STUDIES OF THE EFFECT OF TRANSITION METAL ION ON THE BIOLOGICAL ACTIVITY OF SOME NOVEL ORGANIC COMPOUNDS

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SUMMARY

This project was aimed at the synthesis of some chromone derived ligands and their transition metal complexes of biological significance. The ligands **1–17** were synthesized by the reactions of 6,8-dichloro-3-formylchromone and 4-aminobenzoic hydrazide, 4-brmobenzoic hydrazide 4-chlorobenzoic hydrazide, , 4-hydroxybenzoic hydrazide, isonicotinic hydrazide, nicotinic hydrazide, 2,5-dichlorophenylhydrazine, 2,5-diflororophenylhydrazine, 2,3,5,6-tetraflororophenylhydrazine, *m*-tolylhydrazine hydrochloride, 4-methoxyphenylhydrazine hydrochloride and 4-hydrazinobenzoic acid, semicabazide, 4-phenylsemicarbazide, 4,4-diphenylsemicarbazide, thiosemicarbazide and 4-phenylthiosemicarbazide respectively. The structures of all the ligands were determined by analytical and spectral (IR, ^{1}H-NMR) techniques.

These ligands **1–17** were used for synthesizing their copper (II) complexes **18 – 34**. All the synthesized metal complexes were characterized by elemental analyses, molar conductance, magnetic moment, IR, electronic spectral and thermal data and were found to be six-coordinated exhibiting octahedral geomatry.

The synthesized ligands and their transition metal complexes were screened for their *in vitro* antibacterial activity against four Gram-negative (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Shigella flexneri)* and two Gram-positive (*Bacillus subtilis, Staphylococcus aureus)* bacterial strains by the agar-well diffusion method. The ligands were found to exhibit either no or low to moderate activity against one or more of the bacterial species. Whereas, all the metal complexes exhibited varied activity against different bacteria. The ligands, which were inactive before complexation became active and less active ones became more active upon coordination with copper ions. The complexes **18 - 22**, **27 - 28** and **30 - 33** showed comparatively much higher activity. However, the metal complex **34** was found to be the most active one.

CERTIFICATE

We certify that Syed Khalid Aftab has compiled this thesis according to the Regulations for Higher Degrees of University of Sargodha. We also certify that the research work embodied in this thesis entitled "STUDIES OF THE EFFECT OF TRANSITION METAL IONS ON THE BIOLOGICAL ACTIVITY OF SOME NOVEL ORGANIC COMPOUND" has been carried out by Mr. Khalid Aftab under our supervision and is worth presenting to Department of Chemistry University of Sargodha, Sargodha (Pakistan) for the award of the degree of *Doctor of Philosophy.*

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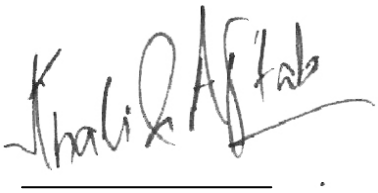
DECLARATION

I hereby declare that the work described in this thesis was carried out by me under the supervision of Prof. Dr. Mohammad Saeed Iqbal and Prof. Dr. Zahid H Chohan, at the Department of Chemistry University of Sargodha, Sargodha (Pakistan).

I also hereby declare that the substance of this thesis has neither been submitted elsewhere nor is being concurrently submitted for any other degree. I further declare that the thesis embodies the results of my own research or advanced studies and that it has been composed by myself. Where appropriate, I have made acknowledgement to the work of others.



Syed Khalid Aftab

DEDICATION

TO THE MOST DEAREST PERSONALITY OF MINE OUR PROPHET "MOHAMMAD" (Salallah-o-alaeihe-wa-alaehe-wasalim)

AND TO THE SWEET MEMORY OF MY FATHER

AND MY MOTHER (Under whom feet lies the paradise of mine)

CHAPTER 1

INTRODUCTION

INTRODUCTION

In this work reaction of chromone moiety with various hydrazides, hydrazines and thiosemicarbazides have been investigated. The products have further been complexed with copper (II) ions and the complexes were subjected to biological activity testing. An introduction to the chromones and their chemical reactivity is presented as follows.

1.1 Chromones

Chromone (or 1,4-benzopyrone) is a derivative of benzopyran (Fig. 1) with a substituted keto group on the pyran ring. Chromone constitute one of the major classes of naturally occuring compounds and interest in their chemistry countinues because of their usefulness as biologically active agent *[1]*. The chromone structure is an important part of several flavonoids.

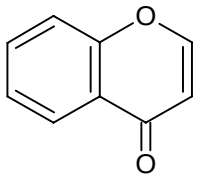


Fig. 1: Structure of chromone

The chromone moiety forms the important component of the pharmacophores of a number of biologically active molecules of synthetic as well as natural origin and many of them have useful medicinal applications. The famous anti-asthmatics, such as cromolyn sodium (or cromolyn) and nedocromil, are commonly grouped together as chromones (also called cromoglycates). These chromones are potent in preventing both early and late responses to inhaled allergens, such as pollen, and reducing airway reactivity to a range of inhaled irritants, such as sulfur dioxide and cold air *[1]*.

1.2 Importance of chromones in medicinal chemistry

Chromones have drawn considerable interest from synthetic organic and medicinal chemists *[2]*, particularly 3-formylchromone has emerged as a valuable synthon for incorporation of the chromone moiety into a number of molecular frameworks *[3]*. However, its synthetic utility is limited due to ease of opening of the chromone ring *[3,4]* and strategies are being worked out to deal with this problem *[5]*. Reaction of 3-formylchromones with aromatic amino acids has also been studied by *Groweiss et al [6]*. The synthetic significance of 4-oxo-4*H*-[1]-benzopyran-3-carboxaldehydes (4-oxochromene-3-carboxaldehydes, 3-formylchromones) (Fig 2) comes from their usefulness as reactive agents and valuable precursors for many different heterocycles. They contain three electron deficient sites (C-2, C-4, CHO) suitable for nucleophilic attack and as a consequence of competition between these centers, various types of compounds can form upon the reaction of (4-oxochromene-3-carboxaldehydes, 3-formylchromones) with strong nucleophiles *[7]*.

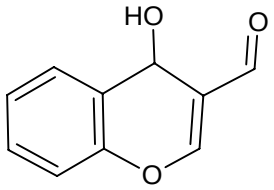


Fig. 2: Structure of 3-formylchromones

Reaction between equimolar quantities of 3-formylchromone and a primary aromatic amine in benzene leads to a mixture of the 3-(aryliminomethyl) chromone and 2-arylamino-3-(arylaminomethylene) chroman-4-one, making the isolation of pure compounds difficult *[8,9]*. The reason for this rather unusual ring addition of a second aromatic amine molecule to the imine is the formation of the stable ketoamine hydrogen bond *[10]*. Alcohols, thiols and amines are sufficiently nucleophilic to add to 3-(aryliminomethyl) chromones *[9,11,12]*. A much improved yield of 3-(aryliminomethyl) chromones can be obtained from the condensation of reactants in

the presence of 4-toluenesulfonic acid *[9,13]*. Pure 3-(aryliminomethyl) chromones can be prepared from 2-alkoxy-3-(arylaminomethylene) chroman-4-ones via elimination of a molecule of alcohol by heating the compounds to their melting point under vacuum *[12]*. Primary aromatic amines having a nucleophilic functionality at their *ortho* position react with 3-formylchromones giving fused seven-membered heterocycles *[14-16]*, 3-(aryliminomethyl) chromones*[14-16]* or dihydrotetraazanulenes*[17,18]*.

