NOVEL CONJUGATED TRIAZINE CENTRED METAL DERIVATIVES FOR PHOTOPHYSICAL STUDIES AND BIOMEDICAL APPLICATIONS

The chemistry of metal complexes with multidentate ligands such as Schiff bases of *s*-triazine moiety has recently gained more attention because of their use as models in biological systems. They proved outstanding pharmalogical actions such as antimicrobial, antituberclosis, antiplatelet, antioxidant, anti-inflammatory, anticancer, antimalarial, analgesi. In complex formation and novel preparation and application is obtained due to molecular conjugation. Aromatic molecules are conjugated molecules with one or more rings fused together in planar form to confer extra stability. Electronic features of conjugated molecules include their ability to absorb and emit light, formation and transport of charge, and the capability to accept or donate one or more electrons as semiconductors.

Basing on the primary objective of the research, symmetric triazines are chosen. These are a very important class of six-membered aromatic heterocyclic compounds receiving much attention in scientific research. There has been a growing interest on synthesis, structural studies and applications of transition metal complexes of symmetric triazines along with curcumin towards pharmacological properties particularly in application to cytotoxic activities. The tri-substituted 1,3,5-triazines are used as important core structure in many chemotherapeutic agents due to their interesting pharmacological properties including anticancer, anti-angiogenesis, anti-HIV, antimalarial, anti-trypanosome, antibacterial and antimicrobial activity. The *s*-triazine compounds also exhibit anti-ulcer, anti-depressant, antiviral and anti-oxidant activity.

The primary objective of this research work is to characterize the structural, electronic and fluorescent properties of novel conjugated organic molecules. The synthesized molecular materials are made therefore to undergo photophyical studies and evaluated based on emission properties. From the results obtained from molecular docking studies, a serious biomedical study is made on different complexes towards various cell-lines of ovarian cancer, lung cancer, skin cancer, oral and gastric cancer. A good evolution of results is attained in biomedical field.

CHAPTER I

GENERAL INTRODUCTION

This chapter provides an introduction to triazine based curcumin compounds, their chemistry, biological importance, optoelectronic properties and biomedical applications.

The objectives and scope of the present study has also been discussed in order to justify the choice and significance of the work reported in the thesis. This chapter is an outcome of a concerted effort to prove that the novelty is inherent in synthesis and applications of the complexes.

CHAPTER II

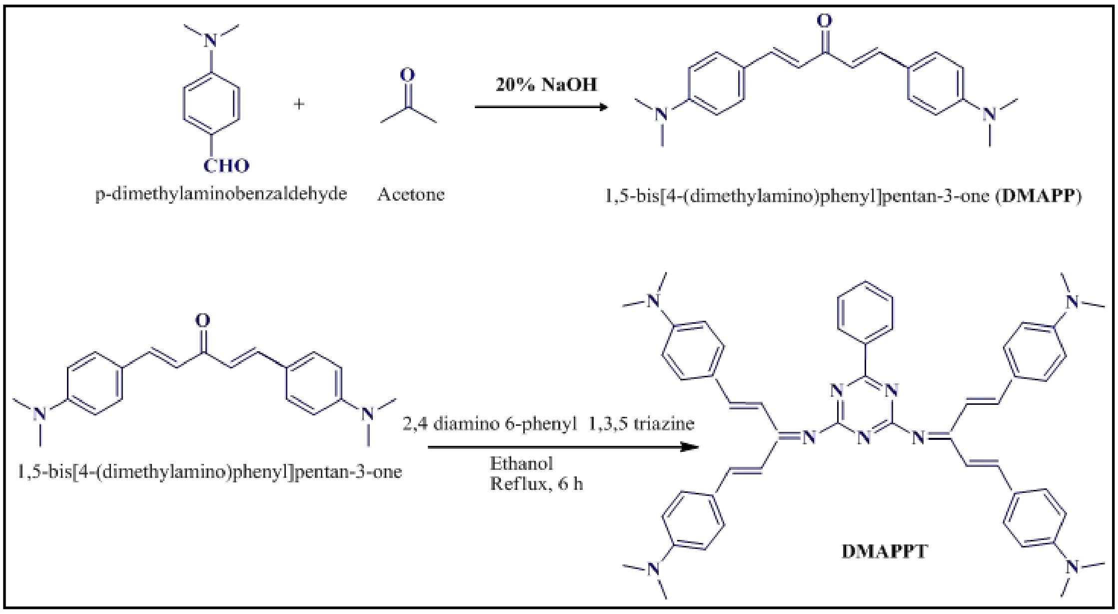
GENERAL EXPERIMENTAL METHODS AND TECHNIQUES

This chapter presents the details of the preparation of precursors and experimental techniques used for the characterization of ligand and its metal complexes. It also includes details about the general experimental methods, analytical procedures, magnetic measurements and spectroscopic techniques like FT-IR, UV-Vis, ^{1}H-NMR, EI-Mass, ESR, CV, XRD, SEM and EDAX studies used for the work. The details of the general experimental technique, adopted analytical procedures, materials employed along with purification of solvents, procedures for measurement of photophysical studies and biological activities alike described in this chapter. Details of the synthesized triazine and curcumin based ligands and their complexes were given in the relevant chapters. A specific summary is made on antimicrobial and anti-oxidant studies; methodical study to be done on anti-tubercular, anti-diabetic and anti-cancer activities is highlighted.

CHAPTER III

TRIAZINE CENTRED METAL COMPLEXES FOR STRUCTURAL STUDIES TOWARDS ANTI-TUBERCULAR AND ANTI-CANCER ACTIVITIES

A series of metal complexes of the type [ML.H_2O.Cl_2], where M = Cu(II), Co(II), and Ni(II) has been synthesized from [1,5-bis(4-(dimethylamino) phenyl) penta-1,4-die-3ylidene)-6-phenyl-1,3,5-triazine-2,4-diamine] (DMAPPT) **L_1 (Scheme 1)**. The molar conductance of the complexes in 10^{-3}M ethanol is in the range 5-9 Ω^{-1} cm^2 mol^{-1} indicating that all the complexes are non electrolytes.



Scheme 1. Synthesis of ligand (DMAPPT) **L_1**

The IR spectrum of the ligand (**L_1**) shows a strong peak at 1655 cm^{-1} which is characteristic of the azomethine group ν(C=N) and it is shifted to lower frequency range of 1618-1585 cm^{-1} upon complexation which indicates that the azomethine nitrogen is one of the coordinating sites. Peaks at 3512–3446 cm^{-1} are attributed to υ(O-H) stretching vibrations of water molecules and indicate the presence of hydrogen bonding which is absent in the ligand. The peaks due to ν(M-O) appear at 513-572 cm^{-1} and peaks at 456-479 cm^{-1} are attributed to ν(M-N) stretching vibrations.

The electronic spectrum of Co(II) complex shows bands at 246 nm and 386 nm which are attributed to $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ transitions. The band at 462 nm is assigned to $^4T_{1g}$ (F) → $^4T_{1g}$ (P) transition. The magnetic moments value is 4.8 BM which agrees with the octahedral coordination geometry. Ni(II) complex exhibits a sharp band of high intensity at 341 nm due to $n\rightarrow\pi^*$ transition and a broad band at 462 nm due to $^3A_{2g}$ (F) → $^3T_{1g}$ (P) transition respectively. The magnetic moments value is of 3.21-3.31 BM, observed for octahedral coordination geometry. The Cu(II) complex shows a sharp bands of high intensity at 246 and 283 nm due to $\pi\rightarrow\pi^*$ transitions and the band 333 nm is due to $n\rightarrow\pi^*$ transition. The band at 481 nm is due to $^2E_g\rightarrow{}^2T_{2g}$ transition required for Cu(II) octahedral complexes.

