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PG & RESEARCH DEPARTMENT OF CHEMISTRY
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As per the regulations of Madurai Kamaraj University, Madurai, **Mrs. A. ARUNADEVI (F9773),** Former Research Scholar (Full-Time) of Chemistry, VHNSN College(Autonomous), Virudhunagar, will defend her thesis at a Public Viva-Voce Examination through **Video Conference mode** using **Google Meet Platform**.

Title of the Thesis

SYNTHESIS, CHARACTERIZATION, DNA BINDING AND CLEAVAGE STUDIES OF BIOLOGICALLY ACTIVE AMINO ACID BASED COORDINATION COMPOUNDS OF TRANSITION METALS

Date & Time

9.11.2020 (Monday) at 10.30 am

Venue

Department of Chemistry, MBA LAB, VHNSN College (Autonomous)
Virudhunagar-626001

Video Conference Platform

Google Meet

Meeting ID

https://meet.google.com/zyy-hgag-xtx

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The Synopsis of the thesis is available in the College Website and a copy of the thesis is available in the Department Library, for reference. Faculty members, Scholars and Students are most welcome to attend the Viva-Voce Examination and take part in the discussion.

ALL ARE CORDIALLY INVITED

Place: Virudhunagar
Date: 27.10.2020

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SYNTHESIS, CHARACTERIZATION, DNA BINDING AND CLEAVAGE STUDIES OF BIOLOGICALLY ACTIVE AMINO ACID BASED COORDINATION COMPOUNDS OF TRANSITION METALS

SYNOPSIS of the thesis submitted to Madurai Kamaraj University in partial fulfillment for the requirements of the Degree of

DOCTOR OF PHILOSOPHY IN CHEMISTRY

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September– 2019

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Nowadays, developing metal-based drug is emerging as an active area of scientific research because of its much importance in medicinal field. They are used as medicines for the treatment of diabetes, antiinflammatory, cancer and cardiovascular diseases. Schiff base metal complexes have been amongst the most broadly studied coordination compounds. Several Schiff bases and their transition metal complexes have also been used as drugs as bactericidal, fungicidal, antitubercular and antiviral agents *etc*. This is due to the steric and electronic effects of substituents in the coordination zone amplifying the activity of the metal and other groups on Schiff base ligands. In addition, Schiff bases containing halogen groups and their metal complexes have a special interest due to their antimicrobial properties. Mainly, transition metal complexes have been of interest in the field of cancer research owing to the fact that they exhibit unique spectral and electrochemical signatures, as well as the ability of their ligands to be modulated to DNA binding and cleaving abilities. The most popular metal analogues on the market today are those that contain platinum and ruthenium based compounds. Other metal analogues containing copper, nickel, cobalt, zinc and vanadium are still under development.

In this potent area, recent years have observed a great deal of interest in the synthesis and characterization of transition metal chelates of pyrazolone derivatives. Many drugs and medicines contain a pyrazole ring system. There is much interest on the chemistry of pyrazolone Schiff base compounds. Among the pyrazolone derivatives, 4-aminoantipyrine derived organic and inorganic compounds are extensively studied due to the interest of their biological activities. Moreover, the mixed ligand complexes are models for finding applications in perceptive many biological systems. In this regard, the presence of coordination sites belonging to nitrogen heteroatomic rings such as indole, pyrazole, imidazole, 1,10-phenanthroline, pyridine *etc*., possesses remarkable DNA binding tendency. Among these 1,10-phenanthroline, imidazole and indole derivatives are important moieties which have attracted considerable attention for their versatility in exhibiting electronic properties and high cleavage efficiency.

In this aspect, biologically imperative compounds of amino acids are much interest in chemistry. Amino acids contain an amine group, a carboxylic acid group and aside chain that varies between different amino acids. These molecules are particularly form complexes with metal ions due to their potential donor atoms in -NH_2 and –COOH groups. Importantly, presence of multiple functional groups in the amino acid can lead to the development of various biological properties. In addition, amino acids compose the building blocks of proteins and are chemical species essential for performing number of biological purposes, as exemplified by the role of enzymes.