There are two possible pathways for the formation of 3-(aryliminomethyl) chromones. The first is a straightforward 1,2-addition of the amine to the aldehyde function of **3**, while the second is a 1,4-addition of the amine with concomitant opening of the pyrone ring and subsequent recyclization of the intermediate. In spite of a thorough investigation of the reaction of 3-formylchromones with primary aromatic amines, the exact mechanism of the formation of products is not clear *[19]*.

A detailed investigation, including the reaction kinetics, of reaction between 6-substituted 3-formylchromones and aromatic amino acids has been reported *[19]*. This study contributed a lot to the clarification of preparation of imines, enamines and their mutual transformation in the reaction of weak *N*-nucleophiles with aldehydes. Some of the prepared compounds were active against mycobacterial strains, in photosynthesis *[20]* and have a hereditary bleaching effect (similar to antibiotics) on the plastid system of *Euglena gracilis [21]*.

Reaction of 6-substituted 3-formylchromones with aromatic amino acids in aprotic solvents yielded only 3-(arylaminomethylene)-2-hydroxychroman-4-ones at room temperature or under reflux. Changing the solvent, reaction temperature or amount of catalyst did not affect the yield of enamines. This was surprising, as the authors expected the formation of 3-(aryliminomethyl) chromones when the reactions were carried out under reflux. Increase in reaction time also did not help to produce imines. This difference between reactivity of anilines *[8,9]* and aromatic

amino acids has been explained in terms of lower nucleophility and basicity of arylamino acids in comparison with aniline derivatives. In the case of anilines having a nucleophilic functionality at *ortho* position, fused seven-membered heterocycles were prepared *[14,16]*. Formation of this type of compounds was not verified in case of 3-aminosalicylic acid.

Protic solvents led to different reaction products than those found in dry aprotic ones. 2-Alkoxy-3-(arylaminomethylene) chroman-4-ones were synthesized by reaction of 3-formylchromones with aromatic amino acids in the presence of 4-toluenesulfonic acid as catalyst in an alcoholic reaction medium at room temperature. 4-Aminobenzoic and 4-aminohippuric acids yielded also 2-alkoxyderivatives when the reaction was carried out under reflux. Aminosalicylic acids reacted differently. 3-(Aryliminomethyl) chromones were isolated from reactions performed at reflux for 3 h. The imines are sparingly soluble in solvents, and precipitate from the reaction mixture. Prolonged heating of the reaction mixture for more than 20 h led to polymerization of the imine. The prepared 3-(aryliminomethyl) chromones did not give 2-alkoxy-3-(arylaminomethylene) chroman-4-ones after 3 days standing at room temperature or after 6 h reflux in alcohol. These synthetic results contradict the previously proposed mechanism for the conversion of 2-substituted-3-(arylaminomethylene) chroman-4-ones from 3-(aryliminomethyl) chromones *[9,12]* and are supported by kinetic study of the above process. In general, 3-formylchromones readily react with primary amines in an alcoholic medium yielding an enamine-adduct which rarely reacts further to give the corresponding Schiff base *[13,16,17]*.

The structure-activity correlations for the isoflavonoid compounds have indicated certain features desirable for the antitumor properties of a chromone motif, with a double bond between C2-C3 positions and a side chain containing a phenyl ring having metal chelating ability *[22-24]*. These structural requirements can easily be built in 3-formylchromone a versatile synthon having antiinflammatory and anticancer activities, by condensing it with various amines in alcoholic

medium. Such compounds are capable of forming metal complexes with several transition metal ions among which copper is particularly effective in yielding moieties with potent radical scavenging properties *[25]*. Thus the review of literature highlights contradictory behaviour of chromones with respect to their reactions with amino compounds.

1.3 Biological role of copper

The study of the biological role of metal ions has a long history in medicine, in pharmacology and in toxicology, but it is only recently that the extent and variety of metal ion involvement has been appreciated. For example, amongst the transition metalloelements, V, Cr, Mn, Fe, Co, Ni, Cu, Zn and Mo have been shown to be essential to life and Au, Ag, Pt, Pd, Ir, Os, Ti, and others have either been used in therapy or claimed to be of therapeutic value.

One aspect of the behavior *"in vivo"* of metals which cannot be over-emphasized is that their chemistry is essentially that of the complexed ion, irrespective of whether more polar ions such as Na^+ or K^+ or more covalent species such as Au (III) or Pt (II) are being considered. Properties such as the effective size and solubility of a metal ion *in vivo* are a function of ligand and solvent present as well as of the metal ions themselves. Further, the correct metal ion balance in various *in vivo* compartments is important for the functioning of specific metal containing sites in many enzymes and proteins. For example, if the concentrations of some metal ions are raised considerably above the normmal, blocking of transport sites can occur and symptoms more normally attributed to depletion of certain metal ions can appear. A corollary to this is that metal ion distribution can be affected by alterations in the *in vivo* concentration of naturally occurring low molecular weight ligands or of complexing sites in proteins. An example where this may be the case is in rheumatoid arthritis, where serum histidine levels and albumin thiol levels are significantly lower than in normal subjects. However, as yet little is known about the effect this has on the disease process.

In biological fluids, there are large numbers of complexing species for metal ions. For example, in serum there are many more complexing sites for iron or copper in amino acids, low molecular-weight peptides and proteins than there are metal ions, so that the chemistry of these ions *in vivo* is that of ions present in an excess of competing complexing groups. Thus, considerable impetus has been given recently to the study of complexing in determining likely distributions and reactivities of transition metal ions in such fluids.

There are a wide range of microenvironments present *in vivo* and each would be expected to affect the chemistry of the metal in much the same way as would a change in the solvent or the absorption of the metal ions on a surface. For example, when transition metals such as copper react with amino acids, quite different complexes are formed in ethanol to those which are formed in water, both because of changes in the solubility of the complexes and because of the different strengths of water and ethanol as ligands. The *"in vivo"* microenvironments are often separated by membrane barriers which can be penetrated by specific complexes rather than by the ion or other complexes of different size and charge. The understanding of these problems has been the subject of inorganic bi-ochemistry. A better understanding of inorganic chemistry and with the advent of more powerful physical techniques would mean that many more problems could be investigated with a reasonable chance of success. This concept is difficult to apply absolute terms but it is useful in a relative sense. For example, copper (II) is 'harder' than copper (I). Therefore, the latter will complex more readily with thiol ligands and a redox enzyme such as caeruloplasmin in which both oxidation states of copper are required.

