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.

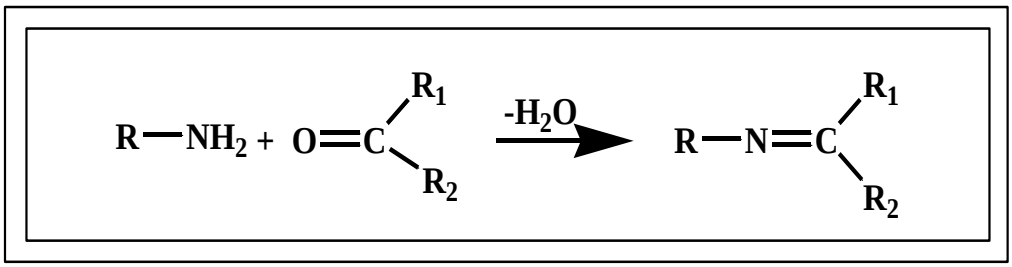

Fig.1.1. Condensation reaction for Schiff base formation.

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:

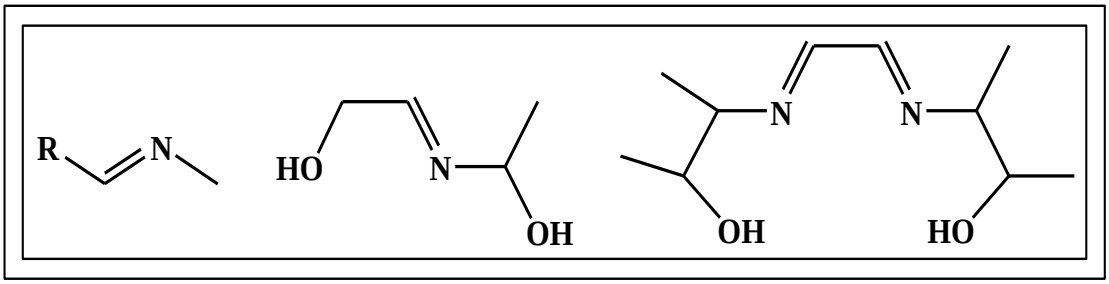

Fig.1.2. Schiff bases with different denticity.

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.

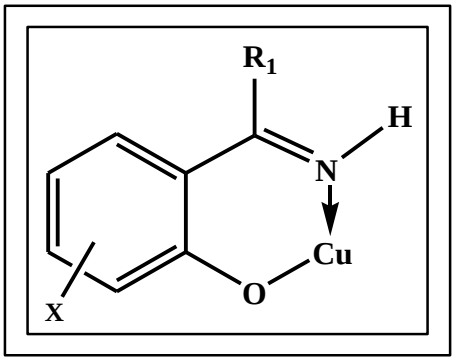

Fig.1.3. Structure of bis(salicyladimino)Cu(II).

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.

