{20}N_2O_8).2H_2O]$. In Fig.5.1. three dimensional layout of the complex molecule has been displayed. Fig.5.2. disclosed the supramolecular framework of the complex formed via both intramolecular and intermolecular H-bonding between coordinated $H_2O$ molecules and the guest $H_2O$ molecules.

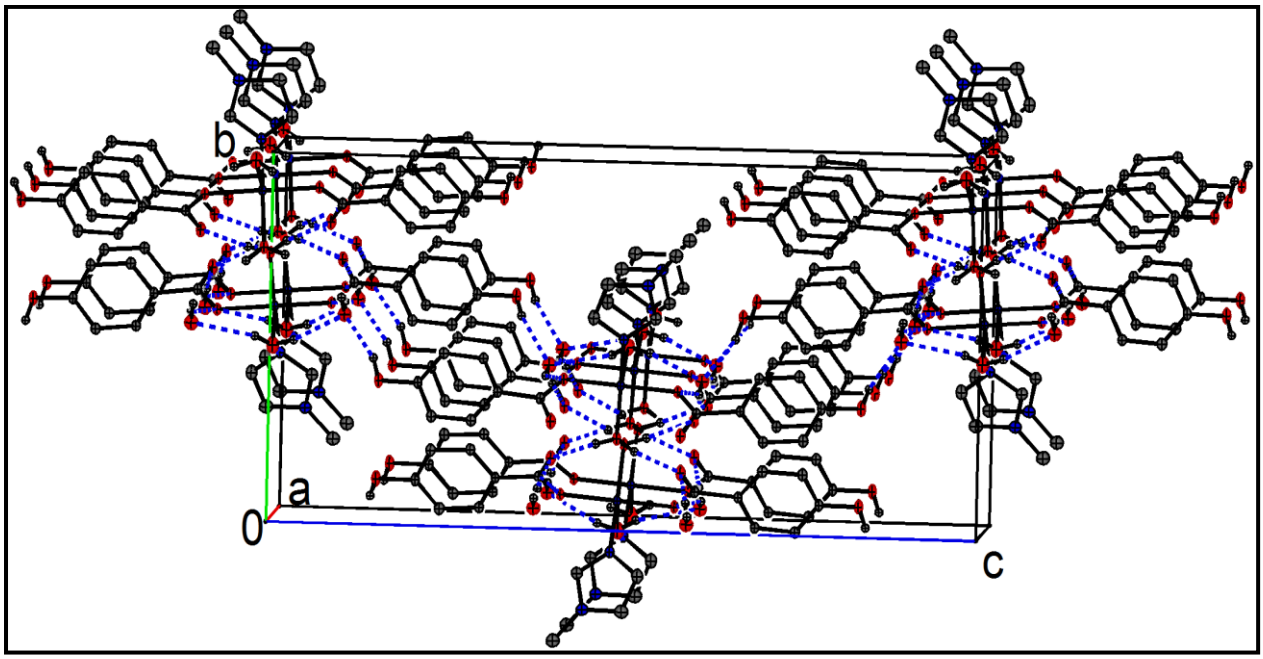


**Fig.5.2.** Crystal packing and hydrogen bonding within the framework.

### 5.3.1.2. FTIR Spectroscopic study:

Infra-red spectra always offer productive details regarding metal ligand bonding. The FTIR spectrum of the synthesized complex has been depicted in Fig.5.3. and it showed two characteristic bands emerging at 1595 $cm^{-1}$ and 1389 $cm^{-1}$ for asymmetric ($\upsilon_{as}$) and symmetric ($\upsilon_s$) stretching of (COO-) group respectively [12-13]. The large separation value of $\Delta\upsilon = \upsilon_{as} - \upsilon_s = 206$ $cm^{-1}$ implies un-symmetrical monodentate behavior of the -COO group. A broad band was noticed in the region 3222-3459 $cm^{-1}$ which is related to the presence of coordinated $H_2O$ molecules in the structure of the complex meanwhile the presence of Cu-N bond was confirmed by the appearance of peak at 632 $cm^{-1}$. The data of IR spectra supports the structure obtained from single crystal XRD.

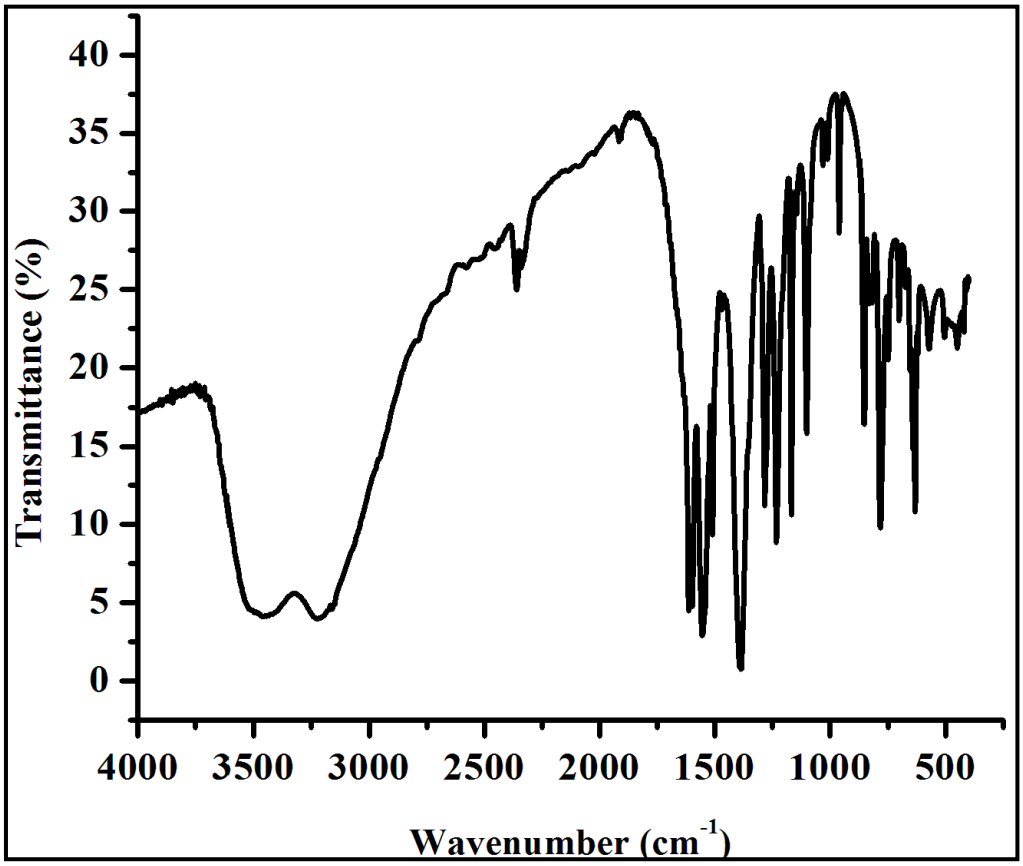


**Fig.5.3.** FTIR spectrum of the complex**.**

### 5.3.1.3. Magnetic moment and EPR analysis:

The magnetic moment, $\mu_{eff}$ of the complex was found to be 1.92 B.M and it is in well acceptance of the presence of an unpaired electron in synthesized Cu (II) compound [14]. The $\mu_{eff}$ value is greatly propped by the X-band EPR spectrum. The recorded EPR spectrum is characteristic of square pyramidal geometry. A perusal of the *g*-values [$g_e < g_\perp < g_\parallel$ (2.002 < 2.15 < 2.215)] suggests that $d_{x^2-y^2}$ orbital contains the unpaired electron having the electronic configuration $(e_g)^4(a_{1g})^2(b_{2g})^2(b_1)^1$.

### 5.3.2. Molecular Docking Analysis:

The molecular Docking investigation revealed a high binding affinity of the compounds with the membrane receptor proteins. Among them Kappa opioid receptor and mu opioid receptors showed highest interaction of -8.2kcal/mol and -8.4kcal/mol (Fig.5.4) both molecular view and secondary protein structure). Such a range of binding affinity predicts spontaneous reaction when present in biological system. The opioid receptors like kappa, delta and mu are all effective in Central Nervous system, especially mu opioid receptor are against to morphine [15]. Thus, the torment assuaging action of the compound can be anticipated.

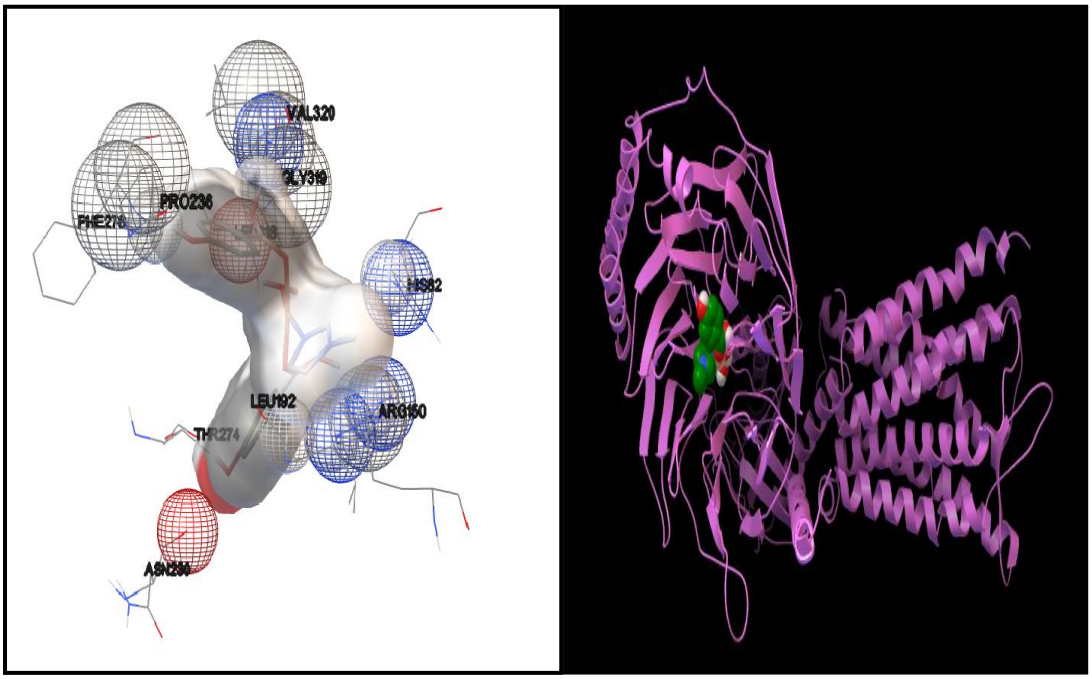


**Fig.5.4.** Molecular Docking interaction

### 5.3.3. DNA cleavage study:

The DNA cleavage reaction was investigated by gel electrophoresis technique. The DNA cleavage activity was determined by monitoring the transformation of supercoiled pBR322 DNA (Form I) to nicked circular form (Form II) and linear form (Form III) [16]. To perform cleavage reaction, solutions of pBR 322 DNA were prepared in Tris-HCl buffer and each reaction mixture contains 20μM DNA, 200μM $H_2O_2$ and 5-15μM synthesized complex. After incubation for 2 hours, 2 μl of loading buffer and 1% agarose gel (containing 1 μg/cm$^3$ EB) was added to each reaction mixture tube. The electrophoresis was performed in Tris-acetic acid-EDTA buffer for 2h at 50V. After the completion of electrophoresis, the gel was captured photographically under UV light.

The electrophoresis study clearly showed that the Cu(II) complex have acted on DNA as there was noticeable difference in bands of the complex with respect to band of the control DNA (Fig.5.5). The synthesized complex is highly efficient to convert supercoiled form of DNA (Form I) into nicked circular form (Form II). The appearance of smears in the gel photograph supporting radical cleavage. As the synthesized metal complex was found to cleave DNA, so it can be inferred that the Cu(II) complex will inhibit the growth of pathogens.

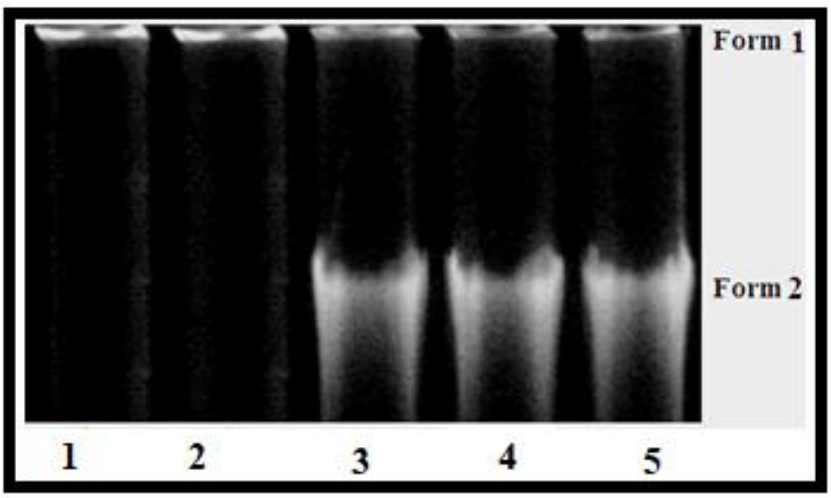


**Fig. 5.5.** Changes in the agarose gel electrophoretic pattern of pBR322 plasmid DNA induced by $H_2O_2$ Cu(II) complex. Lane (1): DNA control, Lane (2): DNA + $H_2O_2$, Lane (3-5): DNA+ Cu(II) complex + $H_2O_2$, [complex] = 5, 10, 15μM respectively

### 5.3.4. *In vitro* antioxidant activity

In the present study, novel Cu (II) complex shows high antioxidant activity compared to respective standard compounds (Fig.5.6.). DPPH activity has been extensively used for screening free radical scavenging activity at its low concentration. Cu (II) complex shows DPPH activity in a dose dependent manner. The highest dose 20 µg/ml concentration shows the highest percent of inhibition (81.63 ± 2.52 µg/ml) has been demonstrated in Fig.5.6. Similar results were found in case of hydroxyl radical and hydrogen peroxide radical scavenging assay. The percent of inhibition in case of hydroxyl radical (62.56 ± 4.34 µg/ml) is much higher than the standard compound Mannitol (31.31 ± 0.85 µg/ml) and hydrogen peroxide (65.81 ± 5.27 µg/ml) also much higher than standard sodium pyruvate (7.64 ± 0.68 µg/ml). Hypochlorous acid, superoxide anion, singlet oxygen, nitric oxide, peroxinitrite and lipid peroxidation shows similar results where percent of inhibition gradually increases in a dose dependent manner and shows better scavenging activities than standard compound each. The better antioxidant activity of any compound has potential and reduces the risk of oxidative damages with least side effects [17]. Therefore, in the present study in practice several antioxidant and free radica scavenging activities were carried out with our sample of interest. Now days, due to the environmental pollutions human beings are exposed to $H_2O_2$, which may enter in our body by normal physiological function. Hydrogen peroxide directly or indirectly converted to hydrogen and water and may produced hydroxyl radical (.OH) that can cause lipid peroxidation and DNA damage. Therefore, scavenging activity of $H_2O_2$ by novel Cu (II) complex proves beneficial for health. On the other hand, peroxinitrite ($OONO^-$), a reactive nitrogen species alleviating chances of carcinogenesis [18]. Therefore, inhibition of peroxinitrite by Cu (II) complex may prevent the chances of carcinoma. Cu (II) complex also prove that it has the ability to scavenge proxynitrate, hydroxyl radical, superoxide, singlet oxygen and other free radicals that cause the damaging consequence in our biological system. Thus, novel Cu (II) complex might prove to be a solution in hindrance of various diseases related to oxidative stress and can be considered as a good remedial approach.