Copper is a member of the group 1B elements along with gold and silver. In some ways, as a typical transition metal, it is distinct in its properties. Copper can exist under normal conditions in four oxidation states, 0, 1, 2 and 3. Copper (0) (i.e., copper metal) is relatively inert and is used, for example, in water piping. However, in the presence of oxygen and a strong complexing agent, copper (0) dissolves easily - an example of this is the ready solubility of copper metal in

aqueous solutions of amino acids. This is reputedly the therapeutic mechanism of copper bangles - the dissolution of copper (0) by the amino acids in sweat and the subsequent absorption of the copper-amino acid complexes into the body.

Copper (I) forms mainly 4- or 6-coordinate species with essentially tetrahedral or octahedral structures. It is unstable in aqueous solutions unless complexed to soft ligands such as thiols or thiourea, but it is stable in some non-aqueous solvents (acetonitrile) and insoluble copper (I) complexes are readily prepared from aqueous solution. As a d^{10} ion, there are no unpaired electrons, with the result that the detection of copper (I) is more difficult than copper (II) and its *in vivo* role has received much less attention. Nevertheless, soft thiol complexing sites and non-aqueous environments are suitable for copper (I) and it has been identified in specific proteins such as caeruloplasmin and metallothionein.

The most common oxidation state of copper is copper (II) and being a d^9 ion, its complexes are often distorted resulting in unequal bond lengths and interbond angles and a large variety of possible geometries. Thus from biological point of view copper (II) will occupy unique sites. Because of the relative stability of its complexes, labile copper concentrations in natural fluids will need to be kept low, otherwise Cu^{2+} will displace other transition metal ions such as Mn^{2+} or Zn^{2+}. Copper (II) also forms dimeric species, the classic example of this is copper acetate, a dimer with bridging carboxylate groups. Other dimeric complexes also occur in nature for example, in haemocyanin, oxygen is carried as a bridged species between two copper ions and not by a haem group as the name would imply. Copper (III) is relatively rare and is only stabilized by hard ligands such as O^{2-} or F^- in $NaCuO_2$ and K_3CuF_6. Whether copper (III) has any biological role is as yet unknown, but it is possible to form *"in vitro"* a copper (III) albumin complex in which the hard ligand is believed to be an N^- atom in the protein chain. The N^- refers to the formal state of the nitrogen in a metal-nitrogen bond.

The impact of biology on copper chemistry has been largely to stimulate more specific studies of areas where adequate knowledge seemed to exist until the more specific questions relating to particular types of biological activity were posed. For example, the geometry of copper (II) complexes is expected to be distorted from octahedral, but the question of the manner in which the different geometries of amino acids would affect this distortion is less clear. One good example of the empirical classification of this effect is amino acid complexes of type $CuL_2(H_2O)_2$ for which a detailed structural analysis of a wide range of complexes is available *[26]*. The ligand has a considerable effect on the position of the water molecules and each complex studied is different, but broadly they fall into two types as illustrated for alanine (Fig. 3).

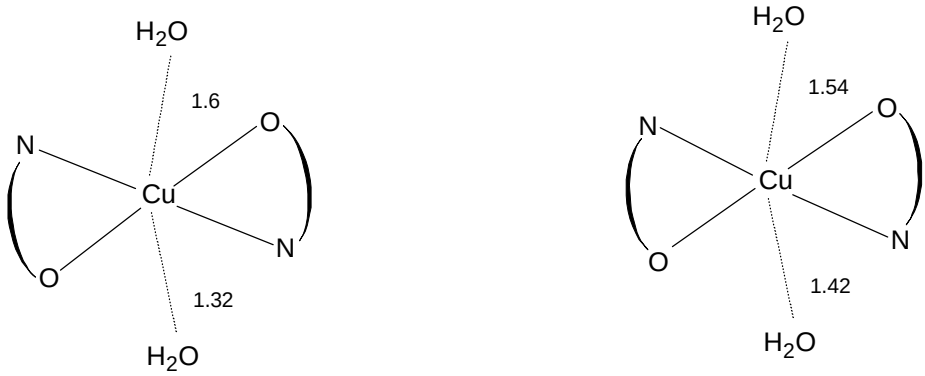


Fig. 3: Geometries of copper (II) in complexes with different bond lengths and angles exhibited by water molecules in *cis* and *trans* bis alanine copper (II) complexes.

Increasing understanding of the biological role of the metal has led to a large increase in the number of studies of model complexes designed to mimic some aspect of the role of copper *in vivo.* For example, copper Schiff-base complexes are believed to be key intermediates in some pyridoxal dependent enzyme processes and possibly in the cross linking of collagen by lysyl oxidase *[27]*. The use of sulphur containing amino acids produces a more complex series of compounds than that found with alkyl amino acids and suggests that, as is the case with most of these systems, much remains to be done before *in vitro* knowledge is adequate to begin an interpretation of *in vivo* behavior *[28]*. The discovery of the nature of the site in blue copper

proteins such as stellocyanin has led to studies of model systems based on this group and the role of copper complexes as oxygen carriers has also been studied. Further, the catalytic role of the copper site can sometimes be effectively studied *in vitro.* For example, copper amino acid complexes can behave as superoxide dismutases.

In medicine, ligands specifically designed to complex and remove copper in Wilson's disease, a condition involving the accumulation of excess copper, have been synthesized. The discovery that copper aspirinate is a more effective and less ulcerogenic anti-inflammatory agent than aspirin has led to the reinvestigation and extension of the chemistry of Cu (II) complexes *[29,30]*.

1.4 Effect of metal complexation on biological activity

The metalloelements, which are present in trace and ultra-trace quantities, play a vital role at the molecular level in a living system. In a healthy body of an adult, the trace and ultra-trace metalloelements weigh less than 10 g in total but life depends upon these elements for more than this Fig. *[31]*. The transition metal ions are responsible for the proper functioning of different enzymes. If their concentration exceeds a certain level then their toxic effects become evident. Certain drugs play a vital role as bio-ligands in the biological systems. Similarly, nitrogen containing bases such as derivatives of pyrrole, pyridine, pyrimidine, pyrazine and purine have a vital role as bio-ligands. Amines such as histamine, carbohydrates such as glucose, and different vitamins such as ascorbic acid are also well recognized bio-ligands.

It has been found that the activity of the bio-metals is attained through the formation of complexes with different bio-ligands and the themodynamic and kinetic properties of the complexes govern the mode of biological action. It is always the case that the permeability, i.e. lipophilicity of drugs is increased through the formation of chelates in *vivo* and drug action is significantly increased due to much more effective penetration of the drug into the site of action.

The knowledge of drug action in *vivo* is, therefore extremely important in designing more special drugs.

