CHAPTER I

DNA cleavage activity by transition metal complexes: an Introduction
I.1. A preamble

The biological importance of metals and their complexes have been proved beyond doubts and created interest in their studies. Precious metals have been used for medicinal purposes for more than 3500 years, literature shows that gold was included in a variety of medicines in Arabia and China\textsuperscript{1-3}. However, the motivation for the use of these metals often had a superstitious or a religious origin. Inorganic elements play diverse biological roles, sodium and potassium are of fundamental importance to neurotransmission characterization and selective ion channels. Lithium is used to manage bipolar disorder. Calcium ions are responsible for the initiation of structural changes in proteins that elicit a variety of neurological processes. Zinc is a major regulator of synaptic transmission and other neuronal processes. Copper has an important role in brain metabolism as it is essential for the activity of CuZn superoxide dismutase (SOD), ceruloplasmin, cytochrome c oxidase, tyrosinase and dopamine b-hydroxylase. Iron is involved in respiration, in the synthesis of DNA and neurotransmission process. All these examples created interest in incorporating metal atoms into drug molecules\textsuperscript{4}.

Since the discovery of the DNA structure in 1953 bioinorganic chemistry related to nucleic acid chemistry has emerged as an exciting interdisciplinary field of science\textsuperscript{5-8}. Transition metal complexes that are capable of cleaving DNA and proteins under physiological conditions are of interests towards developing metal-based anti-cancer agents, artificial restriction enzymes, DNA footprinting agents, and as probes for nucleic acids structure\textsuperscript{9,12}. Metal complexes with their tunable coordination geometry, versatile redox and spectral properties provide wide scope of designing metal-based drug systems that are suitable to target DNA and proteins to control primary and malignant secondary tumours. Several metal complexes have been used as anticancer drugs. For example, iron-bleomycins (Fe-BLMs) and related model complexes are known to show chemical nuclease activity, cleaving DNA in an oxidative manner\textsuperscript{13-16}. The seminal finding by Rosenberg in 1965 that bacterial cell division is inhibited by cisplatin led to the development of one of the most successful antitumor agents in the history of chemotherapy\textsuperscript{17,18}. Metal-based chemotherapeutic drugs like cisplatin and its analogues are known for cytotoxicity but they suffer from side effects along with drug-resistance problems\textsuperscript{19-24}. Among these compounds, only one has been approved by the FDA: oxaliplatin, for the treatment of colorectal cancer.
1.2. Metal complexes as chemical nucleases

The chelating behaviour and other properties of many thiourea and terpyridine have been studied by many researchers. In this overview, much work has been done all over the World. Some of the work is reviewed as follows.

1.2.1. Cu(I) complexes

DNA-cleavage induced by new macrocyclic, azamacrocyclic benzenedimethanimine schiff base dinuclear Cu(I) complexes containing pyridyl pendant arms were reported by Armau et al. Cardo et al. reported the DNA cleavage of binuclear metallo-supramolecular cylinders. Cylinders are based on bis-pyridylimine ligands, which are end-functionalized with short peptides\(^\text{25}\). Murali Krishna et al. reported the DNA cleavage activity of copper(I) complex of cuminaldehyde-4- ethyl-3-thiosemicarbazone\(^\text{26}\). Schweigert et al. reported DNA degradation by DNA-copper-hydroperoxo complexes\(^\text{27}\). Cai et al. reported studies on the synthesis, structure of the schiff base copper(I) complexes and their interaction with DNA\(^\text{28}\). Russo et al. showed Gas-phase theoretical prediction of the metal affinity of copper(I) ion for DNA and RNA bases\(^\text{29}\). Schoentjes et al. reported interaction of double-helical polynuclear copper(I) complexes with double-stranded DNA\(^\text{30}\). Pan et al. reported structure of the *E. coli* DNA complex probed by protein conjugated with 1, 10-phenanthroline copper(I) complex\(^\text{31}\). Meunier et al. reported activity of copper phenanthroline and clip- phen to A:T tracts via linkage to a poly-N-methylpyrrole\(^\text{32}\). Meadows et al. reported a monophenyl phenanthroline complex of copper(I) that binds to DNA\(^\text{33}\). Pruetz et al. studied the effect of pH of buffer on interaction of DNA with copper(I) complex\(^\text{34}\). Ehrenfeld et al. reported copper(I)-bleomycin complex that mediates oxidative DNA strand scission\(^\text{35}\). Veal et al. reported specific DNA cleavage, noncovalent DNA binding of bis(1,10-phenanthroline) and related copper(I) compounds\(^\text{36}\). Devereux et al. reported several copper(I) complexes containing either pyridyl-type ligands such as 1,10-phenanthroline showing specific DNA cleavage\(^\text{37}\). Starosta et al. presented the syntheses, structures and biological properties of a series of copper(I) iodide complexes with aliphatic tris(aminomethyl)phosphanes and 1,10-phenanthroline (phen) or 2,9-dimethyl-1,10-phenanthroline(dmp)\(^\text{38}\). Zhong Han et al. synthesized a novel polynuclear Cu(I)–sulfur cluster, bearing 1,2-dithiolate-o-carborane and 1,10-phenanthroline ligands, studied their in vitro cytotoxicity against a range of epithelial
tumour cells and CT-DNA binding interactions\textsuperscript{39}. Marina Porchia \textit{et al.} described the antiproliferative activity of neutral and charged phosphine/scorpionate copper(I) complexes\textsuperscript{40}. S. Jayashree \textit{et al.} described the synthesis, characterization, thermal decomposition, and antimicrobial Studies of copper(I) complexes of 1,3-Dihydro-4,5-Di(4-Methoxyphenyl)Imidazolin-2-Thione ligands\textsuperscript{41}.

