SYNTHESIS, CHARACTERIZATION, OXIDATIVE DNA CLEAVAGE AND DNA BINDING STUDIES OF MIXED LIGANDS COPPER(II) COMPLEXES

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By

TAN YI HAN

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LIST OF ABBREVIATIONS AND SYMBOLS

AAS	–	Atomic absorption spectroscopy
BPEI	–	Branched polyethyleneimine
byp	–	2,2’-bipyridyl
C	–	Carbon
chrysi	–	5,6-chrysenequinone diimine
Cl	–	Chlorine
-COOH	–	Carboxylic acid
CT-DNA	–	Calf Thymus DNA
DIP	–	4,7-diphenyl-1,10-phenanthroline
dmByp	–	4,4’-dimethyl-2,2’-dipyridyl
DNA	–	Deoxyribonucleic acid
dppz	–	dipyrido[3,2-a;2’,3’-c]phenazine
dpq	–	dipyrido[3,2:2’3’-*f*]quinoxaline
FT-IR	–	Fourier Transform Infrared Spectroscopy
H	–	Hydrogen
H_2O_2	–	Hydrogen peroxide
K_b (M^{-1})	–	Intrinsic binding constant
KBr	–	Potassium bromide
MPA	–	3-mercaptopropionic acid
N	–	Nitrogen
$-NH_2$	–	Amine
O	–	Oxygen
P	–	Phosphorus

PDT	–	Photodynamic therapy
ph	–	*o*-phthalic acid
Phe	–	Phenylalanine
phen	–	1,10-phenanthroline
RNA	–	Ribonucleic acid
ROS	–	Reactive oxygen species
saltrp	–	Salicylidene tryptophan
SC	–	Supercoiled
TATP	–	1,4,8,9-tetra-aza-triphenylene
Thr	–	Threonine
UV	–	Ultra Violet
UV-Vis	–	Ultra Violet-Visible
Val	–	Valine
ε (M^{-1} cm^{-1})	–	Molar absorption coefficient
λ (nm)	–	Wavelength
τ	–	Trigonal distortion parameter

SINTESIS, PENCIRIAN, KAJIAN BELAHAN DNA OKSIDATIF DAN PENGIKATAN DNA UNTUK KOMPLEKS KUPRUM (II) LIGAN CAMPURAN

ABSTRAK

Sebanyak enam kompleks kuprum(II) ternari bagi asid amino dan ligan polipiridil dengan formula am [Cu(AA)(B) Cl]·nH_2O atau [Cu(AA)(B)(H_2O)]Cl·nH_2O, di mana AA = DL-threonina, DL-fenilalanina, dan DL-valina; B = 2,2'-bipiridil, 4,4'-dimethyl-2,2'-dipiridil, dan 1,10-fenantrolina telah disintesiskan. Kompleks ini telah dicirikan dengan analisis unsur CHN, AAS, FT-IR dan UV-Vis serta struktur kompleks ini telah ditentukan secara kristalografi sinar-X. Semua kompleks membentuk geometri koordinatan piramid segiempat terherot. Spektrum penyerapan elektronik bagi kompleks ini menunjukkan keamatan d-d jalur elektronik yang sangat rendah dengan julat 600-635 nm di dalam Tris-HCl/NaCl (5:5 mM) pH 7.2 larutan tampan. Interaksi pengikatan DNA dengan CT-DNA telah dikaji oleh spektrum titratan penyerapan elektronik dan pengukuran kelikatan. Keputusan menunjukkan bahawa kompleks fenantrolina berinteraksi dengan CT-DNA melalui interkalasi manakala kompleks bipiridil mengikat CT-DNA melalui mod pengikatan alur. Pemalar pengikatan intrinsik (K_b) yang dikira bagi semua kompleks adalah dalam julat 0.5-6.2 × 10^5 M^{-1}. Semua kompleks kuprum(II) didapati mempamerkan aktiviti belahan DNA yang efisien pada kepekatan yang rendah dengan kehadiran H_2O_2. Kajian menunjukkan bahawa [Cu(Phe)(Phen) Cl]·3H_2O mempamerkan pengikatan DNA dan aktiviti nukleasa yang paling tinggi dalam kalangan enam kompleks kuprum(II).

SYNTHESIS, CHARACTERIZATION, OXIDATIVE DNA CLEAVAGE AND DNA BINDING STUDIES OF MIXED LIGANDS COPPER(II) COMPLEXES

ABSTRACT

Six ternary copper(II) complexes of amino acids and polypyridyl ligands with a general formula of $[Cu(AA)(B)\ Cl]\cdot nH_2O$ or $[Cu(AA)(B)(H_2O)]Cl\cdot nH_2O$, where AA = DL-threonine, DL-phenylalanine, and DL-valine; B = 2,2'-bipyridyl, 4,4'-dimethyl-2,2'-dipyridyl, and 1,10-phenanthroline are synthesized. The complexes are characterized by CHN elemental analysis, AAS, FT-IR, and UV-Vis and have been structurally characterized by X-ray crystallography. All the complexes formed slightly distorted square-pyramidal coordination geometries. The electronic absorption spectra of the complexes show very low intensity of d-d electronic band in the range of 600-635 nm in Tris-HCl/NaCl (5:5 mM) pH 7.2 buffer solution. The DNA binding interaction with CT-DNA has been investigated by electronic absorption spectral titration and viscosity measurements. The results revealed that the phenanthroline complexes interact with CT-DNA through intercalation while bipyridyl complexes bind to CT-DNA through groove binding mode. The calculated intrinsic binding constant (K_b) of all the complexes are in the range of $0.5\text{-}6.2 \times 10^5\ M^{-1}$. All the copper(II) complexes are found to promote efficient DNA cleavage activities at low concentration in the presence of H_2O_2. The studies show that $[Cu(Phe)(Phen)Cl]\cdot 3H_2O$ exhibits the highest DNA binding and nuclease activity among the six copper(II) complexes.

CHAPTER 1

INTRODUCTION

1.1 Cancer diseases

Cancer can be defined as a disease which cells in the body grow or divide uncontrollably and the ability of these cells to invade other tissues within the body (What, 2013). The cells migrate through lymph and blood system to other part of the body. If the spread of the cells is not controlled, it can cause death. In another meaning, cancer is a disease of gene which is a small part of DNA, whereas DNA is the master molecules of the cells. Majority of the cancer are caused by changes in the cell's DNA through mutation which disrupt the normal function of the gene to produce protein. As a result, these cells become abnormal and divided uncontrollably.

In Malaysia, the cancer disease is growing in a worrying trend. Among all the medically certified deaths, it ranks number four. The annual incidence of cancer is estimated to be around 30000 cases. However, the incidence of cancer is expected to increase due to aging populations. It was reported that there is 4.6 % of people aged 60 above were diagnosed with cases in 1957, the number increased to 5.7 % in 1990 and estimated to reach 9.8 % in 2020 (Lim, 2002).

