SUMMARY AND CONCLUSION

This thesis deals with investigations on some Schiff base metal complexes and mixed ligand metal complexes. Schiff base derived from furyliden-4-aminoantipyrine and 2-amino benzothiazole have been synthesized and characterized. Co(II), Ni(II), Cu(II) and Zn(II) complexes of the same Schiff base and two series of mixed ligand complexes of Co(II), Ni(II), Cu(II) and Zn(II) using the Schiff base, with 1,10 phenanthroline and 2-amino phenol have been synthesized. All the synthesized metal complexes are characterized by analytical, molar conductance, magnetic moment IR, $^1$HNMR, DART MS, electronic, magnetic moment, ESR, cyclic voltammetry, thermal, XRD and SEM. The biological activities of ligand and the metal complexes have also been investigated. This thesis is mainly divided into Six Chapters.

CHAPTER-I

INTRODUCTION

The literature survey covers the work reported in this field from early nineties to till date. Importance of coordination compounds, Schiff bases, methods of synthesis of Schiff bases, coordination behavior, Schiff base transition metal complexes, application of Schiff base metal complexes in various fields such as industry, catalysis, pharmaceutical and biological fields have been illustrated. Importance of compounds chosen for the study, importance of metal ions used, review of works done so far in the proposed field, various spectral characterization methods used have been described. The chapter ends with the scope of the present investigation.
CHAPTER-II

EXPERIMENTAL

The **chapter two** describes the details of chemicals, synthesis of the ligand, complexes, instruments and methods used for the study. Principles of the experimental techniques used are also illustrated.

CHAPTER III

BIOLOGICAL STUDIES

In the **third chapter** various biological studies such as antibacterial, antifungal, DNA binding, DNA cleavage, SOD activity, cytotoxicity, antituberculosis and anticancer studies are enumerated. The antimicrobial activity of the metal complexes was carried out using well diffusion method. DNA binding studies was carried out by UV spectral method using CT DNA. DNA cleavage activity of the complexes was done using agarose gel electrophoresis with pUC18 DNA. Anti tuberculosis assay of mixed ligand complexes synthesized using 1,10 phenanthroline was screened against *M. tuberculosis* H37Rv strain by microplate alamar blue dye assay (MABA) method. Cytotoxicity of the mixed ligand complexes was assayed using *E.coli AB* 1157 strain. *In vitro* cytotoxicity of mixed ligand complexes was carried out using human cervical cancer cell line (HeLa) by MTT assay.

CHAPTER IV

DOCKING

The **fourth chapter** deals with docking studies. Docking predicts the preferred orientation of one molecule to a second when bound to each other to
form a stable complex. The molecular docking tool, Hex 6.0 interface on the windows 7 operating system was used for docking and scoring. The PDB file of the structure for ligand and complex was done by chemoffice software. The structure was minimized and then used for molecular docking. Crystal structure of glucosamine-6-phosphate synthase obtained from protein data bank was used.

CHAPTER V

RESULTS AND DISCUSSION:

It is divided into several parts, dealing with synthesis and characterization of Schiff base and its metal complexes, mixed ligand metal complexes, biological and docking studies.

Furfurylidene-4-aminoantipyrine was prepared by the condensation of furfuraldehyde and 4-aminoantipyrine. An ethanolic solution of 2-aminobenzothiazole was refluxed with the Schiff base obtained in the first stage for 6 h to produce the required Schiff base furfurylidene-4-aminoantipyrine-2-aminobenzothiazole (FAAPBT). The Schiff base ligand has been characterized using IR, UV-Vis., $^1$H NMR and DART mass spectra. Co(II), Ni(II), Cu(II) and Zn(II) complexes of Schiff base (FAAPBT) have been synthesized and characterized on the basis of elemental analysis, molar conductance, magnetic moment and spectral data (IR, $^1$H NMR, UV-Vis., ESR, and DART–MS). Elemental analysis indicates that the found and calculated values are within acceptable limits. The molar conductance values indicate that all the complexes are non-electrolytes. The Schiff base acts as bidentate ligand coordinate through azomethine and imino nitrogen atoms. Two acetate and two water molecules also coordinate to the metal ion. DART mass spectra give the stoichiometry of the metal complexes. UV-Vis., and magnetic moment studies suggest octahedral geometry for all the
complexes. The ESR studies confirm that the complex is mononuclear in nature. Thermal studies of the complexes indicate the presence of two coordinated water molecules in the complexes. Thermal studies also confirm that the metal complexes are thermally more stable than the ligand. The electrochemical studies indicate that the ligand and its complexes show redox behavior. Powder XRD pattern indicates that Co(II) complex is amorphous in nature while FAAPBT, Ni(II), Cu(II) and Zn(II) complexes are nanocrystalline with grain size 43, 22, 28, 43 nm respectively. The SEM micrograph of ligand shows irregularly shaped particles. Co(II) complex shows bar like structure. Faceted micro crystal structure is present in Ni(II) complex. Cu(II) complex shows agglomerated morphology. Bar with layered structure has been present in Zn(II) complex.