In ^{1}H-NMR spectrum of the ligand DMAPPT, signals at 2.8 - 2.10 ppm (δ) correspond to (–CH_3) protons. A characterized singlet at 3.106 ppm (δ) is due to the methyl protons near to hetrocyclic atoms (-N-CH_3). The doublet at 6.809 ppm (δ) and 6.827 ppm (δ) can be attributed due to the proton (-CH=CH-) which is present in the side chain of the ligand. In metal complexes, signals at 2.103-2.968 ppm (δ) and 2.193-2.942 ppm (δ) correspond to (–CH_3) protons respectively. The signals at 8.243-8.257 ppm (δ) and 8.028 ppm (δ) in both the complexes correspond to the phenyl ring proton (-C=CH-) of benzoguanamine. The characteristic dimethyl amino groups are confirmed by chemical shift at 3.1 ppm (δ), corresponding to six protons.

In EPR spectrum of Cu(II) complex, the spin Hamiltonian parameters show $g_{\parallel}$(2.208) is greater than $g_{\perp}$(2.08), suggesting that $d_{x^2-y^2}$ to be the ground state orbital. The covalent character of metal-ligand bond is inferred from g_{iso} value 2.14, which supports the fact that the unpaired electrons lie predominantly in $d_{x^2-y^2}$ orbital. Since $g_{\parallel}$ is greater than $g_{\perp}$, this clearly shows an axial elongation in the octahedral geometry of this particular complex. The observed value of α^2 of the complex is lesser than unity (α^2 = 0.75) which indicates that the complex has covalent character. The observed value of β^2 (1.70) indicates the covalent nature of the complex. The $g_{\parallel}/A_{\parallel}$ (145 cm) ratio reveals that the structure is highly distorted from planarity.

Through the EI-MS spectrum, the molecular ion peak of Co(II) complex is obtained at m/z 938.34 u. It confirms the proposed formula to the corresponding ligand and complex moiety. The base peak is obtained at m/z 78. The series of peaks with values *i.e.,* m/z 211.8, 401, 550, 594 u, are attributed to different fragments of the complex. The molecular ion peak (938.34) is in good agreement with the suggested molecular formula indicated from elemental analysis.

As seen from electrochemical curves, for Co(II) complex, E_{pa} = 0.526 V and E_{pc} = -0.121 V and ΔEp = 0.647V. The ratio of I_{pa}/I_{pc} is not equal to one for the complex and it is quasi-reversible complex. For Cu(II) complex, the ratio of I_{pa}/I_{pc} is equal to less than one therefore it is reversible in nature. In both the complexes it is found that the process taken is a single electron transfer kinetics.

The crystal structure of the complexes were determined by X-ray crystallography. There are peaks for the ligand with 2θ values at 5.10^0, 15.01^0, 24.02^0 and 27.5^0; for Co(II) complex, there are refined peaks at 8.15^0, 15.02^0, 17.5^0, 23.5^0 and 27.5^0. For Cu(II) complex, 2θ values are obtained at 5.10^0, 13.12^0, 15.10^0, 18.15^0, 30.10^0 and 39.45^0. For Ni(II) complex, 2θ values are obtained at 13.12^0, 15.10^0, 18.15^0, 25.10^0 and 29.45^0. The sharpness and intensity of the XRD peaks indicate the more crystalline nature of metal complexes.

SEM and EDAX images show that there is conspicuous change in the morphology of Co(II) complex when coordinated with a metal ion. The characteristic morphologies were observed at 0.1 to 10 µm respectively. The Co(II) complex shows bar like structure at 10 µm and faceted microcrystal at 5 µm. At 0.1 µm, the complex shows ice sponge structure. The result by EDAX has indicated that there are Cobalt, Chlorine, Carbon and Oxygen peaks which complement the proof of the elemental analysis of the complex.

On the basis of elemental analyses, magnetic susceptibility measurements, FT-IR, UV, ESR, CV, EI-Mass, XRD spectral studies, the following structure is proposed for the complexes.

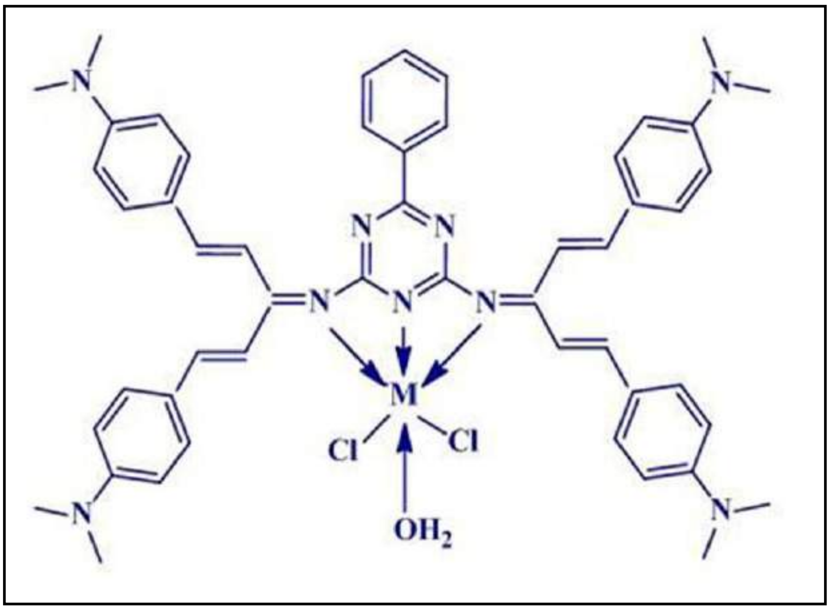


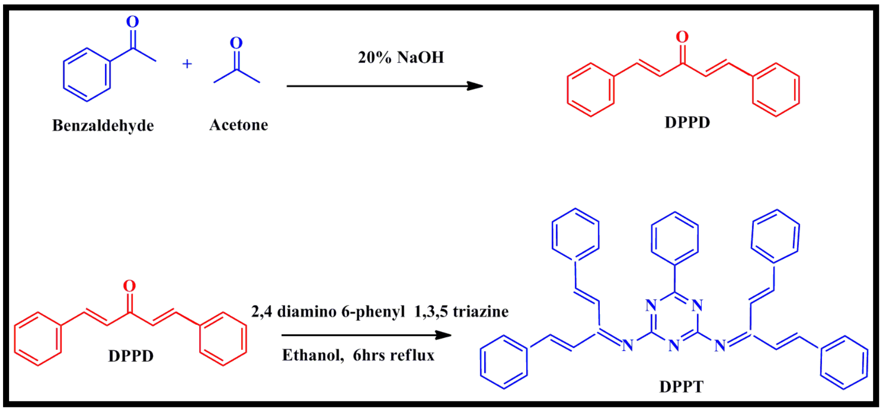
Figure 1. Proposed structure of Metal(II) complexes

The ligand and its metal complexes exhibit an enhanced fluorescent intensity. All the conformers were subjected to docking with the active site of the protein. Binding Energy for the ligand DMAPPT is -460.56 KJ/mol and for [Co-(DMAPPT)$H_2O.Cl_2$] is -456.35 KJ/mol. The docking proved that the ligand interacted with the amino acids: Arg 841, Glu 758, Lys 875, Leu 858, Val 726, Cys 797, Arg 841, Lys 875, Pro 877; and the Co(II) complex interacted with the amino acids: Arg 841, Phe 723, Leu 799. Basing on the docking results, biological activity of Co(II) complex was determined under *in vitro* conditions using *Luciferase Reporter Phage* (LRP) assay against drug sensitive *Mycobacterium tuberculosis* (cell-line:H37Rv) for which **53.69 %** cell-death was obtained at 100µg/ml. For biomedical application, Co(II) complex was screened on ovarian cancer cell-line SKOV3 for which **82.47%** and **67.38%** cell-death was obtained at 1000 and 300 µg/ml respectively. From the results, it is clear that this compound would be better used in drug development towards anti-tubercular and anti-cancer activities.