Based on these considerations, the entire work is planned to provide a discussion on the synthesis of a few novel 4-aminoantipyrine and amino acid based Schiff bases and their Cu(II), Co(II), Ni(II) and Zn(II) complexes which are structurally elucidated by various physicochemical techniques. Their detailed DNA binding, DNA damaging properties, *in vitro* antimicrobial and anticancer activities have been studied. The information obtained on these studies is helpful to develop new metal based therapeutic drugs which are discussed and well presented in Chapters-III to VI.

This thesis has been divided into seven chapters and the contents of each chapter in succinct are delineated hereunder.

CHAPTER-I

Introduction

This chapter presents a general introduction on coordination compounds along with explaining various physicochemical aspects of Schiff base ligands and their metal complexes as well as a brief discussion of their biological activities in medicinal field. It highlights the significance of the bioactive ligands with transition metal complexes along with providing an elaborate literature review regarding the same. The different chelating properties of these complexes and their various biomedical applications are discussed concisely. A brief introduction about the basic structure of DNA, its conformation and different binding modes like intercalation, groove and electrostatic binding are also discussed elaborately. Furthermore, a thorough explanation of DNA cleavage is included. In addition, the needs of *in vitro* biological studies of antimicrobial and anticancer studies are discussed elaborately. This is essential in making medical advances with more detailed analysis and can help in understanding a specific *in vivo* response in any given species. The *in silico* approaches are

also discussed. At the end of this chapter the scope of present work is nicely presented justifying the choice and significance of the work reported in the thesis.

CHAPTER-II

Materials and Methods

This chapter contains the general experimental methods, analytical procedures, and spectroscopic techniques such as FI IR, UV-Vis., NMR, EPR, Mass, powder XRD and electrochemical methods used in this work. It also encloses the experimental techniques used for DNA binding and cleavage, antimicrobial activity, anticancer activity (*in vitro* approach) and *in silico* approach of PASS biological activity predictions, ADME predictions and molecular docking studies.

CHAPTER–III

Histidine Derived Metallointercalators: Synthesis, Characterization, Molecular Docking and Biological Efficacy

This chapter depicts the synthesis of Cu(II), Co(II), Ni(II) and Zn(II) mixed ligand complexes of the type [ML(His)]Cl using the Schiff base ligand (obtained by the condensation of 4-chloro-3-nitrobenzaldehyde and 4-aminoantipyrine) as primary ligand (L) and L-histidine (His) as co-ligand. The synthesized ligand and its mixed ligand complexes have been characterized by the usual analytical and spectral techniques. The magnetic susceptibility and electronic absorption spectral data of the synthesized complexes suggest that the square planar geometry is present around the metal ion. The higher conductivity values of these complexes (52-70 Ω^{-1} mol^{-1} cm^2) indicate that they are electrolytic nature. From the analytical and mass spectral data of the Schiff base ligand and its complexes the stoichiometry of the complexes is found to be of [ML(His)]Cl.

The FT IR spectra of ligand (L) and its metal complexes are compared to know the changes during the complex formation. In the FT IR spectrum of ligand, the bands acquired at 1650 and 1591cm^{-1} are ascribed to the characteristic of carbonyl group in 4-aminoantipyrine and azomethine functional group respectively, which support the formation of ligand from 4-chloro-3-nitrobenzaldehyde and 4-aminoantipyrine. The FT IR spectra of complexes exhibit a shift in the distinctive peak of azomethine and carbonyl functional groups at 1580-1597 cm^{-1} and 1639-1656 cm^{-1} respectively which are assigned to

the coordination of azomethine nitrogen and carbonyl oxygen to metal centre. During the coordination, the additional peaks obtained at ~1460 and ~1386 cm^{-1} are assigned to the asymmetric and symmetric stretching vibrations of carboxylic oxygen and the peaks in the range 3000–3270 cm^{-1} for amine in amino acid moiety. This observation is further supported by M-N and M-O characteristic peaks noted at 433-443 and 536-524 cm^{-1} in all the complexes.