The \textit{in vitro} biological screening of Co(II), Ni(II), Cu(II) and Zn(II) complexes with Schiff base (FAAPBT) were tested against bacterial species \textit{S. aureus}, \textit{E. coli}, \textit{K. pneumoniae}, \textit{S.typhi} and \textit{P. aeruginosa} and fungal species \textit{A. niger}, \textit{R. stolonifer}, \textit{A. flavus}, \textit{R. bataicola} and \textit{C. albicans} by well diffusion method. Antibacterial and antifungal studies of the ligand and complexes indicate that activity increases on complexation with metal ions. The Zn(II) complex shows high antibacterial and antifungal activity. The complexes show significant SOD activity hence can be considered as good model for SOD activity. Among the four synthesized complexes, Cu(II) complex shows high SOD mimetic activity. DNA binding studies with CT DNA using UV absorption spectra indicate that the complexes exhibit stronger binding affinity to DNA by intercalation. DNA cleavage activity was performed by agarose gel electrophoretic assay and noticeably, the complexes exhibit effective DNA cleavage via hydrolytic mechanistic path way. The results indicate that Cu(II) and Ni(II) complex have nicking activity.
The lowest docking score has been obtained for the ligand. Among the complexes, the highest negative score is seen in the Ni(II) complex. The lowest score is found in the Cu(II) complex. The docking score of the complexes are Ni(II) > Co(II) > Zn(II) > Cu(II). The number of hydrogen bonds present is maximum (7) in the Ni(II) complex. The number of hydrogen bonds is equal (3) in all the remaining complexes. The docking studies further indicate that the complexes are more biologically active than the ligand.

Mixed ligand complexes of Co(II), Ni(II), Cu(II) and Zn(II) with FAAPBT and Phen have been synthesized using FAAPBT, 1,10-phenanthroline and metal acetate in 1:1:1 ratio. The mixed ligand complexes are characterized by elemental analysis, molar conductance, magnetic moment and spectral data. Elemental analysis and molar conductance data indicate that the complexes are non-electrolytes.

IR spectra indicate the coordination of azomethine and imine nitrogen atoms. Two acetate groups also coordinate to the metal ion. Comparison of \(^1\)H NMR spectra of ligand and Zn(II) mixed ligand complex indicate the coordination of azomethine group with the metal ion. DART mass spectra give the molecular weight of complexes. UV vis. and magnetic moment studies indicate that all complexes show octahedral geometry except Cu(II) complex which has a distorted octahedral geometry. ESR spectra also confirm distorted octahedral geometry for Cu(II) complex. Electrochemical studies indicate that the mixed ligand complexes show redox behaviour. The thermal stabilities of the complexes are ascertained from thermal studies. Powder XRD studies reveal that all the complexes are nanocrystalline with grain size 19, 20, 14, 12 nm respectively for Co(II), Ni(II), Cu(II) and Zn(II) complexes. SEM micrographs show broken rock like structure for
Co(II) and Cu(II) complexes, irregularly shaped particles have been present for Ni(II) and Zn(II) complexes.