It is worth-mentioning that many drug substances, such as antimicrobial and anticancer drugs exhibit drug action through complexation with the available bio-metals in *vivo [32]*. In absolutely metal-free conditions, they are inactive. Copper metabolism is somehow related with the rheumatoid arthritis. In case of diabetes, an inflammatory disease of the pancreas, chromium metabolism plays an important role. A number of diseases and their remedies are dependent on metabolism of inorganic constituents.

The role of metal ions in the virus replication process is extremely important *[33]*. A virus can penetrate into the host's cell only when it is mediated by some suitable metal ions. For example, link present in the cell wall of the bacterium *E.coli* can coordinate with the sulphur site present in the virus coating. By using any suitable metal complex like $Cd(CN)_3$, which can preferably bind with the 'S' site as virus-(S)-$Cd(CN)_3$, the penetration of the viral DNA into the host cell can be arrested. As a matter of fact, for virus replication, copper and zinc are essentially required, hence by increasing or decreasing their concentration, viral growth may be controlled. Interaction of various metal ions with antibiotics may enhance or suppress their antimicrobial activity but usually in many cases, the pharmacological activity of antibiotics, after complexation with metals, is enhanced as compared to that of the free ligands *[34-36]*. Many of the well-known antibiotics, penicillin, streptomycin, bacitracin, tetracycline, etc. are chelating agents and their action is improved by the presence of small amount of metal ions *[37]*. Binding of metalloelements with polydentate ligands to form ring structure, where the metal atom is part of the ring, is called chelation. In chelates, metal is firmly held by a number of ligand atoms usually nitrogen, oxygen or sulphur through co-ordinate covalent bonds. Some of the chelates are model analogues of certain metalloenzymes *[38,39]*. Furthermore, some of the chelates develop considerable antimicrobial activity *[40]*. Chelate compounds obtained from Schiff bases are

convenient for the study of change in structure and associated biological activity, since varying a substituent of the metal ring permits variation in the three dimensional structure of the molecule *[41-45]*. It has been demonstrated through several studies *[46-71]* that the biological activity of chelating compounds is enhanced on chelation with a metal atom. Some of the inactive ligands developed such properties upon chelation *[72-88]*. The antitumor activity of some Schiff bases has been attributed to their ability to chelate with trace transition metal *[89-91]*. Several explanations have been suggested for this enhancement in activity of metal complexation. Generally, it has been observed that transition metal complexes have greater activity and less toxic effects.

Some antibiotics are delicately balanced so as to be able to compete successfully with the metal binding agents of bacteria while not disturbing the metal processing by the host. The chelating properties of antibiotics may be used in metal transport across membrane or to attach the antibiotics to a spacific site from where it can interfere with the growth of bacteria *[92]*. Tetracyclines form an important group of antibiotics. Their activity appears to result from their ability to chelate metals. The extent for antibacterial activity parallels the ability to form stable chelates. It has been shown in a study that tetracycline and cycloserine bindings to metal ions suppress their antimicrobial activity because the associative pH changes alter the intra- and inter-molecular interactions. A similar correlation has been drawn between active tetracycline and the ability to form 2:1 complexes with Cu (II), Ni (II) and Zn (II) *[31]*. Qualitative and quantitative differences in biological activities have been observed among metal chelates, differing in the metal ion or in the ligand. Metal chelates during chemical synthesis can be varied in size, charge distribution, stereochemistry, redox potentials and other physical properties *[93]*. This property provides important information for suitable drug design.

1.5 Physical techniques used for characterization

1.5.1 Infrared spectroscopy

Infrared spectroscopy is an extremely powerful technique for both qualitative and quantitative analysis *[94]*. The I.R spectrum of any substance is interpreted by the use of known group frequencies. It is the one of the most useful technique for the detection of functional group in pure compounds and mixtures, for the identification of the substances *[95]*. I.R spectroscopy involves different types of twisting, bending, rotational and vibrational motions of the atomic group in a molecule. I.R radiations have not sufficient energy to cause the excitation of electrons, however it cause atoms or groups of atoms to vibrate faster about covalent bond or the bond which connect them. The compound absorbs infrared energy in the particular region of spectrum, upon interaction with infrared radiation at particular wavelength. A highly complex absorption spectrum is obtained which is the characteristic of the functional group comprising the molecule and overall configuration of the atom as well *[96-98]*. Infrared region consists of three parts, near, mid and far infrared regions.

The near I.R region which meets the visible region at about 12,500 $cm^{-1}$and extend to about 4,000 cm^{-1}. This region is found in many absorption bands resulting from harmonic overtones of fundamental bands and combination bands often associated with hydrogen atoms. Among these are the first overtones of the O-H and N-H stretching vibrations near 7140 cm^{-1} and 6667 cm^{-1} respectively. Bands resulting from C-H stretching, and deformation vibration of alkyl groups are found at 4548 cm^{-1} and 3850 cm^{-1}.

Middle-infrared region is divided into the “group frequency “region at 4000 cm^{-1} and extend to about 1300 cm^{-1} and the “finger print “region at 1300 cm^{-1} and extend to about 650 cm^{-1}.

Far-IR region is between 667 cm^{-1} and extend to about 10 cm^{-1} that contains the bending vibrations of carbon, nitrogen, oxygen and fluorine with atoms heavier than mass 19 and

additional bending motion in cyclic or unsaturated systems *[99,100]*. The IR spectrum can give a perfect picture of the structural formula without a chemical investigation *[101]*.

1.5.2 Nuclear magnetic resonance

NMR spectroscopy is at present the most powerful technique available for structural determinations. Due to NMR spectroscopy it becomes possible to define the environment of all commonly occurring functional groups, as well as of fragments (such as hydrogen atoms attach to carbon), which are not otherwise accessible to other spectroscopic or analytical techniques *[102,103]*. More sophisticated applications often yield kinetic and thermodynamic parameters of certain types of chemical processes and others, it often gives accurate information about the relative positions of groups of magnetic nuclei within molecules *[104]*. NMR technique is useful in study of the structure of diamagnetic substances. The paramagnetic substances, like Cu (II) complexes do not produce useful NMR signals. On complexation with metal atoms the chemical shifts of the protons in the vicinity of the metal atom are also shifted which are use to characterize the complexes.

1.5.3 Electronic absorption spectra

The spectra of transition metal complexes depend on the transition of unpaired electrons from the ground state to an excited state. Most of the transition metal complexes are colored; the color is observed due to d-d transitions in the visible region *[105]*.

The electronic structure of coordination compounds is mainly described by the molecular orbital theory. The atomic overlap in metal-ligand bonds allows *d*-electrons to penetrate from the central atom to the ligand. In coordination compounds, the central atom and the ligands are generally separated systems interacting only weakly with each other. Thus electron transfer between two parts of a molecule can be observed in both directions but the transfer from the ligand has a higher electronegativity than the central ion and the transfer is possible in reverse direction if ligand possesses empty orbitals *[106]*. The transitions are affected by the effect of ligands on the

energies of the *d*- orbitals of metal ions. Since octahedral, square-planar and tetrahedral fields cause splitting of *d*-orbitals in different ways, the geometry will have a pronounced effect upon *d-d* transitions in a metal complex. Thus spectral data of these transitions provide useful information about the structure of the complexes.