CHAPTER IV

COBALT BASED NOVEL CONJUGATED TRIAZINE CENTRED METAL COMPLEXES TOWARDS ANTI-MICROBIAL AND ANTI-CANCER ACTIVITIES

Lung cancer remains one of the primary fatality causes in most countries. To respond to the need, a new series of Co(II), Cu(II) and Ru(III) complexes were prepared with chelating ligand derived from benzaldehyde and acetone in combination with benzoguanamine. Based on the analytical and spectral data, it is concluded that the ligand acts as tridentate forming octahedral geometry with metal ions in case of $[ML_2]Cl_2$ type metal(II) complexes. Metal complexes were found to have molar conductance values in the range of 154-200 Ω^{-1} cm^{-1} mol^{-1} suggesting its ionic nature and of 1:2 electrolytic nature.



Scheme 2. Synthesis of ligand (DPPT) L_2

The IR spectra shows that the coordination through azomethine nitrogen was supported by shifting of ν(C=N) towards higher frequencies observed at 1672 cm^{-1} in free ligand, while in complexes, shifted to lower frequencies of 1626-1656 cm^{-1}, which suggests that the coordination of azomethine nitrogen to the metal ion. The ligand shows a strong peak at 1543 cm^{-1} which characteristic of the ν(C=N) group in s-triazine. This band is shifted to lower frequency of 1520-1480 cm^{-1} upon complexation which indicates that the above group has one of the coordinating atoms in the ligand. Bands in the region 543-558 cm^{-1} are assigned to ν(M-N). Hence it is concluded that the ligand acts as a tridentate forming octahedral geometry with the metal ions in case of $[ML_2]Cl_2$ type.

The electronic spectrum of Co(II) complex exhibit three d-d bands. Around 18484 cm^{-1} and 17271 cm^{-1} are assignable to $^4T_{1g}$ (F) → $^4T_{2g}$ (P) transition, and band at 12870 cm^{-1} is assignable to $^4T_{1g} \rightarrow {}^4A_{2g}$ (F) transition respectively. The magnetic moment for Co(II) complex is in 4.71 BM range supporting octahedral geometry. The Cu(II) complex under investigation displays two absorption bands at 14556 cm^{-1} and 14306 cm^{-1}, which are assigned to $^2E_g \rightarrow {}^2T_{2g}$ transition respectively. The magnetic moment for all complexes is well within the range expected for distorted octahedral geometry around the central metal ion. Ru(III) reflects a peak value with 671nm (14903 cm^{-1}) due to d-d transition and magnetic moment value (5.12 BM) comply with the octahedral nature of the complex .

In ^{1}H-NMR spectra of all metal complexes, the chemical shift at 3.055-3.674 ppm (δ) in **1**, 3.353 ppm(δ) in **2** and 3.361 ppm(δ) in **3** refer to -N-CH protons of triazine moiety in metal complexes. The multiplet at 7.102-7.787 ppm (δ) in **1**, 7.120-7.829 ppm(δ) in **2** and 7.141-7.845 ppm(δ) in **3** have been assigned to the protons on benzene rings. The singlet obtained due to the chemical shift at 6.330-6.834 ppm (δ) relate to -CH=CH- protons in **1**. In Schiff base, peak appears at 9.785-9.933 ppm(δ) is attributable to azomethine (-N=CH-) protons which deshielded in the spectrum of Co(II) complex, indicating the complexation through azomethine nitrogen atom in DPPT complex.

The EI-Mass spectrum a of ligand shows that the molecular ion peak appear at m/z 618.612 and for Co(II) complex at m/z 1298 ($C_{86}H_{66}CoN_{10}$) respectively. The molecular ion peak for Co(II) complex confirms the stoichiometric composition of the Co(II) complex of $[Co(DPPT)_2]Cl_2$ type. A series of the peaks with values i.e., m/z 118,144, 245, 387, 480 u, are attributed due to fragment patterns of $[Co(DPPT)_2]Cl_2$.

EPR spectrum of Cu(II) complex shows a well resolved anisotropic broad signal. The spin Hamiltonian parameters of Cu(II) complex, $g_{\parallel}$ (2.28) is greater than $g_{\perp}$(2.07), suggests that the $d_{x^2-y^2}$ to be the ground state orbital. Since observed spectral parameters show that the value of $g_{\parallel}$ is greater than $g_{\perp}$, this clearly shows an axial elongation in the octahedral geometry of this particular complex. The observed value of α^2 of the complex is lesser than unity ($\alpha^2 = 0.75$) which indicates that the complex has covalent character. The $g_{\parallel}/A_{\parallel}$ (145 cm) ratio reveals the structure is highly distorted from planarity.

XRD analysis of Co(II) complex revealed four sharp and two moderate reflections at 5^0, 7.5^0, 10^0, 18^0 and 22^0 wherein the ligand shows only two peaks at 18^0 and 22^0, verifying the complexation of the metal ion of Co(II) with DPPT. XRD analysis of Cu(II) complex revealed seven moderate reflections at 9^0, 28^0, 37^0, 48^0, 52^0 and 69^0 confirming the complexation of the metal ion of Cu(II) with DPPT.

The cyclic voltammogram of Cu(II) complex exhibits a well-defined redox process due to the formation of Cu(II)/Cu(I) couple at E_{pa} = 0.8 V and the corresponding cathodic peak at E_{pc} = -0.7 V and ΔEp = 0.1V. The ratio of I_{pa}/I_{pc} is equal to less than one for Cu(II) complex. Hence it’s a reversible complex and also it was found that the process was simple one electron process.

The characteristic morphologies were observed with the image resolution of 10 µm to 0.1 µm respectively. SEM images of the complex in the resolution of 0.1-0.5 µm are viewed as flower type particles, and from 1µm -10 µm the images appear to be rocky in nature. There is conspicuous change in the morphology of Co(II) complex when coordinated with a metal ion. The elemental composition of Co(II) complex is identified with Energy Dispersive X-ray Analysis (EDAX). The analytical results indicated that there are Cobalt, Chlorine, Oxygen and Carbon peaks which relects the stoichiometric analysis of the complex.

On the basis of the above physico-chemical evidences, the following structure has been assigned for the metal complexes of $[M(DPPT)2]Cl_2$ type.

