SYNTHESIS AND CHARACTERIZATION OF SCHIFF BASE TRANSITION METAL COMPLEXES - DNA INTERACTION STUDIES

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The various branches of chemistry are occurring in nowadays but, inorganic chemistry is most important of medicinal field during the lack of challenges for newly spreader diseases. The numerous of viruses, bacteria and fungi are also increases due to the environmental changes. In the past decay years is mainly due to co-ordination chemistry of co-ordination compounds has always been a challenge to the inorganic chemists. The important area of Co-ordination chemistry is widely applicable for the biological processes and determination of new drugs. Because transition metals have most one of the immense number of extensively differing biological processes. Some of these processes are quite specific in their metal ion requirements, in that only certain metal ions in specified oxidation states accomplish the necessary catalytic structural requirement. Metal ions are vital role in biological processes of human beings. Many new and exciting developments in the field of biochemistry create interest out of inorganic chemists to court in the new area called “Bio-inorganic chemistry”. One of the principle ethics of bio-inorganic chemistry is the synthesis of metal complexes that have the ability to mimic the functional properties of natural metallo proteins (ex. Hemoglobin, Cobalamine). Proteins, some vitamins and enzymes contain metal ions in their structure involving macromolecular ligands (such as organic macrocyclic rings). The co-ordination of metal complexes forming with multi-dentate ligands, such forming chelates system of metals. The schiff base ligands containing nitrogen atom, it is the one of the most important of the element in biological processes. Basically the human being system containing DNA which contains back bone of the nitrogen compounds. It is well known that deoxyribonucleic acid (DNA) plays an important role in the life processes since it contains all the genetic information for cellular functions. However DNA is the primary intracellular target of anticancer drugs due to the interaction between small molecules and DNA. This is due to their possible applications as new therapeutic agents and their photochemical properties which make the potential probes of DNA structure and conformation. So the nitrogen atom reduces the activity of pathogens produced diseases. The elements present in Nitrogen, Oxygen and Sulphur containing compounds are highly active against pathogens. The Schiff base metal complexes are

active against microorganisms, because the schiff base ligands containing azomethine or imine presence of nitrogen atoms considerable chemical and biological processes.

This Ph.D thesis is mainly concerned with the synthesis and characterization of novelty Schiff base ligand encapsulated transition metal complexes.

These ligands are 2-hydroxy-1-naphthaldehyde and 2-2' (ethylene dioxy) bis ethylenediamine (L_1), 2-hydroxy-1-naphthaldimino- 4-amino antipyrine (L_2**),** (Z)-N-(3-bromo-5-fluorobenzylidene)-2,5-Dimethoxyaniline(L_3),3-fluoro-4-methoxybenzaldehyde-2,2'-(ethylenedioxy) bis ethylenedimine (L_4**)** and 3-((2-aminophenylimino)methyl)naphthalen-2-ol (L_5) are synthesized and its characters have been studied by spectral data. The metal complexes have been amalgamation with these newly formed ligands. The co-ordinated metals Copper (II), Manganese (II), Cobalt (II), Vanadium (V) and Ruthenium (III) were used for the synthesis. These are transition metals widely used for the biological and industrial purposes. These ligands provide great structural diversity during complexation. After complete the complexation of ligands and its metal complexes studied on their biological activity. Shows metal complexes are highly active against micro organism in-vivo method. And the finally the complexes are highly interact with DNA base pairs.

This thesis quintessence deals with the newly synthesis of Schiff bases and their metal complexes of biometals such as Cu(II), Co(II), Mn(II), Ru (III) and VO(IV). They have been synthesized and deliberate for structural illumination, physical properties, antimicrobial, antioxidant and DNA binding studies. The thesis comprises of seven chapters and the contents of each chapter in concise are delineated hereunder.

Summary and Future scope

Metal complexes as well as Transition metal complexes correspond to an important class of compounds since of their applications in extensive range areas from fabric sciences to biological sciences. Metal complexes are well-known to accelerate the drug achievement and the effectiveness of a therapeutic agent can often be enhanced by co-ordination with a metal ion. The medicinal uses and application of metals and metal complexes are increasing experimental and commercial importance. The imperative criteria for the development of transition metal complexes used as chemotherapeutic agents are the aptitude to bring about DNA binding. This information encouraged to design, amalgamate, structural distinguish and to investigate the antimicrobial,

antioxidant and DNA binding nature of Schiff base ligands and its Copper (II), Cobalt (II), Oxovanadium (IV), Manganese(II) and Ruthinium (III) complexes, having 2-hydroxy- 1-napthaldehyde , 2-bromo-4-fluorumethoxy Benzaldehyde and 3-bromo-4-fluorobenzaldehyde based schiff bases as main ligands.

This thesis quintessence deals with the newly synthesis of Schiff bases and their metal complexes of biometals such as Cu(II), Co(II), Mn(II), Ru (III) and VO(IV). They have been synthesized and deliberate for structural illumination, physical properties, antimicrobial, antioxidant and DNA binding studies. The thesis comprises of seven chapters and the contents of each chapter in concise are delineated hereunder.

