CHAPTER-I

BRIEF PROLOGUE ON APPLICATIONS OF SCHIFF BASE METAL COMPLEXES

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1.1 Introduction to Bioinorganic Chemistry

“Bioinorganic Chemistry” is an access for inorganic chemistry and biochemistry, i.e. it illustrate the mutual relationship between these two sub-disciplines, with focus upon the function of inorganic “substances” in living systems, including the transport, speciation and eventually, mineralisation of inorganic materials and including in medicinal therapy and diagnosis. These “substances” can be metal ions (such as K⁺, ferrous and ferric), composite ions (e.g. molybdate), coordination compounds (like cisplatin and carbonyl technetium), or inorganic molecules such as CO, NO, O³.
Studies on the complex formation of metal ions with a number of biomolecules or biologically active ligands have in fact, attracted a lot of interest during the last few years because they act as models for the interactions of metalloenzymes [1] and other complicated proteins [2] in the biological systems. Thus, the bioinorganic chemistry of essential transition metal ion and its complexes is a topic of increasing interest [3, 4] because the study of the interactions of transition metal with nucleotides offers a unique opportunity for understanding various properties of metal complexes such as the carcinogenicity [5] and the antineoplastic activity recently detected in some transition metal complexes [6]. Furthermore, development in the field of bioinorganic chemistry has also led to an increased interest in complexes of N, O donor ligands since it has been recognized that many of these complexes may serve as models for biologically important species having N and O as bonding sites [1].

1.2 Inorganic Chemistry

Inorganic reaction perhaps played an important role in the formation and development of organic “life molecules” in prebiotic area (terrestrial and/or extraterrestrial) and from beginning of life on Earth. Inorganic chemistry is involved in structure and function of all life present on Earth, belonging to one of the three main branches, viz. bacteria. Many biologically active compounds used as drugs possess modified pharmacological and toxicological potentials when administered in the form of metal based compounds. Various metal ions potentially and commonly used are cobalt, copper, nickel and zinc because of forming low molecular weight complexes and therefore, prove to be more beneficial against several diseases.
Biological metal ions play key roles in the structural organization and activation of certain enzymes, which are involved in the transfer of genetic information from DNA, leading to the synthesis of specific proteins.

1.2.1 Essential Elements in life

No common element is toxic at levels normally encountered though almost anything can be harmful at too high levels. All the known toxic elements, which are currently of much concern in environmental pollution problems are extremely rare in abundance in the earth crust.

Fig 1.1 Essential elements in life

A Essential elements are absolutely essential or necessary for life processes.

A Trace elements are also necessary for life processes.

A Non-essential elements are not essential. If they are absent other elements may serve the same function.

A Toxic elements disturb the natural functions of the biological system.
1.2.2 Transition metal complexes

Transition metal complexes have attracted attentions of inorganic, metallo-organic as well as bio-inorganic chemists because of their extensive applications in wide ranging areas from material to biological sciences.

Biologically relevant metal complexes have several requirements in terms of their synthetic design. First, biologically active metal complexes should have sufficiently high thermodynamic stability to deliver the metal to the active site. The metal-ligand binding should be hydrolytically stable. The kinetics with which the metal ion undergoes ligation or delegation reactions is of great importance. The molecular weight of the metal complex is also critical. The compounds of low molecular weight with neutral charge and some water solubility are soluble in almost any medium and may slip through biological membranes by passive diffusion.

Complexes of transition metal ions with multidentate organic ligands have been the subject of intensive research because they not only have interesting spectral and magnetic properties, but also possess a diverse spectrum of biological activities [7-12]. These complexes often possess remarkable and unique spectroscopic, photophysical and electrochemical properties which may be exploited in sensory and diagnostic applications and there have been a number of reviews [13-18] on the utilisation of transition metal complexes as ion and molecular sensors. Based on the widely varied coordination environment of the transition metal complexes and variation in the identities of the coordinating ligands, synthesis of such complexes with desired molecular geometry can be realized. It is well known that several metal
chelates have been shown to inhibit tumor growth [19] and some drugs even exhibit increased activity when administered as metal complexes [20-22].

Thus, the study of the coordination of transition metal ions with different types of ligands has been amplified by the recent developments in the field of bioinorganic chemistry and medicines [23]. The rich diversity of transition metal coordination chemistry therefore, provides exciting prospects for the design of novel coordination ligands having unique structures, valuable functional characteristics [24-30] and significant efforts directed toward the design of specific architectures formed by the self-assembly processes have been carried out in a number of fields of synthetic chemistry [31-33]. In many cases, transition metal ions and their complexes play a central role in controlling the reactivity and mechanism of the chemical reactions of interest. The unique ability of transition metal ions and their complexes to control the chemistry of environmental, industrial, and biological processes has increased the importance of clarifying their mechanistic behavior in simple and complex chemical processes. The knowledge of coordination chemistry is essential for the understanding of the structural and functional features of various biomolecules like metalloproteins, its medical application ranges from the development of MRI contrasting agents, radiopharmaceutical chemotherapeutics to the treatment of metal toxicity [23].