The DNA binding behaviors of these amino acid mixed ligand complexes were investigated by electronic absorption spectra, cyclic voltammetry and viscosity measurements. Electronic absorption titration study of the interaction of complexes with calf thymus DNA (CT DNA) reveals that the complexes can bind to DNA. Here [CuL(His)]Cl exhibits the highest binding affinity. The cyclic voltammetry of the complexes recorded in the absence and presence of CT DNA in 5 mM Tris-HCl/50 mM NaCl buffer solution confirms that they can bind to CT DNA *via* intercalation binding mode which has also been verified by the viscosity experiments. Based on the above findings, the ability of the complexes to affect the DNA helix is investigated. The cleavage study is examined by gel electrophoresis. It is found that the plasmid pUC18 DNA cleavage activity of the complexes is increased in the presence of H_2O_2 activator. Moreover, the *in vitro* antimicrobial activity of the complexes has been carried out by broth microdilution method. It signifies the activity of the ligand which has been improved by complexation. Finally, the *in silico* studies of PASS biological activity predictions and ADME-Tox properties of ligand and molecular docking studies of the synthesized ligand and its complexes are also carried out.

CHAPTER-IV

Biological and Molecular Docking Studies of DNA Targeted 4-aminoantipyrine Incorporating Mixed Ligand Complexes Having Histidine as Co-ligand

In this chapter, it is proposed to synthesize mixed ligand amino acid based transition metal complexes with slight modification of complex system by increasing the ratio of co-ligand synthesized in Chapter-III, for screening the biological potentials of the complexes which have been investigated for their binding with CT DNA using electronic absorption spectroscopy, cyclic voltammetry and viscosity measurement. The physicochemical studies of the synthesized metal complexes reveal the octahedral geometry for Co(II), Ni(II) and Zn(II) complexes whereas Cu(II) complex exhibits distorted octahedral geometry. But the

metal complexes described in Chapter-III exhibit square planar geometry. This change in geometry may be due to the modification of the co-ligand ratio (Chapter-III).

The low molar conductivity values (12–19 Ω^{-1} cm^2 mol^{-1}) indicate that the synthesized complexes are non-electrolytic nature. The magnetic susceptibility measurements at room temperature reveal the monomeric nature of the complexes. The electronic absorption spectrum of ligand shows two absorption characteristic bands, the weak band at 38911 cm^{-1} due to the $\pi \rightarrow \pi^*$ stacking interaction of π electrons present in the aromatic ring containing C=C and azomethine group of HC=N and the strong band at 27624 cm^{-1} for the $n \rightarrow \pi^*$ transition of non-bonded electrons available in the azomethine group. In all the complexes these characteristic bands of $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ are slightly changed either in position or intensity due to the result of coordination of ligand to the metal ion. The metal complexes show extra important band of d-d transition and this d-d transition band is useful to predict the geometry of the complexes. The electronic spectrum of copper complex shows the d-d band at 11696 cm^{-1} which is assigned to $^2E_g \rightarrow {}^2T_{2g}$ transition. This d-d transition suggests the distorted octahedral geometry of the copper complex. The cobalt complex shows d-d band at 12270 cm^{-1} which is assigned to $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ transition as the result of octahedral geometry. Furthermore, the UV-Vis spectrum of the Ni(II) complex reveals a d-d band at 12330 cm^{-1}, assigned to $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ transition attributed to octahedral geometry. In addition, FT IR, NMR, EPR and ESI-mass spectral and other analytical data confirm the geometry of the metal complexes $[ML(His)_2]$.