The in vitro biological screening was done against bacterial species S. aureus, E. coli and P. aeruginosa and fungal species A. niger, A. flavus and C. albicans by well diffusion method. The studies indicate that the antimicrobial activity increases with increase in concentration of the complexes. The complexes show significant SOD and DNA cleavage activities. The SOD activities of the complexes are in the order Cu(II) > Ni(II) > Zn(II) > Co(II). The Cu(II) complex completely cleaves DNA. The results of cytotoxicity assay using E. coli AB 1157 strain indicate that, Cu(II) complex shows more activity, Co(II) and Zn(II) complexes show moderate activity, while Ni(II) complex does not show any activity. Anti tuberculosis assay of FAAPBT and complexes indicate that Cu(II) and Zn(II) complexes are effective growth inhibitors for mycobacterium tuberculosis strain. The Co(II) and Ni(II) complexes show moderate activity. In vitro cytotoxicity assay using human cervical cancer cell line (HeLa) indicate that the Cu(II) complex shows higher activity with IC$_{50}$ value 8.3 µM. The Zn(II) complex exhibits good activity with IC$_{50}$ value 8.33 µM. The Co(II) complex have IC$_{50}$ value at 72.35 µM. The ligand and Ni(II) complex show very low activity.

The highest docking score has been obtained for the Cu(II) complex. The docking score is similar in Co(II), Ni(II) and Zn(II) complexes. The antimicrobial screening results of the complexes are in accordance in with the docking results. The binding pattern of the complexes has been ascertained from the structural analysis of docked structures. The docking results also indicate that the Cu(II) complex can act as a anti cancer agent. This is in correlation with the results
obtained for anti cancer activity of the Cu(II) complex. The number of hydrogen bonds present is similar in all the complexes.

Mixed ligand Co(II), Ni(II), Cu(II) and Zn(II) complexes with Schiff base (FAAPBT) have been synthesized using FAAPBT, 2-aminophenol and metal acetate in 1:1:1 ratio. The mixed ligand complexes are characterized by elemental analysis, molar conductance, magnetic moment and spectral data. Elemental analysis results show that the found and calculated values are within acceptable limits. Molar conductance data confirm non electrolytic nature of complexes. IR spectra indicate the coordination of two water molecules. Comparison of $^1$H NMR spectra of ligand and Zn(II) complex indicate the coordination of azomethine group with the metal ion. In the $^1$H NMR spectrum of the Zn(II) complex, the azomethine proton signal is shifted downfield compared to the free ligand due to the deshielding of the azomethine group on coordination with Zn(II) ion. DART mass spectra give the molecular weight of the complexes. The physico-chemical data suggest octahedral geometry for Co(II), Ni(II) and Zn(II) complexes and distorted octahedral geometry for Cu(II) complex. ESR parameters of Cu(II) complex also support distorted octahedral geometry. Thermal decomposition and thermal stability of complexes are known from thermal studies. Powder XRD studies reveal that all the complexes are amorphous. The SEM micrographs show spherical structured particles for Co(II) complex, irregularly shaped particles for Ni(II) complex and cauliflower like morphologies for both Cu(II) and Zn(II) complexes.

The in vitro biological activity of the complexes were tested against bacterial species S. aureus, E. coli, P. aeruginosa and fungal species A. niger, A. flavus and C. albicans by well diffusion method. Among the complexes, Cu(II) complex has higher antimicrobial activity. The mixed ligand complexes show
significant SOD, DNA binding and DNA cleavage activities. The Cu(II) complex shows highest superoxide radical scavenging activity followed by the Zn(II) complex. The Co(II) and Ni(II) complexes show moderate activity. The DNA cleavage activity of the mixed ligand complexes are given as Cu(II) > Co(II) > Zn(II) > Ni(II). Cytotoxicity assay of the mixed ligand metal(II) complexes shows that Co(II) and Ni(II) complexes are more toxic, Zn(II) complex is moderately active whereas the Cu(II) complex is inactive. The anticancer activity by the MTT assay shows that the Cu(II) mixed ligand complex has very good inhibitory (0.8 µM) effect on the growth of human cervical carcinoma (HeLa) cells. The Zn(II) complex possesses moderate (68.81µM) growth inhibition activity while the Co(II) and Zn(II) complex have low activity.

The structural analysis of docked structures gives significant details about the binding pattern of the complexes. According to the docking studies, highest negative score is present for the Cu(II) complex. The results show that the Cu(II) complex has the ability to act as anticancer agent. The Cu(II) complex adopts an acceptable confirmation with in the active site of PDB ID1jxa and significant binding interactions have been noticed from the figures. The docking results obtained are in good agreement with antimicrobial results.