Copper (II) complexes are usually blue or green. Exceptions to this are generally caused by strong ultraviolet and charge transfer bands tailing off into the blue and visible spectrum thus causing the substances to appear red or brown. The blue or green colors are due to the presence of Cu (II), *d-d* absorption band in 600-900 nm region of the spectrum. The envelopes of these bands are generally unsymmetrical seeming to encompass several overlapping transitions, but definite resolution into the proper number of sub-bands with correct location is difficult *[107]*.

Complexes with four, five or six donors or with marked tetrahedral distortions are accompanied by bond length changes and deviations from expected ligand geometry *[108-110]*. So-called stepped or umbrella distortions are well documented with deviations from planarity and unexpected bond lengths in the aromatic ring of salicylaldiminato (salim) groups also being claimed *[111,112]*. A variety of geometries which are adopted by the same complex or by complexes which have minor changes in constituents or in packing arrangements. Thus bis(N-methylsalim) Cu (II) is perfectly planar in one crystal form *[113]*, planar but stepped in another *[114]* and square-pyramidal and dimeric in a third *[115]*. None of these crystals contains solvent or other adduct molecules. Bis(N-ethylsalim) Cu (II), in contrast, adopts a partially tetrahedral molecular structure in the three crystallographically independent molecules in two different crystalline forms *[116-118]*. If the R-group on the nitrogen is switched to n-propyl the copper complex is planar again *[119]* but i-propyl derivative is partially tetrahedral *[120]*. Thus, apart from packing considerations or the intra-molecular steric interactions thought to give rise to molecular stepping *[121,122]*, there seem to be electronic influences also at work. It has been postulated that substituents which load or help stabilise electron density on the oxygen donors

bring about molecular stepping in otherwise planar complexes. Electron density loaded onto nitrogen donors, on the other hand, induces a tetrahedral distortion at copper. Additionally it has been postulated that a decrease in the ligand field at the four donor atoms contributes to an increase in co-ordination number from four to five or six *[112]*, the extra donors arising from the sharing of atoms between metal centres, the incorporation of solvent molecules or even from interactions of the charge transfer type with adjacent aromatic rings. It has also been noticed that hydrogen bonding to the donor oxygen atoms leads to a return to four-coordinate planar stereochemistry at copper and an increased overall planarity of the complex *[112]*. More accurate re-determinations of structures, such as those undertaken by Kamenar and his co-workers *[123,126]*, help test these hypotheses more rigorously.

1.5.4 Magnetic moments

The measurement of magnetic moment is one of the most useful methods available to the coordination chemists for studying the electronic structure of a transition metal complex. It provides fundamental information about the bonding and stereochemistry of the metal complexes *[127]*. The magnetic properties of the coordination compounds are based on the effect of the ligands on the spectroscopic terms of the metal ions. The Gouy's method is the simplest method for measuring the magnetic moments. It consists of a suspension of a uniform rod in a non-homogeneous field of about 5000 Oersteds and measuring the force exerted on it by a conventional weighing technique. The calibrants usually used are $HgCo(CNS)_4$ and $Ni(en)_3$ (S_2O_3) which are easy to prepare, do not decompose or absorb moisture and pack well in the sample tube. Their susceptibilities at 20°C are 16.44 x 10^{-6} and 11.03 x 10^{-6} c.g.s units, respectively. They decrease to 0.05 x 10^{-6} and 0.04 x 10^{-6} at per degree temperature rise *[128]*.

All the substances possess magnetic properties and thus are affected by the application of a magnetic field. The substances may be diamagnetic when a reduction is caused in the applied magnetic filed and paramagnetic when increase is caused in the magnetic filed. The molar

susceptibility, a measure of magnetic moment, of a substance is an algebraic sum of the susceptibilities of the component atoms, ions or molecules. The susceptibility per gram atom of a paramagnetic metal ion in a particular compound is determined by measuring the molar susceptibility of the compounds and applying diamagnetic corrections for the other ions or molecules in the compounds. The diamagnetic corrections can be estimated by various methods *[129]*, however Pascal's constants are more often used to calculate the corrections. The magnetic susceptibility and moments can generally be calculated by using the following relationships *[130]*.

$$X = \{K_2\nu + (2gl \div H^2)\, w\} \div W - A + Bw \div W$$

Where
K = Susceptibility per unit volume
H = Integration over the whole length of the specimen
w = Weight in grams
W = Total weight of specimen in grams
l = Length of the specimen
$\mu e = 2.828\, X_A T$ Bohr Magnetons (B.M.)
X_A = Molar susceptibilities (susceptibilities of diamagnetic group or ligands)
T = Temperature (°K)

1.5.5 Conductivity measurements

It is the technique in which electrical resistance of a solution is used to measure the total ionic conductivity of a solution as a function of the concentration of substrate in the sample solution *[131]*. Conductivity can also be explained as the power of electrolyte to conduct electric current. Conductance is the reciprocal of the resistance. Its units are Mohs or Ohm^{-1} and represented by Λ.

$\Lambda = 1/R$ where Λ = conductivity (conductance)

R = resistance

Conductivity is affected by different factors like concentration and temperature. Conductivity increases by increasing concentration, and concentration decreases by dilution because on

dilution the ionic mobility increases and inter-ionic attraction decreases. The conductivity of a solution of an electrolyte generally increases at temperature (2-3 %) for one-degree rise in temperature. The increase in conductance with rise in temperature is due to the speed of ions with which they move towards the electrodes. Velocity of the solvent decreases with the increase in temperature which makes the ions to move freely towards the electrodes *[132,133]*. Conductivity measurements are important in determining the lipophilicity of complexes. The neutral complexes show enhanced lipophilicity.

1.5.6 Thermal analysis

Practically all chemical reactions are accompanied by a heat effect and the course of a reaction can be followed by observing the heat librated or absorbed. Such type of measurements can be made by titration in a small adiabatic colorimeter. A suitable colorimeter can easily be assembled from a pair of small Dewar flasks or insulated beakers *[134-136]*. Now with the development of technology thermal analysis using instruments, for measurement of temperature at transititon state, weight loss in materials, energies of transitions, dimensional changes etc, have become possible. The most common technique used in various laboratories are thermogravimetry (TG), differential thermal analysis (DTA), differential scenning colorimetry (DSC) and evolved gass analysis (EGA) etc *[137]*.