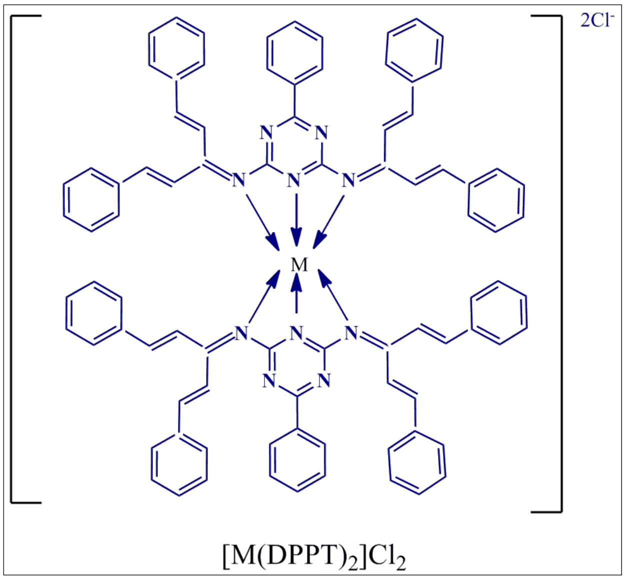


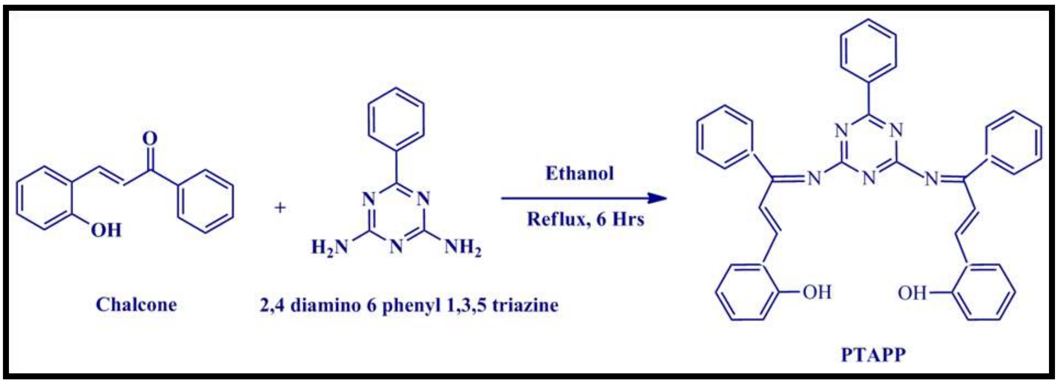
Fig.2. Proposed structure of metal(II) complexes

Significant differences in the positions of emission maximum establish the complexation process. Such enhancement of fluorescence through complexation is of much interest as it opens up the opportunity for photochemical applications. All the conformers were subjected to docking with the active site of the protein. Binding Energy for the ligand DPPT is found to be -408.85 KJ/mol and for [Co-(DPPT)$_2$].Cl$_2$ is -536.21 KJ/mol. Docking results proved that the igand interacted with the following amino acids: Leu 799, Leu858, Ile878; and the [Co-(DPPT)$_2$].Cl$_2$ complex interacted with the following amino acids: Cys797, Phe 723. [Co-(DPPT)$_2$].Cl$_2$ shows better antimicrobial activity than the ligand. All the synthesized compounds were screened *in vitro* against A549 cell-line for lung cancer. Experimental study demonstrated that the Cobalt substituted derivative exhibited more potent anticancer activity than other substituted derivatives. Co(II) complex shows a close proximity observed (**1.544, 100µg/ml**), compared to the world standard cis-platin (**1.123, 100µg/ml**). It is notable that docking between Co(II) complex and the protein EGF kinase (2J5F) was found to have the lowest binding energy *i.e.*, **-536.21 KJ/mol**. Hence this compound is the ideal candidate for further studies with regard to the biomedical applications. Structure–activity relationship in this compound lays foundations to develop more potent compounds as it leads to chemotherapy.

Chapter V

Metal complexes of Chalcone substituted triazine moiety and their Structural, Molecular Docking studies and Biological Applications

1,3,5-Triazine is considered to be a remarkable synthon in supramolecular Chemistry due to its chemical interactions. Triazine based ligand [(2,2’-(6-phenyl-1,3,5-triazine-2,4-diyl)bis(azanylylidene)) bis(3-phenylprop-1-en-1-yl-3-ylidene))diphenol)] (PTAPP) **L_3** (**Scheme 3**) and its Mn(II), Co(II), and Cu(II) complexes have been synthesized from benzoguanamine. Based on the analytical and spectral data, it is concluded that the ligand acts as multidentate forming square pyramidal geometry with metal ions in case of [ML]Cl type metal(II) complexes. Metal(II) complexes were found to have molar conductance values in the range of 154-200 Ω^{-1} cm^{-1} mol^{-1} suggesting its ionic nature and of 1:2 electrolytic nature.



Scheme 3. Synthesis of ligand (PTAPP) L_3

In IR spectra of ligand and metal complexes, the coordination through azomethine nitrogen was supported by higher frequencies of ν(C=N) observed at 1681cm^{-1} in free ligand, while in complexes, it is shifted to lower frequencies of 1606-1617 cm^{-1}, which suggests that the coordination of azomethine nitrogen to the metal ion. The peak at 1547 cm^{-1} corresponds to ν(C=N) of triazine ring which confirms the condensation reaction between the chalcone and 6-phenyl-2, 4-diamino triazine. It is shifted to lower frequency range at 1459-1480 cm^{-1}. The peak at 1246 cm^{-1} in the ligand is an indication of the presence of ν(C-O), which is moved to higher frequencies 1405-1459 cm^{-1} after complexation, this shift confirms the participation of phenolic oxygen of the ligand in ν(C-O-M) bond formation. The peaks at 439-447 cm^{-1} and 520-580 cm^{-1} correspond to ν(M-O) and ν(M-N) respectively, confirming the coordination of metal(II) ion to the ligand.

The electronic spectrum of Mn(II) complex shows a broad band at 572 nm (17482 cm^{-1}) which is one of the spin-forbidden transitions of Mn(II) high spin complex. The d^5 configuration makes Mn(II) ion susceptible to Jahn-Teller distortion in an environment of symmetry resulting in square pyramidal geometry. The Co(II) complex displays three d-d absorption bands at 235 nm (42553 cm^{-1}), 295 nm (33898 cm^{-1}) and 345 nm (28985 cm^{-1}) assigned to π-π*, n-π* and $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ transitions indicating a square pyramidal geometry around Co(II) ion. The Cu(II) complex displays three bands at 240 nm (41666 cm^{-1}), 298 nm (33557 cm^{-1}) and 358 nm (27932 cm^{-1}) which are assigned to π-π*, n-π* and $^2E_g \rightarrow {}^2T_{2g}$ transitions. Its μ_{eff} value appears at 1.84-1.95 BM which supports square planar geometry around Cu(II).

The ^{1}H-NMR spectrum of ligand PTAPP shows a broad peak due to the two phenolic protons at 5.32 ppm (δ). Two doublet peaks at 5.81 ppm (δ) and 7.14 ppm (δ) are due to the four alkene protons of the chalcone. In metal(II) complexes, signals at 2.528 ppm (δ) and 2.512 ppm (δ) correspond to methyl protons in cobalt(II) and copper(II) complexes respectively. The singlet obtained due to the chemical shift at 6.8 ppm (δ) and 6.771-6.941 ppm (δ) relate to -CH=CH- protons. The multiplet at 7.50 and 7.285-7.958 ppm (δ) are assigned to the protons on benzene rings. The signals at 8.265 ppm (δ) and 8.043-8.255 ppm (δ) in both the complexes correspond to the phenyl ring proton (-C=CH-) of benzoguanamine.

EI-Mass spectrum for the ligand is observed at 599.912 which is closer to the calculated mass. The molecular ion peaks in the mass spectra are observed at m/z 689.234 (**1**), 694.53 (**2**) and 701.3916 (**3**) which are in close agreement with the calculated mass of M^+ ions of complexes, corresponding to molecular ion formula $MnC_{39}H_{29}N_5O_2^+$, $CoC_{39}H_{29}N_5O_2^+$ and $CuC_{39}H_{29}N_5O_2^+$ respectively. The spectrum of [Mn(PTAPP)]Cl is taken for analysis in detail, in which the spectrum records the presence of fragments with m/z values at 77, 148, 183, and 209.7 attributable to different fragments of the complex.