A rational control of the nuclearity of transition metal complexes is important to design systems with the desired properties, as some of these applications require the presence of more than one metal centre in the particular complex. Indeed, binuclear complexes may have very different reactivity than
mononuclear counterparts, thereby enabling transformations inaccessible to single metal ions [34]. In case of the ligand dependent assembly of different metal ions, polydentate Schiff base ligands have always been a good choice for preparing binuclear complexes [35–38].

1.3 Development of a new anticancer agent

Cisplatin was introduced in to clinical use in the UK in 1979 and it is claimed to be the most effective drug for the chemotherapy testicular and ovarian cancers 5.9 % of patients with testicular cancer and 30 % with ovarian cancer were cured in clinical trials using cisplatin in combination with vinblastine. Unlike most other alkylating agents, cisplatin forms intrastrand crosslinks predominately in the major groove of DNA with preferential interaction between the N-7 atoms of adjacent guanines which results in the local denaturation of the DNA chain. Cisplatin has also shown activity towards many other tumours including cancers of the lung, bladder, head and neck. Major side effects of cisplatin are leukopaenia, extreme nausea and renal dysfunction that are usually dose limiting. Many analogues have been prepared in the hope of reducing the side effects of cisplatin. Of these, carboplatin is now in clinical use and offers lower side effects towards the kidneys and nervous system.

The development of a new anticancer agent is a multi-stage process and includes steps such as synthesis, characterization, proof of biological activity, pre-clinical and clinical screenings. Testing for the biological activity requires the measurement of the biological effect in in vitro (in the cancer cell lines) and in vivo
(in animals) screens. If larger pharmaceutical companies do not take over extended work in the area of metal complexes, many developments will be vanished.

There are three methods to develop new tumor-inhibiting complexes:

i. Through the synthesis of classical and non classical derivatives of cisplatin,

ii. Synthesizing tumor-inhibiting non-platinum complexes,

iii. Synthesizing the platinum complexes linked to carrier systems that have the ability to accumulate the drug in organs and tissues.

The first possibility seems to be less promising because it will lead to drugs that will not much differ from cisplatin. However, attempts can be made to reduce the toxic side effects in comparison to the parent compound, as was the case in the development of carboplatin, or change the tumor selectivity.

The second approach aims at compounds with a biologically important metal ion other than platinum. Here, the range of activity can be changed owing to different chemical properties.

Finally, the linkage of platinum complex molecules carrier is a concept known as drug targeting. The aim of this approach is to synthesize a platinum drug that possesses high selectivity towards malignant cells. It can be done for tumors containing biochemical targets different in structure or quantity from the normal tissues.

The major problem in the development of anticancer drugs is the large leap from the preclinical in vitro and in vivo studies to clinical trials. The cause for this is the great difference between the experimental animal models and the individual
patient tumors, making the therapeutic situation of the cancer patients much more complex.

Nowadays Schiff base complexes play a vital role and it has been probe for anti-tumor properties. Hence in this present research, we decided to investigate on synthesis of Schiff base metal complexes.

1.4 Chemistry of Schiff’s base

A Schiff’s base, named after Hugo Schiff, is a compound with a functional group that contains a carbon-nitrogen double bond with the nitrogen atom connected to an aryl or alkyl group, not hydrogen. Schiff’s bases have the general formula R_1R_2C=NR_3, where R is an organic side chain. These are the compounds containing characteristic -C=N- (azomethine) group, so called azomethines (Fig. 1.2).

![Fig 1.2 General Structure of azomethine](image)

Schiff’s bases are usually synthesized from the condensation of primary amines and active carbonyl groups by nucleophilic addition forming a hemiaminal, followed by a dehydration to generate an imine [39].

The electrophilic carbon atoms of aldehydes and ketones can be targets of nucleophilic attack by amines. The end result of this reaction is a compound in which the C=O double bond is replaced by a C=N double bond. This type of compound is known as an imine, or Schiff base. First, the amine nitrogen acts as a nucleophile, attacking the electrophilic carbonyl carbon of aldehydes or ketones. In
the next step, the nitrogen is deprotonated, and the electrons from this N-H bond push the oxygen off of the carbon, leaving a compound with a C=N double bond (an imine) and a water molecule displaced. The end result of this reaction (Fig. 1.3) is a compound in which the C=O double bond is replaced by a C=N double bond. This type of compound is known as an imine, or Schiff’s base [39].