In thermogravimetry the change in mass of the sample is recorded as a function of temperature. It provides the analyst with quantitative measurements of change in weight associated with any transition. TG can directly record the loss in weight with time or temperature due to dehydration or decomposition. Thermogravimetric curves are characteristic of a given compound or system because of the unique sequence of physiochemical reactions which occurs over definite temperature ranges. In order for a mass change to be detected, a volatile component must be evolved or absorbed by the sample. The former is the usual mode of measurement, but many examples are also known for the latter. Since elevated temperatures are normaly required for the

evolution of volatile materials, mass-change measurements are made at increasing rather than decreasing temperatures. Routine measurements can be made at temperature range from ambient to 1500 °C with inert atmosphere *[138]*.

In DTA the temperature of test sample with respect to temperature of a thermally inert material is measured. Any transition, which the sample undergoes, will result in liberation or absorption of energy by the sample with the corresponding deviation of its temperature from that of the reference. The difference in temperature between the sample and the inert reference substance will be observed when changes involve heat of reaction, such as chemical reactions, phase changes or structural changes occurring in the sample, DTA curves are useful both quantitatively and qualitatively for reaching at some conclusions. The position and the shapes of the peaks can be used to determine the changes in composition of the sample due to dehydration, decomposition and polymerization, viz., physiochemical aspects etc. The shape of the DTA curve can also be used in evaluating the kinetics of a reaction under carefully controlled conditions. It is important to note that thermal analysis is affected by experimental conditions and deviations caused by instrumental factors. Thermal analyses provide important information regarding thermal stability of substances and thus indirectly provide clues to the structure and composition of the substances.

1.5.7 Microanalysis

CHN analysis, commonly known as microanylisis is a basic tool in determination of elemental composition of materials. So it is the first step in characterization of new substances. The microanylisis is usually performed by use of automated methods and instrumentation.

1.5.8 Anti-microbial activity

The sensitivity of bacteria to antimicrobials is determined by different microbiologic methods. The term antimicrobial agents may be used to designate any substance of natural, semi-synthetic or synthetic nature which kills free living, commonest or pathogenic microorganisms while

causing little or no injury to the host *[139]*. The antimicrobial susceptibility test involves various types of in vitro techniques. Two type of susceptibility tests were used by Flemming *[140]*, which are still being used, they are dilution and agar diffusion methods.

Dilution method

For estimates of antimicrobial activity, dilutions of the antimicrobial agents may be incorporated into broth or nutrient agar and then inoculated with standard suspension of the test organism. Different types of dilution tests are: (a) Broth dilution test, (b) Tube dilution test, (c) Agar dilution test and (d) Plate dilution test.

Dilution tests are used to determine the minimal inhibitory concentration (MIC) or minimal lethal concentration (MLC), i.e. the lowest concentration of an antimicrobial of an antimicrobial required to inhibit or "kill" a particular microorganism. The primary advantage of dilution test is that it provides both qualitative and quantitative estimate of the activity of an antimicrob. In clinical laboratories, quantitative data is needed occasionally whereas qualitative information is needed frequently.

Agar diffusion method

In this technique *[141-143]* a nutrient agar plate is inoculated and the antimicrobial diffuses from a reservoir into the agar medium. As the microorganisms grow, they are exposed to a continuous gradient of decreasing concentrations of the antimicrobial at increasing distances from the reservoir. The reservoir can be formed by filling a ditch or a well cut from the agar plate, by filling a cylinder placed onto the agar surface, or by applying filter-paper discs that contain the antimicrobial agent. Although the methods for applying the antimicrobial differ, the principle of all agar diffusion tests is the same, that is, the larger the zone of inhibition the greater will be the degree of susceptibility of the test organism. Generally agar diffusion tests are used for qualitatively distinguishing between susceptible and resistant strains, but they may be

standardized to provide nearly quantitative information. If the amount of drug in the reservoir is held constant and all other variables are standardized, the size of the zone of inhibitions is a measure of the degree of susceptibility of the test organism. The accuracy of the method may be influenced by pH and size of inoculum, composition and thickness of the agar medium, timing of disc application to plate, time and temperature of incubation and the accuracy of the stated quantity of the anti-microbe present on the paper disc; it is of practical clinical importance in indicating which anti-bacterial agent to employ and the general level of the dose required to produce the therapeutic effect.

Sterilization of equipments

Sterilization is a process used to kill or remove all kind of microorganisms from the article. Sterilization of an article can be achieved by exposure to lethal physical and chemical agents or in case of heat sensitive solutions sterilization can be achieved by filtration using membrane filters.

1.6 Aims of the study

The relationship between metal ions and biological activity of certain systems is obvious and a subject of great interest. It has been demonstrated through several studies that biologically inactive compounds become active *[72-88]* and less biologically active compounds become more active *[46-71]* upon coordination / chelation with the metal ions. The apparent role played by metal ions in the induction or enhancement of biological activity of the organic compounds / ligands is therefore definite, but how, is still a matter of conjecture.

There has been a large number of studies involving the synthesis of new compounds and possibility of altering their biological activity via coordination to metal ions *[144-147]*. The present work is an extension to such studies and deals with the synthesis and antibacterial activity of Cu (II) complexes of some new compounds derived from 6,8-dicloro-3-formylchromone and hydrazides, hydrazines semicarbazides and thiosemicarbazides.

In order to get an insight into the versatile behavior of chromones towards amino compounds and their potential as ligands to produce complexes the present study was designed. The interaction between metal ions and such biologically active ligands represents an important route in designing new metal-based antibacterial, antifungal and anticancer therapies against different kinds of bacteria, fungi, and cancer-associated viruses that become resistant to the use of conventional drugs *[148,149]*. It was, therefore, thought worthwhile to synthesize some chromone derivatives and their complexes and study their biological activities with a view to design new metal-based compounds that could fight more aggressively against such bacterial strains, which become resistant to certain presently available and commonly used antimicrobial agents.

CHAPTER 2

EXPERIMENTAL

EXPERIMENTAL

2.1 Materials

Reagents and solvents

All reagents and solvents were obtained from the supplier and used without further purification. The list of the reagents, chemicals and solvents used in this study is given in Table 1.