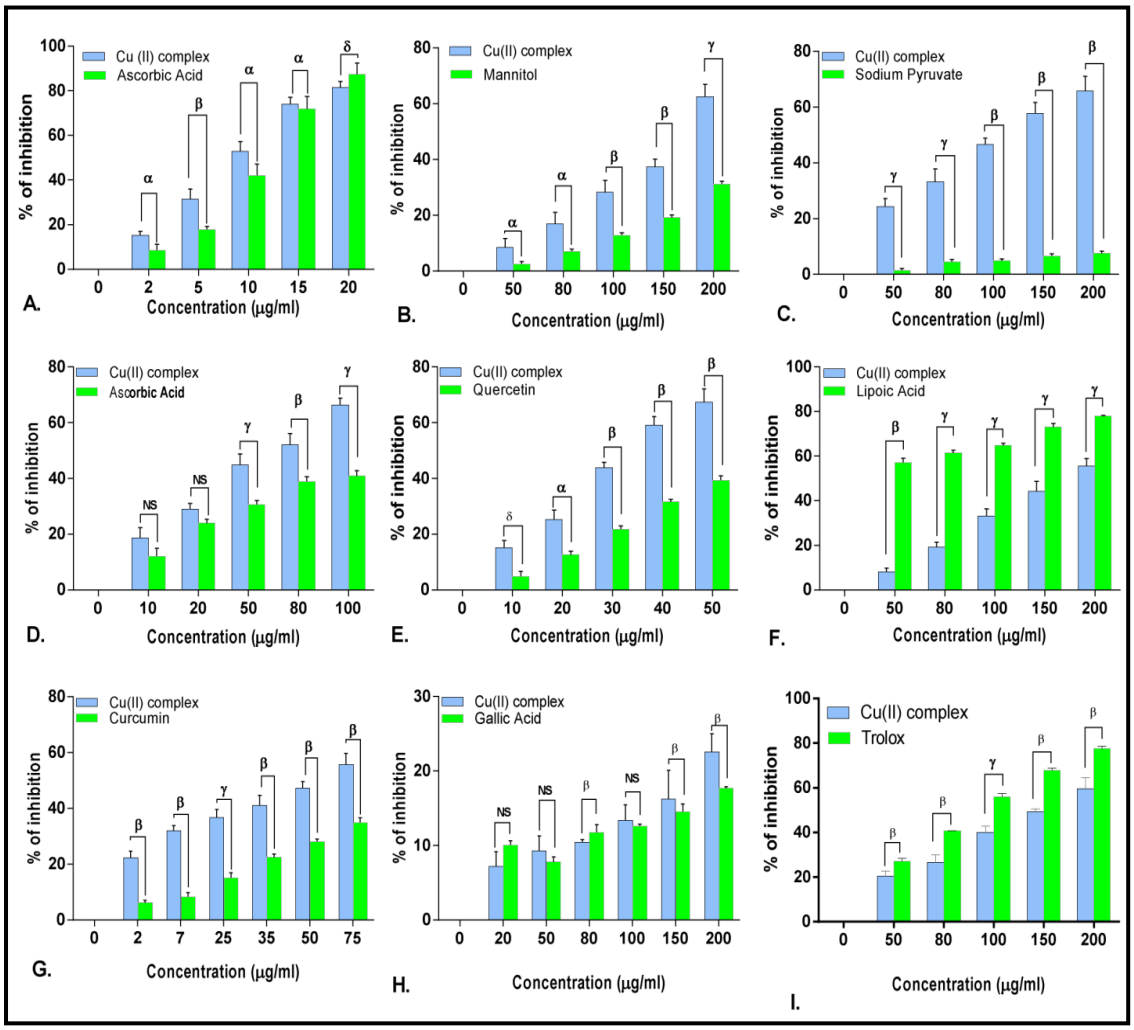


**Fig.5.6.** Antioxidant activity of Cu (II) complex. (A) DPPH activity; (B) Hydroxyl radical activity; (C) Hydrogen peroxide; (D) Hypochlorous acid activity; (E) Superoxide anion; (F) Singletoxygen; (G) Nitric oxide; (H) Peroxinitrite; (I) Lipid peroxidation. Data expressed as mean ±S.D (n = 6). α p< 0.05; β p< 0.01; γ p< 0.001; NS - Non significant when compared with standard.

### 5.4. Conclusion:

The blue colored newly synthesized metal-organic hybrid complex has Parallelopiped shaped orthorhombic crystals with space group $P2_12_12_1$. The three-dimensional structure of the complex was stabilized due to extensive inter- molecular hydrogen bonding interactions. The compound shows a great probability of being an analgesic drug although it can have a long way to go to establish it also as a standard drug. In the present study it is clear that the novel Cu (II) complex aided in the revival of oxidative stress.

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# CHAPTER-VI

# IONIC LIQUID TAGGED AZO-AZOMETHINE BASED Zn(II) COMPLEX: SYNTHESIS, CHARACTERIZATION, BIOLOGICAL ACTIVITY

## 6.1. Introduction

Azo compounds containing conjugated chromophoric azo (-N=N-) group are an important class of organic colorants which are mostly studied during recent years [1-2]. These colorant compounds are widely used as a class of dyes due to their versatile application in various fields such as the dyeing of textile fiber, coloring of different materials, plastics, cosmetics industries, biological-medical studies and advanced application in organic synthesis [3-4]. Other applications of azo dyes includes in emerging technologies like liquid crystals, Organic photoconductors and nonlinear optics [5]. Furthermore, these compounds were found to possess a variety of biological activities such as antibacterial, antifungal, antiviral to anti-inflammatory [6-9]. Interestingly, Azo compounds are involve in a variety of biological reactions, such as inhibition of DNA, RNA and protein synthesis, nitrogen fixation and carcinogenesis [10]. In addition, the "privilege ligand" Schiff bases, which are formed by the condensation of carbonyl compound and primary amine, are important intermediates for the synthesis of some bioactive compounds [11]. In Schiff bases, it has been suggested that the azomehine linkage (-C=N) are responsible for many biological activities like antibacterial, antitumor, antiviral, anti HIV-1, herbicidal etc [12-16]. Also among the various organic chelating ligands, the Schiff bases bearing azomethine linkage is an excellent class of chelating ligands which are able to coordinate various metal ions and stabilize them in various oxidation states and enabling the application of Schiff base metal complexes in variety of catalytic organic transformations [17]. The Schiff base metal complexes are also used in many analytical fields such as colorometric sensing of ions, corrosion inhibitors and metal extractions etc [18]. During past decade, considerable attention has been paid to the synthesis and study of azo-azomethine compounds containing hydroxyl groups for intermolecular proton transfer reaction [19]. The

thermochromic and/ or potochromic behavior of these classes of compounds can be exploited from the electronic structure of such dyes [20]. In our ongoing interest in the stynthesis and DNA binding activity of metal complexes, we report here the synthesis and physico-chemical properties of ionic liquid tagged azo-azomethine ligand by condensation of azo-coupled salicyldehyde with amino functionalized ionic liquid followed by complexation.

## 6.2. Experimental Section:

### 6.2.1. Synthesis of Azo derivative of Salicylaldehyde (1a):

The azo-derivative of salicylaldehyde was prepared by diazotization of salicyldehyde with 4-chloro aniline. In a typical experiment, salicyldehyde (1.22gm, 10 mmol) was dissolved in water (15 ml) containing 0.4 gm (10 mmol) of NaOH and 4.24 gm (40 mmol) of $Na_2CO_3$ during the period of 30 minutes at 0°C in a 50 ml beaker. In another beaker, 4-chloro aniline (10mmol) was dissolved in water by adding concentrated HCl in a requisite amount. The solution was then cooled on an ice bath at 0°C and solid $NaNO_2$ (10 mmol) was added to the solution slowly with constant stirring. The salicylaldehyde solution was added drop wise to the resulting diazonium chloride solution with constant stirring and the temperature was maintained at 0°C during the coupling time. The reaction mixture was stirred for one hour at 0°C and then allowed to warm slowly to room temperature. The product was collected by filtration and washed with 100ml of 10% NaCl solution.

**2-hydroxy 5-(4-chlorophenylazo)benzaldehyde:** yellow solid. Yield 82%, M.P-168-171°C, (IR, KBr $cm^{-1}$); 3300 (O-H), 1668 (CHO), 1590 (N=N), 1399, 940 (N=N, bending); Anal. calcd. for $C_{13}H_9ClN_2O_2$: C, 59.88; H, 3.45; Cl, 13.60; N, 10.74; O, 12.28. Found: C, 59.60; H, 3.24; Cl, 13.10; N, 10.59; O, 12.03%.

### 6.2.2. Synthesis of amino functionalized ionic liquid (1b):

The amino functionalized ionic liquid has been prepared by following the literature procedure given elsewhere [21]. 1-methylimidazole (4.1 gm, 0.05mol) was taken in 20 ml of acetonitrile and 2-bromoethylamine hydrobromide (10.25 gm, 0.05mol) was added to it. The resulting reaction mixture was then heated with stirring at 80°C for 4 h. After completion of the reaction, the solvent was removed by distillation and the oily product was washed with ethanol. The oily residue was dissolved in 40 ml of $CH_3CN/H_2O$ (1:1, v/v) oily and $KPF_6$ (9.20 gm, 0.05mol) was added to the solution. The mixture was left for 24 h at room temperature and NaOH (2 gm, 0.05mol) was added to neutralize the solution. Solvents were then removed by vacuum distillation and the product was washed successively with $CHCl_3$ (20 ml) followed by methanol (2 ml). The salts thus separated were filtered and solvents were evaporated. The yellow oil thus obtained was washed with chloroform and diethyl ether. After drying for several hours under vacuum, the desired product 1-(2-Aminoethyl)-3-methylimidazolium hexafluoro-phosphate ([2-aemim] $[PF_6]$) has been obtained as yellow oil.

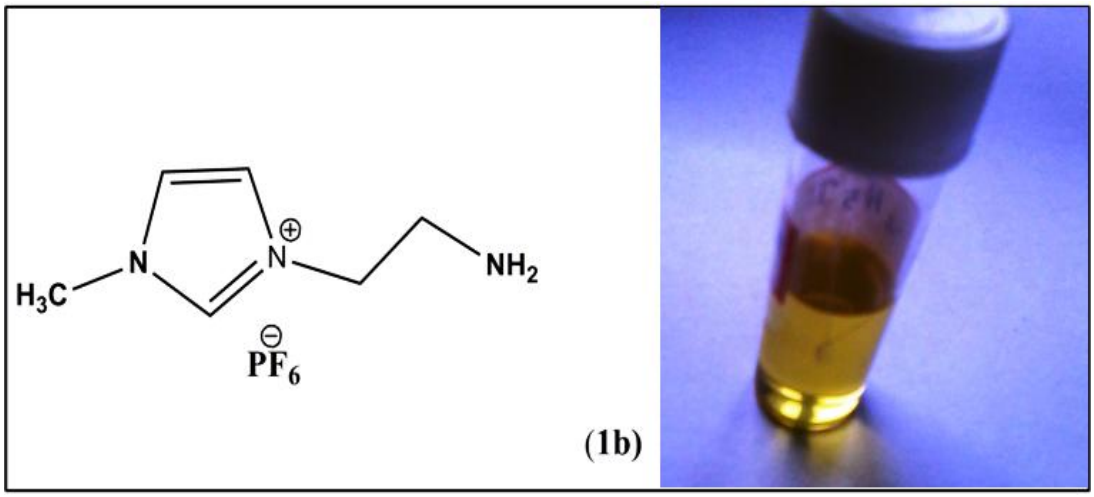



**Fig.6.1.** Structure of [2-aemim]$PF_6$.

**1-(2-Aminoethyl)-3-methylimidazolium hexafluoro-phosphate, ([2aemim] $[PF_6]$) (1b)**: Yellow oil; 9.35 g (yield, 69 %). Anal. Calcd. for $C_6H_{12}F_6N_3P$ (271): C, 26.58; H, 4.46; N, 15.50. Found: C, 26.32; H, 4.42; N, 15.36 %. FT-

IR (KBr, $cm^{-1}$): 3429 (N-H), 3106, 2365, 1617 (C=N), 1175, 846 (P-F). $^{1}H$ NMR (400 MHz, $D_2O$) δ: 3.26 (t, 2H, $NH_2$-C$H_2$); 3.83 (s, 3H, C$H_3$), 4.44 (t, 1H, N-C$H_2$), 7.39 (s, 1H, NC*H*), 7.48 (s, 1H, NC*H*), 8.52 (s, 2H, N$H_2$), 8.79 (s, 1H, N(*H*)CN); $^{13}C$-NMR (400 MHz, $D_2O$) δ: 136.87, 124.53, 122.97, 53.35, 36.01, 27.88. ESI-MS ($CH_3OH$, *m/z*): 126.20 [$(M\text{-}PF_6)^+$]. The FT-IR and ESI-MS spectra are given below.