\textbf{1.2.2. Terpyridine metal(II) complexes}

Rajalakshmi \textit{et al.} reported three Cu(II) complexes containing the tridentate ligand bitpy, which bears biologically relevant benzimidazolyl head group, as one of the ligands. Because of the presence of the planar benzimidazolyl group in the bitpy ligand, the complexes exhibited intercalative mode of binding with DNA. All the three complexes possessed DNA condensing ability. The DNA condensing ability of the complexes was in the order 2 > 1 > 3. The DNA condensation induced by these three complexes was found to be reversed in the presence of 1 M NaCl\textsuperscript{42}. Pengfei \textit{et al.} reported the DNA cleavage ability and DNA-binding affinity of the complexes depended on the substituent group on the terpyridine ligands\textsuperscript{43}. Patel \textit{et al.} reported that terpyridine Cu(II) complexes bind to DNA by an intercalative mode and all the complexes are showed moderate oxidative DNA cleavage\textsuperscript{44}. Suntharalingam \textit{et al.} reported trinuclear Cu(II) complexes using terpyridine as ligands, nuclease potential of the three complexes was investigated by using circular plasmid DNA as a substrate and analysing the products by agarose-gel electrophoresis. The cleaving activity was found to be dependent on the number of copper centres present (cleaving potency was in the order: tri-copper >di-copper >mono-copper). Interestingly, the tri-copper complex was able to cleave DNA without the need of external co-reductants\textsuperscript{45}. Sovan \textit{et al.} reported pyrinyl-terpyridine Cu(II) complexes showing that an intercalative mode of binging to DNA and DNA cleavage is dependent on the substitution on the terpyridine\textsuperscript{46}. Manikandamathavan \textit{et al.} reported three mononuclear Cu(II) complexes containing terpyridine, showed the effect of coordinated ligands on DNA cleavage in presence of H\textsubscript{2}O\textsubscript{2} and DNA binding affinity by an intercalative mode\textsuperscript{47}. Abdi \textit{et al.} reported that the binding mode of the Cu(II) complexes of terpyridine to DNA is of an intercalation nature with the planar dppz ligands located between the base pairs of double-stranded DNA\textsuperscript{48}. Basu \textit{et al.} reported the six mononuclear Fe(II) complexes containing terpyridine. The complexes bind to DNA in a partial intercalative mode. The pytp complex efficiently photo-cleaves DNA in green light via superoxide and hydroxyl
radical formation\textsuperscript{49}. Maithy et al. reported seven mononuclear 3d metal [Cu(II), Co(II), Fe(II), Zn(II)] complexes containing ferrocenyl terpyridine as main ligand. The positively charged complexes bind to DNA through electrostatic interactions. Except Cu(II) complex, the other complexes do not show any chemical nuclease activity, but all the complexes exhibit significant plasmid DNA photo cleavage activity in visible light via a photo redox pathway\textsuperscript{50}.

I.3. Elements of DNA structure

The discovery of the double-helical structure of DNA by Watson and Crick in 1953 based on the analysis of X-ray diffraction pattern coupled with careful model building, in which two anti-parallel polynucleotide polymeric chains wound around each other in helical fashion, was a momentous event in science\textsuperscript{51}. This has given rise to entirely new discipline and influenced the course of many established ones. Understanding of the storage and utilization of a cell’s genetic information made possible by this discovery. DNA has been studied for more than half a century with a view to reaching different goals, more particularly in order to develop probes, reagents, or drugs targeting DNA particularly for treatment of cancer. Chemically, nucleic acids are polymeric esters of phosphoric acid. The monomeric unit is called nucleotide which is composed of phosphate group, nitrogen bases (purine or pyrimidine bases \textit{viz.} adenine, guanine, cytosine and thymine) and deoxyribose sugar for DNA\textsuperscript{52}. Nucleosides, the glycosylated nitrogen bases are linked by 3', 5' phosphodiester bonds to form polymeric chain (or strand) of a nucleic acid. The planar bases of each strand stack one above the other in the centre of the helix and the sugar-phosphate backbone is on the outside and carries negative charges on the phosphate groups. This negative charge gets neutralized \textit{in vitro} by the binding of metal ions, typically by Na\textsuperscript{+}. Some positively charged proteins also provide neutralizing force. Between the sugar-phosphate backbone lies the major and minor grooves, which also follow a helical path. The strands are joined noncovalently by hydrogen bonding between the bases on opposite strands to form base pair. Adenine (A) is paired with thymine (T) by two hydrogen bonds; guanine (G) is paired with cytosine (C) by three hydrogen bonds (Fig. 1.1). Mainly, there are three forms of DNA known as A-DNA, B-DNA and Z-DNA (Fig. 1.2). DNA exists in biological systems mainly in so-called B-form, which has a right-handed helical structure, where the base pairs are perpendicular to the helix axis\textsuperscript{53,54}. 