Table 1: List of chemicals

Reagent/Solvents	Grade	Source
6,8-Dichloro-3-formylchromone	AR Grade	Aldrich
4-Aminobenzoic hydrazide	AR Grade	Aldrich
4-Bromobenzoic hydrazide	AR Grade	Aldrich
4-Chlorobenzoic hydrazide	AR Grade	Aldrich
4-Hydroxy benzoic hydrazide	AR Grade	Aldrich
Isonicotinic hydrazide	AR Grade	Aldrich
Nicotinic hydrazide	AR Grade	Aldrich
2,5-Dichlorophenyl hydrazine	AR Grade	Aldrich
2,5-Difluorophenyl hydrazine	AR Grade	Aldrich
2,3,5,6-Tetrafluorophenyl hydrazine	AR Grade	Aldrich
m-Metatolyl (methyl phenyl) hydrazine HCl	AR Grade	Aldrich
4-Methoxyphenyl hydrazine HCl	AR Grade	Aldrich
4-Hydrazinobenzoic acid	AR Grade	Aldrich
Semicarbazide	AR Grade	Aldrich
4-Phenylsemicarbazide	AR Grade	Aldrich
4,4-Diphenylsemicarbazide	AR Grade	Aldrich
Thiosemicarbazide	AR Grade	Aldrich
4-Phenyl-3-thiosemicarbazide	AR Grade	Aldrich
Ethanol	GR Grade	E Merck
Methanol	GR Grade	E Merck
H_2SO_4	GR Grade	E Merck
$CuCl_2.2H_2O$	Extra Pure	E Merck

2.2 METHODS

2.2.1 Preparation of ligands

General procedure for the preparation of ligands

To a stirred solution of 6,8-dichloro-3-formylchromone (0.01 mole) in ethanol (30-40 mL) was added the appropriate hydrazide or aryl hydrazine (0.01 mole). The resultant mixture in each case was heated under reflux for 3-4 h. The solid formed during refluxing was collected by suction-filtration. Thorough washing with hot ethanol and drying the required product obtained in pure form was collected and kept air tight for further use. The ligands L_{15}-L_{19} were prepared by a slight modification in this method with addition of 2-3 drops of concentrated H_2SO_4 as catalyst.

Characterization of ligands

All ligands were characterized by their physical (solubility, melting/decomposition), analytical and spectral (electronic absorption, FT-IR, mass spectral and nuclear magnetic resonance) data.

Solubility

Solubility of the ligands was checked in water, dimethylsulfoxide (DMSO), N, N-dimethylformamide (DMF), and other common organic solvents by shaking a small amount of the ligand with the solvent in a test tube.

Melting /decomposition points

The melting /decomposition point of the ligands were determined by placing a crystal of the ligand in a glass capillary tube and heating it in a slower rate using GallenKamp melting point apparatus and were reported as uncorrected.

Electronic absorption spectra

Electronic absorption spectra in the UV-Visible range were recorded on Irmeco U-2020 spectrophotometer between 400-1200 nm by using DMF as the solvent.

FT-IR

FT-IR spectra of the ligands were obtained using KBr discs and Nujol mull techniques on IR Spectrophotometer FTIR-8101M Shimadzu in the 4000–500 cm^{-1} range. The important IR bands, ν(CH=N) symmetric and asymmetric stretching, ν(amino,–$NH_{2\ smy.str}$), ν(amino,–$NH_{2\ asmy.str}$) and ν(chromen –C=O), ν(carboxlic –OH), indicating the formation of ligands were studied.

Mass spectra

Mass spectra of the ligands were obtained using JEOL MS Route spectrometer using EI ionization.

NMR

^{1}H-NMR spectra of the ligands were recorded in DMSO–d_6, δ, on a R10-Perkin Elmer spectrometer, using tetramethylsilane (TMS) as a standard.

2.2.2 Preparation of copper (II) complexes

General procedure for the preparation of copper (II) complexes

Copper (II) complexes were prepared by mixing the respective ligand (0.02 mol) in methanol (15 – 20 mL) with the solution of $CuCl_2.5H_2O$ (0.01 mol) in warm methanol (5 – 10 mL). The mixture was refluxed for 1 hour. The complex was isolated after reduction of volume by evaporation and filtration under vacuum, the solid was thoroughly washed with hot methanol to get purified product.

Characterization of copper (II) complexes

All complexes were characterized by their color, solubility, melting/decomposition points, conductivity, watercontent, magnetic moments, thermal, microanalytical, electronic absorption, and FT-IR data.

Solubility

Solubility of the metal (II) complexes was checked in water, dimethylsulfoxide (DMSO), N, N-dimethylformamide (DMF), and other common organic solvents by shaking a small amount of the complex with the solvent in a test tube.

Melting /decomposition points

The melting /decomposition point of the complexes were determined by placing a crystal of the ligand in a glass capillary tube and heating it in a slower rate using GallenKamp melting point apparatus and were reported as uncorrected.

Conductivity measurements

Conductivity measurements of the copper (II) complexes were made on a conductivity meter YSI model-32 (Milwaukee) in DMF (0.01g/100 mL) at room temperature.

Water content

Water content was determined by using Metrohn Herizall E55 Karl Fischer titrator using methanol as medium.

Magnetic moments

Magnetic moments of the metal (II) complexes under investigation were determined at room temperature by the Gouy's balance constructed in this laboratory using Hg (II) tetrathiocyanatocobaltate as a caliberant *[150]* [145].

Thermal analysis

Thermal analysis was carried out on Universal SDT– Q600 thermal analyzer on TGA, DSC and DTA modes from ambient to 1000°C.

Microanalysis

Carbon, hydrogen and nitrogen analysis of the copper (II) complexes were carried out by using CHN–932 (Leco) automatic elemental analyzer.

Electronic absorption spectra

Electronic absorption spectra of the copper (II) complexes in the UV-Visible range 190–900 nm were recorded on Irmeco U–2020 spectrophotometer in DMSO using a 1 cm quartz cell.

FT-IR

FT-IR spectra of the copper (II) complexes were obtained using KBr discs and Nujol mull techniques on IR Spectrophotometer FTIR-8101M Shimadzu in the 4000–500 cm^{-1} range. The important IR bands, ν(CH=N) symmetric and asymmetric stretching, ν(amino,–$NH_{2\ smy.str}$), ν(amino,–$NH_{2\ asmy.str}$), ν(chromen –C=O), ν(carboxlic –OH) and ν(M-N), ν(M-O), ν(M-Cl) and ν(M-S) indicating the formation of copper (II) complexes were studied.

2.3 Antibacterial activity studies

Paper disc diffusion and serial dilution methods were used for determination of antibacterial activity as follows:

Following growth media used in this study.

Procedure

2.3.1 Sterilization of equipments

The equipments (petri dishes, flasks, pipettes, tweezers, forceps and wire loops etc.) were first thoroughly washed with detergent and distilled water then sterilized by dry heat method in a hot air oven at 200°C for two h. The sterilized equipments were kept under laminar air flow where subsequent procedure was completed. Other materials and media were sterilized by autoclaving at 121°C for 20 min at one bar pressure.

2.3.2 Media preparation and challenge test

a) Media preparation

It was ensured that the media flasks were thoroughly clean and free from cracks. The flasks were rinsed with distilled water. Distilled water was added by means of a measuring cylinder (500 mL). According to manufacture's instructions, a weighed quantity of dehydrated powder media tryptic soy broth Table 2 was transferred to flasks. To this distilled water was added by means of a measuring cylinder and warmed slightly. The volume was made up with distilled water to 500 mL and pH was adjusted to 7.3 ± 0.2

Table 2: Composition of tryptic soy broth (MERCK)

Ingredients	Composition
Peptone from casein	17 g
Peptone from soymeal	3.0 g
D(+)Glucose	2.5 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	2.5 g
Distilled water	1000 mL
pH	7.3 $\pm$ 0.2 at 25°C

with 0.1 M NaOH before sterilization. The broth was dispensed in the test tubes and sterilized in an autoclave at 121°C for 20 min at one bar pressure. The media were cooled to room temperature and pH was checked. The media thus prepared were incubated at 37°C for 24 h to check the sterility.

b) Microbiological challenge to test the media

This experiment was carried out to challenge the test media to ensure that the test equipment and media do not inhibit the growth of micro organisms and the test media to be used were capable of supporting the growth of micro organisms. Every batch of media listed in the table was challenged with an inoculum of < 100 cfu of the appropriate micro organism.