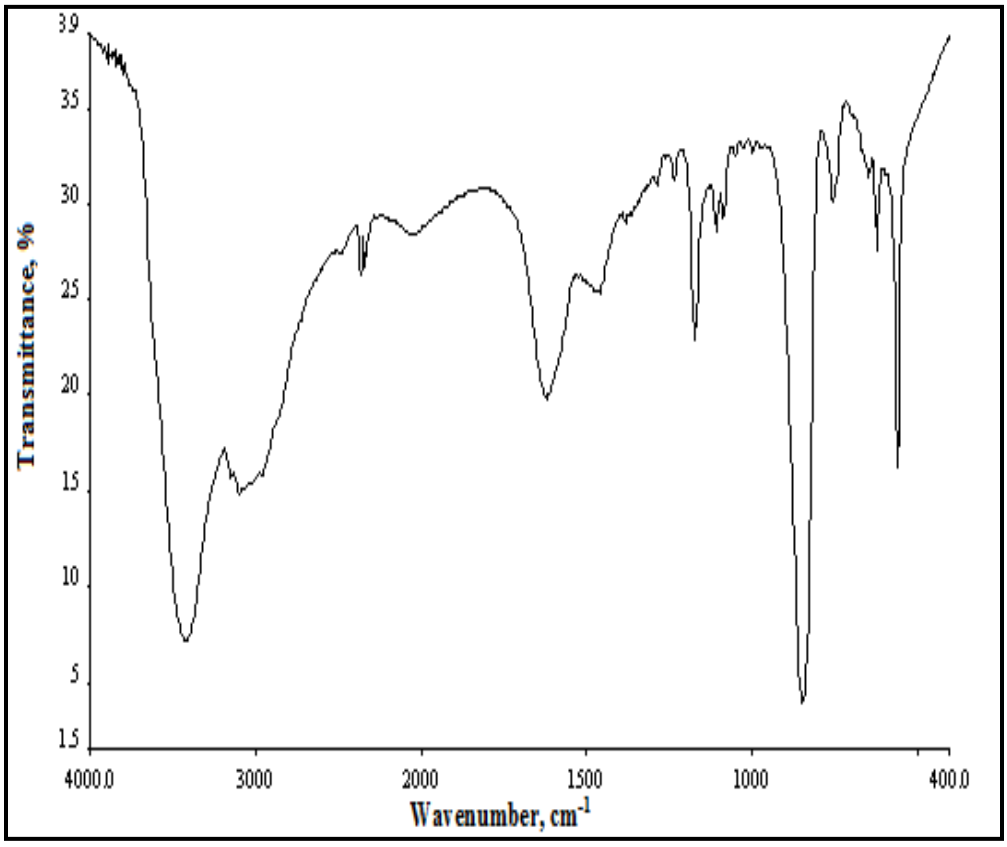



**Fig.6.2.** FT-IR spectrum of [2-aemim]$PF_6$.

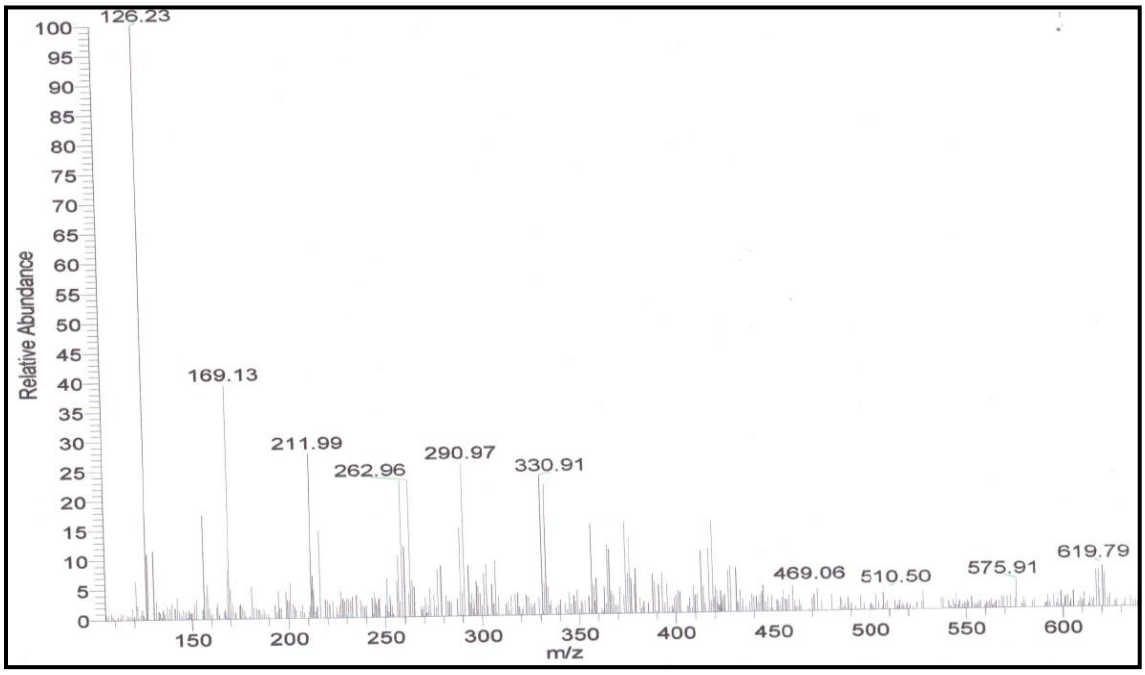
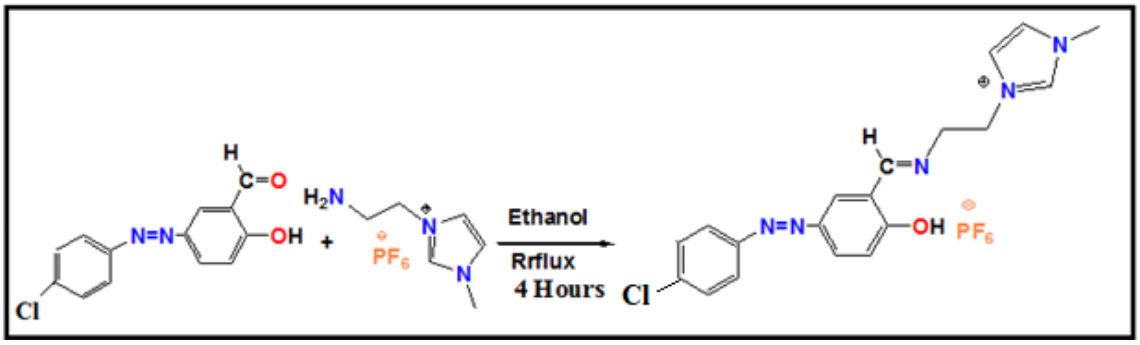



**Fig. 6.3**. ESI-MS spectrum of [2-aemim]$PF_6$.

### 6.2.3. Synthesis of ionic liquid tagged azo-azomethine ligand (L1):

In a typical reaction procedure 1-(2-Aminoethyl)-3-methylimidazolium hexafluoro-phosphate, ([2aemim] [$PF_6$]) (5 mmol) in absolute ethanol was added to stirring solution of azo-coupled salicyldehyde precursor, (5mmol) in 20 ml of absolute ethanol during a period of 10 min [22]. The reaction mixture was then refluxed in an oil bath for 6 h at 90°C with constant stirring with the help of magnetic stirrer. The solution was cooled to room temperature and the solid product thus obtained was filtered. The solid product was washed with little ethanol and diethyl ether respectively. Finally the resulted ionic liquid tagged azo-azomethine derivatives were recyrstallized from hot ethanol solution and dried over silica under vacuum.

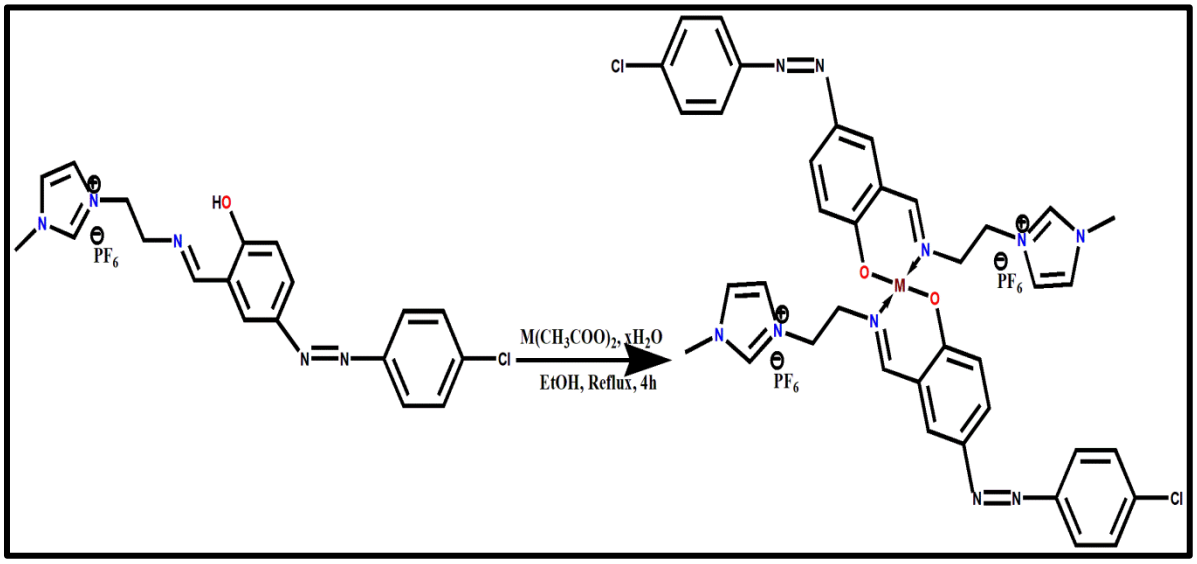



**Scheme.6.1**. Synthesis of ionic liquid tagged azo-azomethine ligand

**1-[2-(2-hydroxy-3-methoxy-5-(4-chlorophenylazo) benzaldeneamino)ethyl]-3-methyl-3H-imidazole-3-ium hexafluorophosphate:** brown solid. Yield: 62%, (IR, KBr $cm^{-1}$); 3200 (O-H), 1645 (C=N), 1614 (C=C), 1453 (N=N), 1168 (N=N, bending); 838 ($PF_6$). $^1$H NMR (300 MHz, $d_6$-DMSO, ppm): δ 3.37 (s, 3H, $NCH_3$), 3.69 (t, 2H, N-$CH_2$ $J_1$=6.00 Hz, $J_2$=6.00 Hz), 4.61 (t, 2H, N-$CH_2$, $J_1$=6.00 Hz, $J_2$=5.10 Hz), 7.62 (s, 1H, NCH), 7.73 (s, 1H, NCH), 9.17 (s, 1H, N(H) CN), 8.56 (s, 1H, HC=N), 13.50 (s, 1H, broad, OH), 7.41-7.89 (m, 6H, Ar-H) ) [62-65]. Anal. calcd. for $C_{20}H_{21}ClF_6N_5O_2P$: C, 44.17; H, 3.89; Cl, 6.52; F, 20.96; N, 12.88; O, 5.88; P, 5.70. Found: C, 44.02; H, 3.67; Cl, 6.32; F, 20.77; N, 12.56; O, 5.78; P, 5.61%.

### 6.2.1.4. Synthesis of Zn(II) metal complex:

Hot ethanolic solution of Zn(II) acetate was added dropwise to the equimolar ethanolic solution of synthesized ligand L1. The resulting mixture was then stirred and refluxed (at 40-50 °C) for 2 h whereupon the Zn(II) metal complex was precipitated out. The dark brown colored solid complex was separated by filtration, washed with ethanol and dried in vacuum desiccator.




**Scheme.6.2**. Synthesis of azo-azomethine ligand based Zn(II) complex.

### 6.3. Results and discussion:

The formation of the investigated azo-azomethine ligand based Zn(II) complex is represented in Scheme.6.2.

### 6.3.1. Characterization of the ligand and its Zn(II) complex:

The newly synthesized ligand and its corresponding Zn(II) complex have been characterized by different analytical and spectroscopic techniques (IR, NMR, UV-Visible *etc.*).

#### 6.3.1.1. IR spectral studies:

In order to ascertain the binding mode in the synthesized azo imidazole ligand and its metal complex, the IR spectra of the compounds were closely analyzed [23-27]. In the solid state IR spectrum of the ligand (L1) a strong band at 3200cm$^{-1}$ can be assigned to stretching frequency of OH group. However this OH band gets disaapered in the spectra of complex suggesting the involvement of OH group in complexation. The appearance of absorption band at 1645cm$^{-1}$ in the spectra of L1can be assigned to the stretching vibration of azomethine linkage ν(-C=N). This band gets shifted to 1629 cm$^{-1}$ in the synthesized complex indicating the participation of the azomethine nitrogen in

complexation The presence of band at 1616 $cm^{-1}$ can be assigned to ν(C=C) stretching frequency. Also, the N=N stretching frequency of the synthesized compound is found at 1453$cm^{-1}$ and the N=N bending vibration is found at 1168 $cm^{-1}$. Band at 838 $cm^{-1}$ can be assigned for stretching vibration of $PF_6$ group. The participation of phenolic O and azomethine N in complexation are confirmed by the development of two non-ligand bands in the spectra of complex at 515 $cm^{-1}$ and 412 $cm^{-1}$ due to ν(Zn-O) and ν(Zn-N), respectively.

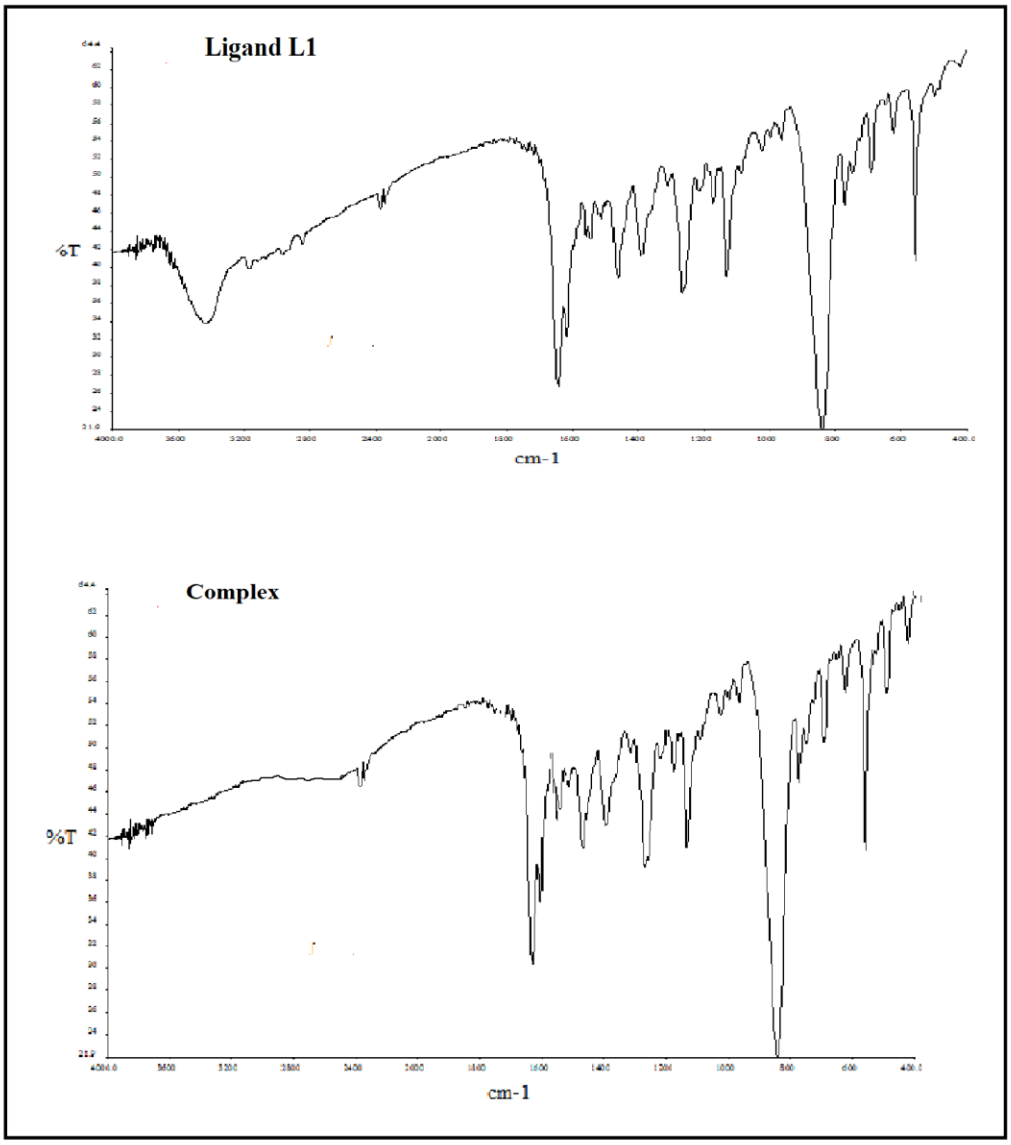



**Fig.6.4.** FTIR spectra of ligand and its Zn(II) complex.

### 6.3.1.2. $^{1}$H NMR spectral studies:

The $^{1}$H-NMR spectra of the synthesized ligand recorded in DMSO-$d_6$ at ambient temperature, displays a group of signals corresponding to the hydrogen. In the $^{1}$H-NMR spectrum of L1, the signal corresponds to OH proton exhibited a slightly broad singlet peak at δ 13.50 ppm and the signal for CH=N proton appeared as a singlet at δ 8.56 ppm. The appearance of the peaks like singlet at δ 7.62 ppm for (NCH) proton, singlet at δ 9.17 ppm for N (H) CN proton is in good agreement with the proposed structure for L1. Signal for $NCH_3$ protons appeared at δ 3.37. The aromatic protons appeared as multiplet in the range δ 7.41-7.89 ppm. The signal at 13.50 ppm (-OH gr.) get disappeared in the complex while the signal at 8.56 ppm (-CH=N) shifted to 8.18ppm [28-29]. Both these observation confirms complexation.