1.4.1 Mechanism of Schiff base

Mechanistically, the formation of an imine involves two steps [40]. Usually carbinolamine [A] undergoes an acid catalyzed mechanism, as it is an alcohol. Formation of Schiff base seems to be nucleophilic addition reactions in which the amines act as nucleophile. In this reaction, dehydration of carbinolamine [A] is the rate determining step (fig 1.4).

Fig 1.3 Formation of Schiff base

Fig 1.4 Mechanism of Schiff base
Usually, the Schiff bases are obtained by the reaction of primary amines with aldehydes and ketones. Primary amines with simple R group give imines, which rapidly decompose, dimerize or polymerize. The stability of Schiff bases can be enhanced by insertion of an aryl group in amines. Generally, aldehydes are more reactive than ketones and order of their reactivity is $1^o > 2^o > 3^o$.

There is also a continuing interest on Schiff bases because of the presence of hard nitrogen or oxygen and soft sulphur donor atoms in the backbone of these ligands, they readily coordinate with wide range of transition metal ions yielding stable and intensely coloured metal complexes, some of them exhibits interesting physical and chemical properties.

1.4.2 Schiff Base as Coordinating Ligands (Chelating ligands)

Schiff base ligands coordinate to a metal through the imine nitrogen and another group, usually oxygen, situated on the original aldehyde. When a diamine was first combined with two equivalence of aromatic aldehyde, the ligands came into being. The ligands feature consists of two covalent and two coordinate covalent sites situated in a planar array. This makes the ligands ideal for the equatorial coordination of transition metals, leaving the two axial sites open for ancillary ligands. They are very much like porphyrins in this regard, but unlike porphyrins the Schiff base ligands are easy to prepare and are relatively inexpensive. Schiff bases are among the most general N ligands, because the basicity of the sp$^2$-hybridized N lone pair, although lower than that of amines (sp$^3$ hybridization), is well suited to form complexes with metal ions. The salicylidene imine group is prone to undergo an acid-catalyzed hydrolysis, reverting to the corresponding salicylaldehyde and diamine in the presence of water. However, the stability of the Schiff base group
increases considerably upon coordination with a metal ion and formation of the Schiff base-metal complex. For this reason, in contrast to the free ligand, the Schiff base-metal complex can be used in wet solvents or even in aqueous media without undergoing hydrolysis. By incorporating additional groups around the phenol portion of the ligand, such as tert-Butyl, the ligands can be made highly soluble in aromatic and aliphatic solvents. Incorporation of hydrophilic groups may also lead to ligands that are soluble in water and alcohols.

Amino pyridines find wide application in pharmacological industry and in analytical chemical laboratories. They serve as good anaesthetic agent and hence used in the preparation of drugs for certain brain disease, particularly 4- aminopyridines is an effective medicine in the treatment for multiple sclerosis [41]. As the aminopyridine improves the transmission of nerve impulses down damaged axons it drastically improves the conditions of patients suffering from spinal-cord injury [42]. Metal complexes of amino pyridine are proved to have improved pharmacological and therapeutic effects because of the following factors

- Considerable reduction of drug resistance on complexation.
- Form complexes with potentially active and biologically essential elements.
- Metal complexes may act as a vehicle for the activation of ligand, which is the principal of cytotoxic agent and
- The long-term side effects of therapeutic agents can be avoided.

In-spite of their wide ranging applications, few works has been expended on aminopyridines and hence the molecule has been considered for synthesizing an effective and potentially active Schiff bases.
Ligands bearing one or more chelating 2,2’-bipyridine or 1,10-phenanthroline units have attracted considerable interest as building blocks in the preparation of transition metals [43].

The coordination compounds of Schiff bases of terephthalaldehyde have been extensively studied [44], reports are scanty on Schiff bases derived from terephthalaldehyde and ortho substituted aromatic amines. Presence of such groups at suitable position markedly influences their coordination characteristics.

1.5 Applications of Schiff Base metal complexes

The coordination chemistry of transition metal complexes of symmetrical Schiff base ligands has attracted much attention in recent years due the fact that the ligands around central metal ions in natural systems are symmetrical and it has extensive variety of applications in many fields such as fields of inorganic chemistry, biochemistry, environmental chemistry, inorganic and analytical chemistry [45].