1.1.1 An introduction to Deoxyribonucleic Acid (DNA)

Deoxyribonucleic acid (DNA) was discovered serendipitously by a Swiss chemist, Friederich Miescher in 1869, when he tried to determine the chemical composition of the cells. DNA is a nucleic acid that functions as the hereditary material in humans and almost all the other organisms. Besides RNA and protein,

DNA is among the three major macromolecules that are vital to the earth's ecosystem. Most of the DNA located in the cell nucleus (nuclear DNA) and a small amount of DNA can be found in the mitochondria (mitochondrial DNA).

Basically, double helix DNA consists of two complimentary long polymeric chains of simple units called nucleotides which twisted against each other, with backbones made of sugar residues and phosphate groups joined by ester bonds. Nucleotides are the building blocks of nucleic acids. These two DNA strands run in anti-parallel directions which always assembled in the 3’ to 5’ direction. A phosphodiester linkage exists between a phosphate group on one nucleotide and the sugar molecule on the adjacent nucleotide. The DNA double helix is stabilized by hydrogen bonds between the nucleotides and the base-stacking interaction among the aromatic nucleobases.

The sequence of the four nucleobases along the backbone encodes the hereditary information. The information in DNA is stored as a code and read using the genetic code, which specifies the sequence of the amino acids within proteins. The code is made up of four chemical bases, that is, adenine (A), cytosine (C), guanine (G) and thymine (T). These four nitrogen-containing bases are divided into purines (adenine and guanine) and pyrimidines (cytosine and thymine). Adenine binds to thymine and guanine binds to cytosine to form a complementary base pair. Each base pair is attached to a sugar molecule and a phosphate molecule to form a complete nucleotide.

The sugar phosphate backbone of paired strands defines the helical grooves, within which the edges of the heterocyclic bases are exposed. These grooves are adjacent to the base pairs and may provide a binding site. The biologically relevant

B-form structure of the DNA double helix is characterized by a shallow, wide major groove and a deep, narrow minor groove where both grooves offer a number of hydrogen binding sites. The chemical structure of a DNA double helix is illustrated in Figure 1.1.

Numerous reports suggested that DNA is the primary intracellular target for most of the anticancer and antiviral therapies according to cell biologist (Kumar *et al.*, 2008*a*). The interaction between DNA and small molecules through binding can lead to DNA damage in cancer cells, blocking the distribution and growth of cancer cells, hence resulting in the cancer cell death. This damage could cause major pathological changes in living organisms (Kumar & Arunachalam, 2009). Therefore, it is vital to develop potential anticancer agents that are capable of binding and cleaving DNA under physiological conditions (Rajendiran *et al.*, 2007; Raman & Selvan, 2011; Chen *et al.*, 2011; Kashanian *et al.*, 2012).

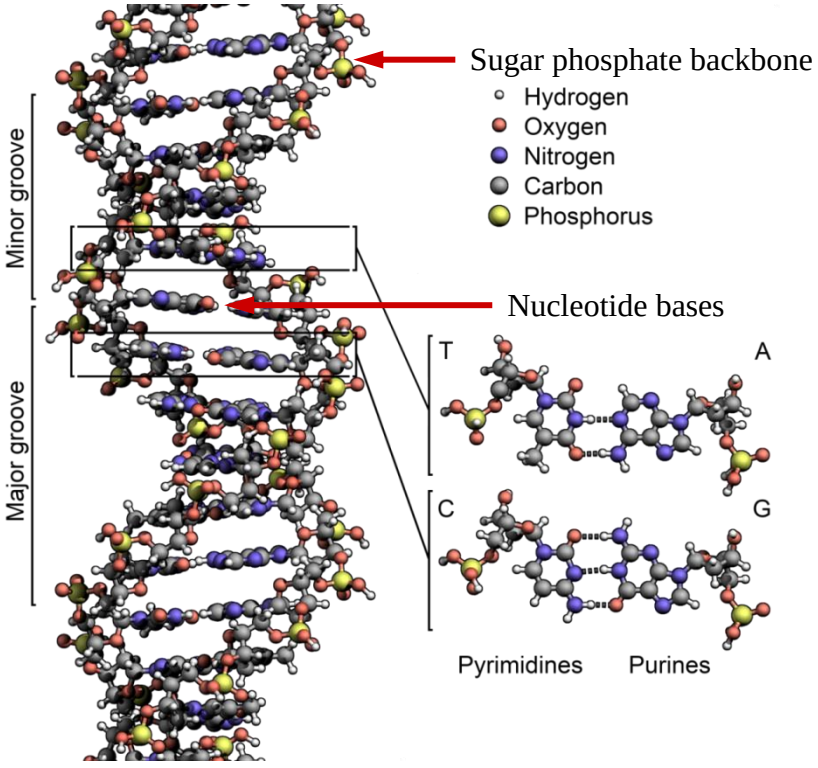


Figure 1.1: Chemical structure of a DNA double helix (DNA, 2011)

1.2 Background of nucleolytic activity of transition metal complexes

The DNA cleavage process is regarded as one of the vital process in the ecosystem. The function of anticancer drugs is its capability to cause damage to cells such as bleomycin in the DNA. This damage can cause apoptosis which leads to the death of the affected cell. (Chen & Stubbe, 2005).

Transition metal nucleolytic activity has undergone intensive studies which lead to the breakthrough in effective interaction of anticancer agents with DNA and further induces DNA cleavage in the presence of co-factor. The research on nucleolytic activity is very important in attaining the formation of DNA sequence and forbidding the replication of cancer cell (Yang *et al.*, 2004*a*). Several transition metal complexes such as copper, ruthenium, cobalt, zinc, nickel, vanadium and manganese complexes have been reported to exhibit DNA cleavage activities. Such complexes could interact with DNA and promote the breakage of DNA strands by applicable methods. For instance, the DNA double strands of cancer genes break after the strands are cleaved and the capability for replication of cancer gene is damaged (Raman *et al.*, 2007).

In order to monitor the progress of DNA cleavage by transition metal complexes, agarose gel electrophoresis is the most common method applied in biochemistry and molecular biology to demonstrate damage incurred on DNA. Plasmid pBR322 DNA is one of the commonly used DNA strains which is a double helix DNA and exists in supercoiled form. In general, the supercoiled DNA (Form I) will relax and convert to nicked form (Form II) when the DNA scission occurs in one strand of the supercoiled DNA. On the other hand, if the DNA scission takes place on both strands, a linear form (Form III) will be generated. These three forms exhibit

different migration rate in the gel electrophoresis (Figure 1.2). The supercoiled form will be the fastest to migrate while the nicked form is the slowest. The linear form migrates in the intermediate which is between supercoiled and nicked forms. The movement of DNA bands is achieved by moving the negatively charged nucleic acid molecules through an agarose gel matrix with the application of an electric field (electrophoresis).

The efficiency of DNA cleavage is varied with some complexes able to induce both single and double strand scissions whereas some complexes can only bring about single scission. Generally, DNA cleavage can be categorized in three different types which are hydrolytic DNA cleavage, photochemical DNA cleavage and oxidative DNA cleavage (Li *et al.*, 2010). Photochemical and oxidative DNA cleavages are quite closely related.