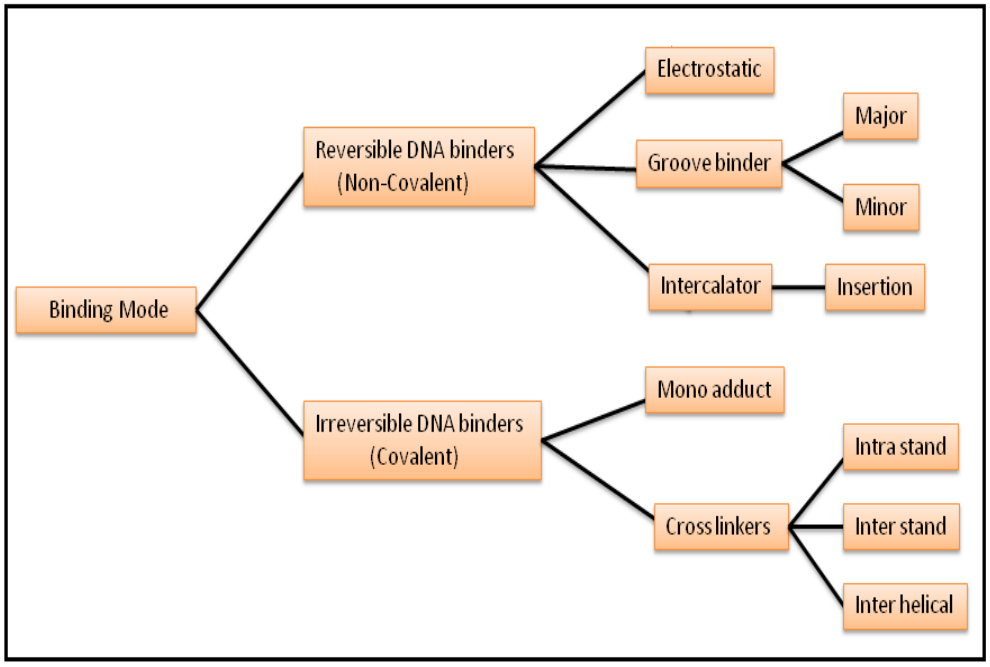

Fig.1.4. Modes of interaction of metal complex with DNA.

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].

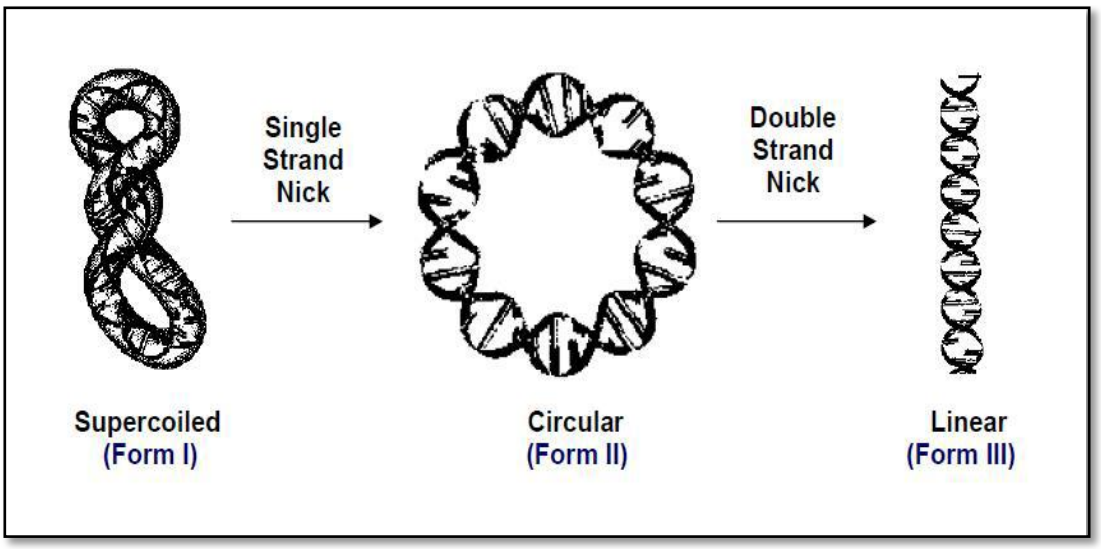

Fig.1.5. Representation of DNA cleavage process.

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].

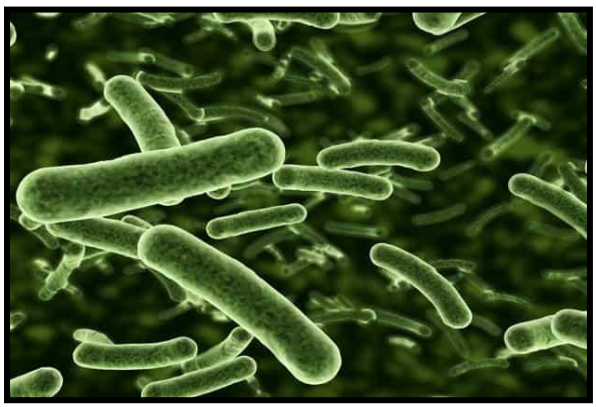

Fig 1.9. *Escherichia coli* bacteria.[131]

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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