The interaction of the complexes with DNA has been effectively examined and explored by UV–Visible spectroscopy, cyclic voltammetry, and viscosity measurements of the synthesized compounds. Based on these studies, the binding mode is confirmed. The results obtained from these techniques reveal predominant intercalation of the compounds within DNA. Among all the coordination compounds, $[CuL(His)_2]$ complex shows comparatively greater binding as evident from its greater intrinsic binding constant value. The plasmid pUC18 DNA cleavage activity of the complexes is significantly increased in the presence of the oxidant (H_2O_2). The antimicrobial activities of the free ligand and its metal complexes are screened in DMSO using the broth microdilution method to evaluate their minimum inhibitory concentration (MIC) against a few pathogens. The cytotoxic potential of all the synthesized complexes against the selected cancer cell lines and normal cell line has been carried out and the outcome of it suggests that the complexes exhibit cytotoxic activity

which is compared with the activity of cisplatin. The fascinating thing is that the complexes show considerably less selectivity towards the normal cell line. This recommends that the complexes are sensitive towards the cancer affected cell lines than the normal cell line. In addition, *in silico* approach of molecular docking studies were executed to understand the nature of binding and binding affinity of the synthesized complexes with protein (PDB ID: 6COX) and DNA (PDB ID: 1BNA).

CHAPTER-V

DNA Interactions, Molecular Docking and *In Vitro* Biological Critiques of Pyrazolone Based Schiff Base Complexes Having Tryptophan as Co-ligand: Synthesis and Their Characterization

This chapter deals with preparation, characterization, DNA binding, DNA cleavage, molecular docking, *in vitro* antimicrobial and cytotoxic studies of pyrazolone based Schiff base mixed ligand complexes incorporating L-tryptophan and its chelates with a few transition metals like Cu(II), Co(II), Ni(II) and Zn(II). It describes the modification of metal complexes synthesized in previous chapter by introducing the co-ligand L-tryptophan instead of L-histidine which is used for further investigation. The structural features of the synthesized complexes have been arrived from their elemental analysis, magnetic susceptibility, molar conductance, mass, IR, UV–Vis., NMR and EPR spectral studies. The elemental analyses of the complexes confirm to the stoichiometry of the complex $[ML(Try)_2]$, where M= Cu(II), Co(II), Ni(II) and Zn(II) and Try = L-tryptophan. The electrical conductance studies of the complexes (12-16 Ω^{-1} mol^{-1} cm^2) in DMSO indicate their non-electrolytic nature.

In ^1H NMR, the ligand exhibits the multiplet at 7.2-8.4 ppm which is attributed to protons in the phenyl ring and also it displays the peaks at 2.5, 3.2 and 9.3 ppm for C-CH_3, N-CH_3 and HC=N respectively. The corresponding $[ZnL(Try)_2]$ complex shows the signals for aromatic and aliphatic protons with chemical shift values in accordance with the proposed structure. Compared to ligand, there is a signal at 9.9 ppm in downfield region, which might be attributed to the azomethine group of the Schiff base ligand involving coordination with metal ion. In addition to this, the observed peak at 10.1 ppm, which may be either indole –NH or protonated carboxylic group of the tryptophan because both appeared approximately in the same region of the NMR spectrum. In the case of amino acid, the characteristic signals

of CH, NH_2 and CH_2 appeared at 4.9, 6.8 and 3.52 ppm respectively confirming the presence of tryptophan in metal complexes. The peaks with chemical shift near 7.1–8.4 ppm were ascribed to aromatic protons of the Zn(II) complex. The obtained data of ^1H NMR are in close concord with the proposed structure. The EPR spectra of the copper(II) complex exhibit the g-tensor factors of $g_{\|}(2.23) > g_{\perp}(2.04) > g_e(2.0023)$ which indicate that the Cu(II) metal having unpaired electron predominantly localizes in the ground state $d_{x^2-y^2}$ orbital and also provide $A_{\|}(163) > A_{\perp}(66)$ revealing that the complex is present in axially elongated octahedral geometry.