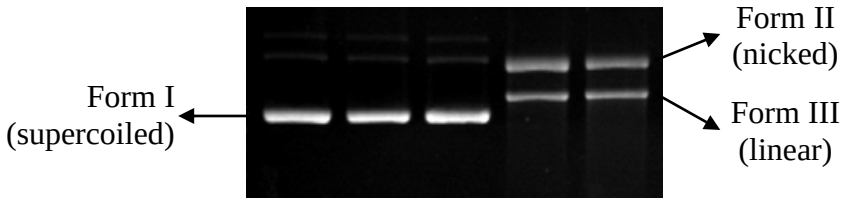


Figure 1.2: Supercoiled, nicked and linear DNA bands in gel electrophoresis diagram

1.2.1 Hydrolytic DNA cleavage

Hydrolytic DNA cleavage involves the hydrolysis of phosphodiester bond. The transition metal complexes can promote DNA cleavage directly by cleaving the P-O bond in phosphodiester of DNA, leading to fragmentation of DNA which could be then religated. Hydrolytic cleavage can occur without any reductants or oxidants. The active species of hydrolytic cleavage are capable of mimicking restriction enzymes.

The hydrolysis reaction is made possible by the existence of Lewis acids which in the case is the metal ions. The function of these Lewis acids is to activate the phosphate group towards the nucleophilic attack, where Lewis acids withdraw the electron and weakening the P-O bond, water or hydroxide acts as nucleophile to attack and break the bond. Generally, the DNA hydrolysis reaction mechanism is the nucleophilic attack. The nucleophile attacked the DNA phosphate backbone which resulted in the formation of five coordinate intermediates. These intermediates will be stabilized by the catalyst as shown in Figure 1.3. As for subsequent cleavage of either the 3'-PO or the 5'-PO, it will result in a strand scission (Mancin *et al.*, 2005).

Among the transition metal complexes that are used as synthetic hydrolases, zinc(II) and copper(II) are suitable for hydrolytic DNA cleavage owing to their strong Lewis acid properties. Li and co-workers studied the DNA cleavage activity of four new copper(II) complexes and found that all complexes could cleave plasmid DNA effectively through hydrolytic pathway (Li *et al.*, 2010).

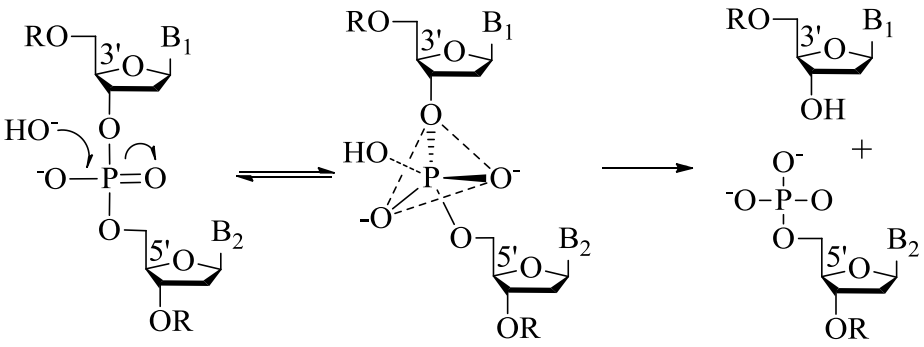


Figure 1.3: Proposed reaction mechanism for the hydrolytic cleavage of DNA (Mancin *et al.*, 2005)

1.2.2 Photochemical DNA cleavage

Photochemical cleavage of DNA is a type of oxidative DNA cleavage on photoirradiation which plays a significant role in photodynamic therapy (PDT) of

cancer (Patra *et al.*, 2007; Sasmal *et al.*, 2007). It can be categorized into two types which are the DNA cleavage through the generation of reactive oxygen species (ROS), or by photochemical means and direct UV-induced DNA damage. The later induced DNA damage happens through the formation of mutagenic and cytotoxic DNA lesions. This formation is possible due to the presence of two pyrimidine residues which are thymine and cytosine. Both the pyrimidine residues react through a direct UV-promoted dimerization. (Sinha & Häder, 2002).

In the oxidative and photochemical DNA cleavage, reactive intermediates are produced, which are the two similar pyrimidine residues. As for the photochemical DNA cleavage, either the metal complexes or the organic compounds are responsible for producing the reactive intermediates. In most of the situation, DNA-cleaving agents produce extremely reactive intermediates that cause damage to DNA. DNA cleaving agents itself neither directly responsible nor attack on the DNA (Burrows & Muller, 1998). In turn, strand scission happens in results of the auto-oxidation reactions from the damaged DNA.

There are two types of DNA damage initiated by photosensitization (Figure 1.4), a one electron process (Type I process) and a pathway involving singlet oxygen (Type II process). In the Type I process, through the excitation of the cleaving agent, it generates sequentially a superoxide radical from molecular oxygen via an electron transfer step. Due to the properties of superoxide which is a poor oxidant, it could act as a strong reductant. The DNA damage observed through this pathway is mainly guanine oxidation, formed via guanine radical cations. This results in the formation of base labile sites in the DNA. In a Type II process, a contrast to superoxide, the photo excited compound generates singlet oxygen, which modifies guanine residues. There are two pathways that can be distinguished in Type II process (Figure 1.5). A

Diels-Alder reaction with singlet oxygen results in the formation of 4,8-digydro-4-hydroxy-8-oxo-dG, and further reduction in 8-oxo-dG. A [2+2] cycloaddition with singlet oxygen results after a cascade of reactions in the formation of cyanuric acid. The modified residues are base labile positions in the DNA and alkaline work-up is required to initiate strand breaks (Paillous & Vicendo, 1993).

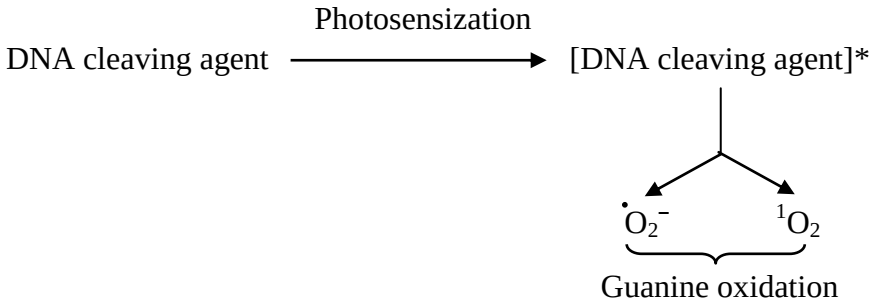


Figure 1.4: Type I and Type II photochemical cleavage of DNA (Paillous & Vicendo, 1993)