Chapter-I

General introduction

In this chapter deals with universal introduction of coordination chemistry, history of coordination compounds and Schiff base compounds in presence of coordination chemistry, mechanism for Schiff base formation, explain the basicity character of the Schiff base ligands, biological important of Schiff base ligands and the biological revolution of transition metals using Copper, Cobalt, Manganese, Ruthenium and Vanadium for complexation of Schiff base ligands. Diversity applications of Schiff bases complexes due to catalytic, biological application such as anti-bacterial, anti fungal, anti-oxidant, anti-inflammetry, anti-viral, anti-tumor etc., agricultural applications and other field application of Schiff base complexes deals with their chapter, literature survey of the earlier work in this field covers the period from past half of decades to the end of 2019. It also presents what is meant by literature survey and its introduction. A brief literature survey is present in this chapter. Finally at the end of this chapter the scope of the present work is presented justifying the choice and consequence of the work reported in the thesis.

Chapter- II

General experimental methods and techniques

In Chapter-II describes the general experimental methods, spectral characterization procedures, anti microbial activity, anti oxidant and DNA binding procedures. The Physical

measurements, molar conductance and structural elucidation by spectrum like UV-Vis, FT-IR, ^{1}H-NMR, EPR, Mass and Spectrofluorophotometer used in this work. The anti-oxidant studies carried out by using DPPH free radical. The Stock solution of DPPH prepared at 300 μg/mL. The DNA binding studies carried out by using CT-DNA was purchased from Bangalore. The DNA cleavage studies were done using gel documentation system by using Tris-HCl buffer at pH-7.0.

Chapter-III

DNA interaction Study of newly synthesis of 2-hydroxy-1-napthaldimino-2, 2'(ethylenedioxy)bis ethylamine(L_1) its transition metal complexes and their biological activity.

In this chapter deals novelty synthesized ligand of Synthesis of 2-hydroxy-1-naphthaldimino-2-2, (ethylene dioxy) bis ethylamine (L_1) synthesized from 2-hydroxy-1-napthaldehyde condensed with 2-2'-(ethylene dioxy) bis ethylamine by conventional method. The metal complexes were synthesized by this ligand using Mn(II), Ru(III), Cu(II) and V(IV) transition metals. The analytical data of the complexes correspond well with the general formula $M(L_1)_n$. The molar conductance studiestells about the non-electrolytic nature of these complexes. The structural elucidation is confirmed by the spectral techniques like UV-Visible spectroscopic gives an octahedral geometry of Mn(II) and Ru (III) complexes, and distorted octahedral structure of Cu(II) and VO(IV) complexes. The FI-IR spectrum is confirmed the M-L bonding nature, band at 1618-1635 cm^{-1} assigned to the imine (C=N) linkage. Finally the M-N and M-O bond present at 418-489cm^{-1} and 501-503cm^{-1} respectively. But the Oxo Vanadayl(V=O) complex confirmed by the frequency of 920.53 cm^{-1}. the electronic spectrum provides the bands shown in the range of 240 and 347nm for the electron trasition occur π-π * and n-π * respectively. Mn(II) and Ru(III) complexes are octahedral and Cu(II), VO(IV) complexes are distorted octaherdral geometry is confirmed by the humbled peak of the absorption band present in the electronic spectrum The fast atom bombardment mass spectra of the ligand and its complexes confirm the stoichiometry of metal complexes as being of the $M(L_1)_n$ type. The ^{1}H- NMR spectrum, the phenolic OH present at 3.67 (1H), 6.87-7.80 for (ArH) of naphthalene ring and = 8.656 for (s, 2H) of azomethine Proton present in the L_1 compound. The EPR Spectrum provides the Vanadium metal presents at Oxo

vanadyl nature of complexes and other metal oxidation could be explained. The ESI-mass spectrum of (L_1) ion peak observed at m/z 454.51 is confirmed the formation of ligand for prepared ratio. All metal complexes exhibit the parent peak due to confirmed complexation. The synthesized metal complexes are higher activity than the free ligand of Antimicrobial and anti-oxidant activity. The DNA binding studies of Cu(II) and Ru (III) complexes are studied. The binding of DNA with Metal complexes by intercalation mode is proved by absorption technique.

Chapter-IV

Synthesis of (E)-3-((2-hydroxynaphthalen-1-yl)-4, 5, dimethyl-1-phenyl-1H-pyrrole-2(3H-one) (L_2) Schiff base ligand and its transition metal complexes of their biological activities and DNA interaction studies.