Application of many new analytical devices requires the presence of organic reagents as essential compounds of the measuring system. They are used, e.g., in optical and electrochemical sensors, as well as in various chromatographic methods, to enable detection of enhance selectivity and sensitivity [46-48]. On the other hand, Schiff base metal complexes resulted from the reaction of transition metal carbonyls and N-containing heterocycles have been the subject of considerable interest due to their photochemical, electronic properties and potential for industrial applications have led to many studies for the use as photo-sensitizers, catalysts and conducting coordinating polymer. Carbonyl derivatives of transition metals, are useful
intermediates in the synthesis of important coordination compounds and have applications in catalysis of important reactions such as epoxidation, carbonylation, hydrogenation and hydroformylation reactions [49].

Furthermore, complexes of Schiff bases showed promising biological activity and biological modeling applications [50]. Coordination of such compounds with metal ions, such as copper, cobalt, nickel, manganese and vanadium, often enhances their activities[51, 52]. In biological field it is used as anticancer [53], as antitumor [54], as antituberculous [55], as antimalarial [56], as antiphlogostic, as nematocide and they also found to have clinical, therapeutic [57] and analytical applications.

Apart from this applications, Schiff base and its metal complexes have been used in various fields such as materials synthesis [58], photochemistry, magnetism [59], medical imaging [60,61] etc.,

Fig 1.5 Schematic Diagram for Branches of Applications of Schiff base metal complexes
Much current effort is being devoted to the synthesis of Schiff base complexes from organic molecules in view of preparing compounds with tunable chemical and physicochemical properties. The Schiff base with its chelating properties it has more pharmacological properties. Schiff base ligands have wide applications in biological field, as antidepressants, antimicrobial, antitumor, antiphlogogistic, nematocide, and other medicinal agents have been reported based on these compounds.

Although several kinds of metal ions are found, the main reason for the preference of transition metal ions like Cu(II), Co(II), Ni(II) and Mn(II) ions over the other metal is ultimately due to their unique features such as the flexibility to adopt more than one coordination geometries and the ability to exist in multiple oxidation states.

It has been shown that the copper complexes of the nonsteroid anti-inflammatory drugs are more active compared to the free ligands and the inorganic copper salts. The binuclear copper complex of the well known NSAIDS drug, diclofenac was found to inhibit the activity of lipoxygenase [62]. Another interesting feature of the copper(II) complex shows not only anti-inflammatory but also antiulcer, anticarcinogenic, anticonvulsant, antidiabetic and analgesic properties [63]. There are also copper(II) complexes that possess antimalarial activity. Furthermore, a copper(II) complex of pyridine-2-carboxamidrazone appeared to have potent antimalarial activity. In general, aminopyridine copper(II) complexes show antimicrobial activity against both types, Gram(+) (Staphylococcus aureus, Bacillus subtilis) and Gram(-) (Escherichia coli, Pseudomonas euruginosa) [64,65].
Various Co(III) complexes have been reported with antimicrobial activities. For instance, a Co(III) complex of the known antiulcer drug famotidine turned out to have greater antimicrobial activity against E. coli and M. lysodeikticus than the metal free drug [66]. The Schiff base metal complexes of substituted pyridine and aromatic aldehyde have recently shown activity against Gram(+) and Gram(-) bacterial strains and fungi C. Albicans [67,68]. Cobalt(II) complexes not only show antimicrobial but also antifungal activities. Cobalt(III) complexes have been described as hypoxia selective antitumor agents. The concept of such a design is based on the fact that the tumor cells develop resistance to chemotherapeutic agents under anaerobic conditions and they may be reduced under hypoxic conditions to Co(II) species followed by loss of neutral ligand [69].

Nickel complexes are used in heterogeneous catalysis, electroplating, and in making pigments and ceramics. The Ni(II) complex of benzoic acid derivative acts as a stabilizer against oxidation of polybutadiene [70]. A number of nickel complexes of Schiff bases have been seen to possess fungicidal and bacterial activity.

On the contrary, Mn(II) complexes are much cheaper and less harmful, but little Mn(II) complexes have been used as DNA intercalator and also fabrication of DNA electrochemical biosensor [71].

Hence, we concentrate more on pharmacological applications of Schiff base ligands and its metal complexes.
1.6 Antimicrobial Activity

For the treatment of diseases inhibitory chemicals were employed to kill micro-organisms or prevent their growth are called antimicrobial agents. These are classified according to their application and spectrum of activity, as germicides that kill micro-organisms, whereas micro-biostatic agents inhibit the growth of pathogens and enable the leucocytes and other defense mechanism of the host to cope up with static invaders. The germicides may exhibit selective toxicity depending on their spectrum of activity. They may act as viricides (killing viruses), bacteriocides (killing bacteria), algicides (killing algae) or fungicides (killing fungi).