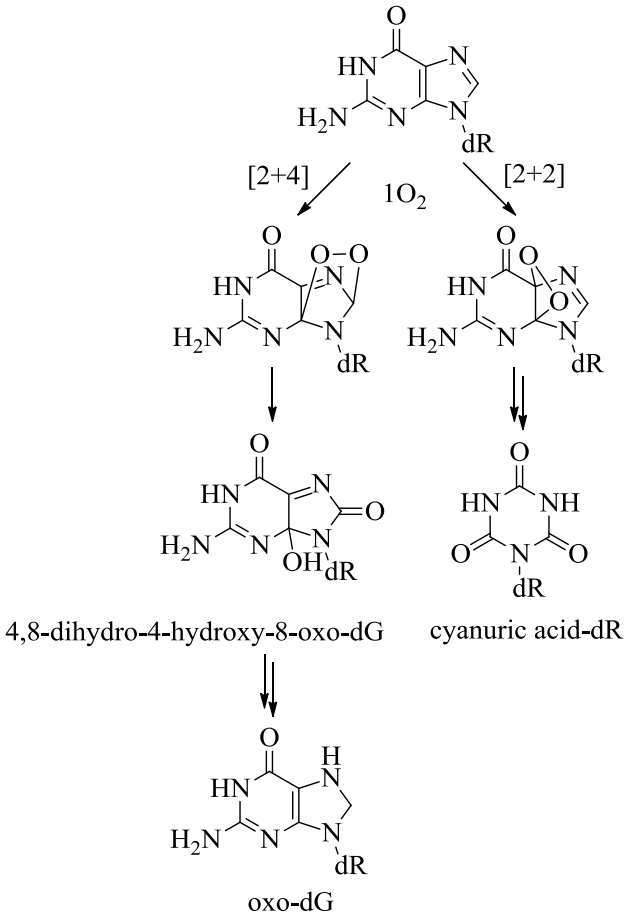


Figure 1.5: Different reaction pathways of guanine with singlet oxygen (Paillous & Vicendo, 1993)

1.2.3 Oxidative DNA cleavage in the presence of H_2O_2/MPA/Ascorbic acid

In oxidative DNA cleavage, a transition metal complex cannot induce the cleavage activity directly but indirectly through generating ROS by a redox active metal complex such as hydroxyl radical and singlet (Jiang *et al.*, 2007). Oxidative DNA cleavage takes place in the presence of additional oxidizing or reducing agents such as hydrogen peroxide (H_2O_2), 3-mercaptopropionic acid (MPA) and ascorbic acid (Sasmal *et al.*, 2007).

A massive number of oxidative DNA cleavage reagents have been utilized with great success for DNA foot-printing and as chemotherapeutic agents. There are some strong reductive agents act as inducing agents to produce radicals that are necessary being involved into the system of DNA cleavage reaction (Yang *et al.*, 2004*a*). The oxidative process results in either oxidation of nucleobases or degradation of sugar moiety by abstracting sugar hydrogen atom. Among four nucleobases, the guanine with purine base is the most susceptible for oxidation process (Patra *et al.*, 2007; Rao *et al.*, 2007; Sancheti *et al.*, 2012).

The interaction of these intermediates with the sugar moiety (Pogozelski & Tullius, 1998) or with nucleobases (Burrows & Muller, 1998) can result in direct strand cleavage or the formation of labile sites in the DNA. Besides these oxygen based intermediates, metal bound active intermediates are also known, such as intermediates in the catalytic cycle of iron bleomycin (*vide infra*). Figure 1.6 shows the proposed oxidative DNA cleavage mechanism of guanine by copper(II) complex in the presence of H_2O_2.

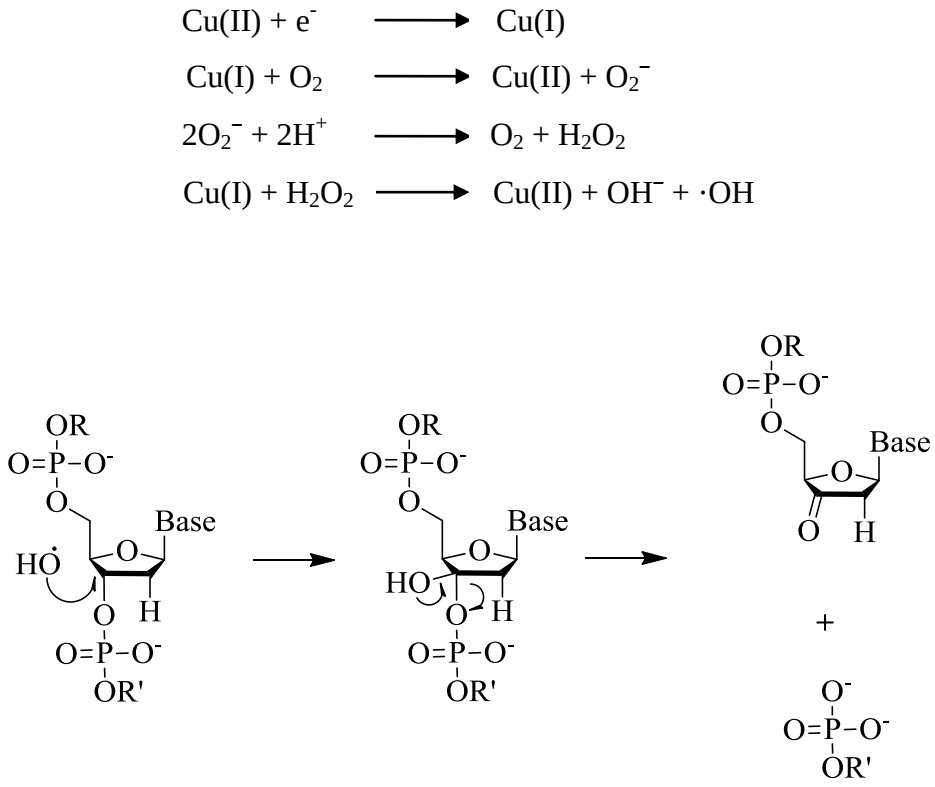


Figure 1.6: Proposed oxidative DNA cleavage mechanism of guanine by copper(II) complex in the presence of H_2O_2 (Burrows & Muller, 1998)

1.3 DNA interaction with transition metal complexes

Since DNA is the basis for the storage, transmission and expression of genetic information, any reaction or damage caused to it will have important consequences. There has been a large interest in exploring the factors that determine kinship and selectivity in binding small molecules with DNA. A quantitative understanding of such factors that determine the recognition of DNA sites would be valuable in designing small molecules that binds to specific sites in DNA and applies it in chemotherapy. A number of metal chelates mostly polypyridyls have been used as probes of DNA structure in solution, as agents for mediation of strand scission of duplex DNA and as chemotherapeutic agents (Lippard, 1978; Hirohama *et al.*, 2005).

Transition metal complexes need to bind with DNA before they can cleave the DNA. Thus, there are several experimental techniques to determine the binding modes of complexes with DNA such as absorbance spectroscopy, fluorescent dye displacement assays, thermal stability, Scatchard analysis, viscometric titration, circular dichroism spectroscopy, electric linear dichroism, footprinting, fourier transform infrared spectroscopy, multi-dimensional nuclear magnetic resonance, X-ray crystallography, confocal microscopy, *Taq* polymerase stop assay, and topoisomerase inhibition. The determination of the physical properties of DNA and small molecules is a practical tool as these properties change significantly with complex formation (Reddy *et al.*, 1999).