In this Chapter describes Synthesis of (E)-3-((2-hydroxynaphthalen-1-yl)-4, 5, dimethyl-1-phenyl-1H-pyrrole-2(3H-one) (L_2), Schiff base ligand and its transition metal complexes of their biological activities and DNA- interaction was studied. The newly synthesized ligand by conventional method using 2-hydroxy-1-napthaldehyde condensed with 4-Aminoantipyrine. The metal complexes have synthesized by using Mn(II), Ru(III), Cu(II) and Co(II) transition metals. The L_2 and Metal complexes was characterized by elemental analysis, spectroscopic measurements (FT-infrared, electronic spectroscopy, ^{1}H-NMR, EPR and Mass spectroscopy). Elemental analysis of the metal complexes was suggested that the stoichiometry metal-ligand ratio is 1:2. The molar conductance values indicating synthesis complexes all are non-electrolyte nature. The FT-IR spectral data is confirmed by the functional group of imine frequency υ(C=N) present at the region of 1645 cm^{-1}. The ligand act as bi-dentative manner. The region of 3419-3440 cm^{-1} a broad band peaks confirmed the water molecule co-ordinate with metal atom. The electronic spectra suggest an octahedral geometry for all Schiff base complexes. The ^{1}H-NMR spectrum tells all the protons were found as to be in their expected region. The observed signal at 10.40 ppm was assigned to the proton of phenolic -OH group. This peak is due to hydrogen bonded phenolic protons and the integration is generally less than 2.51 ppm due to this intra-molecular hydrogen bonding. The ketone double bond present in 2.41 ppm. Signal for the methane proton of the azomethine group for Schiff base –N=CH- were observed at 8.34ppm. In the region of 6.98 –6.42 ppm chemical shift shifts downfield were assigned for the deshielding of hydrogen of the aromatic ring. The Schiff base and its metal chelates have been screened for their invitro test antibacterial

activity against three bacteria, gram-positive (Staphylococcus aureus) and gram-negative (Klebsiella Pheneuammonia and Salmonella typhi). Two strains of fungus (Aspergillus Niger and Candida albicans). All the synthesized ligand and its metal complexes are inactive against gram negative bacteria (Salmonella and Klebsiella). The Cu (II), Mn (II) and Ru (III) complexes highly active against gram positive bacteria (Staph.aureus). The metal chelates were shown to possess more anti fungal activity compare then antibacterial activity and antioxidant properties. The complexes are highly active than the free Schiff-base ligand. The binding nature of metal complexes of Cu(II) and Ru(III) with DNA by intercalation mode is confirmed by the absorption spectroscopic method.

Chapter-V

Biological evaluation of newly synthesized (Z)-N- 3-fluoro-4-methoxy benzaldehyde-2,2'-(ethylene dioxy) bis ethylenedimine (L_3) schiff base ligand and its transition metal complexes and DNA interaction studies.

In Chapter-V deals an interesting bi-dentate Schiff base ligand (Z)-N-3-fluoro-4-methoxy benzaldehyde-2,2'-(ethylene dioxy) bis ethylenedimine (L_3**)** was prepared by the condensation of 3-Fluoro-4-Methoxy Benzaldehyde with 2,2'-(ethylene dioxy) bis ethylenediamine. The metal complexes were synthesized by the L_3 using the Cu (II), Co(II), Mn (II) and Ru (III) transition metals by reflux for room temperature. Structural investigation of the ligand and its metal complexes were analyzed by elemental analysis, FT-IR, UV- Visible, ^{1}H-NMR and Fluorescence spectroscopy. The bands appearing in the FT-IR spectra of the ligand at 1581 cm^{-1} is assigned to the stretching vibration of (-C=N-) azomethine. the formation of co-ordination and covalent bonds between the donor atoms –N, –O and the central metal ions (M-N and M-O) the range between the 501-556 cm-1 and 748-756 cm^{-1} respectively. the presence of a broad band at 3331-3387 cm^{-1} which corresponds to the stretching vibration of the OH group of the ligand under investigation. The electronic absorption spectrum of the Cu (II) complex showed two bands at (359 nm) 27855cm^{-1}, (322 nm) 31055 cm^{-1} and (303 nm) 33003cm^{-1} corresponding to the transition $^2B_{1g}$ $^2A_{1g}$, $^2B_{1g}$ $^2B_{2g}$ and $^2B_{1g}$ ^2E_g which supports octahedral geometry. The Mn (II) complex has a d^5 electronic configuration the absorption values shows 31250cm^{-1}(320nm), 29069cm^{-1} (344nm) and