1.6.1 Classification of Antibacterial Agents

The antibacterial agents are classified in three categories

(i) Antibiotics and chemically synthesized chemotherapeutic agents

(ii) Non-antibiotic chemotherapeutic agents (Disinfectants, antiseptics and preservatives)

(iii) Immunological products.

1.6.1.1 Antibiotics

They are produced by micro-organisms or they might be fully or partly prepared by chemical synthesis. They inhibit the growth of micro-organisms in minimal concentrations. Antibiotics may be of microbial origin or purely synthetic or semisynthetic [72]. They can be classified by manner of biosynthesis or chemical structure.

Synthetic antimicrobial agents include sulfonamides, diamino pyrimidine derivatives, antitubercular compounds, nitrofuran compounds, 4-quinoline antibacterials, imidazole derivatives, flucytosine etc.
A Streptomycin

Streptomycin is an antibiotic (antimycobacterial) drug, the first of a class of drugs and it was the first antibiotic remedy for tuberculosis. It is derived from the actinobacterium Streptomyces griseus. Streptomycin is a bactericidal antibiotic [73]. Streptomycin cannot be given orally, but must be administered by regular intramuscular injections. Adverse effects of this medicine are ototoxicity, nephrotoxicity, fetal auditory toxicity and neuromuscular paralysis. At low concentrations, however, Streptomycin inhibits the growth of bacteria by inducing prokaryotic ribosomes to misread mRNA [74]. Streptomycin is an antibiotic that inhibits both Gram-positive and Gram-negative bacteria [75] and is therefore a useful broad-spectrum antibiotic. Hence streptomycin has been chosen as standard for this research against antibacterial activity.

Fig 1.6 Structure of Streptomycin

1.6.1.2 Non-antibiotics

The second category of antibacterial agents includes non-antibiotic chemotherapeutic agents [76-78] which are as follows:

- Acids and their derivatives
- Alcohols and related compounds
- Chlorination and compound containing chlorine
Iodine containing compounds
Heavy metals
Oxidising agents
Dyes
8-Hydroxyquinolines
Surface active agents

1.6.1.3 Immunological products

Certain immunological products such as vaccines and monoclonal antibodies are used to control the diseases as a prophylactic measure.

1.6.2 Mode of Action

Antimicrobial drugs interfere chemically with the synthesis of function of vital components of micro organisms. The differences of cellular structure and functions of cell provide us with selective toxicity of chemotherapeutic agents against bacteria. Antimicrobial drugs may either kill microorganisms outright or simply prevent their growth. There are various ways in which these agents exhibit their antimicrobial activity [79-81] and they may inhibit

1. Cell-wall synthesis
2. Protein synthesis
3. Nucleic acid synthesis
4. Enzymatic activity
5. Folate metabolism or
6. Damage cytoplasmic membrane
A number of drugs are metal-binding agents. The chelates are the active form of drugs. The site of action within the cell or on the cell surface has not been established. The site of action of oxine and its analogs has been suggested inside the bacterial cell [82] or on cell surface [83].

![Mechanism of Antibacterial Activity](image)

**Fig 1.7 Mechanism of Antibacterial Activity**

Four different microbial species were used to screen the possible antibacterial activity of the synthesized binuclear Schiff base metal complexes of the species used.

1.6.3 Bacteria

The bacteria are microscopic organisms with relatively simple and primitive forms of prokaryotic type. Danish Physician Christian Grams, discovered the differential staining technique known as Gram staining, which differentiates the bacteria into two groups “Gram positive” and “Gram negative”, based on the
structural differences in their cell walls. The bacteria that retain the crystal violet dye do so because of a thick layer of peptidoglycan and are called Gram-positive bacteria. In contrast, Gram-negative bacteria do not retain the violet dye and are colored red or pink. Compared with Gram-positive bacteria, Gram-negative bacteria are more resistant against antibodies because of their impenetrable cell wall.

Also, 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, which loose the crystal violet, are counter-stained by saffranin and hence appear red in colour.

![Cell wall of Gram positive and Gram negative bacteria](image)

**Fig 1.8 Cell wall of Gram positive and Gram negative bacteria**

1.6.3.1 Staphylococcus aureus

The word staphylococcus is derived from the Greek language (Gr. Staphylo = bunch of grapes; Gr. Coccus = a grain or berry), while the species name is derived from Latin language (L. aureus = golden). Staphylococcus is differentiated from
micrococcus and another genus of the same family by its ability to utilize glucose, mannitol and pyruvate anaerobically. Cells of staphylococci, which are slightly smaller than those of Micrococi, are found on the skin or mucus membrane of the animal body.