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1.4. Different modes of interaction of small molecules with DNA

Binding of small molecules to DNA has shown significant promise over the past few decades as diagnostic probes, reactive agents and in therapeutics. The unique double helical structure of ds-DNA offers mainly two fundamentally different modes of DNA binding associated with small molecules that interact with DNA. They are covalent and non-covalent. Covalent binding in DNA is irreversible and invariably leads to complete inhibition of DNA replication processes causing subsequent cell death. An example of covalent bonding to DNA is cisplatin with the platinum atom binding covalently to the N7 position of purines to form 1,2- or 1,3-intrastrand crosslinks, and interstrand crosslinks. Non-covalent binding of small molecules to DNA is reversible. Non-covalently bound drugs mostly fall under the following three classes (Fig. 1.3)\textsuperscript{54,55} First one is non-specific electrostatic interaction of positively charged metal ions like Na\textsuperscript{+}, Ca\textsuperscript{2+} or Mg\textsuperscript{2+} or metal complexes with the negatively charged phosphate back-bone leading to surface aggregation. This
electrostatic interaction is the weakest mode of binding. DNA groove binding is second one, which occurs through hydrophobic, van der Waals and hydrogen bonding interactions. Natural antiviral antibiotics distamycin reversibly binds in the minor groove of DNA by hydrogen bonds, van der Waals contacts, and electrostatic interactions with a strong preference for adenine-thymine (AT)-rich sequences containing at least four AT base pairs\textsuperscript{56}. Natural antitumor antibiotic bleomycin is known to bind through the DNA minor groove by pyrimidine moiety in collaboration with the bithiazole tail\textsuperscript{57}. The positively charged groups on the bithiazole tail enhance the binding of bleomycin to DNA electrostatically.\textsuperscript{10} Importantly, groove binders can be designed to span over a number of base pairs and hence can exhibit high degree of sequence-specific recognition sites of nucleic acids, a precondition for anti-cancer therapy and as ‘foot-printing’ agent for DNA- or RNA binding proteins. The third and the strongest among the non-covalent one is the intercalation of planar molecules between the DNA base-pairs, which are stacked perpendicular to the primary axis of the double helix. The complex, among other factors, is thought to be stabilized by $\pi-\pi$ stacking interactions between the drug and DNA bases. Intercalators introduce strong structural perturbations in DNA. Owing to their extended planar structures, ethidium bromide, acridine, proflavin and methylene blue intercalate into DNA base-pairs\textsuperscript{58}. Transition metal complexes like [Ru(bpy)$_2$(tactp)]$^{2+}$ and [Ru(bpy)$_2$(dppz)]$^{2+}$ having planar ring show intercalative mode of binding to DNA\textsuperscript{59}. Additionally, chiral nature of DNA helix gives direct selective binding of chiral compounds. The $\Delta$-enantiomer of [Ru(phen)$_3$]$^{2+}$ prefers binding to DNA in the intercalative binding mode, while the complementary $\Lambda$-enantiomer favors the minor groove binding mode (Fig I.4)\textsuperscript{56}.

I. 5. Types of DNA cleavage

Cleavage of DNA strand can occur by targeting the basic building blocks of DNA, \textit{viz.} nucleobase, sugar or phosphodiester units. Hydrolytic cleavage of DNA involves phosphodiester linkages leading to DNA strand scission. Oxidation of sugar or
nucleobase leading to DNA strand scission is known as oxidative cleavage of DNA. Lewis acidity of the central metal ion, along with its binding affinity towards oxygen (hard) atom and substituional lability are the key criteria for hydrolases. On the other hand, in addition to the principal cleaving agents, presence of co-reactants like reducing or oxidizing agents or light or molecular oxygen is required for the oxidative DNA cleavage reaction.\textsuperscript{50} Ionizing radiation, photooxidation, hydroperoxides, hydroxyl radical, singlet oxygen or many other transient radical species and oxidizing agents can initiate oxidative DNA cleavage.\textsuperscript{61}

\subsection*{1.5.1. Artificial nucleases}

Artificial nucleic acid cleaving agents are gaining considerable attention in last decades due to their potential applications in molecular biology, as structural probes and therapeutic agents.\textsuperscript{62} Although natural enzymes have been used extensively for many applications, but their large size and limited sequence selectivity prevent them from being used for general purposes. The restriction enzymes, which are widely used for the synthesis of recombinant DNA, have a specificity of only 4 – 8 base pairs. Further development of artificial nucleases requiring a specificity of more than 8 base pairs is needed.\textsuperscript{63} Thus designing and developing artificial nucleases that show such specificity of more than 8 base pairs are of interests.

\subsection*{1.5.2. Hydrolytic cleavage of DNA}

The building blocks of DNA are linked through phosphodiester functional groups. Its remarkable hydrolytic stability necessitates the use of enzyme catalysts (nucleases) to mediate strand scission of the phosphate ester backbone \textit{in vivo}. The half-lives of DNA phosphodiester bonds, when extrapolated to physiological conditions, is estimated to be approximately $10^6$ years.\textsuperscript{64} Such extraordinary hydrolytic stability, which ensures the preservation of the genetic information, poses a real challenge in promoting the hydrolysis of stable phosphodiester bond at the reasonably time scale of few hours to few minutes. Large negative charge of the polyanionic backbone that inhibits attack of
nucleophiles hinder the hydrolysis of phosphodiester bond and so neutralization of the charge by bound metal cofactors is one of several mechanisms used by natural nuclease enzymes like purple acid phosphatase, P1 nuclease, S1 nuclease, endonuclease IV (Fig. 1.5)\(^65\). Other than the charge neutralization by metals, Lewis acidity of the central metal ion, affinity towards oxygen (hard) atom and substitutional lability are also the key criteria for the effective hydrolysis. Lewis acidic metal ions such as Mg(II), Ca(II), Co(II), Cu(II) and Zn(II) have been used to cleave the phosphodiester bonds due to the formation of active hydroxide ion as nucleophile. The essential chemical steps involved in DNA hydrolysis are: (i) nucleophilic attack of oxygen of water to phosphorus to give a five coordinate phosphate intermediate; (ii) cleavage of either the P–O3’ or P–O5’ bond (usually P–O3’ cleavage in the enzymatic process) causes a strand scission, yielding the R–OH and R–O–PO\(_3\)(H\(_2\)) termini (Fig. 1.6)\(^66\).