Basically, metal complexes could bind to DNA via covalent and non-covalent interactions (Patel *et al.*, 2010*a*; Lakshmipraba *et al.*, 2011). Covalent binding is irreversible and takes place when the metal complexes bind directly to one or more atoms, which are located at the surface of the DNA molecule. The replacement of labile ligand of metal complexes by a DNA nucleobase occurs in covalent binding with the coordination through guanine N7. Conversely, the non-covalent DNA interaction is reversible and takes place along the outer site of the DNA helix, either in major or minor groove. There are three different categories of non-covalent DNA interactions, which are intercalation, groove (surface) and electrostatic binding of cationic transition metal complexes (Peyratout *et al.*, 1995; Ni *et al.*, 2006; Kumar *et al.*, 2007, 2009; Jia *et al.*, 2010; Dixit *et al.*, 2011).

1.3.1 Intercalation

The intercalation binding involves the insertion of a planar polycyclic aromatic ring system into a π-stack between two DNA base pair (Ihmels & Otto,

2005; Keene *et al.*, 2009; Anbu & Kandaswamy, 2011*a*; Suntharalingam & Vilar, 2011). Figure 1.7 demonstrates the example of an intercalation binding mode of ellipticine with DNA (Boer *et al.*, 2009). There are several significant driving forces for this binding mode such as dipole-dipole interactions, electrostatic factors, π-stacking and dispersive interactions of the guest molecules with the aromatic nucleic bases (Ihmels & Otto, 2005). The stacking interaction stabilizes this mode of binding and is thus less sensitive to the ionic strength relative to the other two binding modes.

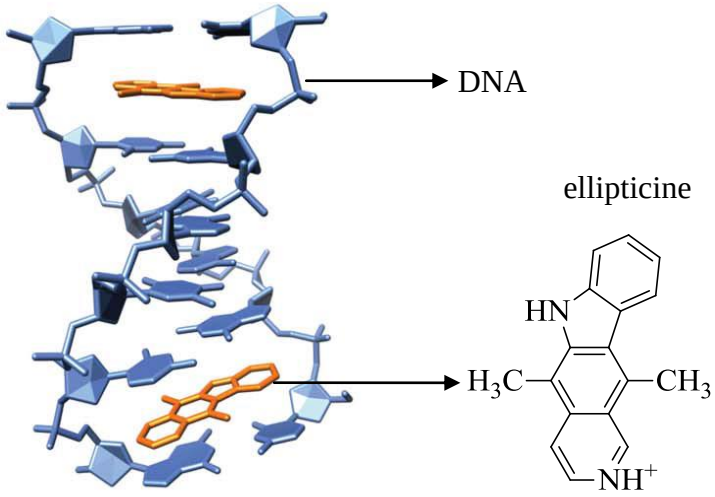


Figure 1.7: Intercalation binding of ellipticine with DNA (Boer *et al.*, 2009)

Metallointercalators has acquired an enormous interest since the interaction of DNA with aromatic ligands platinum complexes which is coplanar with metal coordination sites and has been reported by Howe-Grant and co-workers (Howe-Grant & Lippard, 1979). Metallointercalators play an important role not only in probing nucleic acid structure and function but also in the intercalation process itself (Majumder *et al.*, 2002).

The ligands in the transition metal complexes are essential in the binding to DNA in which the aromatic moieties are able to enhance the DNA binding and cleavage activities. The presence of a large planar aromatic ligand such as

dipyrido[3,2-a;2',3'-c]phenazine (dppz) of transition metal complex favours intercalation binding to DNA (Haq *et al.*, 1995; Majumder *et al.*, 2002; Rajalakshmi *et al.*, 2011; Reddy & Raju, 2012). The intercalation binding to DNA by planar organic compounds was first proposed by Lerman (Lerman, 1961). Indeed with less extended aromatic systems, the intercalation is usually prevented through clashing of the ancillary ligands with the phosphodiester backbone, so that only partial intercalation can occur as in the case for $[Ru(phen)_3]^{2+}$ (Lincoln & Norden,1998). Besides the existence of planar aromatic ligands, solubility of the complex in water is also one of the desirable properties to act as a metallointercalator (Majumder *et al.*, 2002).

1.3.2 Groove-binding

There are two different sizes of grooves in DNA helix, the major and minor grooves, which provide binding sites for guest molecules. Examples of major groove binding by Δ-$[Rh(byp)_2chrysi]^{3+}$ (chrysi = 5,6-chrysenequinone diimine) (Sato & Yamagishi, 2007) to DNA and minor groove binding by distamycin (Boer *et al.*, 2009) to DNA are illustrated in Figure 1.8. Major groove-binding is preferred by relatively large molecules such as protein, while smaller ligands preferably bind to the minor groove binding sites (Ihmels & Otto, 2005). Non-planar ligands or ligands without extended planar aromatic system, particularly octahedral transition metal complexes, promote the groove binding with hydrogen bonding and π-π stacking interactions (Rajalakshmi *et al.*, 2011; Suntharalingam & Vilar, 2011).

Groove binding is the interaction of the molecules approach within van der Waals contact and reside in the DNA groove. Hydrophobic and hydrogen-bonding are usually important components of this binding process and provide stabilization.

Transition metal complex with at least two aromatic or heterocyclic rings are usually a groove binder. Minor groove binders may have some advantages compared to the major groove binder as they have been reported to exhibit excellent potential as therapeutic agents. Some examples of naturally occurring minor groove binders are Hoechst 33258, netropsin, distamycin and anthramycin (Mei & Barton, 1986; Reddy *et al.*, 1999; Ihmels & Otto, 2005).