![Staphylococcus aureus](image)

**Fig 1.9 Staphylococcus aureus**

1.6.3.2 Bacillus subtilis

Originally named Vibrio subtilis in 1835, this organism was renamed Bacillus subtilis in 1872. Bacillus subtilis are also known as the hay bacillus or grass bacillus. Bacillus subtilis bacteria were one of the first bacteria to be studied. These bacteria are a good model for cellular development and differentiation.

Bacillus subtilis cells are rod-shaped, Gram-positive bacteria that are naturally found in soil and vegetation. Bacillus subtilis grow in the mesophilic temperature range and the optimal temperature is 25-35 °C. B. subtilis is only known to cause disease in severely immune compromised patients and can conversely be used as a probiotic in healthy individuals. It rarely causes food poisoning.
1.6.3.3 Escherichia coli

They are Gram-negative rods, motile with peritrichate flagella or nonmotile. They do not form spores. All are sometimes (i.e. from rarely to, invariably) found in intestinal treatment of man or lower animals. Escherichia in 1885 discovered Escherichia coli which is a commensal of the human intestine and is found in the sewage, water or soil contaminated by faecal matters. E. coli are generally non-pathogenic and are incriminated as pathogens, because in certain instance some strains have been found to produce septicemia, inflammation of liver and gall bladder, appendix and other infections and this species is a recognized pathogen in the veterinary field.
1.6.3.4 Klebsiella pneumoniae

Klebsiella pneumoniae is a gram negative bacterium, nonmotile, usually encapsulated rod shaped bacteria. Klebsiella pneumoniae is a very common pathogen that is encountered by many health care providers. It can cause destructive changes to human lungs if aspirated. As a general rule, Klebsiella infections are seen mostly in people with a weakened immune system.

![Fig 1.12 Klebsiella pneumoniae](image)

Various methods have been used from time to time by several workers to evaluate the antimicrobial activity. The evaluation can be done by the following methods:

A Turbidometric method.

A Agar streak dilution method.

A Serial dilution method.

A Agar diffusion method.

Following Techniques are used as agar diffusion method:

Agar Cup method.

Agar Ditch method.

Paper Disc method.
Fig. 1.13 Showing cylinder cup method (Agar Diffusion Technique) with essential arrangement (diagrammatic)

The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic and develop research to better understand the genetic mechanisms of resistance and to continue studies to develop new drugs, either synthetic or natural.

In general, when the antimicrobial activity of metal complexes is concerned, the following five principal factors may be considered:

- The chelate effect, i.e. multidentate Schiff base ligands, such as the quinolones, show higher antimicrobial efficiency towards complexes with monodentate ligands.
- The nature of the ligands.
- The total charge of the complex; generally the antimicrobial efficiency decreases in the order cationic > neutral > anionic complex.
- The nature of the counter ion in the case of the ionic complexes.
The nuclearity of the metal center in the complex; dinuclear centers are more active than mononuclear ones.

The antimicrobial activities of metal complexes depends more on the metal centre itself than on the geometry around the metal ion.

Generally, drug combinations have proven to be an essential feature of antimicrobial treatment due to a number of important considerations: (i) they increase activity through the use of compounds with synergistic or additive activity; (ii) they thwart drug resistance; (iii) they decrease required doses, reducing both cost and the chances of toxic side effects; (iv) they increase the spectrum of activity.

Several metal complexes are known to accelerate the drug action and the efficacy of the organic therapeutic agent. The efficacy of the various organic therapeutic agents can often be enhanced upon coordination with a suitable metal ion. There is a real perceived need for the discovery of new compounds endowed with antimicrobial activities. The newly prepared compounds should be more effective and possibly act through a distinct mechanism from those of well-known classes of antimicrobial agents to which many clinically relevant pathogens are now resistant.

1.7 DNA Interaction Chemistry

1.7.1 Intercalating agents

These agents have a flat shaped structure usually containing three or four fused aromatic rings. The molecular structure allows them to insert between the hydrophobic faces of the base pairs of DNA perpendicular to the axis of the helix. The insertion is held by interactions with DNA base pairs via hydrogen bonding and
van der Waals forces. Most examples of this class of anticancer drug were originally discovered from natural sources in screening programmes that tested antibiotics for cytotoxic activity [84-88].

1.7.2 DNA Binding Modes

Transition metal complexes having unique electronic and spectroscopic signatures which offer a multitude of coordination geometries and mechanism of cytotoxic action which is related to DNA binding affinity [89] and can also vary accordingly as the biological activity is strongly dependent on structure–activity relationship. Besides this, metal complexes also utilize or create open coordination positions for DNA binding and hydrolysis generates reactive oxygen-containing species or other radicals for DNA oxidation [90].