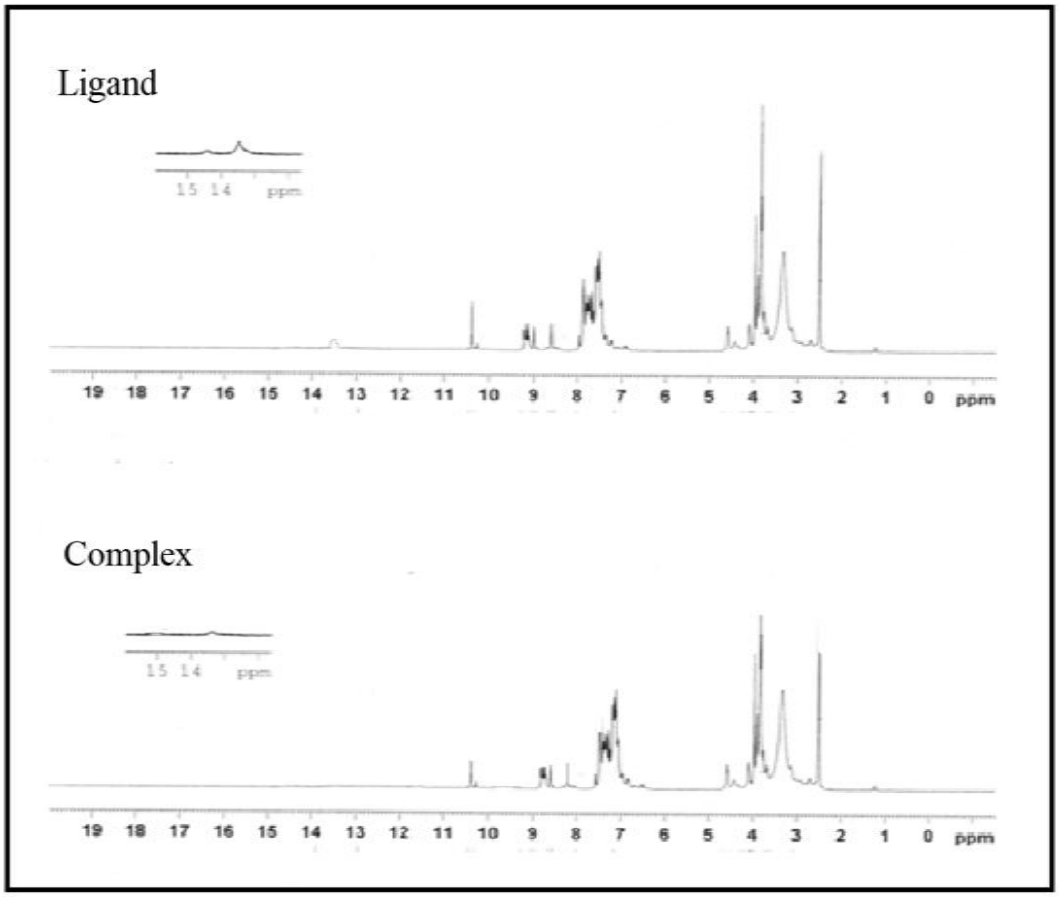



**Fig.6.5.** NMR spectra of Ligand and its Zn(II) complex.

### 6.3.1.3. Electronic absorption spectra:

UV-Visible spectrum of the synthesized azo-azomethine derivative was recorded in Methanol at room temperature. The electronic absorption spectra of the synthesized ligand displayed three peaks appear at 275 nm, 335 nm and 438 nm; these peaks were attributed to $\pi \rightarrow \pi^*$ transition of the aromatic rings, $n \rightarrow \pi^*$ transition of azomethine moiety and $n \rightarrow \pi^*$ transition of azo nitrogen (–N=N–), respectively [30-34]. Upon complexation the peaks for azomethine moiety and azo nitrogen (–N=N–) shifted to 355 nm and 460 nm respectively.

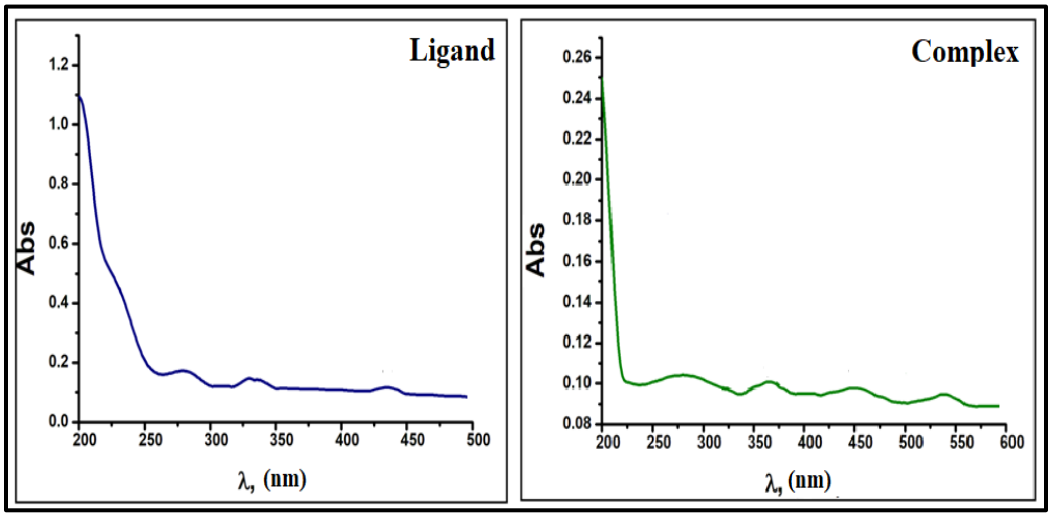



**Fig.6.6.** Absorption spectra of azo-azomethine based ligand and its Zn(II) complex.

### 6.3.2. DNA binding study:

### 6.3.2.1. Electronic absorption titration:

To study the binding mode of the metal complexes with DNA, electronic absorption spectroscopy serves as a most common tool. When a metal complex binds to DNA through intercalative mode (because of strong stacking interaction between an aromatic chromophore and DNA base pairs) results into shift in both absorbance and wavelength. Absorption spectrum of the complex in presence of DNA is shown in Fig.6.7.

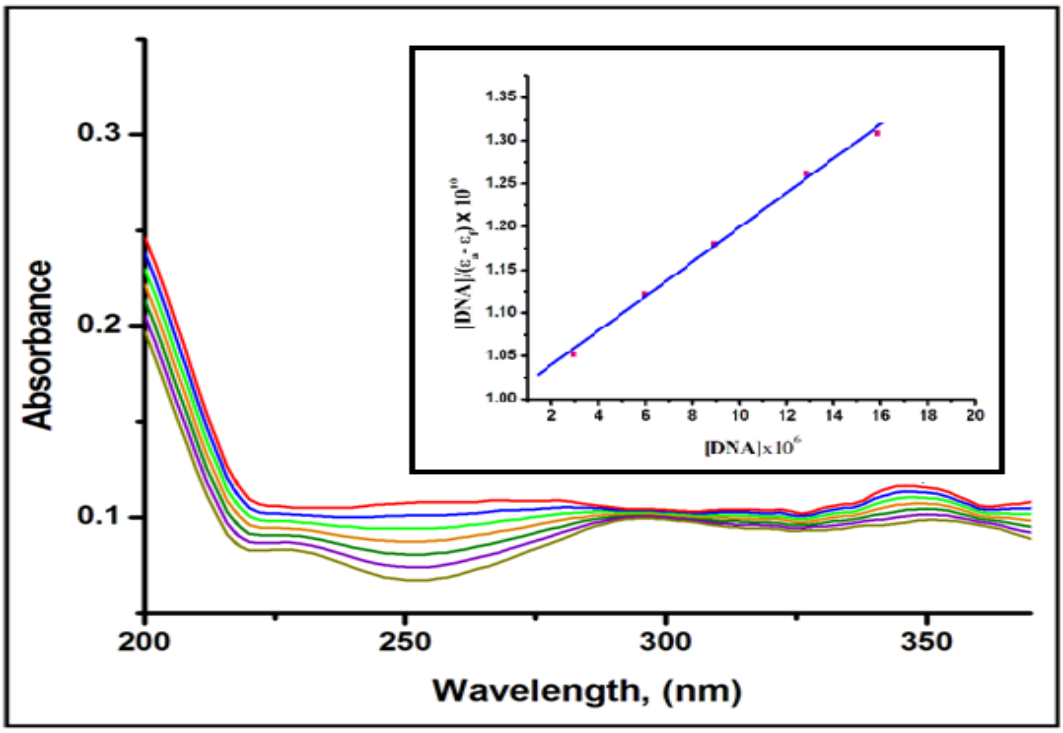



**Fig.6.7.** Absorption spectra of Zn(II) complex (in absence and presence of increasing amount of CT DNA; Inset: plot for binding constant ($K_b$).

On successive addition of DNA in the complex solution chamber, change in both absorbance and wavelength (2.5 nm for azomethine band) were observed suggesting an intecalative mode of binding. In order to calculate the binding ability of metal complex to CT-DNA, the intrinsic binding constant ($K_b$) was determined using Wolfe-Shimer equation [35]:

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f)$$

and the value of $K_b$ is $(1.26\pm0.02) \times 10^4\ M^{-1}$.

### 6.3.2.2. Fluorescence emission spectroscopy:

To affirm the mode of binding of synthesized complex to CT-DNA, a competitive binding experiment was performed using Ethidium Bromide displacement strategy. EB-DNA couple shows an intense emission band at 592 nm because of intercalation of the planar phenanthridine ring of EB between adjacent DNA base pairs. A noticeable

quenching of the EB-DNA emission band may be seen if a foreign molecule having the ability to intercalate to DNA equally or stronger than EB, is added into EB-DNA solution [35].

The addition of complex solution in EB-DNA solution resulted in significant quenching in the emission intensity (shown in Fig.6.8). This lowering in emission intensity of EB-DNA couple upon addition of complex suggests displacement of EB which can be delineated as intercalative mode of binding. The quenching constant or quenching strength ($K_{sv}$) of synthesized complex toward EB-DNA conjugate was further calculated using the classical Stern–Volmer equation and calculated value of $K_{sv}$ for the synthesized complex is $(1.8\pm0.3)\times10^4$ $M^{-1}$.

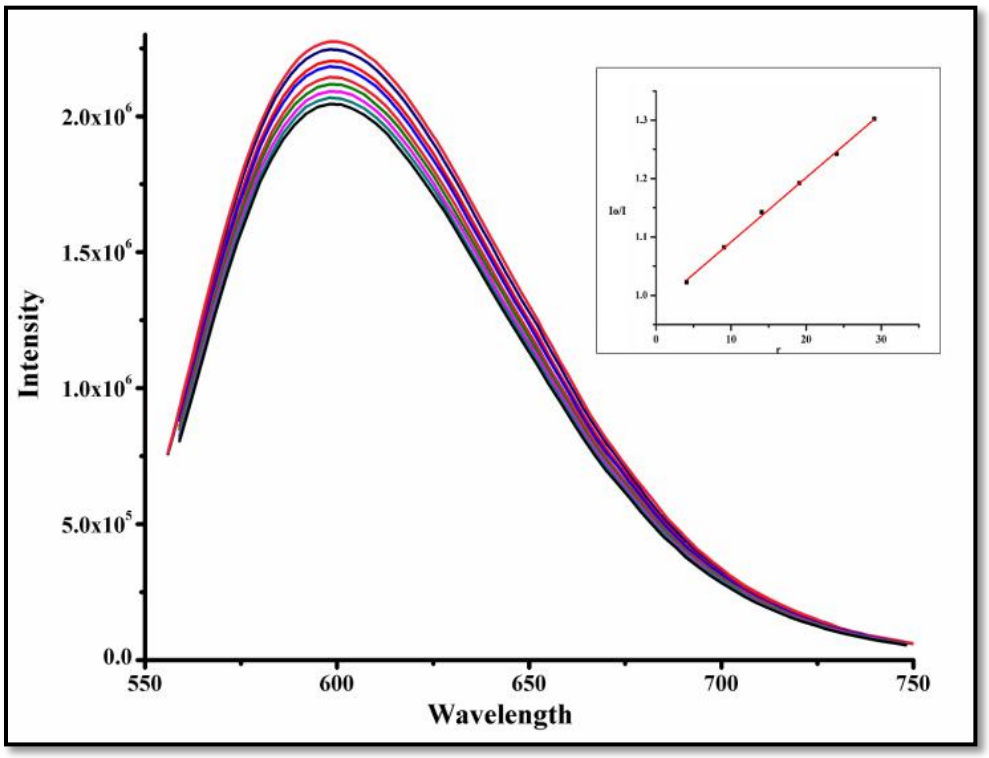



**Fig.6.8.** Emission spectra from EB bound to the DNA in the absence and presence of increasing amount of complex (0–30 μM), Inset: plot for quenching constant (Ksv).

### 6.3.3. Molecular docking study:

Prediction of protein-molecules interaction through molecular docking studies plays an important role to screen the potent molecules having an ability to alter the activity of biological receptor/enzymes. Thus Molecular docking plays an important role for drug design and it aims to achieve an optimize conformation for both protein and drug with relative orientation between them such that the free energy of the overall system is minimized [35]. In this context, we used molecular docking between the synthesized complex with receptor of breast cancer mutant 3hb5-oxidoreductase and diabetic molecular target 11-β-hydrosteroid dehydrogenase type 1 (shown in Fig. 6.9.). The docking study showed a favourable interaction between the metal complex and the receptors 11-β- hydrosteroid dehydrogenase 1 (2BEL) and Receptor breast cancer (3hb5). Based on the interaction (binding affinity) it can be proposed that the synthesized complex could alter the activity of 2BEL involved in glucose metabolism and breast cancer (3hb5) receptors and thus may have a potential to be used as antidiabetic and anticancer agents.