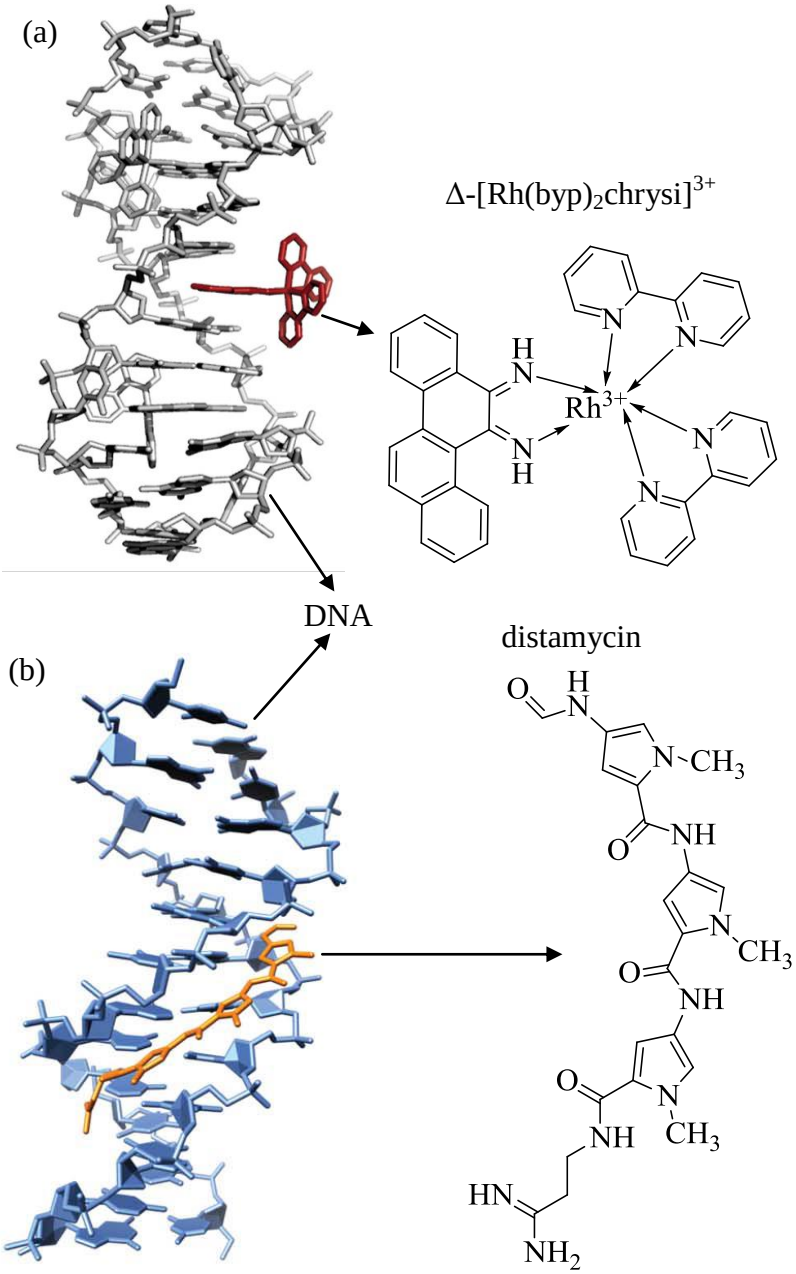


Figure 1.8: Major (a) and minor (b) groove binding with DNA (Sato & Yamagishi, 2007; Boer *et al.*, 2009)

1.3.3 Electrostatic binding

Electrostatic binding involves the complexes with positively charged and the DNA phosphate sugar backbone with negatively charged. This association mode was proposed for $[Ru(byp)_3]^{2+}$ (Figure 1.9), due to the luminescent enhancement of this complex upon binding to DNA. Cations such as Mg^{2+} usually interact in this way.

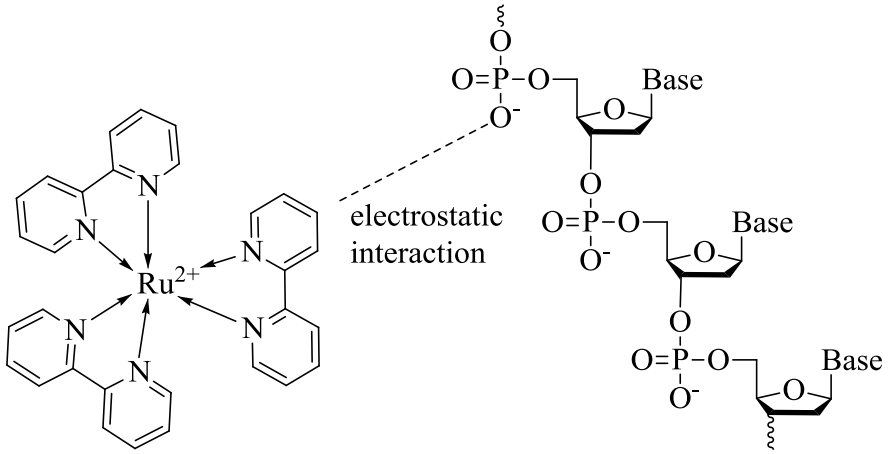


Figure 1.9: Electrostatic binding by $[Ru(byp)_3]^{2+}$ to DNA

1.4 Transition metal complexes

In general, a transition metal complex is a substance that contains at least one complex ion, a species consisting of a centre transition metal cation that is coordinated to one or more ligands which displays diverse chemical, optical and magnetic properties. Coordination compounds are crucial in our lives; the study of them has contributed to the highest degree of understanding the chemical bond in inorganic chemistry. They play an important role in catalysis, material synthesis, photochemistry and biological systems.

Transition metals are crucial in a huge number of widely differing biological processes. Some of these processes are quite unique in their metal ion requirements,

which only certain metal ions in specified oxidation states can achieve the necessary catalytic structural requirement. Metal ion dependent processes are found throughout the life science and vary tremendously in their function and complexity. It is now appreciated that metal ions control an enormous range of processes in biology. Many new and exciting developments in the field of biochemistry create interest out of inorganic chemists to court in the new area called "Bioinorganic Chemistry".

1.4.1 Biological and medicinal applications of transition metal complexes

Transition metal complexes have attracted the attention of many researchers as they have a broad diversity of technological and industrial applications in wide ranging fields from material sciences to biological sciences. In the past century, the studies on DNA interactions by transition metal complexes were thought to be a peculiar choice and not so common (Sancheti *et al.*, 2012). However, in the last few decades, the investigation on the interaction of transition metal complexes with DNA have become a dynamic area of research and acquired prominence owing to their significance in relation to the design and development of novel artificial nucleases for molecular biology, biotechnology and medicine (Wang *et al.*, 2007; Kumar *et al.*, 2008*b*; Patel *et al.*, 2010*a*; Raman & Selvan, 2011; Dong *et al.*, 2011).

The combination of toxicology, pharmacology, chemistry and biochemistry elements have caused medicinal inorganic chemistry to be regarded as a multidisciplinary field. Transition metal complexes possess marvellous potential that can be applied in genomic research, sequence specific binding of nucleic acids, footprinting studies and as diagnostic and therapeutic agents in medicinal industry. They are extensively used in clinical application as anticancer and antiviral agents due to their potential to comprise a class of chemotherapeutics (Brabec & Nováková,

2006; Patra *et al.*, 2009*a*, 2009*b*; Arias *et al.*, 2009; Chen *et al.*, 2011). Metal complexes have been studied generally for these applications because of their electrochemical properties and distinctive spectroscopic. Besides that, another significant reason is that DNA binding and cleavage ability of a metal complex can be tuned by altering the ligand environment. This will enable the study of mechanism of the metal ion toxicity. (Raman *et al.*, 2010*a*; Dong *et al.*, 2011; Anbu & Kandaswamy, 2011*a*).

These days, cancer has become the leading cause of death in most of the countries. Therefore, it is crucial to develop new anticancer therapies in medicinal chemistry. Transition metal complexes are renowned to stimulate drug action and the effectiveness of a therapeutic agent can always be enhanced against coordination with a metal ion. In addition, the pharmacological activity is not only highly relying on the nature of the metal ion but also the donor sequence of the ligands since different ligands show different biological properties (Khan *et al.*, 2009).