![Fig. 1.6 Different steps involved in the hydrolysis of phosphodiester bond](image)

In the case of DNA, the breakdown of the intermediate is rate limiting. The most effective metal ions have been shown to be lanthanide ions (Ln(III), Eu(III), Dy(III), Ce(IV)) as well as Zn(II), Ca(II), Cu(II) and to some extent Fe(III). Barton’s group first reported metal-activated hydrolysis of supercoiled DNA in 1987 by Zn(II) and Cd(II)-phenanthroline complexes\(^67\). Another interesting development from Barton’s group was combination of both DNA-binding and reactive moieties: (i) a rhodium intercalator which binds in the major groove with high affinity and (ii) a tethered metallopeptide (Fig. 1.7)\(^68\). The complex promotes plasmid DNA cleavage with a rate constant of 2.5 \(\times\) 10\(^5\) s\(^{-1}\) at pH 6 and 37 °C.

![Fig. 1.7 Metal peptide conjugate species Ru(phen)\(_2\)bpy-peptide.](image)
temperature. Cowan et al. reported degradation of plasmid DNA by a mixture of \( \text{Cu}^{2+} \) and kanamycin\(^6\). Dhar and co-workers reported \([\text{Cu(dpq)}_2(\text{H}_2\text{O})](\text{ClO}_4)_2\) complex as a potent DNA hydrolase\(^7\).

### 1.5.3. Oxidative DNA cleavage: general overview

Oxidative cleavage of DNA can be achieved by targeting either the nucleobases or the hydrogen atom of the deoxyribose sugar (Fig 1.8). The DNA-cleavage by different metallonucleases is largely determined by the ability of the compound to generate active oxygen species (ROS). Most reactive intermediates are produced in an aerobic environment or in the presence of co-oxidants. The type of oxidative cleavage, which needs cofactor like reducing agents, is known as “chemical nuclease” activity as exemplified by Fe(II)-bleomycin, Fe(II)-EDTA, \([\text{Cu}(\text{phen})_2]^{2+}\) metalloporphyrins, Ni-peptides, etc\(^{5,6,8,71,72}\). Photo-oxidation of DNA by synthetic nucleases involves an initial oxidation of either a nucleobase or sugar residue. Thus, oxidative cleavage of DNA can be broadly divided into two categories: (i) chemical nuclease activity in presence of external agents like reducing agents and (ii) photo-induced DNA cleavage in presence of light. In most cases, the photoexcited photosensitisier generates ROS like singlet oxygen or \( \text{^1OH} \) radical (hydroxyl radical). The \( \text{^1OH} \) radical being an extremely strong and highly diffusible oxidant with redox potential of 2.8 V, can mediate DNA damage by adding to double bonds of DNA bases or abstracting hydrogen atoms from the sugar moiety\(^{74}\). Transition metal ions in a variety of ligand environments play a key role in the redox cycle to form ROS.

![Fig. 1.8 Different types of Oxidative DNA cleavage](image)

### 1.6. Chemical nuclease activity

Chemical nucleases are redox active coordination complexes that cleave DNA by an oxidative pathway. It is of paramount importance in the field of molecular biology for the
development of DNA footprinting agents and sequence selective targeting of nucleic acids. For example, a copper complex of 1,10-phenanthroline is used in DNA-footprinting experiments and such agents are important for investigating DNA–protein interactions\textsuperscript{75}. Chemical nucleases are able to oxidize the sugar units by hydrogen abstraction. The bleomycins (BLMs) are a group of glycopeptide-derived natural products isolated from \textit{Streptomyces verticillus} that are clinically employed in the treatment of several neoplastic diseases including squamous cell carcinomas, non-Hodgkin’s lymphomas, testicular carcinomas and ovarian cancer (Fig. I.9)\textsuperscript{76,76a} The overall structure of this agent can be thought of as containing four distinct regions consisting of (i) an N-terminal domain, which is responsible for metal binding, oxygen activation, and site-selective DNA cleavage, connected via (ii) a methylvalerate-threonine linker peptide to (iii) a C-terminal domain, containing a bithiazole moiety which provides the majority of the DNA binding affinity, and (iv) a disaccharide moiety consisting of gulose and mannose sugars connected to the metal binding domain. The disaccharide may influence metal ion binding, cell surface recognition, and the selective accumulation of bleomycin in some cells and possibly provides a pocket for oxygen activation. In the presence of molecular oxygen, BLMs show their antitumoral effects through a sequence-selective, metal-dependent oxidative cleavage of DNA and RNA. Although Fe(II)-bleomycin was the first of the metallobleomycins demonstrated to effect DNA strand scission, several other metallobleomycins