DNA is an important genetic material in organisms and the basis of gene expression. Small molecules can interact with DNA [91-93] through four type of bindings they are electrostatic binding/external binding, intercalative binding and groove binding (figure 1.14).

Surface binders interact electrostatically and/or via hydrogen bonds to the phosphate backbone on DNA. The binding is often non-specific and is difficult to observe directly. Typically only indirect binding is detectable, for example as a change in the backbone configuration. Electrostatic interaction happens in the case of positively charged molecules. They electrostatically interact with the negatively charged phosphates backbone of DNA chain. Electrostatic attraction is generally weak under physiological conditions [94].
Intercalation is typically observed for cationic molecules having planar aromatic rings [95]. Intercalative binding results when small molecules or the drug intercalate into the nonpolar interior of the DNA helix between two neighbouring bases. Aromatic group is stacked between the base pairs in this type of binding and this happens when ligands of an appropriate size and chemical nature fit themselves in between base pairs of DNA. This leads to increased distance between the bases and local unwinding. Often, the stacking is very little influenced in the rest of the helix. The intercalation is stabilized by stacking interactions, i.e. \( \pi-\pi \) interactions between the aromatic rings. The ligands suitable for intercalation are mostly polycyclic, aromatic and planar and therefore often make good nucleic acid stains. There is also a current interest in designing and synthesising DNA strand, as these molecules might function as chemotherapeutic agents.

Binding within the major groove of the double helix is rare for small molecules [96]. Groove binding interactions involve direct interactions of the bound molecule with edges of base pairs in either of the major (G-C) or minor (A-T) grooves of the nucleic acids. Minor groove binders are typically long elongated structures with a curvature that fits the curvature of the minor groove. The ligand is fitted between the narrow walls of the groove and stabilized via hydrogen bonds and van der waals interactions. The minor groove also has a certain flexibility to accommodate for ligands that do not have a perfect fit, since this allows the molecule to adjust its structure to follow the groove as it twists around the central axis of the helix [97,98]. Binding in the minor groove requires substantially less distortion of the DNA compared with intercalative binding. Major
groove binders utilize the numerous possibilities for specific hydrogen bonds with donors and acceptors on the nucleic bases providing the basis for both complex stabilization and sequence specificity. Many proteins bind to DNA in the major groove.

The frequently used methods to provide insight into the binding modes of small molecules are UV-vis spectroscopy, fluorescence spectroscopy, circular dichroism and linear dichroism. Binding to DNA will often cause a change in the absorption maximum or peak extinction coefficient. But this is insufficient to determine a binding mode, equilibrium binding constants can be determined based on the concentration dependence of any observed shifts. Fluorescent small molecules exhibit changes in wavelength or quantum yield upon binding and able to act as energy acceptors from the DNA bases. Circular dichroism measures the differential absorption of right- and left-handed circularly polarized light. Circular dichroism is an effective technique for an assessment of DNA-binding mode. When a ligand bound to the chiral DNA, CD is induced [99,100]. The actual sign and magnitude of the induced CD signal is complicated and depends on the binding mode, DNA sequence, and orientation of the transition dipole of the ligand. However, intercalators will often exhibit lower intensity CD spectra compared with groove binders and this is most likely due to the fact that a groove binder contacts a larger part of the helix, around 4–6 base pairs. In contrast, a simple intercalator only contacts two base pairs.
Fig 1.14 Example for modes of metal complex binding with DNA

1.7.3 Cleavage of DNA

Schiff base complexes have suitable biometric properties that can mimic the structural features of the active sites, and they have been widely used in various fields such as biochemical reaction and biological regulator. The humankind has attempted to reproduce those biological activities with artificial compounds using many metal complexes for the interactions with biological molecules. DNA cleavage can be achieved by inorganic complexes and these complexes are called inorganic nucleases.

Fig 1.15 Plasmid pUC18
DNA can be further classified into genomic DNA (present in all living organisms) and plasmid pUC18 DNA (found mostly in bacteria, or mitochondria/chloroplasts in higher organisms). In most biological systems, plasmid DNA is present as supercoiled form (Form I) (Figure 1.16). This form is important for gene expression, DNA replication and recombination. If there is a nicking in one of the strands, it will result in loss of supercoils and the DNA will be in nicked form (Form II), or also called open circular form. If there is a nicking in both the strands at the (nearly) same points, the DNA will arrange itself into a linear form (Form III).