DNA binding ability of these complexes has been investigated by electronic absorption titration, cyclic voltammetry and viscometry titration. These results reveal that the complexes effectively bind with DNA *via* an intercalative mode. The $[CuL(Try)_2]$ complex exhibits greater intrinsic binding constant of 5.6×10^5 M^{-1}. The results obtained from *in vitro* antibacterial and antifungal tests together show that all the complexes are effectively active towards bacteria and fungi. It has been found that the activities of the complexes are higher than Schiff base ligand. Further, the synthesized complexes were investigated for their *in vitro* cytotoxicity against two cancer cell lines namely, Human Liver Cancer cell line (HepG2), MCF-7 (human breast adenocarcinoma) along with NHDF (normal human dermal fibroblasts). The results suggest that the tested complexes exhibit better cytotoxic activity and selectivity towards the cancer cell lines and comparatively show low toxicity to NHDF cell line. This suggests that the presence of pyrazole and indole moieties plays an important role in the activity of the complexes which may be further modified to be a better anticancer agent. The results obtained from this work would be very useful to understand the mechanism of interactions of the small molecules binding to DNA and helpful in the development of their potential applications in biological, pharmaceutical and physiological fields in future.

CHAPTER-VI

Synthesis, Characterization of Indole Derived N, O Bidentate Ligand and Its Mononuclear Transition Metal Complexes: *In Silico* and *In Vitro* Biological Screening, Molecular Docking and Macromolecule Interaction Studies

Chapter-VI discusses the synthesis, characterization, DNA interactions, *in vitro* antimicrobial and cytotoxicity studies, *in silico* studies of PASS biological activity predictions of ligand, Swiss ADME properties, molecular docking studies of indole based Schiff base complexes with the ratio of 1:2 (metal: primary ligand) and mixed ligand Schiff

base complexes with the ratio of 1:1:2 (metal: primary ligand: co-ligand) condensed from 4-chloro-3-nitrobenzaldehyde and L-tryptophan (primary ligand) and espousing 1, 10-phenanthroline (co-ligand). These complexes could ultimately help to design newer drugs to develop useful DNA structural probes with efficient DNA recognition and cleaving agents. The physicochemical studies and spectral data reveal that the complexes $[ML_2]$ (1:2) adopt square planar geometry and complexes $[ML(1,10\text{-phen})_2]Cl$ (1:1:2) ratio adopt octahedral geometry around the metal ions. The DMSO solution of the metal complexes shows lower molar conductance values (12.3-18.0 Ω^{-1} mol^{-1} cm^2) for complexes of $[ML_2]$ type and higher (38.0-44.1 Ω^{-1} mol^{-1} cm^2) for complexes $[ML(1,10\text{-phen})_2]Cl$ due to the non-electrolytic and electrolytic nature of the synthesized complexes respectively, which illustrate the absence and presence of anion in the outer sphere of the complexes.

The interaction between the metal complexes of the Schiff base and CT DNA was investigated by electronic absorption titration, fluorescence titration, cyclic voltammetry and viscosity measurements. From these obtained results, $[CuL(1,10\text{-phen})_2]Cl$ complex has higher binding constant value and shows superior binding ability compared to other synthesized complexes. Thus, the binding order depends upon nature of co-ligand, presence of aromatic nature of ligand, size and ionic nature on the metal ion. Moreover the complexes of the type $[ML(1,10\text{-phen})_2]Cl$ have planar co-ligands comparatively showing good binding affinity towards the CT DNA. This suggests that conjugation and planarity play a significant role in the activity of the complexes. Gel electrophoresis investigation reveals that the synthesized complexes are efficient metallonucleases in the presence of hydrogen peroxide as activator. The results of antimicrobial activity show that the metal complexes are more effective against tested bacterial (*B. subtilis, S. aureus, P. aeruginosa, K. pneumoniae* and *S. typhi*) and fungal (*A. niger, A. flavus, C. lunata, R. bataticola* and *C. albicans*) species than that of the respective primary ligand under identical experimental conditions due to the enhancement of lipophilicity character of metal complexes. Among the synthesized complexes $[CuL(1,10\text{-phen})_2]Cl$ shows slightly higher activity compared to other metal chelates which may be due to easy of penetration to cell membrane. The additional factors of solubility, planarity, conjugation and also the bond length between the metal and ligand increase the antimicrobial activity. Furthermore, the *in vitro* cytotoxicity behavior of highly active Cu(II) complexes $[CuL_2]$ and $[CuL(1,10\text{-phen})_2]Cl$ against two cancer cell lines MCF-7 (human breast cancer cell line) and HepG2 (human liver cancer cell line) and one normal cell line NHDF (Normal Human Dermal Fibroblasts) through a colorimetric viability