\textbf{Fig. I.9.} Structure of bleomycin

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including complexes of Cu(I), Mn(II), VO(IV), Co(III), Ru(II), and Ni(III) also mediate DNA cleavage through various forms of activation, even though less well characterized^{32b}. Fe(II)-bleomycin, which is assumed to be the therapeutically active form of metallobleomycin, is formed by Fe(II) cofactor and bleomycin. After binding to the minor groove of DNA, the drug is activated either by molecular oxygen and two electrons, or by H$_2$O$_2$. The actual identity of the oxidizing species in activated bleomycin has been an issue of debate. The DNA cleavage product analysis predicts initial H-4’ abstraction in oxidative damage of DNA by Fe(II)-BLM. Sigman et al. demonstrated the chemical nuclease activity of 1,10-phenanthroline–copper species Cu(phen)$_2$]$^+$ while studying the mechanism of inhibition of E.coli DNA polymerase by 1,10-phenanthroline (Fig. 1.10). The nuclease activity of Cul(phen)$_2$ proceeds by an ordered mechanism in which the freely diffusing CuII(phen)$_2$ is first reduced to the CuI complex, which then binds reversibly to DNA. Hydrogen peroxide oxidizes this non-covalently bound CuI complex to generate the reactive species that are responsible for DNA strand scission. The oxidizing species could be radical or a copper-bound oxidant such as [CuO]$^+$, [CuOH]$^{2+}$ or CuOOH. Cul(phen)$_2$ is also reported to cleave DNA by a minor pathway that begins with abstraction of H-4’. The reactions involving copper-bis(phen) species are schematically shown below (Fig. 1.11).

\[
\begin{align*}
2 \text{Cu}^{II}(\text{phen})_2 + 2\text{RS}^- & \rightarrow 2 \text{Cu}^I(\text{phen})_2 + \text{RSSR} \\
\text{Cu}^{II}(\text{phen})_2 + \text{O}_2^- & \rightarrow \text{Cu}^I(\text{phen})_2 + \text{O}_2 \\
\text{Cu}^I(\text{phen})_2 + \text{DNA} & \rightarrow \text{Cu}^I(\text{phen})_2-\text{DNA} \\
\text{Cu}^I(\text{phen})_2-\text{DNA} + \text{H}_2\text{O}_2 & \rightarrow [(\text{phen})_2\text{Cu}^{III}-\text{OH}]\text{DNA} + \text{OH}^- 
\end{align*}
\]

**Fig. 1.10** Schematic representation of [Cu(phen)$_2$]$^+$

**Fig. 1.11.** Proposed reactions involving copper-bis(phen) species.

The reaction of [FeIIEDTA]$^{2-}$ with H$_2$O$_2$ is well-known as a technique for studying the structure of DNA, which occur through formation of hydroxyl radical by the typical
Fenton-type reaction\textsuperscript{73}. The enedynes give site-specific DNA breaks after abstraction of hydrogen atoms from the deoxyribose of DNA\textsuperscript{76}. Development of these artificial and selective DNA cleavers is a challenging area in the rational design of antitumoral and antiviral agents as well as in the field of molecular biology. Considerable progress has already been made for the development of highly efficient and highly specific DNA cleaving agents.

I.7. Thiourea ligands

I.7.1 Biological activity of thiourea ligands.

Urea, a naturally occurring compound, became the first organic compound which was synthesized in lab by Wohler in 1928, and played important physiological and biological roles in animal kingdom. Replacement of oxygen atom in urea by sulphur atom produces Thiourea which has been successfully used in many diseases. Mitchell et al explained that ‘Thiourea’ the sulphur analogue of urea has been known for over a century and a quarter during which time it has found a variety of uses, some within the biological field. Most noted of these have been their employments as a plant growth stimulator to break bud dormancy and increase crop yield (1920-40) and more recently as a therapeutic agent to treat thyroid dysfunction (1940-50)\textsuperscript{77}. Yao et al. reported the synthesis of aryl thiourea derivatives and studied their antiproliferative activities against HCT116 and MDA-MB-231 cell lines, and their inhibitory activities against the phosphorylation of VEGFR were evaluated and described. Some of the compounds showed significant activities against both cell lines and VEGFR\textsuperscript{78}. Venkatachelam et al. synthesized various analogues of indolyl, naphthyl and phenylethyl substituted halopyridyl, thiazolyl and benzothiazolyl thioureas and examined their in vitro effects on the high affinity IgE receptor/FeERI-mediated mast cell leukotriene release. Compound N-[2-(4-hydroxyphenyl)ethyl]-N’-[2-(pyridyl)]thiourea (IC\textsubscript{50}=8.5 \mu M) was the most active pyridyl thiourea agents. Notably, the introduction of electron withdrawing or donating groups had a marked impact on the biological activity of these thiourea derivatives\textsuperscript{79}. Vinicius et al. conducted survey on global view on HIV Infection, the data showed that thiourea is a very important functional group for anti-HIV and anti-TB drug discovery, the development of substances containing thiourea moiety and showing a potential activity against both diseases could be considered an important research field, which should be particularly
orientated towards the improvement of therapeutic options for HIV-TB co-infected patients\textsuperscript{80}.