The ESR spectrum of Cu(II) complex **(3)** exhibits anisotropic signals with $g_{\|}$ = 2.201 and $g_{\perp}$ = 2.06. The observed spectral parameters show $g_{\|}$ (2.201) > $g_{\perp}$ (2.06), which is a characteristic of an axially elongated square planar geometry. The covalent character of metal-ligand bond is inferred from the g_{iso} value, which also supports that the unpaired electrons lie predominantly in the $d_{x^2-y^2}$ orbital. The α^2 values suggest appreciable in-plane covalency in the molecule. The calculated value of ($g_{\|}/A_{\|}$) for the complexes is characteristic of distorted structure. The existence of unpaired electron in the d_{x2-y2} orbital corresponds to square planar geometry around the Cu(II) center. It is further confirmed by the value of G (axial symmetry parameter), which is calculated with the help of $g_{\|}$ and $g_{\perp}$.

Powder X-ray diffraction patterns of the metal complexes show the sharp crystalline peaks. XRD analysis of Mn(II) complex revealed two well-defined peaks with 2θ values at 22.02^0 and 40.02^0 and for the Co(II) complex there are three peaks with 2θ values at 8.12^0, 17.5^0 and 25.2^0. The sharpness and intensity of the XRD peaks indicate the more crystalline nature of metal complexes.

SEM image of [Co(PTAPP)]Cl complex in the resolution of 400 μm is viewed as needle type particles. There is conspicuous change in the morphology of Cu(II) complex when coordinated with a metal ion. In the complex the characteristic morphologies were observed with the image resolution of 0.2 to 1 μm respectively. The Energy Dispersive X-ray Analysis (EDAX) results indicated that there are Cobalt, Chlorine, Carbon and Oxygen peaks which correlate well with the elemental analysis of the complex.

From cyclicvoltomogram curves, for complexes a reversible character was found by the ratio between anodic and cathodic current (I_{pa}/I_{pc}). For Mn(II) and Co(II) complexes, E_{pa} = 0.38V and E_{pc} = -0.1V and ΔEp = 0.48 V. The ratio of I_{pa}/I_{pc} shows that the complexes are quasi-reversible. For Cu(II) complex, the E_{pa} = 0.025v and E_{pc} = 0.1. The ratio of anodic-to-cathodic peak current is less than one. This establishes the electrode process as diffusion controlled. The peak to peak separation (ΔEP=0.075) indicates a reversible one electron transfer redox behavior in the case of [Cu(PTAPP)]Cl complex.

On the basis of the above physico-chemical evidences, the following structure has been assigned for the metal complexes of [M(PTAPP]Cl type.

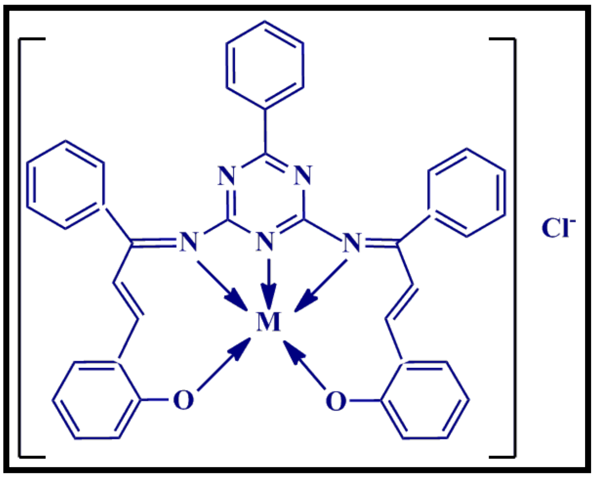


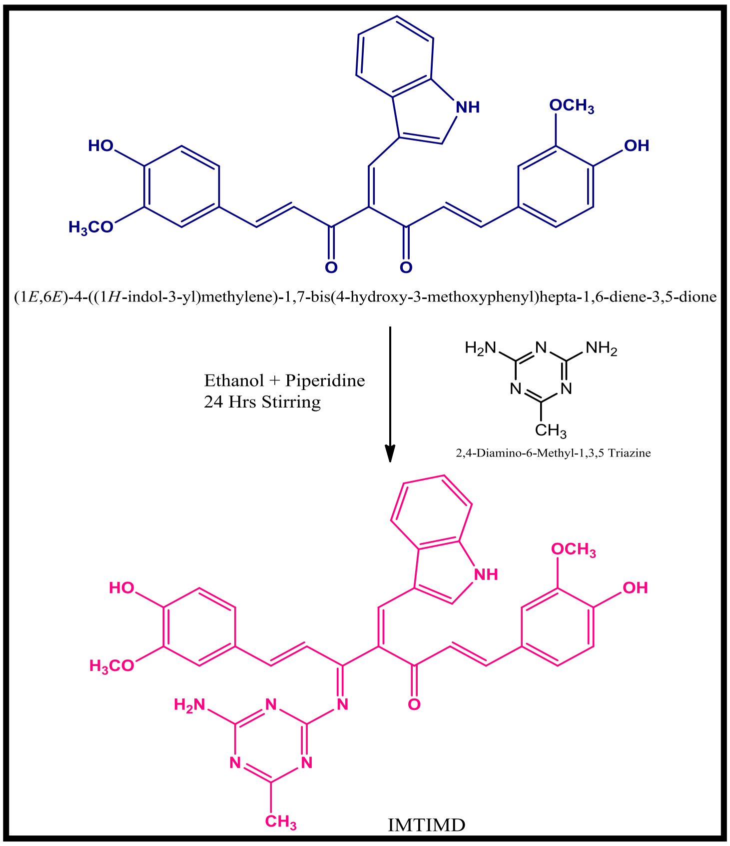
Fig.3. Proposed structure of metal(II) complexes

The metal complexes exhibit strong fluorescence with high emission maxima values compared to the ligand. The molecular docking results reveal that the binding energy for the ligand PTAPP is **-358.22 KJ/mol** and for [Co(PTAPP)]Cl is **-358.12 KJ/mol**. Docking results reveal that the ligand interacted with the following amino acids: Lys 322, Arg 343, Thr 377 and Ala 318. The Mn(II) complex interacted with the following amino acid: Arg 252. All the complexes were screened for anti-oxidant activity using DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay, wherein [Ru(PTAPP)]Cl shows IC_{50} at **11.16 µg/ml** and the standard drug (Ascorbic acid) is **13.38 µg/ml.** Co(II) and Cu(II) complexes were screened for in vitro anti-diabetic activity using alpha-amylase assay, for which the IC_{50} value of [(Co-PTAPP]Cl was found to be **34.511 µg/ml** and the standard drug (Acarbose) was **21.016 µg/ml**.

CHAPTER VI

CURCUMIN INDOLE-BASED TRIAZINE CENTRED METAL COMPLEXES TOWARDS STRUCTURAL STUDIES AND BIOMEDICAL APPLICATIONS TO GASTRIC AND ORAL CANCER CELL-LINES

Curcumin is a promising natural bioactive compound. The current chapter focuses on diverse molecular targets towards gastric and oral cancer. Transition metal complexes of curcumin based ligand of the type ML [where M = Co(II), Cu(II), Ru(III); L = IMTIMD] have been synthesized and characterized. Spectroscopic and other analytical data of the complexes suggest octahedral geometry to the molecules. The molar conductance value of the metal complexes was measured to establish the charge of the metal(II) complexes and it varies from 4-10 $\Omega^{-1}cm^2 mol^{-1}$ implies that all the complexes are non-electrolytes.



Scheme 4. Synthesis of ligand (IMTIMD) L_4

The IR spectrum of the ligand (IMTIMD) shows a strong peak at 1629 cm^{-1}, which is characteristic of the azomethine group and it is shifted to lower frequency of 1509-1490 cm^{-1} upon complexation which indicates that the azomethine nitrogen is one of the coordinating sites. The peak at 1721 cm^{-1} in the ligand is a characteristic mark of stretching frequency of keto group in curcumin. In the case of the metal complexes this band is shifted to 1693-1636 cm^{-1} region which supports the coordination of carbonyl oxygen with the metal ion. The broad bands due to –OH group in complexes appear at 3570-3322 cm^{-1} indicating the chelation of –OH group which is absent in ligand, showing the absence of water molecules in ligand. The bands at 418-501 cm^{-1} and 534-557 cm^{-1}, are attributed to stretching bands of M-N and M-O respectively.

