CHAPTER – VI
SUMMARY AND FUTURE SCOPE

Transition metal complexes represent an important class of compounds because of their applications in wide range areas from Material Sciences to Biological Sciences. Metal complexes are well-known to accelerate the drug action and the efficiency of a therapeutic agent can often be enhanced by coordination with a metal ion. The medicinal uses and application of metals and metal complexes are of increasing clinical and commercial importance. The important criteria for the development of transition metal complexes as chemotherapeutic agents are the ability to bring about DNA cleavage. A large number of transition metal complexes, because of their redox properties, have been found to promote DNA cleavage. Transition metal complexes have been reported to bring about DNA cleavage either oxidatively, hydrolytically and photolytically. A large number of Cu(II), Co(II), Ni(II), Mn(II) and Zn(II) complexes have been shown to promote oxidative DNA cleavage in the presence of a co-reagent. This fact prompted to design, synthesize, structural characterize and to investigate the DNA cleavage ability of Schiff base mixed ligand Cu(II), Ni(II), Co(II), Mn(II) and Zn(II) metal complexes, having 2,4-dihydroxybenzophenone/2-hydroxy-4-methoxybenzophenone derived Schiff bases as main ligands and 2-amino phenol/2-aminobenzoic acid/ o-phenylenediamine as co-ligands.

The coordination metal complexes are gaining increasing importance in the design of respiratory, slow release and long acting drugs. Metal ions are therefore known to accelerate drug actions. The efficacies of few therapeutic agents are known to increase upon co-ordination. Few metal complexes are known to exhibit remarkable antitumour, antifungal, antiviral and special biological activities. Although most antibiotics do not need metal ions for their biological activities, there are a number of antibiotics that require metal ions to function properly, such as
bleomycin, streptonigrin and bacitracin. It was reported that the coordinated metal ions in these antibiotics play an important role in maintaining proper structure and/or function of these antibiotics thereby increasing antibacterial activities of the drugs. Schiff bases are characterized by –N=CH– (imine) groups which have biological activities such as antibacterial, antifungal and antitumoral.

Bearing these facts in mind, the present work aims to provide a brief discussion on the antimicrobial activities of mixed ligand complexes having 2, 4-dihydroxybenzophenone/2-hydroxy-4-methoxybenzophenone derived Schiff bases as main ligands and 2-aminophenol/2-aminobenzoic acid/o-phenylenediamine as co-ligands. Hence this work has widened the scope of developing a novel biologically active Schiff bases and the metal complexes as promising DNA binding and antibacterial agents. The research has led to the discovery of a series of compounds for further pharmacological investigation. Detailed study on synthesis, spectral characterization, antimicrobial and nuclease activities of few novel bioactive Schiff base ligands derived from the condensation of 2,4-dihydroxybenzophenone/2-hydroxy-4-methoxybenzophenone with aniline and their metal(II) complexes are discussed in this thesis. Also mixed ligand complexes using the above bioactive ligands as main ligands are presented in this thesis. This thesis is divided into five chapters. The essences of these chapters are delineated hereunder.

Chapter - I

This chapter highlights a general introduction of bioactive Schiff base ligands and their beneficial applications in various areas. A brief introduction on the structure of DNA and its different binding modes such as intercalation, groove binding and insertion are also discussed. A brief discussion on DNA cleavage is also included. The survey of earlier work in this field covers the period from early sixties to the middle of 2013. The synthesis of substituted benzophenone derived Schiff base ligand and its applications in different fields are also discussed. These views tempted to
synthesize 2,4–dihydroxybenzophenone / 2–hydroxy–4–methoxybenzophenone derived Schiff base ligands and their mixed ligand complexes. The scope of the present work and the possible applications of synthesized Schiff base ligands and their metal(II) complexes in various fields are also discussed in this chapter.

Chapter - II

This chapter describes the general experimental procedures, analytical techniques and various spectra such as FT–IR, UV–vis., ¹H–NMR, EPR, FAB–Mass, XRD, SEM and TGA/DTA analyses used in the study. In this chapter, the detailed experimental procedures for in vitro antimicrobial activity and oxidative DNA cleavage are given. Moreover, the experimental conditions and spectral techniques used to investigate the DNA binding ability of the synthesized metal(II) complexes with CT–DNA are reported in this chapter.

Chapter - III

This chapter is further dived into two parts:

Part - I

In this part, the Schiff base ligand, 4–[phenyl(phenylimino)methyl]benzene–1,3–diol has been synthesized by the condensation reaction of 2,4–dihydorxybenzophenone [2,4–dihydroxyphenyl]phenylmethanone] with aniline. Its metal(II) complexes of Mn, Co, Ni, Cu and Zn have been synthesized and structurally characterized by elemental analysis, FT–IR, UV–vis., ¹H–NMR, EPR, XRD, SEM and TGA/DTA analysis. From the obtained results, it is found that the Schiff base ligand coordinates to M(II) ions in a bidentate manner. The vibrational and ¹H–NMR spectral studies show that the ligand coordinates to M(II) ion through azomethine–N
and deprotonated phenolic–OH groups. Electronic absorption studies coupled with magnetic moment values suggest the possibility of octahedral / tetrahedral environment with mononuclear nature having two water molecules in the coordination sphere. Thermogravimetric (TGA/DTA) analyses have been studied in the temperature range of ambient to 1100 °C under stable air conditions and the presence of coordinated water molecules in the metal(II) complexes is confirmed from thermal (TGA/DTA) study. From powder XRD and SEM of metal(II) complexes display sharp crystalline peaks with well-defined crystalline and homogeneous uniform particle size.