1.7.2 Applications of thiourea ligands

Metal complexes of thiourea and substituted thiourea ligands have been extensively used in various fields of chemistry and biology. Mufakkar \textit{et al.} reported copper(I) complexes of thioureas having the general formulae [CuLnBr] and [CuLn]Br [where, n = 1 - 4 and L = thiourea (Tu), N-methylthiourea (Metu), N-ethylthiourea (ETtu), N,N\textsuperscript{\prime}-dipropylthiourea (Dprtu), N,N\textsuperscript{\prime}-dibutylthiourea (Dbtu) or N,N\textsuperscript{\prime}-diphenylthiourea (Dphtu)]. Antimicrobial activities of the complexes showed that only [Cu(ETtu)\textsubscript{3}]Br was effective in inhibiting the growth of all the tested organisms (Gram-positive, Gram-negative bacteria, and Candida sp.), while the other complexes were not effective against all the organisms\textsuperscript{81}. Bowmaker \textit{et al.} reported the copper(I) complexes with substituted thioureas having the general formula [Cu(tu)\textsubscript{4}]\textsubscript{2}(SiF\textsubscript{6}) and discussed the structural aspects of the synthesized complexes\textsuperscript{82}. Safin \textit{et al.} reported the polynuclear and mixed ligand mononuclear copper(I) complexes with substituted thiourea and 1,10-phenantroline and discussed their structural aspects of the synthesized complexes\textsuperscript{83}. Marverti \textit{et al.} reported the bipyridyl complexes of platinum(II) with thiourea, with different substituents on thiourea moiety. They showed that the anti-proliferative efficacy of these drugs was dependent on molecular structure, since it increased with ancillary ligand bulkiness and hydrophobicity of substituents on thiourea moiety. In particular, the presence of two phenyl groups on thiourea moiety confers an outstanding cytotoxicity. The increasing cell growth inhibition along the series of complexes partially paralleled with drug accumulation, particularly in resistant cells, but not with drug intercalation into DNA since all compounds exerted comparable ethidium bromide displacement ability. The cDDP-resistant phenotype seems, at least in part, to be involved in the action of these compounds, since the level of cross-resistance established for most complexes appeared to be in agreement with the observed impairment of drug accumulation in the resistant subline. These findings indicate that resistance to alkylating agents such as cDDP confers low level of cross-resistance to this class of DNA intercalators, which, however, depending on substituents on thiourea moiety may present remarkable cell growth inhibition even of resistant cells. Marverti \textit{et al.} reported the platinum(II) with diphenyl thiourea, the complexes binds to DNA an intercalative mode and the complex causes reactive oxygen species to form and inhibits
topoisomerase II activity to a greater extent in the sensitive than in the resistant line. The impairment of this enzyme led to DNA damage, as shown by the comet assay\textsuperscript{84,85}.

1.8. Terpyridine ligands

Terpyridine (tpy) ligands have been extensively used in coordination chemistry after its first report by Burstall and Morgan in 1938\textsuperscript{86}. In the solid state for minimizing the electrostatic interactions between the nitrogen lone pairs, the three pyridine rings in 2,2':6',2"-terpyridine exhibit transoid configurations about the interannular carbon-carbon bonds, whereas in metal-bonded condition it adopts the cis-cis-configuration. The terpyridine ligands bind to the metal centre in meridional fashion. The 4'-substituted terpyridines are extensively used, by utilizing various properties of the substituents at this position.

I.8.1 Application of terpyridine ligands

Metal complexes of terpyridine and substituted terpyridine ligands have been extensively used in various fields of chemistry and biology (Fig. I.12)\textsuperscript{87,96}. For example, octahedral transition metal complexes having terpyridine ligands have been studied extensively in order to capitalize their unique photophysical properties, electrochemical, emission, magnetic, and optical properties.

The square planar Pt(II) terpyridine and substituted terpyridine complexes have been extensively investigated for their unique luminescent properties offering potential applications in chemosensing for solvents and metal ions, photocatalysis, as well as biological activities, covalent binding to biomolecules with potential applications as antitumor, radiotherapy, antiprotozoal agents and protein probes\textsuperscript{87}. Bertrand and co-workers have reported one square planar Pt(II) complex [Pt(tpy)Cl]Cl, which interacts with human telomeric G-quadruplex-DNA via platination of the adenine residues of the loop of antiparallel G-quadruplex, which depends upon the aromatic surface of the ligand (Fig. I.13). This approach could lead to the discovery of new anticancer drug as the molecule stabilizing the G-quadruplex can lead to an arrest of proliferation of cancer cell\textsuperscript{88}.
Similarly, the potential of (2,2':6',2"-terpyridine) platinum(II) complexes, reported by Katja Becker, acting simultaneously at two different intracellular target hTrxR (reduced thioredoxin reductase) and DNA, make them potent antitumor agents. A range of (2,2':6',2"-terpyridine)platinum(II) complexes such as [Pt(tpy)Cl]Cl. (Fig. I.13 Molecular structure of (hydroxyethanethiolato) (2,2':6',2"-terpyridine)platinum(II), (2-aminoethanethiolato)(2,2':6',2"-terpyridine) platinum(II), (4-picoline)(2,2':6',2"-terpyridine)platinum(II) are shown to possess antiprotoszoal activity in vitro against Leishmania donovani, Trypanosoma cruzi and Trypanosoma brucei, the causative organisms of tropical diseases leishmaniasis and trypanosomiasis. Terpyridine ligands have also found very useful application in the field of supramolecular chemistry which has led to the formation of complexes showing racks, ladders, grids, knots, catenanes as well as dendrimers and due to their versatile chemical and photochemical properties, these metal complexes have been extensively studied. As catalysts, tpy complexes of transition metals have found special interest, and the higher oxidation state of transition metals, e.g. Ru(IV) or Ru(VI), have been applied to the oxidation of alcohols, in the carbonylation of aromatic compounds, and hydroformylation. Although 2,2':6',2"-terpyridine and substituted terpyridine have been extensively used in various fields of chemistry and biochemistry, interestingly, the use of terpyridine ligand in DNA interaction studies is very less. Qin Jiang et al. have reported zinc(II) terpyridine complexes, these terpyridine complexes are show a good nuclease activity. Because of the presence of adenine moiety in complex 1, more significant structural change of DNA and more effective DNA cleavage activity were observed than complex 2.