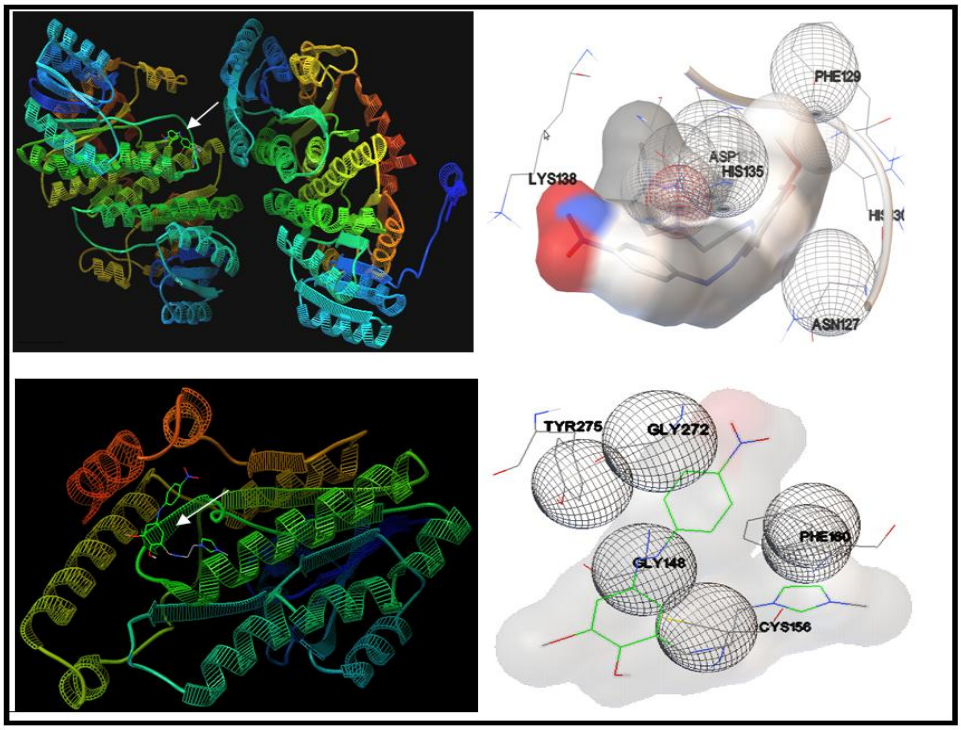



**Fig.6.9.** Molecular docking between the Zn(II) complex and (a) 3hb5, (b)2BEL.

### 7.5.6. Conclusion:

A novel ionic liquid tagged azo-azomethine Zn(II) complex have been prepared by the condensation reaction of azo-coupled salicylaldehyde precursor with amino functionalised ionic liquid and characterized using different analytical and spectroscopic tools (NMR, IR, UV-visible). The synthesized complex was tested for DNA interaction and the results showed that the complexes bind with CT-DNA through intercalative mode. The docking study showed a favourable interaction between the metal complex and the receptors 11-β- hydrosteroid dehydrogenase 1 (2BEL) and Receptor breast cancer (3hb5).

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# CHAPTER VII

## Concluding Remarks

In this present research dissertation synthesis, characterisation and DNA binding ability of some polydentate ligands based transition metal complexes have been described. This research thesis contains seven chapters.

**Chapter I** contains the general overview of polydentate ligands (mainly Schiff base ligand) and their transition metal complexes. It also includes the background and thinking process behind the synthesis and DNA interaction study of transition metal complexes.

**Chapter II** deals with the experimental sections giving the reagents and solvents used to synthesize ligands and their transition metal complexes. The details of instruments used in different spectroscopic and analytical techniques were described briefly in this chapter.

**Chapter III** involves the synthesis of a new Schiff base ligand and its Zn(II) complex. The synthesized Schiff base ligand and its Zn(II) complex have been characterized by different spectral and analytical techniques. Results of the physical measurements exhibit that the Zn(II) metal ion is coordinated by two azomethine nitrogen and two phenolic oxygen atoms, and hence it adopts a tetrahedral geometry. The synthesized complex is stable and non-electrolyte in nature. The ability of the synthesized complex to bind to DNA was monitored by different techniques. The experimental results indicate that the Zn(II) complex interacts remarkably with CT DNA and confirms an intercalative mode of binding to CT DNA. Apart from high binding affinity with DNA at stable energetics, it is also seen that the complex has interaction potential with other biologically important pathways like apoptosis regulation, which hints toward possible involvement with carcinogenesis. In this study, it is also evident that the synthesized complex aided in the recovery of oxidative stress and inhibited lipid peroxidation. On the basis of these facts, antioxidant therapy by synthesized Zn(II) complex alone or in combination with other pharmacological strategies appears as the most reasonable treatment of oxidative stress induced several mental disorders like mental stress, trauma, and anxiety.

**Chapter IV** presents the synthesis and physico-chemical charecterization of a new Co(II) complex. The physico-chemical and spectroscopic results reveal that the synthesized complex is mononuclear and posses tetrahedral geometry around the

Co(II) ion. The results of DNA binding study suggest that the synthesized complex act as an efficient metallointercalators. The result of antibacterial activity showed that both gram positive and gram negative bacterial were susceptible to such metal complex and may provide a support to protect against bacterial disorder. According to molecular docking analysis, proteins like progesterone receptor and Induced myeloid leukemia cell differentiation protein Mcl-1 has high binding affinity with the Co(II) complex. Because of such benevolent biological activities, the synthesized complex may be productive for the design of new metal-based drugs.

In **chapter V** includes the synthesis, characterization of metal-organic hybrid complex of Cu(II). The blue colored complex has Parallelopiped shaped orthorhombic crystals with space group $P2_12_12_1$. The three-dimensional structure of the complex was stabilized due to extensive inter- molecular hydrogen bonding interactions. The compound shows a great probability of being an analgesic drug although it can have a long way to go to establish it as a standard drug. In the present study it is clear that the novel Cu (II) complex aided in the revival of oxidative stress.

**Chapter VI** contains the synthesis and characterisation of ionic liquid tagged azo-azomethine Zn(II) complex. The synthesized complex was tested for DNA interaction and the results showed that the complexes bind with CT-DNA through intercalative mode. The docking study showed a favourable interaction between the metal complex and the receptors 11-β- hydrosteroid dehydrogenase 1 (2BEL) and receptor breast cancer (3hb5).

The results have showed that the synthesized complexes have greater binding affinity toward CT-DNA and their activites can be further modified by tuning the nature of ligand or simply changing the metal ion. This DNA binding strategy should be valuable in finding the powerful agents for probing and targeting nucleic acids. There are two issues that must be addressed precisely in future work:

1) Enacting full control over the selectivity of attack. This will require a more comprehensive analysis of the kinetic and mechanistic profiles of the interaction.

2) Advancement in ligand design to promote recognition and more strong binding to the target nucleic acids. This will yield remarkable advancement in DNA cleavage efficiency also. We have already initiated moleculer modeling analysis which may help in designing new type of ligands and complexes.

# APPENDIX I

## List of Publications

- DNA Binding, amelioration of oxidative stress and molecular docking study of Zn(II) metal complex of a new Schiff base ligand, **Dipu Kumar Mishra** Uttam Kumar Singha, Ananya Das, Somit Dutta, Pallab Kar, Arnab Kumar Chakraborty, Arnab Sen, Biswajit Sinha, *J. Coord. Chem.*, **2018**, *71 (14)*, 1-24.
- DNA Binding and DNA Cleavage activities of newly synthesized Co(II) and Cu(II) complexes of a β-cyclodextrin based Azo functionalized Schiff base, Ananya Das, **Dipu Kumar Mishra**, Pritika Gurung, Vikas Kumar Dakua, Biswajit Sinha, *Aust. J. Chem.*, **2021,** 74(5), 341-350
- Synthesis, characterization and computational study on potential inhibitory action of novel azo imidazole derivatives against COVID-19 main protease (M pro : 6LU7), Abhijit Chhetri, Sailesh Chettri, Pranesh Rai, **Dipu Kumar Mishra**, Biswajit Sinha, Dhiraj Brahman, *Journal of Molecular Structure*, **2021**, 1225(129230), 1-13
- A Pd(II) complex of a β -cyclodextrin-based polydentate ligand: An efficient catalyst for the Suzuki reaction in aqueous media, Ananya Das, **Dipu Kumar Mishra**, Biswajit Sinha, *J. Coord. Chem.*, **2017**, *70*, 3035-3047.
- Solution thermodynamics and taste behaviour of nicotinic acid in aqueous sodium gluconate solutions: A volumetric and rheological perspective, **Dipu Kumar Mishra**, Ananya Das, Amarjit Kamath, Biswajit Sinha, *J. Mol. Liq.*, 2017, *230*, 662-666.

# APPENDIX II

## Seminar, Symposium & Conventions Attended

- **Frontiers in Chemistry 2015,** organized by Department of Chemistry, University of North Bengal, Darjeeling, India on February 17th-18th 2015. (**Presented a Poster**)

- **22nd West Bengal State Science & Technology Congress- 2015,** organized by Department of Science and Technology, Govt. of West Bengal, West Bengal state council of Science and Technology and University of North Bengal, Darjeeling, India on February 28th- March 1st 2015.

- **Science Academies' Lecture Workshop on "Recent Developments on the Theoretical and experimental aspects of advanced materials",** organized by Department of Chemistry, University of North Bengal, September 18th -19th, 2015.

- **Frontiers in Chemistry 2016,** organized by Department of Chemistry, University of North Bengal, Darjeeling, India on March 7th-8th, 2016.

- **19th CRSI National Symposium in Chemistry**, organized by Department of Chemistry, University of North Bengal, Darjeeling, India held from July 14th-16th, 2016. (**Presented a Poster**).

- **Frontiers in Chemistry 2017,** organized by Department of Chemistry, University of North Bengal, Darjeeling, India on February 20th -21st, 2017.

- **National Seminar on "Frontiers in Chemistry 2017-18",** organized by Department of Chemistry, University of North Bengal, Darjeeling, India on September 14th, 2017. (**Presented a Poster**).

- **International Seminar on "Recent Advances in Molecules and Materials",** Jointly organized by Haldia Institute of Technology, Haldia and The Indian Chemical Society from 2nd-3rd August. (Awarded with from 2nd-3rd August. **(Got Young Scientist Award for Best Oral Presentation).**

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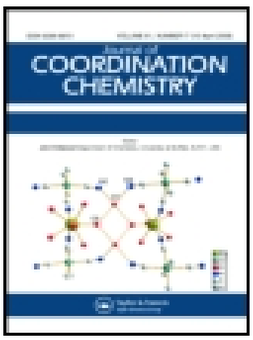

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# DNA Binding, amelioration of oxidative stress, and molecular docking study of Zn(II) metal complex of a new Schiff base ligand

Dipu Kumar Mishra[a], Uttam Kumar Singha[a], Ananya Das[a], Somit Dutta[b], Pallab Kar[c], Arnab Chakraborty[c], Arnab Sen[c] and Biswajit Sinha[a]

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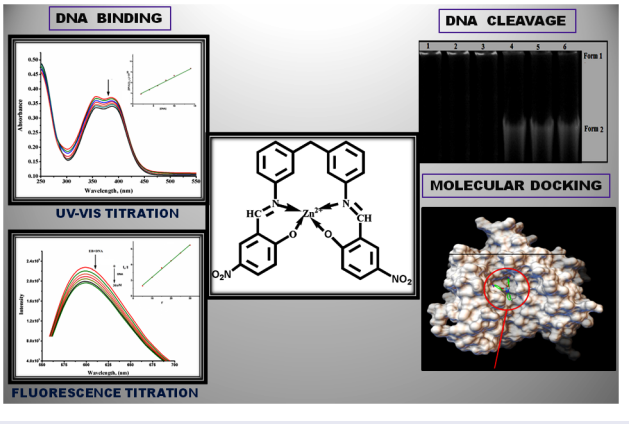
**ABSTRACT**

A new Schiff base ligand, $H_2L$, and its Zn(II) complex were prepared and characterized by different analytical and spectroscopic techniques. The elemental analysis results suggest the stoichiometry of the complex to be 1:1. The molar conductance study shows the non-electrolytic nature of the complex. Infrared spectra reveal that the metal ion is coordinated in tetradentate fashion which was further confirmed by NMR study. The synthesized complex was found to interact with CT-DNA quite efficiently. The DNA binding study of the complex was explored by UV–vis and viscosity measurement. Fluorescence titration studies and the experimental results suggest that the complex might bind to DNA via an intercalative mode. The *in silico* target prediction and molecular docking experiments confirm that, apart from high interaction potentiality with nucleotides, the complex has possible implications in carcinogenesis, too.

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## 1. Introduction

Molecules that interact with DNA have been studied extensively with the aim of designing new types of chemotherapy agents and probes for nucleic acid structures [1, 2]. The study of the interaction of transition metal complexes with DNA has been the main focus of recent investigations. Cisplatin (*cis*-diamminedichloridoplatinum(II)) is the first clinically approved anticancer drug; its various analogs are found to be capable to bind to DNA, halting its replication and induce apoptosis [3]. However, these complexes have so many disadvantages, poor solubility [4], dose-limiting side-effects like nausea, nephrotoxicity, and neurotoxicity [5], and acquired resistance in many cancer types [6]. These basic problems associated with chemotherapy necessitate the evolvement of new anticancer approaches.

Development of new molecules that can obstruct cancerous cells by interacting with DNA in a non-classical mechanism is an example of a new anticancer approach. Transition metal complexes are ideal for these tasks, as their unique characteristics allow specific interactions with DNA and other important biomolecules [7]. Transition metals that are utilized extensively in these fields include platinum [8], ruthenium [9], zinc [10], titanium [11], rhodium [12], copper [13], palladium [14], gold [15], vanadium [16], and iron [17]. Schiff base metal complexes have also gained recognition for their utilities in these biological processes [18]. In recent years, many studies associated with Schiff base metal complexes show promising anticancer activity and are of great interest in both chemistry and biology [19].

Zinc, which is known as "essential trace element," plays an important part in many biological processes. It is essential for the functionality of more than 300 enzymes and vital for the stabilization of DNA and gene expression [20]. It is involved in immune responses, in homeostasis, in apoptosis, and in aging. Zn-binding proteins and enzymes are protective in stress and infections [21]. To explore the chemistry of Zn(II) complexes and DNA interaction, here we have synthesized and characterized a new Zn(II) complex and studied its interaction with calf-thymus DNA (CT DNA) by different physical and spectroscopic techniques.

## 2. Experimental

### 2.1. Materials

3,3′-Methylenedianiline, 2-hydroxy-5-nitro-benzaldehyde, CT DNA, and ethidium bromide (EB) were purchased from Sigma Aldrich. Zn(II) acetate and solvents (included absolute ethyl alcohol, diethylether, and dimethylsulfoxide.) were purchased from Sd Fine Chemicals, India. Solvents were purified prior to their use following standard literature procedures.

### 2.2. Physical measurements

Elemental analyses (C, H, and N) of the ligand and its Zn(II) complex were conducted using a Perkin-Elmer 240 elemental analyzer. NMR spectra were recorded on a Bruker 400 MHz FT-NMR instrument using DMSO-$d_6$ as solvent and tetramethylsilane [$Si(CH_3)_4$] as internal standard. The UV–vis spectra of the ligand and its metal complex were recorded in DMSO using a Jasco V-530 UV–vis spectrophotometer (path-length: 1 cm) while infrared spectra (in the range 400–4000 $cm^{-1}$) were recorded on a Perkin-Elmer FT-IR spectrometer (RX-1) using KBr pellets. Fluorescence emission experiments were carried out Jasco V-530 spectrophotometer at room temperature. Thermal denaturation study of DNA was performed on a

Jasco V-530 double beam UV–vis spectrophotometer. Viscosity studies were carried out using an Ostwald viscometer maintained at constant temperature. Specific conductance of the synthesized complex was measured in DMSO (at room temperature) using a Systronics (India) conductivity meter (TDS-308). The purity of the ligand and its Zn(II) complex was confirmed by TLC. The melting point of the synthesized compounds was determined using open capillary technique.