The electronic spectrum of Co(II) complex (**1**) exhibits absorption bands at 39, 215 cm^{-1} (255 nm) and 33,444 cm^{-1}(299 nm) which are due to n-π* transitions and the band at 426 nm (23,474 cm^{-1}) corresponds to transition due to $^4T_{1g}$ (F)→ $^4T_{1g}$ (P) indicating transition in octahedral field. These transitions, as well as the measured value of the magnetic moment (μ_{eff} = ~4.7 BM) suggest a distorted octahedral geometry around Co(II) ion. The electronic spectrum of Cu(II) complex (**2**) exhibits bands at 22,779 cm^{-1} (439 nm) which can be assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$ transition. The measured value of the magnetic moment (μ_{eff} = ~1.7 BM) suggests a distorted octahedral stereochemistry to the compound. In Ru(III) complex (**3**) the band at 419 nm is assigned to d-d transition. The magnetic moment value lies in the range 1.89-1.98 B.M., which confirms the presence of single unpaired electron in a low spin 4d^5-configuration (t_{2g}^5) in an octahedral environment indicating an octahedral geometry.

In ^{1}H NMR spectrum of ligand, a characteristic singlet at 2.44 δ (3H) is due to the methyl group (-CH$_3$) present in 2,4 diamino-6-methyl-1,3,5 triazine. The singlet at 3.87 ppm (δ) (6H) and 6.74 ppm (δ) (2H) can be attributed due to methoxy group (-OCH$_3$) and hydroxy group (Ar-OH) of phenyl ring present in curcumin respectively. In ^{1}H NMR spectrum of Cu(II) complex, the aromatic protons (-CH=CH-) at ortho position with respect to hydroxyl group of the phenyl ring in curcumin shows a doublet at 6.82 ppm (δ) and 6.84 ppm (δ) (2H). The doublet at

7.1 ppm (δ) (2H) is due to the protons (-CH=CH-) that are present in the side chain of curcumin. The aromatic protons of fused benzene ring of indole-3-carboxaldehyde peaks were obtained at 7.2 and 7.3 ppm (δ) (4H) as multiplet.

EI-Mass spectrum of the [Cu(IMTIMD).$H_2O.Cl_2$] complex shows peak at m/z 754.94, which in good agreement with the calculated mass of the complex, corresponding to the molecular formula $CuC_{34}H_{30}N_6O_5Cl_2$. The spectrum records the presence of fragments with m/z values 602, 493, 585, 177, 117, 109.

In ESR spectrum of Cu(II) complex, the spin Hamiltonian parameters $g_{\|} = 2.26$ and $g_{\perp} = 2.10$, are characteristics of distorted octahedreal geometry. The observed $g_{\|}$ value for the complex is less than 2.3, suggesting significant covalent character of the metal-ligand bond in agreement with the observation of Kivelson and Neiman. By observing g values $g_{\|} > g_{\perp} > g_e$, it is clear that d_{x2-y2} orbital is in the ground state and the d^9 configuration is $(e_g)^4(a_{1g})^2\ (b_{2g})^2\ (b_{1g})^1$. This is also supported from the value of exchange interaction term G. The observed values of α^2 of the complex are less than unity, which indicates that the complex has covalent character.

Using Scanning Electron Microscope (SEM), in Cu(II) complex, the characteristic morphologies were observed with the image resolution 10 μm to 0.2 μm. SEM images of the complex in the resolution 0.2-0.5 μm is viewed as flower type particles. The uniformity and similarity between the particle forms of synthesized complexes, indicate that the existence of morphological phases have a homogeneous matrix. There is conspicuous change in the morphology of Cu(II) complex when coordinated with a metal ion. The elemental composition of Cu(II) complex is identified with Energy Dispersive X-ray Analysis (EDAX). The analytical results indicated that there are Copper, Chlorine and Oxygen peaks which corroborate with the elemental analysis.

From the electrochemical curves of Ru(IMTIMD).$H_2O.Cl_2$] complex, it was found that the complex has a reversible character. It is calculated from the ratio between anodic and cathodic current (I_{pa}/I_{pc}). The $E_{pa} = 0.03V$ and $E_{pc} = -0.02V$, and $\Delta Ep = 0.05V$. The ratio of I_{pa}/I_{pc} is equal to less than one. The complex is therefore reversible in nature and it was found that the process is single electron transfer process.

The crystal structures of Co(II) and Cu(II) complexes were determined by X-ray crystallography. There are four well-defined peaks for Co(II) with 2θ values at 19.44^0, 21.44^0, 25.44^0 and 32.44^0. In Cu(II) complex, there are six well-defined peaks at 7.44^0, 17.44^0, 27.44^0, 31.44^0, 39.44^0 and 55.44^0. The sharpness and intensity of the XRD peaks indicate the more crystalline nature of metal complexes.

On the basis of the above physico-chemical evidences, the following structure has been assigned for the metal complexes of [M(IMTIMD]H_2OCl_2] type.