28409cm^{-1} (352nm) through $^{6}A_{1g}$ $^{4}E_{g}$ (D), $^{6}A_{1g}$ $^{4}A_{2g}$, $^{6}A_{1g}$ $^{4}E_{g}$,, $^{4}A_{1g}$ transition respectively. The bands are assigned to the values are (407nm) 24579cm^{-1} (υ_1), (329nm) 30395cm^{-1}(υ_2) and (315nm) 31746cm^{-1} (υ_3) respectively and the transitions are assigned to $^{3}T_{1g}$ (F) $^{3}T_{2g}$ (υ_1), $^{3}T_{1g}$ (F) $^{3}T_{1g}$ (P) (υ_2) and $^{3}T_{1g}$ (F) $^{3}A_{2g}$ (υ_3). The observed signal at 7.38-7.67 ppm was assigned to the proton of aromatic ring and the 8.10 ppm observed for the (-CH=N-) imine proton conclude for imine formation of the ligand. The methylene (-CH_2-) proton present at 5.65 ppm and the region of 3.90 ppm observed at methoxy (-OCH_3-) proton present in aromatic ring. These observed values are expected to the ligand conformation. The spectroflurophotometer, tells about the excitation wavelength of the ligand (L_3) 459 (nm), the metal complexes also emitted the fluorescent radiation at the wavelength of 460 nm, 482 nm, 502 nm and 475 nm for Cu(II), Co(II), Mn (II) and Ru (III) respectively. Anti-microbial, anti-oxidant and DNA binding interaction activities have been studied. The Ru-L_3 complex has highly reactive against fungal candida albicans. The Ru and Cu-L_3 complexes have highly reactive against as K. Pheumoniea gram positive bacteria than that of free ligand. The Cu (II) and Ru (III) complexes are binding with DNA base pairs by intercalation mode by absorption spectroscopic method. The metal complexes were Cu (II), Co(II) and Mn(II) showed superior activity by cleaving into distinctive nicked and linear form.

Chapter-VI

Pharmacological properties newly synthesized schiff base ligand (1Z, 1'Z)-N, N'-(ethane-1, 2-diylbis(oxy))bis(5-methoxy-2,1-phenylene)bis(1-(2-bromo-4-flourophenylmethanimine (L_4) and its transition metal complexes.

This chapter VI involves newly Synthesis of (1Z, 1'Z)-N, N'-(ethane-1, 2-diylbis(oxy))bis(5-methoxy-2,1-phenylene)bis(1-(2-bromo-4-flourophenylmethanimine. (L_4) and its transition metal complexes and their biological activity and DNA interaction studies were carried out. The (L_4) Schiff base ligand is prepared by coupling of 2, 5- Dimethoxy Aniline and 2-Bromo-4-Fluoro Benzaldehyde. The Cu(II), Co(II), Mn(II) and Ru(III) metals were used by synthesized metal complexes by using newly synthesized ligands. The physical measurements and analytical data were studied. The molar conductance value tells about the non-electrolyte nature of all complexes. The analytical technique used likes, electronic spectroscopy, FT-IR, 1H- NMR, Mass and Spectroflurophotometer. The electronic spectrum provides L_4 shows a band at 277 nm and 221 nm which is assigned to n-π^* and π -π^*transition of the aromatic ring of the benzene. The

position of d-d transitions in the electronic spectrum of a metal complex gives valuable information about the geometry of the complex is confirmed by octahedral geometry. The FT-IR spectra of free Schiff base L_4 shows a band in the region 1618 cm^{-1} characteristics of the ν(C=N). Final evidence of the bonding is also shown by the observation that new bands appear in the spectra of all metal complexes in the low frequency regions at 640-783 and 455-575 cm^{-1} characteristic for ν (M–N) and ν (M–O) vibrations respectively. The broad band at 3300 cm^{-1} due to the intermolecular oxygen atoms is present in water molecule. The aromatic benzene ring present in the newly prepared Schiff base L_4 is confirmed multiplet at δ= 7.0 -7.37, - CH=N at δ = 8.87 , -OCH_3 proton present at δ =2.27, -O-CH_2 at δ =1.43, The azomethine proton (-CH=N) in the deshielding of proton present at the signal. This signal is confirmed the structure of L_4. The Mass spectrum is confirmed the dimeric nature present in this L_4. And the mass spectrum of metal complexes is confirmed M: L ratio is 1:1. All the synthesized metal complexes are fluorescent nature is confirmed the spectrofluorophotometer. The emission band occur at 480 nm for L_4 and the metal complexes also emitted the fluorescent radiation at the wavelength of 527 nm, 411 nm, 560 nm and 566 nm for Cu(II), Co(II), Mn (II) and Ru (III) respectively. Application of biological studies carried out in this chapter, the biological activity of Anti-bacterial, Anti-fungal Anti-oxidant and DNA binding nature of metal complexes. The Ru (III) complex active against Aspergillus flavus, Mn(II) complex active against staph.aureus. The free Schiff base ligand shows less activity against antimicrobial pathogens.

Chapter-VII

Amalgamation of 3-((2-aminophenylimino)methyl)naphthalen-2-ol Schiff base Ligand from Ortho phenylene diamine with 2-hydroxy -1 Naphthaldehyde and its transition metal complexes and their biological Activities