The in vitro antimicrobial activities of Schiff base ligand and its metal(II) complexes were screened against few pathogenic bacterial strains (Escherichia coli, Staphylococcus saprophiticus, Staphylococcus aureus and Pseudomonas aeruginosa) and fungal strains (Aspergillus niger, Enterobacter species and Candida albicans) by well diffusion agar method. From the observed results, all the complexes have much potent activity than free Schiff base ligand (HL₁). The DNA binding studies reveal that Cu(II) complex binds with CT–DNA more strongly than other complexes through an intercalative mode. From the calculated intrinsic binding constant (K₆) and the negative free energy change (∆G) values suggest that the synthesized mixed ligand complexes interact with CT–DNA in a spontaneous manner. Furthermore, the oxidative DNA cleavage activity indicates that Cu(II) complex has moderate activity than other complexes in presence of the oxidant (H₂O₂).

Part - II

This part deals with the synthesis of another NO type Schiff base, in which one of the hydroxy groups is replaced by methoxy group to find out the effect of antimicrobial activities and DNA interaction efficacy. Herein, a bidentate NO type Schiff base ligand of 5–methoxy–2–(phenyl(phenylimino)methyl)phenol has been
obtained by the condensation reaction of 2–hydroxy–4–methoxyphenyl–phenylmethanone with aniline and its metal(II) complexes have been synthesized. The synthesized Schiff base ligand and its metal(II) complexes are structurally characterized by micro elemental and various spectral studies.

The elemental analysis and molar conductance values suggest that the synthesized Schiff base metal(II) complexes have 1:2 (metal: ligand) stoichiometry of [M(L₂)₂(H₂O)ₓ = 0.2] with non–electrolytic nature. From their vibrational studies, free Schiff base ligand (HL₂) coordinates to M(II) ion via azomethine-N and deprotonated phenolic-OH groups and act as bidentate manner. The observed molar conductance, magnetic susceptibility and other spectral studies confirm tetrahedral / octahedral environment around the M(II) ions with mononuclear nature. The presence of coordinated water molecules in all the complexes (except Cu(II) and Zn(II) complexes) have been further confirmed by thermal analysis. The powder X–ray diffraction pattern and SEM show sharp crystalline peaks with microcrystalline and homogeneous particle nature. In vitro antimicrobial activity of Cu(II) complex reveals significant activity than the other complexes against different microorganisms. Cu(II) and Ni(II) complexes show moderate DNA binding and oxidative cleavage activities. The finding should be contributing to the development of novel effective useful DNA probes. All the metal complexes have been showing promise in inhibiting pathogens and are currently under development as potential antibacterial and antifungal drugs.

Chapter - IV

In this chapter, another NO type of Schiff bases having mixed ligands have been synthesized to find out the effect of antimicrobial activities and DNA binding ability. The bioactive mixed ligand MAB type complexes (M = Mn(II), Co(II), Ni(II), Cu(II) and Zn(II); A = Schiff base ligand; B = 2–aminophenol / 2–aminobenzoic acid). The main Schiff base has been synthesized from 4–[phenyl(phenylimino)methyl]benzene–1,3–diol/5–methoxy-2-phenyl(phenylimino)
methyl)phenol with co–ligand (2–amino phenol / 2–aminobenzoic acid). These mixed ligand complexes have been structurally characterized on the basis of FT–IR, UV–vis., ¹H–NMR, FAB–Mass, EPR, XRD, SEM and magnetic studies. The spectral studies suggest that all the synthesized complexes have octahedral geometry with mononuclear non–electrolytic nature. The coordination mode and sites of the ligand to the metal ions have been investigated by comparing the infrared spectra of the free ligand with its metal complexes. Based on the analytical, conductivity measurement, magnetic moment, spectral and thermal studies, it is concluded that all the mixed ligand complexes have octahedral geometry with N₂O₂ environment around the central metal(II) ion.

The thermogram of all the synthesized complexes follows same type of decomposition. Initially there is a weight loss upto 7 % in the temperature range of 110 – 150 °C which indicates the coordinated water molecules are present in all the complexes. It is further confirmed from vibrational and ¹H–NMR spectral studies. Above 200 °C there is a gradual weight loss for the evaporation of organic ligand moiety in a successive steps upto 500 °C and finally leads to form metal oxide as the end residue. Powder XRD and SEM analyses show all the mixed ligand complexes are microcrystalline nature with uniform particle size. From DNA binding studies, it is inferred that all the complexes bind with CT–DNA through an intercalative mode and Cu(II) complexes have moderate oxidative DNA cleavage efficiency than free Schiff base ligands in H₂O₂ medium. From DNA binding experiments, it is concluded that the complexes are moderate intercalators. The oxidative DNA cleavage indicates that Cu(II) complexes show better cleaver than free Schiff base ligands and other complexes. In–vitro antimicrobial activities of free Schiff base ligands and their MAB type mixed ligand complexes are carried out against few pathogenic bacterial and fungal strains by well diffusion method using agar as nutrient at different concentrations. From the results, it is observed that all the complexes show a
remarkable antimicrobial activity at higher concentration and the data reveal that all the complexes are having greater activity than the free ligands.