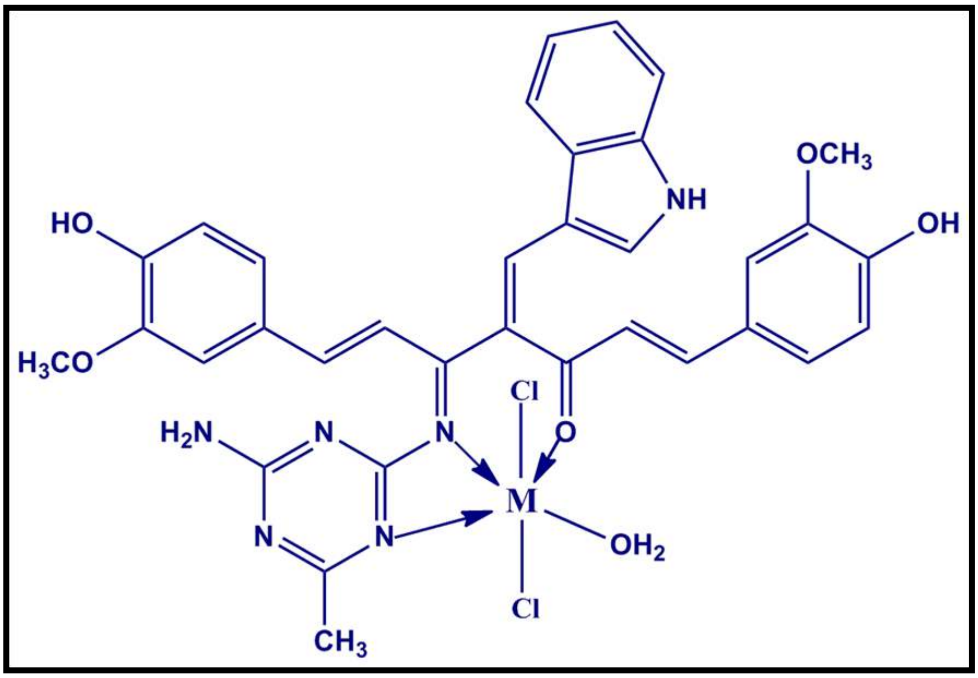


Fig.4. Proposed structure of metal complexes

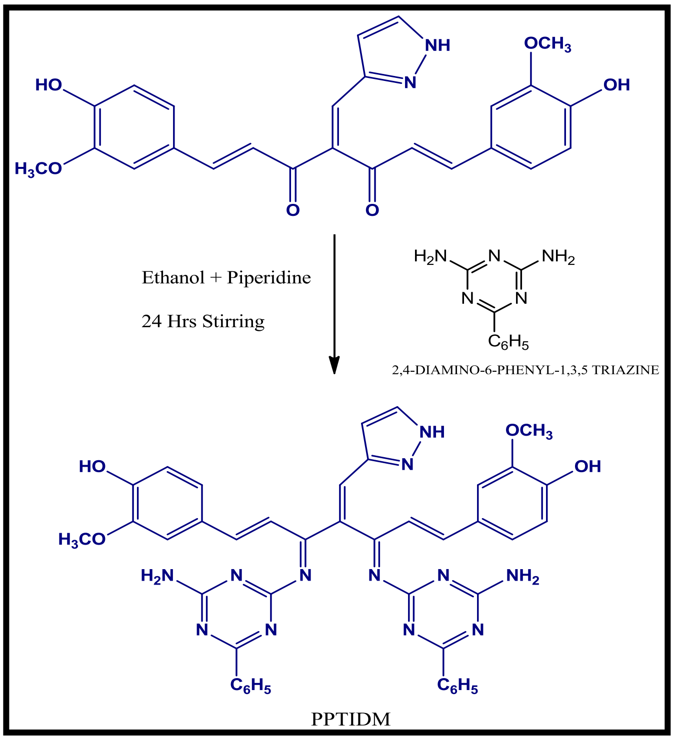
The emission spectral studies show that the ligand (IMTIMD) and its metal complexes were highly fluorescent in nature. Enhancements in the fluorescent intensity of metal complexes show that Schiff base is one of the good chelating agents. Molecular docking was performed using the Hex 8.0 software. Binding Energy for the ligand IMTIMD is -388.91KJ/mol and for [Cu(IMTIMD)H_2OCl_2]Cl is -403.30 KJ/mol. The ligand interacted with the following amino acid: Pro 937; Cu(II) complex interacted with the amino acid: Lys 728. The bonding type in ligand is Alkyl interaction, and in Cu(II) complex it is Pi-cation interaction.

Among the synthesized metal complexes, [Cu(IMTIMD).$H_2O.Cl_2$] has been screened for its *in-vitro* biomedical applications towards anti-cancer activities. MTT assay is employed in both the cases. It exhibited a significant reduction in cell viability towards gastric cancer cell-line CCL-241 and oral cancer cell-line H357. Towards gastric cancer, the result showed maximum inhibition of **61.94±0.93%** at 250µg/ml and **51.86±1.33%** inhibition was observed at 100 µg/ml. In the case of oral cancer, the result showed the maximum inhibition of **76.69%** at 250 µg/ml and **70.95 %** inhibition was observed at 100µg/ml.

CHAPTER VII

CURCUMIN AND IMIDAZOLE-BASED TRIAZINE CENTRED METAL COMPLEXES FOR STRUCTURAL STUDIES AND APPLICATIONS TO ANTI-TUBERCULAR, ORAL AND SKIN CANCER ACTIVITIES

Curcumin being a promising anti-cancer agent and bioactive in treating a number of diseases, this chapter focuses on multiple molecular targets modulated by curcumin towards anti-tubercular and anti-cancer activities. The curcumin and imidazole-based Co(II) (**1**), Cu(II) (**2**) and Ru(III) (**3**) complexes were prepared from [(1-pyrazol-3-yl)methylene)-3,5-bis((4-amino-6-phenyl-1,3,5-triazin-2-yl)imino)hepta-1,6-diene-1,7-diyl)bis(2-methoxyphenol)] (PPTIDM) **L_5** (**Scheme 5**). The curcumin based complexes were obtained in good yield through the reaction of curcumin, imidazole-3-aldehyde and 2,4-diamino-6-phenyl-1,3,5-triazine. The molar conductance value of the metal complexes was measured to establish the charge of the metal(II) complexes and it varies from 3-8 $\Omega^{-1}cm^2$ mol^{-1} implies that all the complexes are non-electrolytes.



Scheme 5. Synthesis of metal(II) complexes

The IR spectrum of the ligand (**L**$_5$) shows a strong peak at 1570 cm^{-1}, which is characteristic of the azomethine group ν(C=N) and it is shifted to lower frequency range of 1498-1553 cm^{-1} upon complexation which indicates that the azomethine nitrogen is one of the coordinating sites. Broad peaks at 3519- 3304cm^{-1} are characteristic of free -OH group present in complexes. The ligand shows a strong band at 834 cm^{-1} and 1490 cm^{-1}, which are characteristics of the ν(C-N) and ν(C=N) groups in s-triazine respectively. These bands are shifted to lower frequency range at 814-805 cm^{-1} and 1464-1444 cm^{-1} upon complexation which indicate that the above groups possess coordinating atoms in the ligand. The peaks due to ν(M-O) appear at 544-532 cm^{-1} and peaks at 488-456 cm^{-1} are attributed to ν(M-N) stretching vibrations.

The Electronic spectrum of Co(II) complex displays band at 41,152 cm^{-1} (243 nm) which is assigned to $\pi\rightarrow\pi^*$ transition. The band at 23,419 cm^{-1} (427 nm) is due to $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ transition. Its magnetic moment value is found to be 4.7 BM which is in the expected range (4.3–5.2 BM) of octahedral complexes. For Cu(II) complex, a band observed in the region of low energy at 23,255cm^{-1} (430 nm) due to $^2E_g \rightarrow {}^2T_{2g}$ transitions and high energy band at 40650 cm^{-1} (246 nm) is due to $\pi\rightarrow\pi^*$ respectively. Its magnetic moment at room temperature is ~1.71 BM, which corroborates with distorted octahedral coordination of Cu(II) ion. The absorption spectrum of Ru(III) complex is characterized by three absorption bands. Bands at 250 nm (40,000 cm^{-1}) and 342 nm (29239 cm^{-1}) are attributed to $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ transitions respectively. The band at 572 nm (17482 cm^{-1}) region has been assigned to d-d transition. The magnetic moment value lies in the range 1.89-1.98 B.M., which confirms the presence of single unpaired electron in a low spin 4d^5-configuration ($t_{2g}{}^5$) in an octahedral environment indicating an octahedral geometry.

The ^1H NMR spectrum of the free ligand showed signals at 2.086 - 2.999 ppm (δ) correspond to ($-CH_3$) protons. A characteristic singlet at 3.87 ppm (δ) and 3.38 ppm (δ) (6H) are observed in the ligand and complex due to methoxy group ($-OCH_3$) of phenyl ring in curcumin. The aromatic protons (-CH=CH-) in imidazole-4-carboxaldehyde showed a doublet at 6.28 ppm (δ) (1H). The singlet at 6.76 ppm (δ) (2H) is due to the hydroxyl group of phenyl ring in curcumin. The doublet at 7.17 ppm (δ) (2H) is due to the protons (-CH=CH-) which is present in the side chain of curcumin observed in the ligand and complex. The aromatic proton (-CH=CH-) of phenyl ring of 2,4 diamino-6-phenyl-1,3,5 triazine at ortho position showed doublet at 8.3 δ (4H). The singlet at 9.087 δ is attributable to azomethine (-N=CH-) or imine protons which deshielded in the spectra of Ru(III) complex, indicating the complexation through azomethine nitrogen atom in [Ru(PPTIDM)Cl_2].