1.4.2 Platinum complexes

In the year 1960s, Rosenberg discovered that platinum complexes were able to inhibit the division of bacterial cells (Rosenberg *et al.*, 1965, 1969). Over the last decades, numerous coordination compounds especially platinum and ruthenium complexes are well-known to be anticancer agents. The very first transition metal anticancer drug was cisplatin [cis-diamminedichloroplatinum(II)], which is a metal coordination compound without any organic unit. It has become one of the most important drugs used for the treatment of cancer in modern medicine (Jaividhya *et al.*, 2012). The discovery of cisplatin has led to a great success in medicinal chemistry

due to its cytotoxicity and ability to bind to DNA covalently (Reddy *et al.*, 2011; Ezadyar *et al.*, 2012).

From a biological and medicinal standpoint, the chemistry of platinum based complexes is very significant. For instance, some platinum complexes such as cisplatin, carboplatin and oxaliplatin are widely used as chemotherapeutic agents for the treatment of ovarian, testicular, lung, head, neck and colorectal cancers (Brabec & Nováková, 2006; Qiao *et al.*, 2011; Patel *et al.*, 2012). Schematic structures of cisplatin, carboplatin and oxaliplatin are illustrated in Figure 1.10. As chemotherapeutic agents, these platinum complexes crosslink the DNA strand in several different ways and subsequently interfere with the cell division by mitosis. The design and development of new complexes that are capable of interacting with nucleic acids and regulating apoptosis have become the favourable strategies for researchers to explore new anticancer drugs for chemotherapy (Qiao *et al.*, 2011).

Tu and co-workers synthesized and characterized two platinum(II) 2,2'-dipyridylamine complexes with both chloride or 1,1-cyclobutanedicarboxylate as leaving groups. It is reported that both complexes could interact with DNA and the leaving groups of the complexes had minor effect on DNA interaction (Tu *et al.*, 2004). Very recently, Patel and co-workers prepared a series of cisplatin analogues of substituted 2,2'-bipyridines, where the complexes bind covalently to DNA and exhibit cytotoxic activity against brine shrimps (Patel *et al.*, 2012). Some of the mixed ligand platinum(II) and palladium(II) complexes with amino acids have shown cytotoxicity against P388 lymphocytic leukemia cells and bonded to calf-thymus DNA. They have been reported to be potential antitumor agents (Paul *et al.*, 1993; Jin & Ranford, 2000).

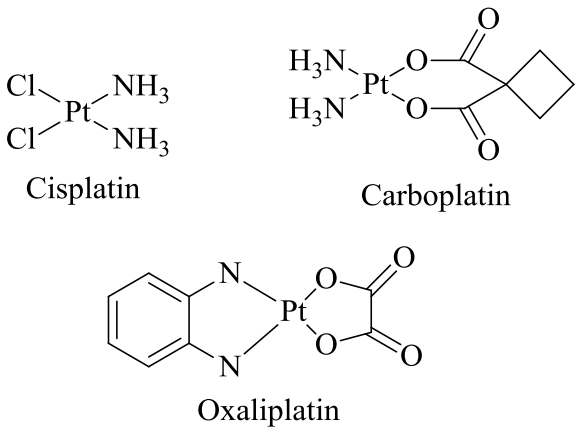


Figure 1.10: Schematic structures of cisplatin, carboplatin and oxaliplatin

1.5 Medicinal applications and biological activities of copper(II) complexes

Next to platinum complexes, copper complexes are one of the potential alternatives to cisplatin as antitumor drugs for chemotherapy. Copper is among the most extensively used transition metals and have found the possibility to be used in medicinal applications for the treatment of various diseases as well as cancer. Over the few decades, lots of inorganic chemists have explored copper(II) complexes due to their extraordinary anticancer activity and lower toxicity than platinum complexes (Patel *et al.*, 2005; Barceló-Oliver *et al.*, 2009; Dong *et al.*, 2011; Jaividhya *et al.*, 2012).

Copper is one of the most interesting bioessential trace elements present in animals and plants, but not in some microorganism with applicable oxidation states of +1 and +2. A number of important redox enzymes like hemocyanins, superoxide dismutase, blue copper proteins etc., contain copper atoms bound to protein molecules. It's oxidative nature and biocompatible properties has gained interest from plenty of inorganic researchers to focus on copper(II) complexes in medicinal applications. Some low molecular weight copper(II) complexes have been proven

useful against numerous diseases such as gastric ulcers, rheumatoid, tuberculosis as well as cancer (Zhang *et al.*, 2006*a*; Ni *et al.*, 2006; Jia *et al.*, 2010). These bioactive compounds not only play a pivotal role in naturally occurring biological systems but were also used as pharmacological agents as well as potential anticancer or cancer inhibiting agents (García-Raso *et al.*, 2003; Patel *et al.*, 2006, 2010; Li *et al.*, 2011; Jaividhya *et al.*, 2012). The anticancer activity of several copper compounds have been screened and found to be active both in vitro and in vivo conditions (Ramakrishnan *et al.*, 2009; Chen *et al.*, 2010; Anbu *et al.*, 2011*b*).

An immense number of copper(II) complexes with different perspectives have been synthesized and their biological activities have been investigated due to the biological relevance of copper (Kumar *et al.*, 2008*b*; Khan *et al.*, 2009; Kumar & Arunachalam, 2009). Copper complexes were reported to produce significant pharmacological effect and act as a potential reagent for DNA cleavage in both oxidative and hydrolytic way with its biologically accessible redox potentials and high nucleobase affinity (Rajendiran *et al.*, 2007; Reddy *et al.*, 2011; Mohamed *et al.*, 2012). Owing to the biological role and potential synergetic activity with the drugs, the development of copper(II) complexes has been explored widely into antibacterial, antiviral, antimicrobial, anti-inflammatory and anticancer agents (Lv *et al.*, 2006; Fountoulaki *et al.*, 2011; Lakshmipraba *et al.*, 2011; Sousa *et al.*, 2012; Chalkidou *et al.*, 2012).

Previous reports showed that many Schiff bases are known to be medicinally important and were used to design medicinal compounds. It was seen that the biological activity of Schiff bases was either increased or decreased upon chelation with metal ions. Bagihalli and co-workers prepared cobalt(II), nickel(II), and copper(II) complexes of Schiff bases derived from 3-substituted-4-amino-5-

mercapto-1,2,4-triazole and 8-formyl-7-hydroxy-4-methylcoumarin which showed potent antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Salmonella typhi* and antifungal activities against *Aspergillus niger*, *Aspergillus flavus* and *Cladosporium* (Bagihalli *et al.*, 2008). The structures of 1,2,4-triazole Schiff base in tautomeric form is depicted Figure 1.11.