(MTT) assay was carried out. The results indicate that the tested copper complexes show inhibitory activity against MCF-7 and HepG2. Moreover these complexes display lower inhibitory rates against NHDF than the standard anticancer drug. The entire result shows that the decrease of cell propagation is found to be dose and time dependent manner.

Theoretically, the different biological activities of synthesized Schiff base ligand were predicted by PASS online software. *In silico* ADME properties of compounds have been calculated by Swiss ADME predictor which shows the biological potential and oral administrative activity of the compounds. Based on PASS biological activity prediction score, the molecular docking studies are also performed for synthesized compounds. The metal complexes have higher binding energy with the target of DNA and protein compared to ligand. Among all the complexes, Cu(II) complexes show higher binding efficiency towards the target than other complexes. The results of this chapter show that the approach of coordinating indole derived analogues and co-ligand with pharmacological active metals like copper, cobalt, nickel and zinc could be a suitable strategy to develop novel therapeutic drugs for the medical treatment.

CHAPTER-VII

Summary and Future Scope

This chapter describes the summary of this thesis and throws light on the future scope of the research. As a conclusion, this thesis work is loyal to the designing of a novel preparative technique for a series of metal based complexes comprising with 4-aminoantipyrine/L-tryptophan based Schiff base ligands and co-ligands of biologically active amino acids (L-histidine and L-tryptophan) and 1,10-phenanthroline with biologically imperative Cu(II), Ni(II), Co(II) and Zn(II) metal ions. They have been characterized structurally by using various analytical and spectral techniques and their detailed DNA binding and damaging properties are studied. In order to investigate the biological inspiration of the Schiff base metal complexes, their antimicrobial screening using broth micro dilution method has been carried out. In addition, the *in vitro* cytotoxicity studies have been deliberated by MTT assay.

The amino acid moieties in Schiff base complexes were synthesized, characterized and evaluated for DNA interactions, molecular docking, and antimicrobial screening and cytotoxicity studies. All of them showed good biological efficacy. Among these, all the

Cu(II) complexes show good DNA binding/cleavage results. In particularly, pyrazolone based N_2O_2 Schiff base Cu(II) complex containing L-tryptophan as co-ligand has good DNA binding/cleavage properties and cytotoxicity activity. The antimicrobial screening study also highlights the efficiency of Cu(II) complexes from Chapters III to VI. Interestingly, the anticancer efficacy of the Cu(II) complexes is selective towards the cancer cell lines and less toxicity towards the normal cell lines. All the copper complexes show diverse results which can be further improved into DNA probes, antimicrobial agents and even anticancer drugs after the *in vivo* approach.

The significant results of the *in vitro* approach of DNA interactions and biological screening studies simulate a growing attention towards the direction of design and development of transition metal based drugs in cancer chemotherapy. Consequently, the elaborate *in vitro* studies utilizing these Cu(II) complexes [CuL(His)]Cl, [CuL$(His)_2$], [CuL$(Try)_2$], [CuL_2], [CuL(1,10-$phen)_2$]Cl, in general and [CuL$(Try)_2$], in particular could lead to a significant outcome in further expanding scope. The innovative findings may occur through a combination of bio–potent ligands and bio-essential metal ions. Therefore, this conceptual demonstration is expected to be a stepping stone for many future clinical applications using *in vivo* approach.