For the molecular ion peak Ru(III) (M^+) ion m/z is observed at 920.32 which is in agreement with the calculated mass of M^+ ion, corresponding to the molecular formula $Ru\text{-}C_{43}H_{36}N_{12}O_4{}^+$. The base peak is observed at m/z 109 which corresponds to a chromone mass fragment. The spectrum records the presence of fragments with m/z 784, 614, 546, 469, 366, 252, 177, 156, 109 values. Thus from fragmentation pattern, the proposed structure is confirmed.

The ESR spectrum of [Cu(PPTIDM)Cl_2] complex indicates anisotropic behavior. The analysis of spectrum gives $g_{||} = 2.30$ and $g_{\perp} = 2.07$. The trend $g|| > g\perp$

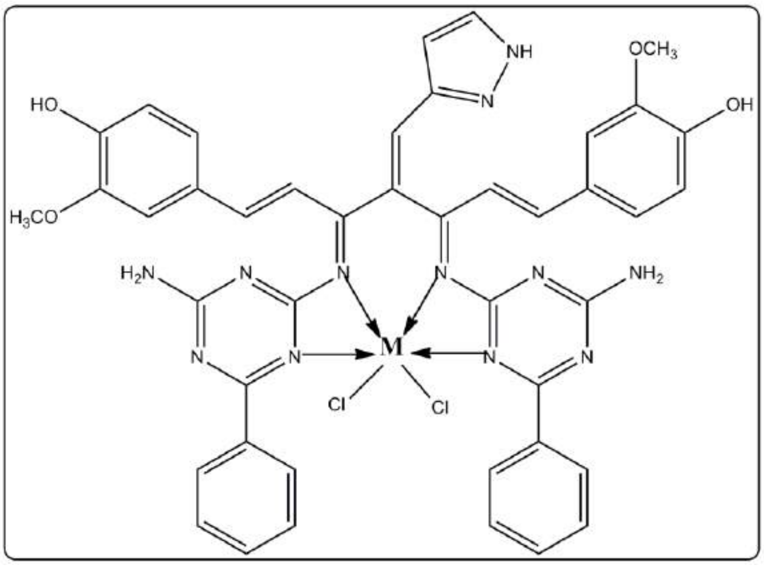
> 2.0023, indicates that the unpaired electron is localized in $d_{x^2-y^2}$ orbital of the Cu(II) ion. The axial geometry pattern for Cu(II) ion has been observed, which is confirmed by the $g_\parallel$ and $g_\perp$ values, which are more than 2.04. The observed spectral parameters show $g_\parallel > g_\perp$ value is characteristic of a distorted octahedral geometry. The calculated magnetic moment for the Cu(II) complex is 1.7 BM as per the relation $\mu^2 = 3/4\ |g|^2$ and is indicative of an unpaired electron. The α^2 value (0.45) indicates appreciable in-plane covalency in the molecule.

The SEM images show Ru(III) complex has granular structure at 0.5 to 1μm range and flower type particles at 5-10 μm. The images display that the particles are agglomerated and unevenly distributed to form a flower-like shape. The micrograph of complex from 0.5μm - 1μm indicate ice rock shaped particles. The average particle size of Ru(III) sample is 5μm at which the complex shows bar alike and faceted microcrystal. The elemental composition of Ru(III) complex is identified with EDAX. The results by EDAX indicate that there are Ruthenium, Chlorine and Oxygen peaks. The elemental analysis of the complex corroborates with the proposed structural components of the complex.

The crystal structure of Ru(III) complex is determined by X-ray crystallography and its crystal structure shows that there are two well-defined peaks with 2θ value at 24.4^0 and 39.55^0. The sharp line diffractogram indicates that Ru(III) complex is crystalline in nature.

From cyclicvoltomogram curves, a reversible character was found by the ratio between anodic and cathodic current (I_{pa}/I_{pc}). For Cu(II) complex $E_{pa} = -0.01$ and $E_{pc} = +0.04$. The ratio of anodic-to-cathodic peak current for the complex (Ipc/Ipa ≈ 1) is less than one which corresponds to a quasi-reversible system with simple one electron process transfer redox behavior. In Ru(III) complex, $E_{pa} = 0.4V$ and $E_{pc} = -0.2V$ and $\Delta Ep = 0.6V$. The ratio of I_{pa}/I_{pc} is equal to less than one, therefore it is reversible in nature and it was found that the process is single electron transfer process.

On the basis of the above physico-chemical evidences, the following structure has been assigned for the metal complexes of $[M(PPTIDM)Cl_2]$ type.



Scheme 5. proposed metal(II) complexes

The emission spectral studies show that the ligand (IMTIMD) and its metal complexes were highly fluorescent in nature. The molecular docking results reveal that the binding energy for the ligand PPTIDM is -447.88 KJ/mol; for [Cu(PPTIDM)Cl_2] is -391.45; and for [Ru(PPTIDM)Cl_2] is -421.56 KJ/mol. The ligand interacted with the following amino acids: Arg 841, Cys 797, Ser 720, Leu 718; the Cu(II) complex interacted with the following amino acids: Lys 745, Arg 841; and Cu(II) complex interacted with Ile 878 , Lys 875, Arg 841 amino acids. Ru(III) complex has been screened for its *in vitro* biomedical applications towards tuberculosis cell-line H37Rv for which **66.47%** inhibition was obtained. The same complex was screened for its *in vitro* applications to oral cancer cell-line H357 for which **89.25%** of cell-death was obtained at 250 μg/ml and **85.58%** at 100 μg/ml. Cu(II) complex was screened for its *in vitro* application towards skin cancer cell-line A375 for which IC_{50} value was **3.82** μg/ml was obtained and the world standard cis-platin is **1.123** μg/ml.

Concluding Remarks

1. The metal complexes synthesized and discussed above exhibit strong fluorescence with high emission maxima values compared to the ligand. Hence, these complexes could be used to form a new class of fluorescence materials

having appreciable photoactive properties which could be used in photonic devices.

2. All the synthesized triazine-based ligands have remarkable anti-bacterial activities. Co(II) complex (**L_2**) is highly potent against almost all the test bacteria. The anti-bacterial activity of metal(II) complexes screened for *in vitro* is in the order : $[Co(DPPT)_2]Cl_2$ > $[Co(DMAPPT)H_2OCl_2]$ > [Mn(PTAPP]Cl.

3. to have potent biomedical activity, pharmacologically active benzoguanamine based derivatives *i.e.*, new Schiff bases were synthesized and characterized. The synthesized metal(II) complexes have been screened for their *in vitro* anti-tubercular, anti-oxidant, anti-diabetic and anti-cancer activities to develop new metal based drugs. To make a mention, $[Co(DPPT)_2]Cl_2$ complex shows a close proximity of medical value (IC_{50}=1.544, 100μg/ml) observed for lung cancer, compared to the world standard cis-platin (IC_{50}=1.123100μg/ml). For the same complex, docking between Co(II) complex and the protein EGF kinase (2J5F) was found to have lowest binding energy (-536.21). Hence this compound is the ideal candidate for further studies with regard to the biological function to develop more potent compounds leading to chemotherapy.

4. curcumin being a lipophilic molecule, it rapidly permeates cell membrane. Being chemopreventive agent, curcumin has been found to modulate multiple molecular pathways through several mechanisms. To have effective biomedical activity, curcumin based complexes were synthesized and screened *in vitro* gastric and oral cancer applications. Remarkably, $[Cu(IMTIMD).H_2O.Cl_2]$ complex showed maximum inhibition for oral caner with inhibition value 76.69% at 250μg/ml and 70.95 % inhibition at 100μg/ml. In the same vein, $[Cu(PPTIDM)Cl_2]$ is screened for its *in vitro* biomedical applications towards skin cancer cell-line A375 for which IC_{50} value is 3.82 compared to cisplatin (1.123) at 100μg/ml.