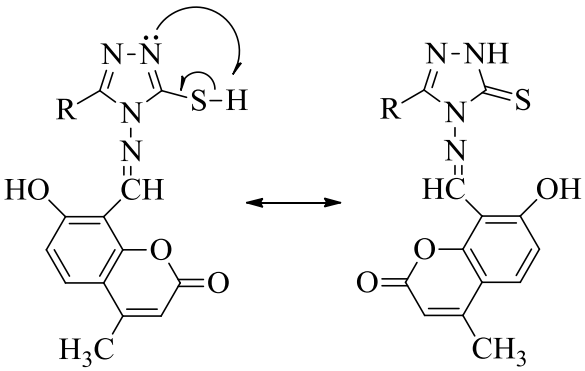
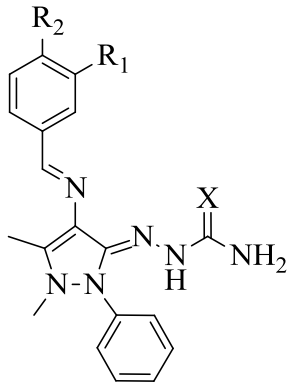


Figure 1.11: 1,2,4-triazole Schiff base in tautomeric form (Bagihalli *et al.*, 2008)

The biological activity of copper(II) and zinc(II) complexes with Schiff bases derived from pyrazole and semicarbazide or thiosemicarbazide (Figure 1.12) have been studied. The synthesized copper complexes binding with DNA through intercalation and photocleavage of plasmid DNA pBR322 was observed. The metal complexes displayed more efficient biological activity than the free ligands (Raman *et al.*, 2010*b*). Sabolová and co-workers prepared a series of copper(II) complexes containing Schiff base derived from salicylaldehyde, and the interaction with calf-thymus has been investigated. The complexes have showed to have intercalative binding with DNA which increased the antimicrobial effect more than 60 times against *Candida albicans* in the presence of ascorbic acid (Sabolová *et al.*, 2011).



$R_1 = OCH_3$, OH; $R_2 = OCH_3$, NO_2; X = O, S

Figure 1.12: Schiff bases structure of pyrazole and semicarbazide/thiosemicarbazide (Raman *et al.*, 2010*b*)

Recently, a new Schiff base ligand, (2-amino-3-((2-hydroxy-1-phenylethylimino)methyl)-4*H*-chromen-4-one, and its chiral complexes (Figure 1.13) containing copper(II) and zinc(II) have been developed by Arjmand group. Both of these complexes bind to calf-thymus DNA via electrostatic groove binding mode. Such copper(II) complex has been observed to promote efficient DNA cleavage with pBR322 DNA in the presence of different activators (Arjmand *et al.*, 2011).

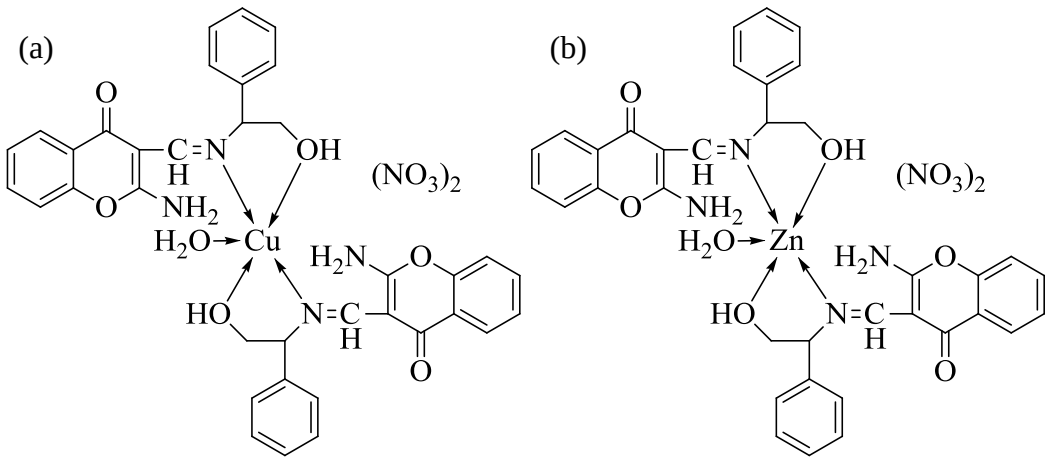


Figure 1.13: Copper(II) (a) and zinc(II) (b) complexes of (2-amino-3-((2-hydroxy-1-phenylethylimino)methyl)-4*H*-chromen-4-one (Arjmand *et al.*, 2011)

1.5.1 Copper(II) complexes of amino acids and its biological applications

Amino acids are biologically important molecules that consist of amino (-NH_2) and carboxyl (-COOH) functional groups, attach with a specific side-chain to each amino acid. Amino acids that comprising of both the amine and carboxylic acid group attached to the first carbon atom which is significant in biochemistry field. Amino acids are the basic structural units of peptides (short polymer chains) or proteins (long polymer chains) that recognize a specific base sequence of DNA by way of hydrogen bond formation (Harada *et al.*, 1996; Kumar & Arunachalam, 2009). The chemical properties of the amino acids are essential in performing an enormous number of biological functions (Stanila *et al.*, 2007; Neelakantan *et al.*, 2008). Proteins not only catalyze most of the reactions in living cells, but also control virtually all cellular processes.

Amino acids which are found in the human body are categorized as essential, non-essential, and conditional amino acids are illustrated in Table 1.1. The naturally occurring amino acids are all in L-form, and the chirality of the side groups is crucial in the specific interaction between the protein and DNA (Chikira *et al.*, 1997).

Table 1.1: Category of amino acids in human body

Essential amino acids	Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan, Valine
Non-essential amino acids	Alanine, Asparagine, Aspartic acid, Glutamic acid, Glycine
Conditional amino acids	Arginine, Cysteine, Glutamine, Ornithine, Proline, Selenocysteine, Serine, Taurine, Tyrosine

Copper(II) complexes with amino acids are known to have potent anti-ulcer activity and anti-inflammatory activities. Earlier reports have exemplified that copper(II) complexes with amino acid or peptide base exhibit good DNA cleavage

activity through oxidative and hydrolytic pathways (Rao *et al.*, 2008). Apart from that, they also show strong superoxide dismutase (SOD) activity (Li *et al.*, 2005). Peptides contain various potential donor centres and act as an effective ligand for many transition metal ions (Tiliakos *et al.*, 2003).

Several reports revealed that first-row transition metal complexes of amino acid Schiff bases also exhibit various biological activities such as antiviral, antitubercular, antifungi and antibacterial activities (Dong *et al.*, 2011). Reddy and co-workers investigated the DNA binding and cleavage abilities of two copper(II) complexes with histamine and amino acids. Both complexes are observed to bind with calf-thymus DNA in intercalative mode with intrinsic binding constants of 2.2-2.7 $\times 10^2$ M^{-1} and cleave plasmid pUC19 DNA (Reddy *et al.*, 2006).

1.5.2 Copper(II) complexes of polypyridyl ligands and its biological applications

Literature reports revealed that an immense number of copper(II) complexes have found to be more active with drugs in the presence of heterocyclic nitrogen donor ligands and gained prominence due to their special electronic properties, peculiar structure and diverse chemical reactivity which result in non-covalent interaction with DNA (Lv *et al.*, 2006; Barve *et al.*, 2009; Dey *et al.*, 2010; Patel *et al.*, 2010*b*; Chen *et al.*, 2011). Numerous polypyridyl based transition metal complexes have been developed over the past few decades for the application as non-radioactive nucleic acid probes and DNA cleaving agents (Hirohama *et al.*, 2005; Anbu *et al.*, 2011*b*).