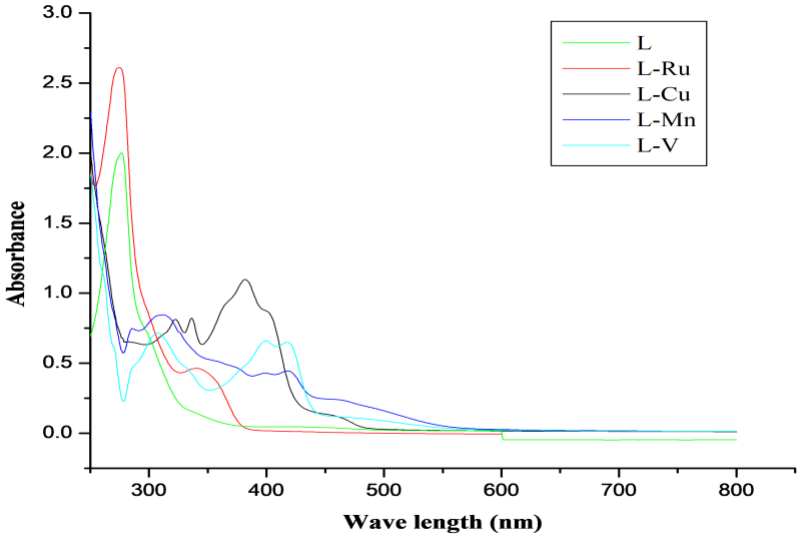
Transition metal complexes of Cu(II), Co(II), Mn (II) and VO(IV) condensed with 3-((2-aminophenylimino)methyl)naphthalen-2-ol and its derived from Ortho phenylene diamine with 2-hydroxy -1 naphthaldehyde have been synthesized and characterized by Electronic ,FT- IR , ^{1}H-NMR , EPR and Mass spectroscopy and screened to establish their potential as antibacterial, antifungal agents and antioxidants and DPPH radical Scavengers. The molar conductance values 8.8- 12.8 $ohm^{-1}cm^2mol^{-1}$ are give the non-electrolytic nature. The ligand and metal complexes are characterized by spectral techniques. FT-IR spectral data gives the functional group of synthesized

metal complexes. The imine (-N=CH) group is the main functional group of the ligand is confirmed the range between 1585-1610 cm^{-1} and the metal complexes is confirmed by (M-O) and (M-N) bond present in complexes arise the peak at 582-780 cm^{-1} and 424-470 cm^{-1}. The electronic spectroscopy gives about the geometrical nature of the complexes. The spectra gives the band at 276 nm assigned the transition of nπ* of imiine group and 228 nm bands assigned the π π* transition provides the aromatic moiety present in the ligand and its metal complexes. The signal at δ=9.45ppm (s, 1H) due to (-N=CH-) azomethine proton (10). The aromatic protons of benzene ring are resonated in the region δ=6.88 – 8.16 ppm (m, ArH) and signal at δ=15.40ppm (s, 1 H) show phenolic OH proton at present in benzene ring, the free amine protons signal at 4.0 ppm (s, 2 H) . Thus the ^{1}H-NMR spectral observations supported the assigned structure of ligand.

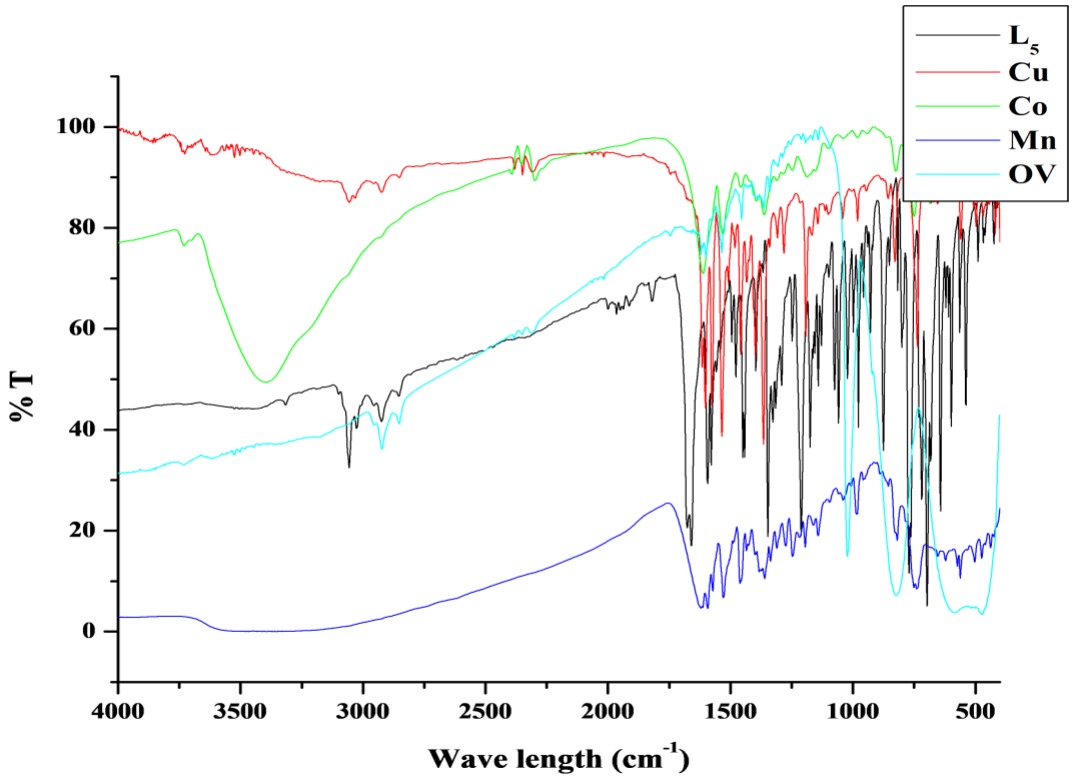
Evaluation of biological properties is investigated by Antimicrobial study for the ligand and its complexes. The complexes are highly active than the ligand. The metal complexes highly active against the (gram positive) S.aureus bacteria and the fungal of Candida albicans and Aspergillu niger. The free radical scavenging activity is hgher for metal complexes is confirmed the complexes prevent the oxidation of the enzymes. The metal complexes binding with CT-DNA via intercalation mode.