### 2.3. Synthesis of Schiff base ligand

A hot ethanolic solution of 3,3′-methylenedianiline (0.1 mol) was refluxed with 2-hydroxy-5-nitro-benzaldehyde (0.2 mol) in a round-bottom flask for 2 h with constant stirring. The resulting yellow solid product was separated by filtration, washed with ethanol followed by diethyl ether, and dried in a vacuum desiccator over anhydrous calcium chloride.

### 2.4. Synthesis of metal complex

Hot ethanolic solution of Zn(II) acetate was added dropwise to the equimolar ethanolic solution of synthesized ligand. The resulting mixture was then stirred and refluxed (at 40–50 °C) for 2 h whereupon the Zn(II) metal complex was precipitated out. The dark yellow solid complex was separated by filtration, washed with ethanol, and dried in a vacuum desiccator.

### 2.5. DNA Interaction study

#### 2.5.1. Absorption study

The DNA binding experiment of the synthesized Zn(II) complex was carried out in Tris-HCl buffer (50 mM, pH 7.2). CT DNA solution gave a ratio of UV absorbance at 260 and 280 nm of about >1.86, informing that DNA was free from protein contamination [16]. The concentration of CT DNA was measured by following the UV absorbance at 260 nm ($\varepsilon_{260}$ = 6600 $M^{-1}$ $cm^{-1}$). Absorption titration experiment was performed with fixed concentration of synthesized complex (10 µM), while the concentration of CT DNA was varied. Because of low solubility of Zn(II) complex in Tris-HCl buffer, the complex was first dissolved in DMSO to prepare a stock solution ($1 \times 10^{-4}$ M) and then diluted by buffer to get the desired concentration. During the study of absorption, equal increment of CT DNA was added at each step to both the complex solution chamber and the reference chamber to eliminate the absorption of free CT-DNA, and the resulting spectra were believed to result from the Zn(II) complex and the CT-DNA-metal complex aggregates. Using the absorption data, the intrinsic binding constant ($K_b$) was measured by plotting [DNA]/($\varepsilon_a - \varepsilon_f$) versus [DNA] using the Wolfe–Shimer equation [19]:

$$[\mathrm{DNA}]/(\varepsilon_a - \varepsilon_f) = [\mathrm{DNA}]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f)$$

where $\varepsilon_a$, $\varepsilon_b$, and $\varepsilon_f$ correspond to apparent extinction coefficient of the Zn(II) complex $A_{obs}$/[complex], extinction coefficient for the complex in fully bound form, and in free form, respectively. The intrinsic binding constant ($K_b$) was obtained from the ratio of slope to intercept.

### 2.5.2. Ethidium bromide (EB) competitive study with fluorescence emission spectroscopy

To examine the ability of the Zn(II) complex to displace EB from DNA-EB conjugate, a competitive study was performed by fluorescence emission spectroscopy. The competitive binding experiment was carried out in Tris-HCl buffer by keeping [DNA]/[EB] = 1.13, while the concentration of the complex was increased gradually [22]. During the titration experiment, samples were excited at a wavelength of 540 nm (*i.e.* $\lambda_{ex}$ = 540 nm), while the emission range was between 550 and 700 nm. The obtained spectra were resolved using the classical Stern–Volmer equation [22], $I_0/I = 1 + K_{SV}r$, where I and $I_0$ are the fluorescence intensities in the presence and absence of the quencher (here Zn(II) complex), respectively, while $K_{sv}$ is quenching constant and r is quencher's concentration.

### 2.5.3. Thermal denaturation study

DNA thermal denaturation study was performed on a Jasco UV–Vis V-530 model spectrophotometer equipped with a temperature controlling programmer. The temperature of the experimental solutions was raised by 1 °C $min^{-1}$. The absorbance for the solutions of CT-DNA (100 µM) both in the absence and presence of Zn(II) complex (10 µM) was monitored continuously at 265 nm [22].

### 2.5.4. Viscosity measurements

Viscosity studies were carried out using an Ostwald capillary-type viscometer thermostated at 25 ± 1 °C. During the measurement, concentration of CT DNA was kept constant (100 µM), while concentration of ligand/complex solution was gradually increased (10–100 µM). The flow times of the solutions were noted with a digital stopwatch with an accuracy of ±0.20 s. Viscosity values were calculated using the relation [23]: $\eta = (t - t^o)/t^o$, where t is the flow time of CT-DNA containing ligand or complex solution and $t^o$ is the flow time of buffer alone in seconds. The obtained data were presented as $(\eta/\eta_o)^{1/3}$ *versus* $1/R$ ($R$ = [DNA]/[Complex]), where $\eta_o$ and $\eta$ is the viscosity of DNA in the absence and in the presence of the ligand or complex, respectively.

### 2.5.5. DNA cleavage study

DNA cleavage study of synthesized ligand and its Schiff base metal complex was guided by agarose gel electrophoresis on supercoiled pBR322 plasmid DNA [24]. The tests were done under aerobic condition using $H_2O_2$ as oxidant. In each reaction mixture, concentration of DNA (20 µM) and $H_2O_2$ (200 µM) was kept constant, while concentration of synthesized complex (5–15 µM) was varied. The mixture was incubated at 37 °C for 2 h. After incubation, 2 µL of loading buffer (having pH 7.0) containing 25% bromophenol blue, 30% glycerol, and 0.25% xylene cyanol was added to each tube and finally each sample solution was loaded on 1% (w/v) agarose gel containing 1 µg/mL EB. The electrophoresis was carried out in a dark chamber for 2 h at 50 V in Tris-acetic acid-EDTA buffer having pH 8.3. After electrophoresis, the gel was photographed under UV light (280 nm).

## 2.6. Molecular modeling analysis

### 2.6.1. Swiss target prediction

The bioactive small molecules, drugs, and metabolites have a tendency to bind with proteins and other macromolecule targets to modulate their activities, which has visible expression in organisms. The Schiff base Zn(II) complex is checked for possible bioactivity with Swiss target prediction web server (http://www.swisstargetprediction.ch).

### 2.6.2. Molecular docking

The target proteins identified by Swiss target prediction were chosen as receptor protein. The X-ray crystallographic structures of these receptor proteins were obtained from RCSB PDB (https://www.rcsb.org). They were used for molecular docking analysis against the synthesized chemical. Molecular docking was performed using Auto Dock Vina. The receptor structures were defined as rigid and the grid dimensions varied from being 80, 80, and 80 for *X*, *Y,* and *Z* dimensions for proteins having PDB ID's 1D3G, 1JY1, 1R2D, 2NL9, and 4LXD while 100, 100, and 100 for 3D2Q and 4CIM, whereas for the protein with PDB ID 2ON9, it is 60, 60, and 60, respectively. The exhaustiveness of the simulation was set to 8. Every protein of interest was made ready by removing the water molecules and by adding polar hydrogen to them. Gasteiger charges were added to the proteins on the basis of electronegativity equilibration and all non-polar hydrogens were merged. This was performed because Auto Dock uses the united atom model to represent molecules. Gasteiger charges were also calculated for the respective complex and all the torsions were allowed to rotate.

## 2.7. Antioxidant activity

### 2.7.1. Determination of *in vitro* antioxidant activity

Antioxidant properties of the Zn(II) complex were evaluated by several *in vitro* biochemical assays. Total antioxidant, DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging, hydroxyl radical scavenging, superoxide radical scavenging, nitric acid radical scavenging, singlet oxygen scavenging, reducing power, $Fe^{2+}$ chelation, peroxynitrite scavenging, and hypochlorous acid scavenging activities were determined by following previously reported methods with minor modification for the evaluation of free radical scavenging properties of Zn(II) complex [25, 26].

### 2.7.2. Determination of erythrocyte membrane stabilizing activity

Erythrocyte membrane stabilizing activity experiment was performed by following the standard method, described by Dey *et al.* [27]. Briefly, different concentrations of the Zn(II) complex (0–200 µg/mL) were added to the mixture of 50 mM phosphate buffer (0.5 mL; pH 7.2), distilled water (1 mL), 10% RBC suspension (0.25 mL PBS), 12 mM EDTA (100 µL), NBT (150 µL of 1% solution), and riboflavin (100 µL). The solutions were then kept under bright light for 30 s and incubated for 30 min at 50 °C, followed by centrifugation at 1000 rpm for 10 min. The absorbance of the supernatant was measured at 562 nm and compared with the standard compound quercetin.

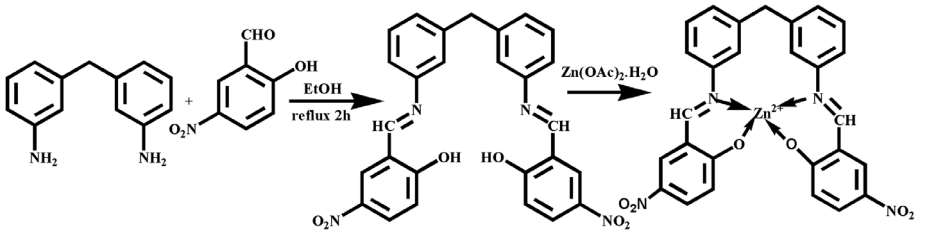


**Figure 1.** Synthesis of Schiff base ligand and its Zn(II) complex.

**Table 1.** Analytical and physical data of Schiff base ligand and its Zn(II) complex.

| Compound | m.p. (°C) | Color (% yield) | Mol. wt. (gm) | % Found (calcd) C | H | N | $\Lambda_m$ $\Omega^{-1}$ $mol^{-1}$ $cm^2$ |
|---|---|---|---|---|---|---|---|
| $H_2L$ ($C_{27}H_{20}N_4O_6$) | 140 | Yellow (84) | 496 | 65.71 (65.32) | 4.16 (4.03) | 11.13 (11.29) | – |
| [Zn(L)] ($C_{27}H_{18}N_4O_6Zn$) | >250 | Dark yellow (78) | 559 | 58.06 (57.96) | 3.41 (3.22) | 9.94 (10.02) | 14.11 |

## 3. Results and discussion

The formation of the investigated Schiff base ligand and its metal complex is represented in Figure 1.

### 3.1. Characterization of the ligand and its Zn(II) complex

The synthesized ligand and its corresponding Zn(II) complex have been characterized by different analytical and spectroscopic techniques (IR, $^1H$ and $^{13}C$ NMR, UV–vis, molar conductance, *etc.*). The analytical data of the synthesized ligand and its Zn(II) complex along with physical properties is listed in Table 1. The ligand and the metal complex are moisture insensitive, air stable, and soluble in DMSO and DMF. The analytical data are in good agreement with calculated values and are consistent with formation of mononuclear Zn(II) complex having metal to ligand ratio 1:1.

#### 3.1.1. Molar conductance

The synthesized Zn(II) complex was dissolved in DMSO solvent and molar conductance of $10^{-3}$ M solution was measured at room temperature (Table 1). The molar conductance value indicates that the metal complex is a non-electrolyte [28].

#### 3.1.2. Infrared spectra

The coordination of the ligand to the metal ion has been studied by comparing the IR data of the ligand with its Zn(II) complex. The IR spectra of synthesized Schiff base and its corresponding Zn(II) complex are shown in Figure S1. There are some appreciable shifts in the stretching frequencies of Zn(II) complex compared to free ligand. The Schiff base shows a characteristic strong band at 1618 $cm^{-1}$ due to the azomethine ν(C=N) [28]. This band shifted to 1599 $cm^{-1}$ in the synthesized complex, indicating participation of the azomethine nitrogen in complexation [28]. A broad band at 3447 $cm^{-1}$ due to phenolic -OH group of ligand

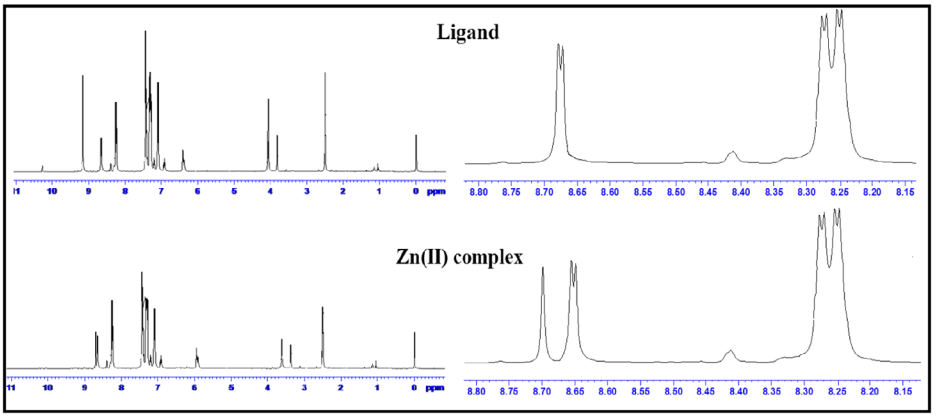


**Figure 2(a).** $^{1}$H NMR spectrum of Schiff base ligand and its Zn(II) complex.

disappeared in the corresponding Zn(II) complex, suggesting the involvement of oxygen atom of -OH group in coordination to Zn(II) ion [28]. The coordination of O atom of phenolic -OH group to metal ion is further proved by the shift in ν(C-O) band of ligand at 1099 cm$^{-1}$ to higher frequency at 1105 cm$^{-1}$ in Zn(II) complex [29]. The participation of phenolic O and azomethine N in complexation are confirmed by the development of two non-ligand bands in the spectra of complex at 535 cm$^{-1}$ and 466 cm$^{-1}$ due to ν(Zn–O) and ν(Zn–N), respectively [28]. Therefore, the above arguments together with the elemental analyses indicated that Schiff base ligand behaves as a dibasic tetradentate ligand coordinated to the metal ion via the azomethine N and deprotonated phenolic O and the complex possibly have a tetrahedral geometry because of d$^{10}$ configuration of Zn(II).

### *3.1.3. NMR Spectra*

The $^{1}$H NMR spectra of the Schiff base ligand and its Zn(II) complex are shown in Figure 2(a). The synthesized Schiff base showed a singlet at 9.17 ppm because of azomethine proton (–CH=N–) [24]. However, in the case of Zn(II) complex, this –CH=N– peak is shifted to 8.70 ppm, suggesting the involvement of azomethine nitrogen in coordination with Zn(II). Moreover, the ligand showed a signal at 10.29 ppm due to hydroxyl (–OH) proton which disappears in the synthesized complex, revealing the deprotonation of –OH group during complexation [30].

The $^{13}$C NMR spectra of both ligand and complex (Figure 2(b)) show peaks at 116.19–153.12 ppm for aromatic carbons and at 42.09 for $-CH_2$. The ligand signals at 167.71 ppm and 169.01 ppm for –CH=N and –C–OH group are shifted upfield to 160.03 ppm and 162.12 ppm for the Zn(II) complex, respectively. There are no appreciable changes in other peaks. So the changes in peak position for –CH=N and –C–OH carbons in the synthesized complex with respect to ligand confirms the complexation.