![Various forms in DNA cleavage](image)

**Fig 1.16 Various forms in DNA cleavage**

DNA cleavage by metal complexes generally proceeds via two major pathways by oxidative pathway and hydrolytic pathway. The DNA cleavage activity of metal complexes can be targeted towards different constituents of DNA: the heterocyclic bases, deoxyribose sugar moiety and phosphodiester linkage.

1.7.3.1 Hydrolytic cleavage

Many metal complexes have been studied to understand their capability in the hydrolytic cleavage of DNA which involves hydrolysis of phosphodiester bond. Nucleophilic activation is required for hydrolytic cleavage of phosphodiester bond
due to unusual stability of the diester bond in DNA. Among several types of DNA cleavage reactions, those occurring under photoactivation are of particular importance in highly targeted chemotherapeutic applications. The reagents showing photo induced DNA cleavage have major advantage over chemical nucleases, as the latter requires a reducing agent and/ or H₂O₂ for its activity. The reagents cleaving DNA on photoactivation generally show localised effect as they are otherwise non toxic and such compounds should be useful in the photodynamic therapy (PDT), which has emerged as a promising tool against cancer. The FDA approved PDT drug photophrin, which is a mixture of hematoporphyrin derivatives and is currently used for the treatment of lung and oesophageal cancers. Hydrolysis by natural nucleases is assumed to proceed via a 5 coordinated transition state [101] and furthermore cleavage on P-O3” produces the breakdown products.

Fig 1.17 Hydrolytic Cleavage
1.7.3.1.1 Mechanism of hydrolytic cleavage

An interest in hydrolytic cleavage comes from the fact that the mechanistic information obtained from such studies would help better understanding of hydrolytic enzymes. Also, the hydrolytic agents could be used to detoxify pesticides and chemical agents, as the pesticides and chemical agents often contain phosphate-ester. The different proposed modes (Figure 1.18) are Lewis acid activation, nucleophile activation, leaving group activation, metal coordinated hydroxides and metal coordinated water molecule [102-104]. When metals with redox chemistry are employed, another form of cleavage-oxidative cleavage could also take place [105].

Fig 1.18 Different proposed activation modes for hydrolysis of phospodiester bonds: (a) Lewis acid activation, (b) nucleophile activation, (c) leaving group activation, (d) metal coordinated hydroxides and (e) metal coordinated water molecule. (M denotes metal complex)
1.7.3.2 Oxidative Cleavage

Oxidative cleavage of DNA takes place in the presence of additives or photo-induced DNA cleavage. Photo-cleavers require the presence of a photo-sensitizer that can be activated on irradiation with UV or visible light. The cleavage can occur either in the nitrogenous base or in the ribose sugar. The agents responsible are called reactive oxygen species (ROS) which include superoxide and hydroxyl radicals, single oxygen and high valence metal oxo species. Since, there are nitrogenous bases and different ROS and the fact that the oxidation could occur either in the base or in the sugar ring, various mechanisms of oxidations are possible. One such oxidative mechanism by Fe$^{II}$ Bleomycin is given in Figure 1.19.

![Diagram of Oxidative Cleavage](image)

**Fig 1.19 Mechanism of oxidative cleavage**
1.7.4 Transition metal complexes as chemical probes for DNA

The design of molecules that exhibit strong binding affinity to DNA is a demanding area of research for chemists. Such molecules can act as excellent chemotherapeutic reagents that exert their biological activity through interactions with DNA [105-110]. Interactions with DNA are not only the factors that determine the biological activity of these molecules, but their reactivity and selectivity are often correlated with their mode of binding with DNA. Therefore a better understanding of the factors that govern the interactions of molecules with DNA has an important role in the rational design of various DNA-targeted chemotherapeutic agents and molecular probes for DNA [111-114]. Stable and inert complexes containing active metal centres are extremely valuable as probes of biological systems.

Our interest in this area is focused for a considerable time on the investigation of coordination chemistry of Schiff base transition metal complexes with binucleating Schiff base ligand from 2,6-diaminopyridine. The term “dinucleating ligands” was first introduced in 1970 by Robson [115] to describe the class of polydentate chelating ligands which able to bind simultaneously two metal ions. Also, recently reported binuclear metal(II) complexes have showed better DNA binding propensity and nuclease activity than the mononuclear Metal(II) analog. This significant rate enhancement of binuclear complexes in phosphate hydrolysis and DNA cleavage activity is due to a synergistic effect that might exist between the metal ions. This stimulated us to design and synthesize new binuclear Schiff base metal(II) complexes to evaluate and understand the factors that show DNA-binding and cleavage properties.
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