Synthesis of 2-hydroxy-1-naphthaldimino-2, 2'-(ethylene dioxy) bis ethylamine (L_1)

The molecular modeling octahedral geometry of metal complexes using L_3

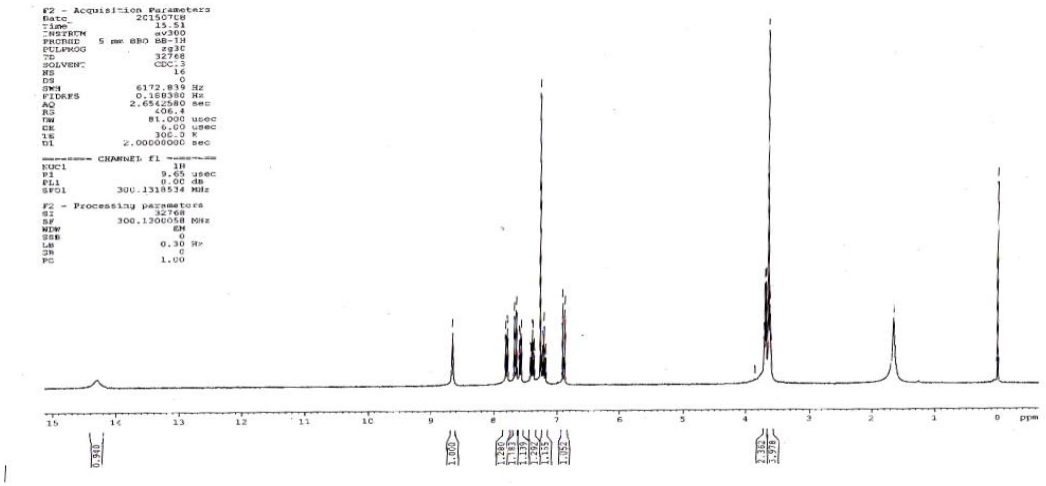


The electronic spectra for L_1 and its metal complexes

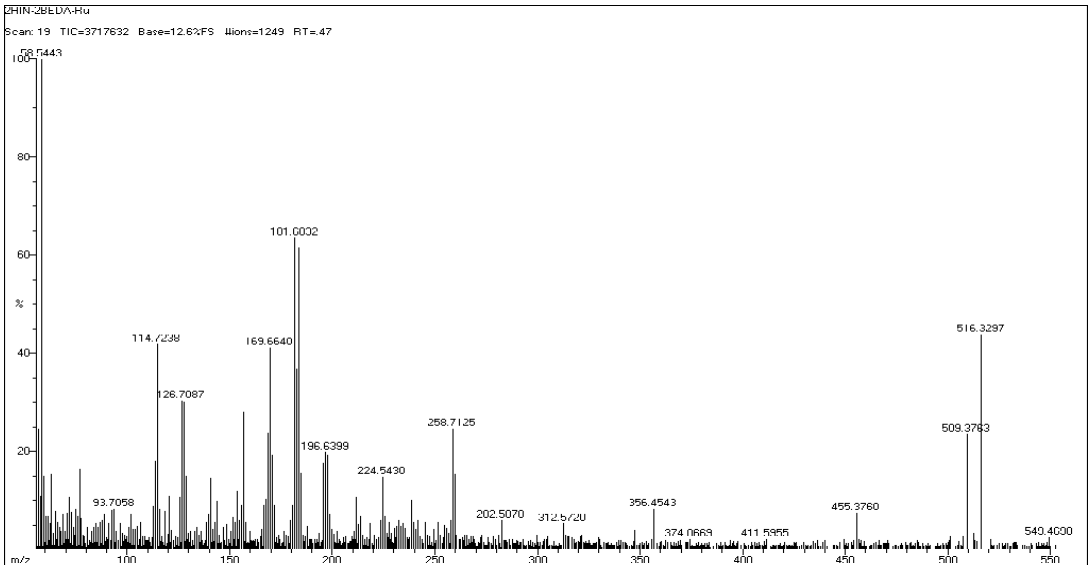


FT-IR Spectrum of L_5 and its metal complexes

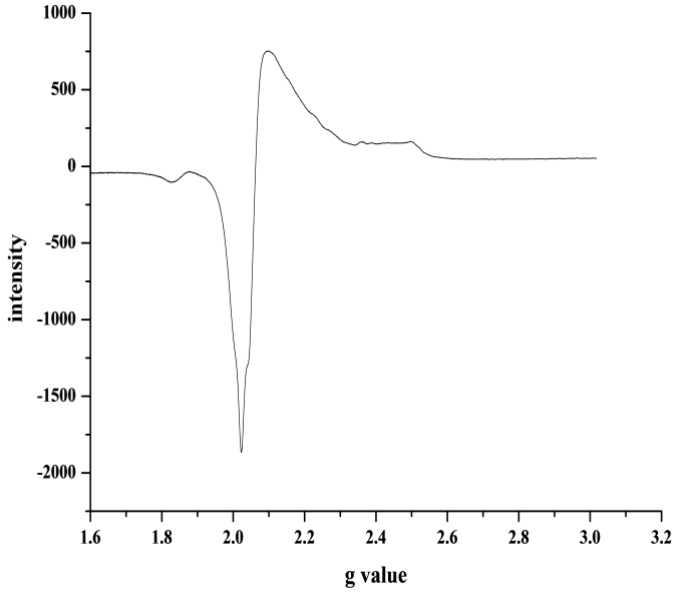
Structure of 1, 2- diphenyl-1-(2, 4, 6-trinitrophenyl) hydrazine free radical