I.9. Antibacterial activity of metal complexes
In thiourea derivatives, the C=S linkage is essential for biological activity, several thiourea have been reported to possess remarkable antibacterial, antifungal, anticancer and antimalarial activities. Accurate determination of bacterial susceptibility to antibiotics is essential to the successful management of bacterial infections and to the comparative analysis of antimicrobial agents. This can be done by a number of techniques, which include the disc diffusion method, the broth dilution assay and the E
tests. Rajesh et al. reported the synthesis of 3,4-Dihydropyrimidin-2(1H)-ones thiourea derivatives and studied their antibacterial activity of compounds, results revealed promising antimicrobial activity at minimum inhibitory concentration of 10–40 μg/ml against selected pathogenic bacteria and fungi\textsuperscript{98}. Zhong et al. reported the acyl thiourea derivatives of chitosan (CS), studied their antimicrobial activity, results indicated that the antimicrobial activities of the acyl thiourea derivatives are much better than that of the parent CS. The minimum value of MIC and MBC of the derivatives against E. coli was 15.62 and 62.49 μg/mL, respectively. All of the acyl thiourea derivatives had a significant inhibitory effect on the fungi in concentrations of 50–500 μg/mL; the maximum inhibitory index was 66.67%. The antifungal activities of the chloracetyl thiourea derivatives of CS are noticeably higher than the acetyl and benzoyl thiourea derivatives. The degree of grafting of the acyl thiourea group in the derivatives was related to antifungal activity; higher substitution resulted in stronger antifungal activity\textsuperscript{99}. Saced et al. reported the antimicrobial activity of the thiourea derivatives of the benzothiazole moiety some of the compounds exhibited the greatest antimicrobial activity. Preliminary study of the structure–activity relationship revealed that electronic factors in benzothiazole rings had a great effect on the antimicrobial activity of these compounds. In preliminary MTT cytotoxicity studies, the thiourea derivatives were found most potent\textsuperscript{100}. Patel et al. reported the synthesis and antibacterial activity of thioureido amide of fluroquinalone, all the compounds showed the significant antibacterial activity against \textit{K. pneumonia}, \textit{S. aureus}, \textit{P. aeruginosa} and \textit{E. coli} bacterial strains\textsuperscript{101}. Many thioureas are known to be medicinally important and used to design medicinal compounds. Recently, a wide varieties of the Cu(I), Cu(II), Ni(II) and Zn(II) complexes of thiourea derivatives including compartmental and macrocyclic ligands were tested \textit{in vitro} for their antibacterial activities against different types of Gram-positive and Gram-negative bacteria using disc diffusion method.\textsuperscript{102,103} For the bacterial organisms, both Gram positive and Gram-negative bacteria were used. Gram-positive and Gram-negative can be differentiated in physical appearance of their cell envelopes. In general, bacteria have the genetic ability to send and acquire resistance to drugs, which are used as therapeutic agents. Even through the pharmaceutical industries have produced a lot of new antibiotics in the last three decades, the resistance of microorganisms to these drugs has increased. The microbial resistance represents a problem and the outlook for the use of antimicrobial drugs in the future is still
uncertain. Therefore, measures must be taken to reduce this problem, for example, to control the use of antibiotic, develop research for better understanding the genetic mechanisms of resistance and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient. For centuries, people have used copper and cobalt ions to inhibit the growth of harmful microbes. The development of more effective chemotherapeutic agent has been the main goals of coordination chemists over the years it has been discovered that metal complexes have enormous impact in medicine. The use of metal complexes capable of enhancing biological activity has become a vibrant and growing area of research among inorganic chemists and biologists over the last few decades. Some metal complexes have been found to have antimicrobial and antiviral properties and could be effective against diseases.\textsuperscript{104}

Many copper complexes biologically active due to their chelating ability and positive redox potential values. Copper(II) complexes exhibited enhanced antibacterial activity against bacteria \textit{Staphylococcus aureus}, \textit{Bacillus subtilis}, \textit{Seratia marcescens}, \textit{Escherichia coli}, \textit{Pseudomonas aeruginosa}, when compared to ligands and metal salts. The ligand 1-phenyl-2,3-dimethyl-4-(N-2-hydroxy-4-methoxy-benzaldehyde)-3-pyrazolin-5-one and their copper complexes show greater bacterial activities against \textit{K. pneumonia}, \textit{S. aureus}, \textit{P. aeruginosa} and \textit{E. coli} bacterial strains as compared to their corresponding ligand systems.\textsuperscript{105}

The thesis consists of seven chapters.

**Chapter 1:** of the thesis presents a concise overview of the chemistry of organic conjugates and metal complexes and their interaction with DNA. It gives an outline of different types of DNA cleavage, DNA cleavage reaction mechanisms and the metal complexes in different types of nuclease activity emphasizing oxidative DNA cleavage activity. The objective of the present investigation is dealt in this chapter.