### *3.1.4. Electronic spectra*

The UV–vis absorption spectra of the Schiff base and its Zn(II) complex were recorded in DMSO ($1 \times 10^{-5}$ M) at room temperature and are depicted in Figure 3. The electronic absorption spectrum of Schiff base contains two bands at 380 and 430 nm. The band at higher

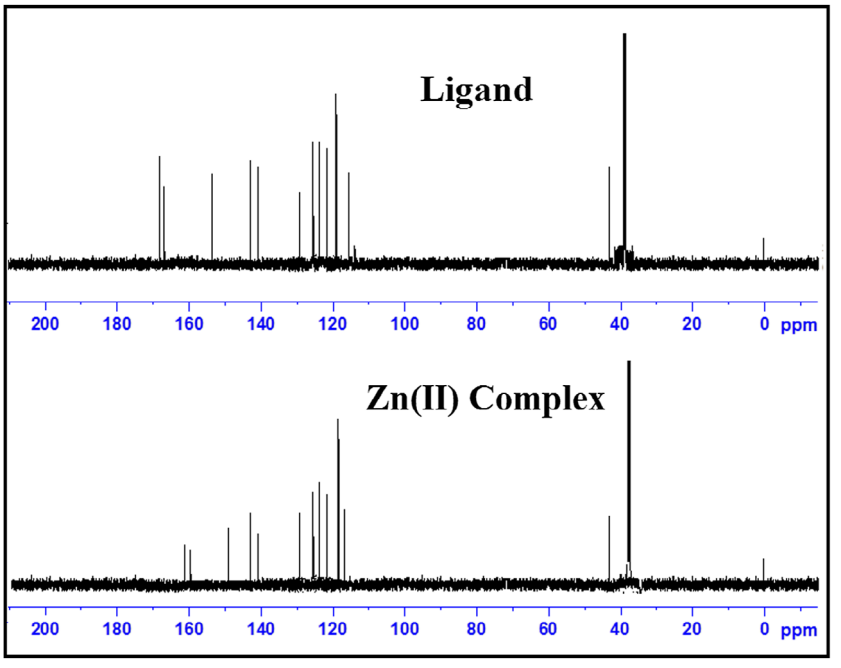


**Figure 2(b).** $^{13}C$ NMR spectrum of Schiff base ligand and its Zn(II) complex.

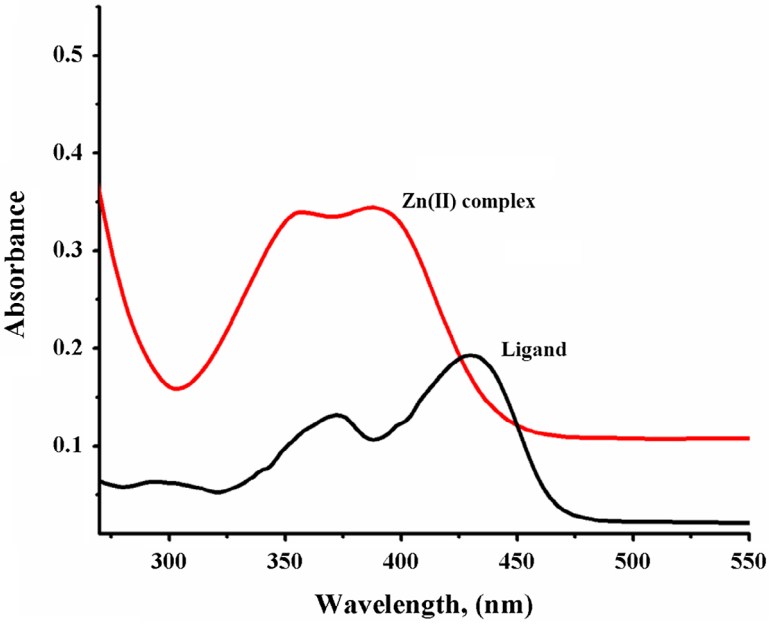


**Figure 3.** UV–vis absorption spectra of Schiff base ligand and its Zn(II) complex in DMSO.

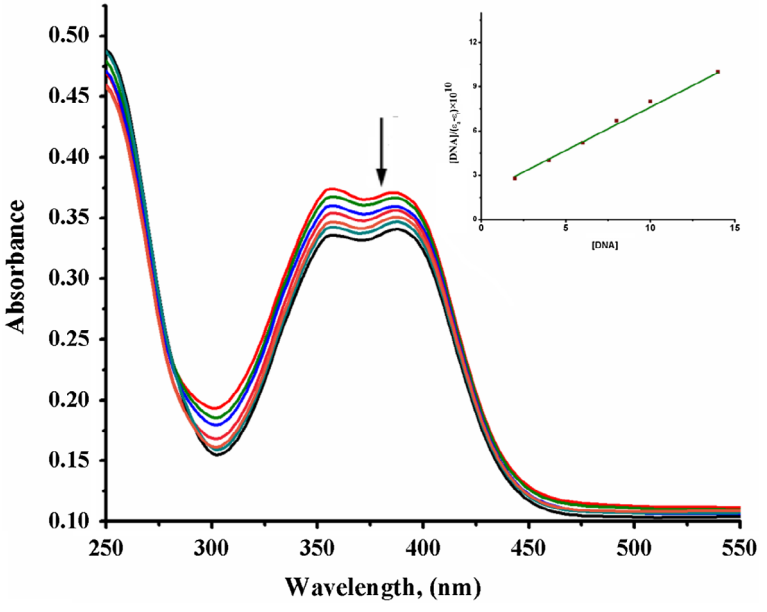


**Figure 4.** Absorption spectra of Zn(II) complex (orange line) in the absence and presence of increasing amount of CT DNA (0–15 μM). Inset: plot for binding constant ($K_b$).

energy (380 nm) is attributed to π-π* transitions of phenyl ring, while the lower energy band at 430 nm is assigned to n-π* transition of azomethine (–CH=N–) group [30]. These absorption bands also exist in the spectrum of Zn(II) complex but undergo blue shift. This shift in the spectra of the synthesized complex supports the coordination of the ligand to Zn(II) ion. The Zn(II) complex because of its $d^{10}$ configuration does not show any d–d transition and hence it's diamagnetic and possibly having tetrahedral geometry.

## 3.2. DNA Binding study

### 3.2.1. Absorption study

Electronic absorption spectroscopy serves as an effective tool to study the binding mode of DNA with metal complexes. The binding behavior of DNA to metal complex is often investigated by the changes in the wavelength and in the absorbance. A metal complex binds to DNA via intercalation usually results in bathochromism (red-shift in wavelength) and hypochromism shift because of strong stacking interaction of DNA base pairs with aromatic chromophore [22]. Here on increasing the amount of DNA in the complex solution resulted in red-shift (1.8 nm for *n*-π* band, Figure 4) and noticeable hypochromicity too, indicating intercalative mode of binding. The intrinsic binding constant ($K_b$) was evaluated using Wolfe–Shimer equation and the value of $K_b$ was 2.30(±0.06) × $10^5$ $M^{-1}$. However, this value is slightly lower than standard EB (1.4 × $10^6$ $M^{-1}$) but mostly higher than other reported Zn(II) complexes [31–33].

### 3.2.2. EB-competitive study with fluorescence emission spectroscopy

EB-DNA conjugate exhibits an intense emission band at 592 nm because of intercalation of the planar phenanthridine ring of EB between adjacent DNA base pairs [35]. A significant quenching of the EB-DNA emission band may be observed if a foreign molecule (which can intercalate to DNA equally or stronger than EB) is added into EB-DNA solution [35]. The synthesized Zn(II) complex does not show any emission band in solution or in the presence of CT DNA or EB when excited at 540 nm. So the observed changes in the emission spectra of EB-DNA solution, when Zn(II) complex is added, are vital to examine the EB-displacement ability of the synthesized complex. The addition of Zn(II) complex in EB-DNA solution causes a significant quenching of the intense emission band at 592 nm shown in Figure 5. The calculated quenching constant ($K_{sv}$) value for the synthesized complex is 3.0(±0.02) × $10^5$ $M^{-1}$, which is mostly higher than other reported Zn(II) complexes [31, 34]. Therefore, the observed quenching suggests remarkable ability of Zn(II) complex to displace intercalator EB from EB-DNA conjugate and divulging indirectly the intercalative mode of binding to DNA [22].

### 3.2.3. Thermal denaturation study

Thermal denaturation study provides information regarding the extent of intercalation [36]. The intercalation of complex into DNA base pairs generally increases the melting temperature ($T_m$) of DNA [22]. Figure 6 shows thermal denaturation profile of CT DNA solution both in the absence and presence of Zn(II) complex. The melting temperature ($T_m$) for CT DNA solution was 79.0 °C. But in the presence of Zn(II) complex, $T_m$ value increases dramatically to 85.0 °C. The increase in melting temperature of CT DNA in the presence of complex ($\Delta T_m$ = 6.0 °C) indicates intercalative binding of Zn(II) complex into DNA [36].

### 3.2.4. Viscosity measurement

Viscosity measurement is a substantial tool to study the binding mode of compounds to DNA. The interaction of DNA with metal complexes via intercalation generally results in an increase of the separating distance of the DNA base pairs present at the intercalation site in order to host the binding compound. As a result, the length of the DNA helix increases, leading to an increase in DNA viscosity [37], while the binding to DNA via non-classic intercalation (like external groove-binding or electrostatic interaction) results in a kink or bend in the DNA-helix. This kink or bend in DNA helix causes slight shortening of the effective length of DNA helix; in such cases, the changes in DNA viscosity is less prominent or there is no change at all [37]. EB is a well-known DNA intercalator that increases the relative viscosity of DNA by lengthening the DNA helix. Upon increasing the concentration of Zn(II) complex in the CT DNA solution, the relative viscosity of DNA solution increases steadily almost similar to the nature as in the case of EB (shown in Figure 7). This increase in viscosity in the case of complex solution suggests that the synthesized Zn(II) complex could bind to DNA via intercalation mode, which is consistent with the UV–vis and fluorescence spectral data.

### 3.2.5. DNA Cleavage study

The DNA cleavage study was guided by gel electrophoresis method with different concentrations of Zn(II) complex in the presence of $H_2O_2$. The ability of the complex to cleave DNA is determined by transformation of DNA from supercoiled form (Form 1) to nicked form (Form 2) and linear form (Form 3). For Form I1, the fastest migration is observed. If only one

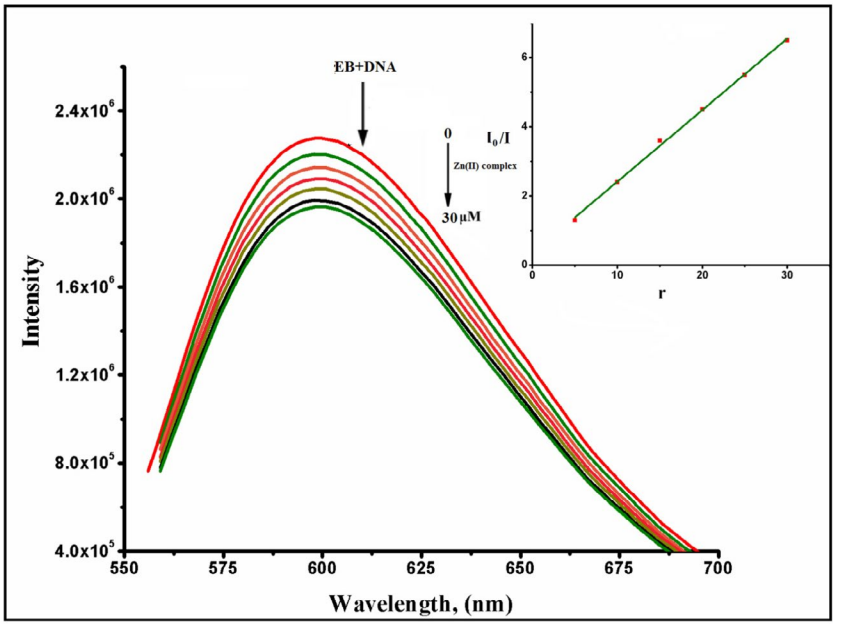


**Figure 5.** Emission spectra from EB bound to the DNA in the absence and presence of increasing amount of Zn(II) complex (0–30 μM), Inset: plot for quenching constant (Ksv).

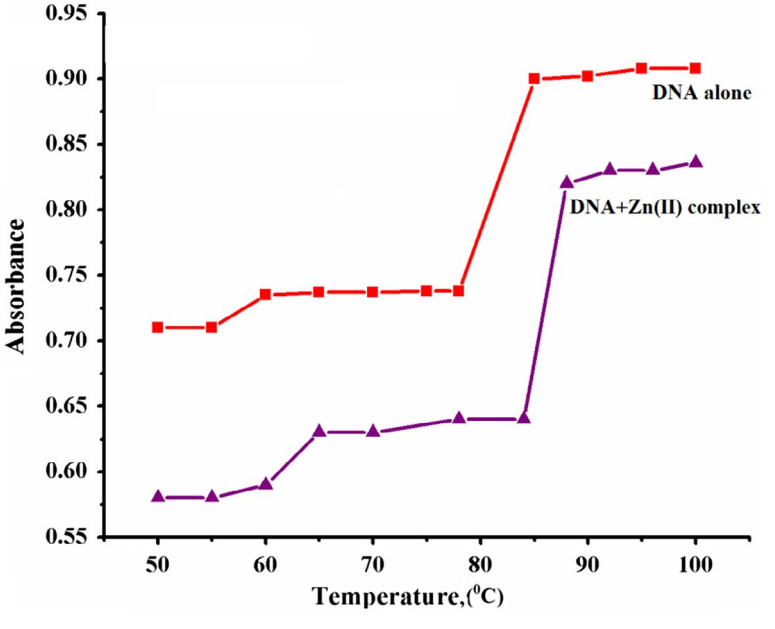


**Figure 6.** Plot of absorbance versus temperature (°C) for the melting of 1) CT DNA alone (red line) and 2) CT DNA + Zn(II) complex (purple line).