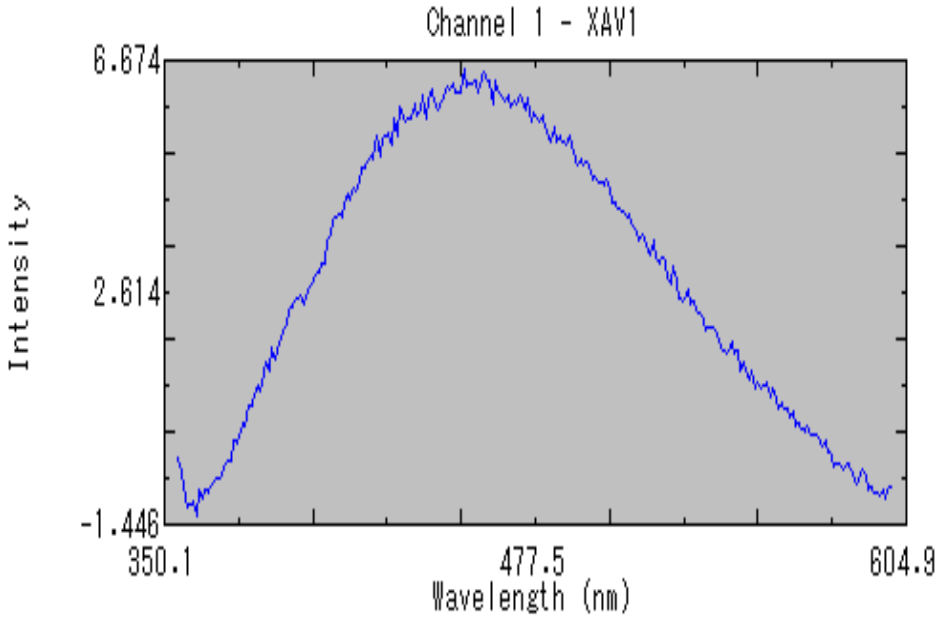
The ^{1}H-NMR spectrum of L_1



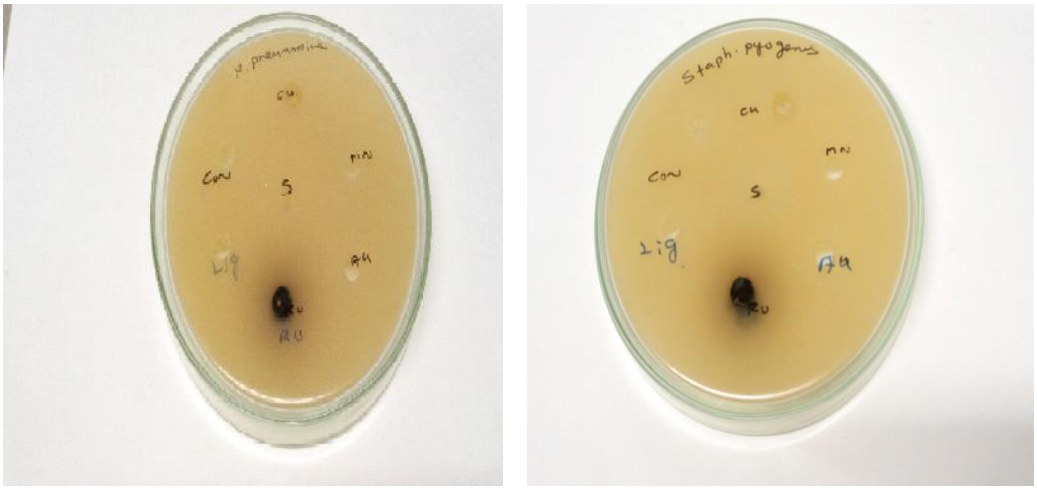
Mass spectrum of L_1– Ru (III) Complex



The EPR spectrum of Cu (II) complex

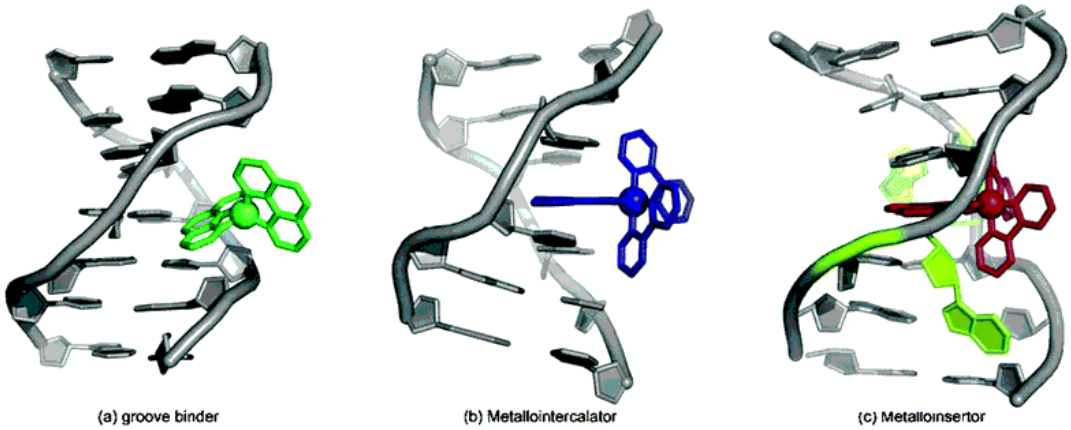


The emission spectrum of the Cu (II) complex



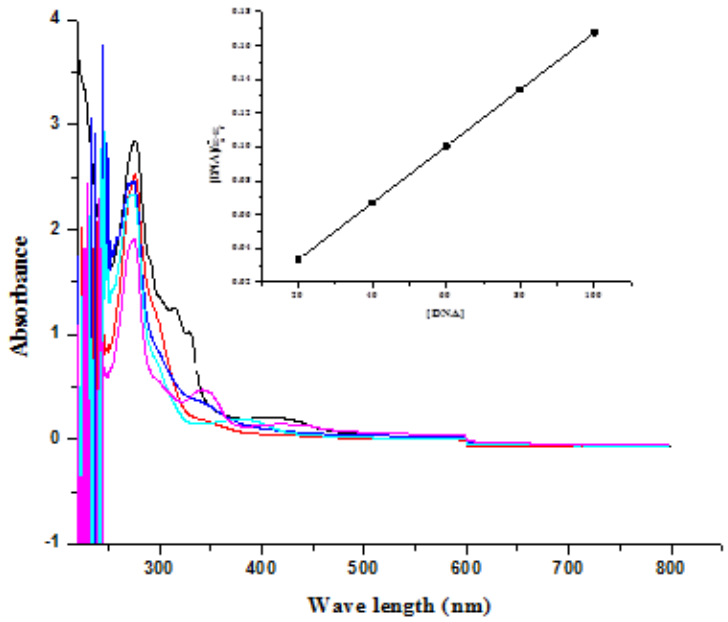
Anti-microbial activity of ligand and its metal complexes

DPPH Radical Scavenging Activity of L_3 compounds at different concentration



The Various mode of metal complex binding with DNA

DNA binding study

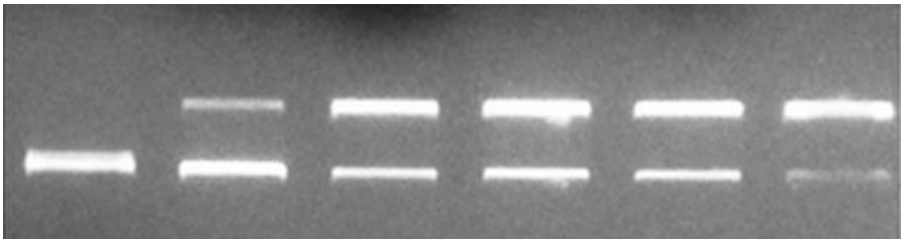


Absorption spectra of Ru (II) complex 1.6x 10^{-3} M in the absence and presence of increasing amounts of CT-DNA (20-100µM)

DNA Cleavage study

Form II

Form I



Lane-1 Lane-2 Lane-3 Lane-4 Lane-5 Lane-6

Gel electrophoresis assay for the cleavage of calf thymus DNA (200 ng) by Ru-complex in the presence of activators – reactive oxygen species (ROS) after 45 min incubation time at different concentration. Lane 1 - DNA control (Calf thymus DNA); Lane 2- DNA + Ru (10μM); Lane 3- DNA + Ru (20μM); Lane 4- DNA + Ru (30μM); Lane 5- DNA + Ru (40μM); Lane 6- DNA + H_2O_2.