**Chapter 2:** describes the synthesis and characterization of 1-Benzyl-3-(4-ethyl-pyridin-2-yl)-thiourea (4ETU) and copper(I) complexes [Cu(4ETU)\textsubscript{2}Cl] \textsubscript{2} (1), [Cu(4ETU)\textsubscript{2}B]Cl \textsubscript{2} (2) where B is a N,N-donor heterocyclic base, viz. 1,10-phenanthroline (phen, 2), by elemental analysis, conductivity measurements and with the help of spectroscopic techniques viz., UV-visible, IR, TGA, NMR, ESI-MS and single crystal data, their DNA binding, cleavage and antibacterial activity studied. The ligand is structurally characterized by single crystal X-ray crystallography. Binding interactions of the complexes with calf thymus (CT) DNA have been investigated by emission, absorption,
viscosity and DNA thermal denaturation studies. The extent of oxidative DNA cleavage activity is accessed by gel electrophoresis followed by gel documentation and calculation processes.

Chapter 3: describes the synthesis and characterization of 1-Benzyl-3-(4-methyl-pyridin-2-yl)-thiourea(4MTU) and 1-Benzyl-3-(6-methyl-pyridin-2-yl)-thiourea (6MTU), copper(I) complexes [Cu(4MTU)₂Cl] (3), [Cu(4MTU)(B)Cl] (4), [Cu(6MTU)₂Cl] (5) and [Cu(6MTU)(B)Cl] (6) where B is a N,N-donor heterocyclic base, viz. 1,10-phenanthroline (phen 3, 6), by elemental analysis, conductivity measurements and with the help of spectroscopic techniques viz., UV-visible, IR, TGA, NMR and ESI-MS, their DNA binding, cleavage and antibacterial activity studied. Binding interactions of the complexes with calf thymus (CT) DNA have been investigated by emission, absorption and viscosity studies. The extent of oxidative DNA cleavage activity is accessed by gel electrophoresis followed by gel documentation and calculation processes.

Chapter 4: describes the synthesis, characterization of 4-(2,6-di(pyridine-2-yl)pyridine-4-yl)quinolone (4qtpy) and 1-(2,6-di(pyridine-2-yl)pyridine-4-yl)quinolone (2qtpy) and seven new 3d metal complexes of general formulation [M(4qtpy)₂] 2(ClO₂) (1-3), [M(2qtpy)₂] 2(ClO₂) (7-13) where, M is Cu(II) (7,10), Co(II) (8,11), Zn(II) (9,12) Fe(II) (13) are synthesized, characterized by elemental analysis, conductivity measurements and with the help of spectroscopic techniques viz., UV-visible, IR, NMR, ESI-MS and single crystal X-ray diffraction method. Complexes 7 and 10 are characterized by single X-ray crystallographic method. The complexes show octahedral (CuN₆) coordination geometry. Their DNA binding, cleavage, antibacterial activity and PsaA Competitive Zn²⁺-binding Assay method studied. Binding interactions of the complexes with calf thymus DNA (CT- DNA) were investigated by UV-visible absorption titration, visometric titration and cyclic voltammetric titration studies. The chemical nuclease activity in the presence of 3-mercaptopropionic acid (MPA) and H₂O₂ was studied by gel electrophoresis method.

Chapter 5: describes the synthesis, characterization of 4-qtpy is 4-(2,6-di(pyridine-2-yl)pyridine-4-yl)quinolone(4qtpy), and 1-(2,6-di(pyridine-2-yl)pyridine-4-yl)quinolone (2qtpy) and six new Ternary copper(II) complexes of general formulation [Cu(4qtpy/2qtpy)(B)] 2(ClO₂) (14-19) where, B is a N, N-donor heterocyclic base, viz.,
1, 10-phenanthroline (phen, 14, 17) dipyrido[3,2-2',3'-f]quinoxaline (dpq, 15, 18) and dipyrido[3,2-a:2',3'-c]phenazine (dppz, 16, 19) are synthesized, characterized by elemental analysis, conductivity measurements and with the help of spectroscopic techniques viz., UV-visible, IR, ESI-MS and single crystal X-ray diffraction method. Complex 17 was characterized by single X-ray crystallographic method. The complex show octahedral (CuN₅O) coordination geometry. Binding interactions of the complexes with calf thymus DNA (CT-DNA) were investigated by UV-visible absorption titration, viscometric titration experiment and cyclic voltammetric titration studies. The chemical nuclease activity in the presence of 3-mercaptopyrrolic acid (MPA) was monitored by gel electrophoresis technique followed by gel documentation system.

Chapter 6: describes the synthesis of novel Benzimidazole derivatives, imidazole derivatives and pyrimidine derivative compounds (4a-i) and characterization by various spectroscopic methods viz., IR, ESI-MS, NMR and single crystal X-ray diffraction technique. DNA cleavage activity was studied, compounds were screened in-vitro for their antibacterial activity against clinical isolates like *Bacillus subtilis*, *Bacillus mycodies*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*. In-vitro antibacterial activities were determined by agar well-diffusion method.

Chapter 7: Summary and Conclusion: The major objective of thesis work is summarized on the developed chemistry of copper(I), copper(II), cobalt(II) zinc(II) and iron(II) complexes showing oxidative DNA cleavage activity. Based on this objective different classes of binary and ternary complexes have been synthesized, characterized from X-ray diffraction and spectral data and their DNA binding, cleavage properties and antibacterial activity studied. Several complexes have been designed to study the oxidative DNA cleavage activity and to explore the structural requirements.

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Chapter I