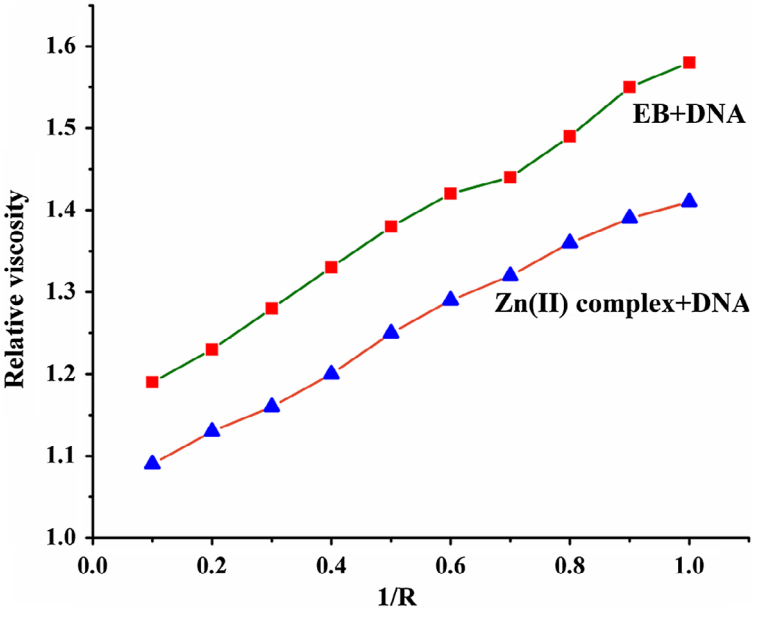


**Figure 7.** Effect of increasing amounts of (a) EB and (b) Zn(II) complex on the relative viscosity of CT DNA.

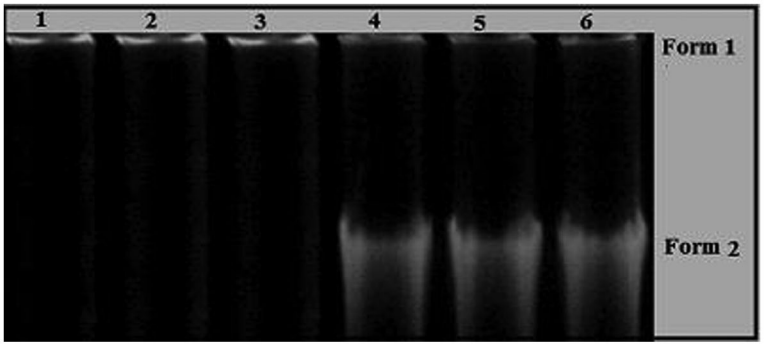


**Figure 8.** Changes in the agarose gel electrophoretic pattern of pBR322 plasmid DNA induced by $H_2O_2$ for ligand and Zn(II) complex. Lane 1: DNA control, Lane 2: DNA + $H_2O_2$, Lane 3: DNA + 10 μM ligand + $H_2O_2$, Lanes 4–6: DNA + Zn(II) complex + $H_2O_2$, [complex] = 5, 10, 15 μM, respectively.

strand is cleaved, then Form 1 relaxes to convert into a slower-moving Form 2. If both strands are cleaved, Form 3 is then generated which moves in between Form 1 and Form 2 [38]. The result of DNA cleavage is shown in Figure 8.

Here for control experiments (lanes 1 and 2) and in the presence of ligand (lane 3), no DNA cleavage is found as there is no metal complex in role, while with increasing the concentration of the synthesized complex (lanes 4–6), the percentage of Form 1 starts to diminish gradually, whereas Form 2 increased. This indicates that the synthesized Zn(II) complex acted on supercoiled plasmid DNA as there was remarkable difference in bands of the complex compared to band of the control DNA. But in the case of ligand, there is no appreciable

change in the bands with respect to control DNA. The experiment is modulated by hydroxyl radical or peroxo species originated from $H_2O_2$. The existence of smears in the gel photograph suggests radical cleavage [39]. The detail mechanism of DNA cleavage by metal complex in the presence of $H_2O_2$ has been reported by various research groups [39, 40]. As the synthesized complex is efficient to cleave the supercoiled plasmid DNA into open circular form, it can be inferred that the complex may inhibit the growth of pathogens by cleaving the genome [24].

## 3.3. Molecular modeling analysis

### 3.3.1. Swiss target prediction

The Swiss prediction report for the synthesized Zn(II) complex showed probable association with biological network. It is seen that among the probable targets, 20% are enzymes and 40% are cytosolic proteins, and there is a huge chunk of another 40% which are unclassified proteins (Figure S2). Among the list of predicted targets, there are important compounds such as BCl-2 like protein 1 which are linked to various cellular activities, especially pro apoptotic functions. Then again, there is Bcl-2-like protein 2 which is also linked to similar function [41]. Apoptosis regulator Bcl-2 is another similar protein which shows interaction with the Zn(II) complex. Induced myeloid leukemia cell differentiation protein Mcl-1 is another protein of BCl-2 family which is essential for apoptosis regulation. All these proteins are essentially related to carcinogenesis pathway [42]. So it is quite likely that the synthesized Zn(II) complex might play a role in general oncology. Moreover, there are other important proteins in this aspect. Microtubule-associated protein Tau promotes microtubule assembly and stability, and might be involved in the establishment and maintenance of neuronal polarity which suggest probable implications in neurodegenerative diseases. They are vital proteins in Alzheimer's disease [43]. These apart from important DNA repair enzymes like Tyrosyl-DNA phosphodiesterase 1 and Muscleblind-like protein groups also seem to have some affinity toward the synthesized complex. These multivaried activities suggest importance of the synthesized complex in biological systems.

### 3.3.2. Molecular docking

The synthesized Schiff base Zn(II) complex has interesting target partners. To better understand their interaction with the complex and to understand which compound has better interaction, we have conducted the molecular docking experiments. Tyrosyl-DNA phosphodiesterase 1 has the best binding affinity with the complex (Table 2). These enzymes have DNA repair capacity and catalyze different hydrolysis reactions in nucleotides. Moreover,

**Table 2.** Binding affinity of the Zn(II) complex with different proteins.

| Protein name | Gene name | PDB ID | Binding affinity (kcal/mol) |
|---|---|---|---|
| Dihydroorotate dehydrogenase (quinone), mitochondrial | DHODH | 1d3 g | −9.2 |
| Tyrosyl-DNA phosphodiesterase 1 | TDP1 | 1jy1 | −10.5 |
| Bcl-2-like protein 1 | BCL2L1 | 1r2d | −9.6 |
| Induced myeloid leukemia cell differentiation protein Mcl-1 | MCL1 | 2nl9 | −10 |
| Microtubule-associated protein tau | MAPT | 2on9 | −7.1 |
| Muscleblind-like protein 1 | MBNL1 | 3d2q | −8.7 |
| Bcl-2-like protein 2 (by homology) | BCL2L2 | 4cim | −9.4 |
| Apoptosis regulator Bcl-2 (by homology) | BCL2 | 4lxd | −8.5 |

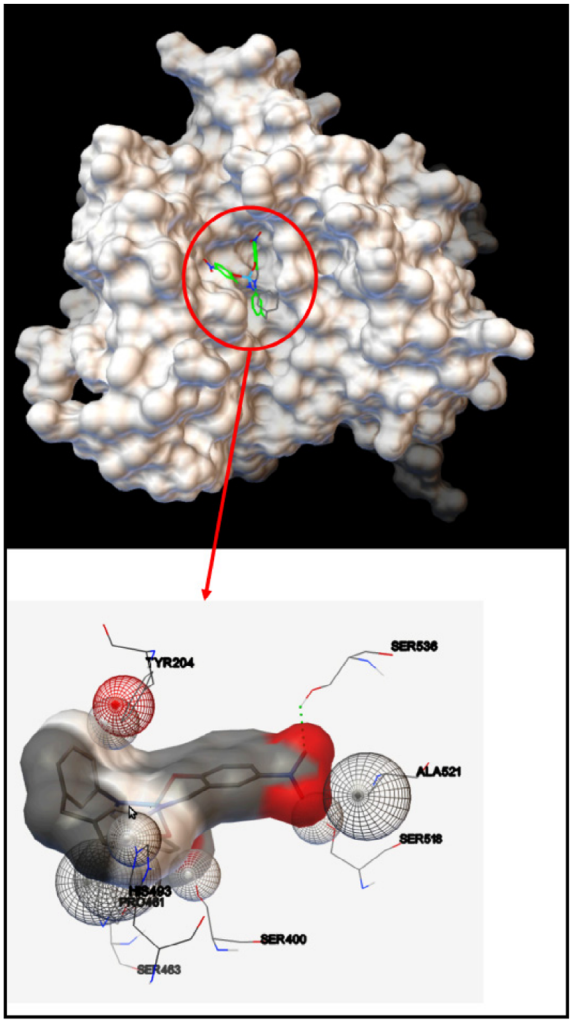


**Figure 9.** Top: the molecular surface view of the protein molecule and the docked complex molecule at its binding site. Bottom: the molecular interaction of the amino acids with the synthesized complex.
Note: The three letter amino acid code with their positions are shown here.

it is seen earlier from DNA interaction study that the Zn(II) complex has different DNA binding and cleaving activity. So, a high affinity of magnitude -10.5 kcal/mol in Auto Dock environment confirms these previous claims. Then again, induced myeloid leukemia cell differentiation protein Mcl-1 has the second best binding affinity. This protein plays a major role in apoptotic regulations. It is already seen that apart from Mcl-1 protein different Bcl-2 family proteins has major activity with the synthesized Zn(II) complex. Proteins like Bcl-2-like protein 1, Apoptosis regulator Bcl-2, and Bcl-2-like protein 2 are all members of Bcl-2 family of proteins, and all have good binding affinity with the synthesized complex (Figure 9). The proteins of Bcl-2 family are all linked to carcinogenesis pathway. So it is confirmed that along from nucleotide interaction the synthesized Zn(II) complex has possible implications in carcinogenesis.

**Table 3.** Percentage of inhibition at highest concentration and $IC_{50}$ values (in parentheses) of Zn(II)-Complex and standard for different antioxidant and free radical scavenging assays.

| Parameter | Zn(II)-Complex | Standard |
|---|---|---|
| DPPH | 43.52 ± 0.04** | 58.5 ± 0.02 |
| | (272.25 ± 1.06**) | (202.06 ± 0.12) |
| Nitric oxide | 57.43 ± 0.48** | 100 ± 0 |
| | (145.42 ± 2.18*) | (60.93 ± 0.04) |
| Hydroxyl radical | 56.9 ± 0.42* | 31.31 ± 0.84 |
| | (129.97 ± 1.05*) | (591.48 ± 9.50) |
| Hydrogen peroxide | 25.14 ± 0.6** | 7.64 ± 0.68 |
| | (744.25 ± 70.57$^{NS}$) | (2147.45 ± 248.44) |
| Hypochlorous acid | 47.52 ± 0.58* | 62.36 ± 0.36 |
| | (214.22 ± 2.13*) | (132.46 ± 4.37) |
| Peroxynitrite | 16.87 ± 0.86$^{NS}$ | 17.68 ± 0.21 |
| | (1034.4 ± 65.65*) | (799.39 ± 36.99) |
| Singlet oxygen | 24.81 ± 1.07*** | 77.97 ± 0.34 |
| | (637.85 ± 36.65**) | (48.43 ± 3.65) |
| Hemolytic | 28.19 ± 0.24** | 73.93 ± 1.01 |
| | (440.12 ± 4.85**) | (78.31 ± 2.35) |
| EMSA | 43.49 ± 0.36* | 78.67 ± 0.67 |
| | (266.02 ± 0.55***) | (67.03 ± 0.64) |

Notes: Units in µg/mL. Data expressed as mean ± S.D.
*$p \leq 0.05$(two sided); **$p \leq 0.01$(two sided); ***$p \leq 0.001$(two sided); $^{NS}p > 0.05$(two sided).

### 3.4. In vitro *antioxidant activity*

The free radical scavenging activity of Zn(II) complex in dose-dependent manner and the differences in the activities compared with standard compound is seen. Half-maximal inhibitory concentrations ($IC_{50}$) of Zn(II) complex are shown in Table 3. The synthesized Zn(II) complex showed lower $IC_{50}$ value than ascorbic acid, mannitol, sodium pyruvate, curcumin, quercetin in DPPH, hydroxyl radical, $H_2O_2$, nitric oxide, superoxide anion, and hypochlorous acid scavenging assay and was found comparable with peroxinitrate shown by gallic acid and lipid peroxidation shown by trolox.

On the other hand, Zn(II) complex showed higher $IC_{50}$ value than lipoic acid and EDTA in singlet oxygen and iron chelation scavenging assay. In the case of total antioxidant activity, Zn(II) complex showed better scavenging activity than the standard ascorbic acid. The reducing power of Zn(II) complex was also determined and found that the reducing capacity of Zn(II) complex extract was increased in a dose-dependent manner comparable to the reference compound ascorbic acid (Figures S3–S7) and ligand.

In the present antioxidant profiling, Zn(II) complex showed potential free radical scavenging activities. The molecule DPPH is a free radical that can accept an electron or hydrogen radical to become stable and reacts with reducing agent to form new bond, changing the color of the solution. The colored DPPH solution was mixed with natural antioxidants. DPPH gives rise to the reduced form with the loss of violet color by the effect of natural antioxidants. Thus, DPPH scavenging activity by Zn(II) complex proves the presence of significant antioxidant properties. But in the case of ligand, the results are not satisfactory.

Human beings are exposed to $H_2O_2$ indirectly. Inhibition of $H_2O_2$ indirectly from the environment is rapidly decomposed into oxygen and water, and this may produce hydroxyl radicals ($OH^{\cdot}$) that can cause lipid peroxidation and DNA damage in the body. Therefore, the ability of Zn(II) complex to scavenge $H_2O_2$ proves beneficial for our health.

Nitric oxide plays an important role as pro-inflammatory mediators. Nitric oxide ($NO^{\cdot}$) is synthesized from the amino acid L-arginine by activation of nitric oxide synthase (NOS). During chronic inflammation, iNOS (calcium-independent isoform of NOS) is activated by LPS (lipopolysaccharide) and produces large amounts of nitric oxide. The active $NO^{\cdot}$ translocates NF-κβ and leads to the formation of cancer. In mitochondria, excess amount of nitric oxide reacts with superoxide radical to produce reactive peroxynitrite radical, which further causes oxidative stress-related disorder. In the present study, it is demonstrated that nitric oxide is down regulated by Zn(II) complex when compared to standard curcumin. Thus, Zn(II) complex might inhibit the inflammation-related disorders.

Hydroxyl radical generated from hydrogen peroxide by Fenton reaction is one of the potent reactive oxygen species in the biological system that react with phospholipids containing polyunsaturated fatty acid moieties of cell membrane and cause damage of cell [44]. Hypochlorous acid is produced from the site of chronic inflammation resulting from the oxidation of $Cl^{-}$ ion by the neutrophil enzyme, myelo-peroxidase. Hypochlorous acid degrades heme prosthetic group and inactivates the antioxidant enzyme catalase. Zn(II) complex was also proved to have the potentiality to scavenge peroxynitrate, hydroxyl radical, superoxide, singlet oxygen, and other free radicals that cause harmful effects in our biological system. In comparison to the ligand, the Zn(II) complex shows far better free radical scavenging activity. Thus, Zn(II) complex might prove to be a key component in prevention of various diseases related to oxidative stress and free radical generation.

## 4. Conclusion

The synthesized Schiff base ligand and its Zn(II) complex have been characterized by different spectral and analytical techniques. Results of the physical measurements exhibit that the Zn(II) metal ion is coordinated by two azomethine nitrogen and two phenolic oxygen atoms, and hence it possibly adopts a tetrahedral geometry. The synthesized complex is stable and non-electrolyte in nature. The ability of the synthesized complex to bind to DNA was monitored by different techniques. The experimental results indicate that the Zn(II) complex interacts remarkably with CT DNA and confirms an intercalative mode of binding to CT DNA. Apart from high binding affinity with DNA at stable energetics, it is also seen that the complex has interaction potential with other biologically important pathways like apoptosis regulation, which hints toward possible involvement with carcinogenesis. In the present study, it is also evident that the synthesized complex aided in the recovery of oxidative stress and inhibited lipid peroxidation. On the basis of these facts, antioxidant therapy by synthesized Zn(II) complex alone or in combination with other pharmacological strategies appears as the most reasonable treatment of oxidative stress induced several mental disorders like mental stress, trauma, and anxiety.

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## Disclosure statement

No potential conflict of interest was reported by the authors.

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