Chapter - V

To extend the study further, two N₃O type Schiff bases and their mixed ligand complexes have been synthesized to explore the effect of antimicrobial and nucleic acid activities under experimental conditions. Mn(II), Co(II), Ni(II), Cu(II) and Zn(II)] mixed ligand complexes have been synthesized using 4–[phenyl(phenylimino)methyl]benzene–1,3–diol/5–methoxy–2–(phenyl (phenylimino) methyl)phenol as main ligand and o–phenylenediamine as co–ligand. They have been structurally characterized by various analytical and spectral studies. The observed elemental analysis and high molar conductance values indicate 1:1:1 (Metal:Schiff base ligand: o–phenylenediamine) stoichiometry ratio with 1:1 electrolytic nature. The measured magnetic moment values coupled with spectral studies confirm mononuclear nature with octahedral environment around the M(II) ions. The presence of coordinated water molecules in all the complexes has been further confirmed from thermal (TGA / DTA) analysis. Powder X–ray diffraction pattern and SEM analysis show sharp crystalline peaks with microcrystalline and homogeneous uniform particle size.

In vitro antimicrobial activity of Cu(II) complexes screened for different microorganisms shows higher activity than the other complexes. All the synthesized mixed ligand MAB complexes show higher biological activities as compared to other mixed ligand systems due to the presence of bulky substituents (phenyl moiety) and electron releasing nature of methoxy (-OCH₃) groups present in benzophenone which enhance the activities. Also, the normal cell process may be affected by the formation of hydrogen bond through the azomethine nitrogen atom with the active centers of cell constituents. DNA binding studies reveal that Cu(II) complexes have reasonable intercalations binding ability with CT–DNA and also Cu(II) and Ni(II) complexes
show considerable oxidative DNA cleavage activities against \textit{pUC–19} DNA in presence of oxidative medium.

The present work is centered on the synthesis of Schiff base mixed ligand Cu(II), Ni(II), Co(II), Mn(II) and Zn(II) metal complexes, having 2,4–dihydroxybenzophenone/2–hydroxy–4–methoxybenzophenone derived Schiff bases as main ligands and 2–amino phenol/2–aminobenzoic acid/ \textit{o}–phenylenediamine as co–ligands and their characterization by different analytical and spectral techniques. A few of these compounds are having good DNA binding and antimicrobial activities. From the available knowledge of literature, it is found that this is the first report on the \textit{in vitro} evaluation of the binding parameters of the above Schiff base mixed ligand complexes with DNA by electrochemical and spectroscopic techniques.

The results of this thesis are of importance in designing new transition metal–based chemical nucleases in nucleic acid chemistry. The UV light–induced DNA cleavage activity is likely to simulate a growing interest towards design and development of analogous metal–based drugs using biologically important metals and ligands to achieve sequence selective recognition of DNA and efficient DNA cleavage for cellular applications which is significant in considering the importance of the results in the chemistry of photodynamic therapy of cancer. Effective DNA cleavage activity is probed for several complexes in this work. The results should be valuable in understanding the mode of the complex with DNA as well as laying a foundation for the rational design of novel, powerful agents for probing and targeting nucleic acids. The present work concludes that metal complexes will be used as good drugs of choice to manage the bacterial and fungal diseases after evaluating the \textit{in vivo} effect of metal complexes on experimental animals and clinical trials.
LIST OF PUBLICATIONS

1. Mixed ligand complexes containing (2-hydroxy-4-methoxyphenyl)(phenyl)methanone and 2-aminophenol: Synthesis and DNA cleavage

2. Mixed ligand complex formation of 2-aminobenzamide with Cu(II) in the presence of some amino acids: Synthesis, structural, biological, pH-metric, spectrophotometric and thermodynamic studies

3. Novel mixed ligand complexes of bioactive Schiff base (E)-4-(phenyl(phenylimino)methyl)benzene-1,3-diol and 2-aminophenol/2-aminobenzoic acid: Synthesis, spectral characterization, antimicrobial and nuclease studies

4. Transition metal complexes with bidentate N$_2$O$_2$ type ligands: Synthesis, characterization, antimicrobial and nuclease activities
LIST OF PAPERS PRESENTED IN CONFERENCES


3. **DNA binding studies of novel water soluble metallic crystals having mixed ligands**, P. Subbaraj, A. Ramu and N. Raman in the National Conference on Recent Advances in Inorganic Chemistry (RAIC-2012) sponsored by UGC, DST and BRNS, organized by the School of Chemistry, Bharathidasan University, Tiruchirappalli, Tamilnadu, March 22 – 24, 2012.