2.3.3 Preparation of cultures

Following bacterial strains were used for determining the nutritive properties of the media and antibacterial activity of the prepared compounds and their metal (II) complexes under investigation. E. coil (ATCC 9027), S. aureus (ATCC6538), P. aeruginosa (ATCC 8739),

a) *Reconstitution of freeze-dried cultures*

The standard bacterial strains were reconstituted as follows:

The ampoule of the strain was placed on a piece of sterile J-cloth and marked a cut across the middle of the tube with a small hand saw. Gentle pressure was applied at the point of the cut, to break open the ampoule. By using sterile forceps the cotton wool plug was removed and discarded into a disinfectant solution (70% IPA). With the aid of a sterile Pasteur pipette, tryptic soy broth (approximately 0.5 mL) was aseptically removed, the cultures were reconstituted and transferred back to the original media container by using the same pipette.

b) *Preparation of culture slopes*

The culture slopes were prepared from the reconstituted freeze-dried culture broths. The cultures were inoculated into tryptic soy agar slopes which were previously pre-incubated overnight at 37°C for 24 h. The Inoculated slopes were incubated at 37°C for 24 h.

2.3.4 Determination of antibacterial activity by paper disc diffusion method

Paper disc diffusion method *[151-153]* was used for the determination of antibacterial activity as follows:

a) Preparation of discs

The ligand / complex in (30 g) DMF solution (0.01mL) was applied on a paper disc prepared from Whatmann / blotting paper (3mm size) with the help of a micropipette. These discs were left in an incubator for 48 h at 37°C and than applied on bacteria grown agar plates.

b) Preparation of agar plates

For this purpose tryptic soy agar Table 3 (27.5 g) was suspended in freshly prepared distilled water (1 L). It was allowed to soak for 15 min and then boiled with constant stirring on water bath until the agar completely dissolved. The mixture was autoclaved for 20 min at 120˚C and then poured into previously washed and sterilized petri dishes and stored at 40˚C for inoculation.

Table 3: Composition of tryptic soy agar (MERCK)

Ingredients	Composition
Peptone from casein	15.0 g
Peptone from soymeal	5.0 g
Sodium chloride	5.0 g
Agar-agar	15.0 g
Distilled water	1000 mL
pH	7.3 $\pm$ 0.2 at 25°C

c) Procedure of inoculation

Inoculation was done with the help of a platinum wire loop. The loop was made red-hot on a flame, allowed to cool in air and then used for the application of previously described bacterial strains.

d) Application of discs

Antibacterial activity of the ligands and metal (II) complexes was determined separately. A sterilized forceps was used for the application of paper discs on the already incubated agar plates. When the discs were applied, these were then incubated at 37˚C for 24 h. The zone of inhibition in diameter was measured.

2.3.5 Determination of MIC

Ligand and their Cu (II) complexes were subjected to microbiological testing by using standard strains of Gram-negative, P. aeruginosa, S. flexneri and Gram-positive, B. subtilis, S. aureus bacteria. Activities of the ligands were compared with those of the Cu (II) complexes and the efficacy was determined by MIC values *[154,155]*.

Procedure

a) The sample (0.1 g) was dissolved in the tryptic soy broth (10 mL) in a test tube and then shaken vigorously with the help of gyro mixer under a laminar flow (preparation A).

b) From preparation A, 1 mL of the mixture was transferred into 9 mL tryptic soy broth (TSB) test tubes in triplicate and mixed thoroughly under a laminar flow (preparation B).

c) The above procedure of serial dilutions was repeated to obtain preparation F.

d) The drug dilutions prepared were as given in Table 4.

e) All drug concentrations obtained by the serial dilution method were inoculated with pre-incubated cultures. All drug dilutions were inoculated with 1 full loop of the appropriate test drug.

f) At the same time positive and negative controls were also run. The positive and negative controls were free from drug. Only the positive control test tube was inoculated with one full loop of the broth culture.

g) After inoculation the dilutions were incubated at 37°C for 24 h. Positive and negative controls were also incubated at 37°C for 24 h, simultaneously.

Table 4: Drug dilutions prepared

Transfer g per mL	Solution	TSB (mL)	Conc. mg per 10 mL	Conc. µg per 10 mL	Preparation code
0.1	Stock	10 mL	100	100000	A
1 mL	A	9 mL	10	10000	B
1 mL	B	9 mL	1	1000	C
1 mL	C	9 mL	0.1	100	D
5 mL	D	5 mL	0.05	50	E
5 mL	E	5 mL	0.025	25	F

h) After incubation the turbidity (growth) was checked in the test tubes and in the control tubes.

i) The test tube in the serial dilution which showed no turbidity was taken as the MIC of the compound.

j) The determination was repeated in order to obtain the exact MIC of a compound. The second set of serial dilutions and concentrations was as follows: Table 5.

Table 5: Serial dilutions and concentrations

Transfer g per mL	Solution	TSB (mL)	Conc. mg per 10 mL	Conc. µg per 10mL	Dilution code
0.1	Stock	10 mL	100	100000	A
1 mL	A	9 mL	10	10000	B
1 mL	B	9 mL	1	1000	C
1 mL	C	9 mL	0.1	100	D
3 mL	D	7 mL	0.07	70	E
2 mL	E	8 mL	0.05	50	F

CHAPTER 3

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

3.1 Preparation of ligands

All of the ligands (1–12) were easily obtained by refluxing the equimolar (0.01 mol) quantities of 6,8-dichloro-3-formylchromone and the respective hydrazide or aryl hydrazine for 3–4 h in ethanol (30-40 mL). The ligands (13–17) were obtained by the addition of 2–3 drops of concentrated H_2SO_4. All of the isolated ligands were characterized by melting/decomp. points and spectroscopic data. The microanalysis and melting points are given in Table 5. UV-Visible, FTIR, mass and nuclear magnetic resonance spectral data are given in Tables 7, 8, 9 and 10, respectively. The purity of the products was checked by thin layer chromatography and the spots were visualized under ultraviolet light at 254 and 366 nm or by exposing to iodine vapors. The presence of single spot at different *rf* values than those of the starting materials indicated the purity of the ligand. The structures of all the synthesized ligands were established through UV-Visible, FT-IR, mass and ^{1}H-NMR spectral data. All the ligands were stable at room temperature.

3.1.1 Characterization of the ligands 1–17

3.1.1.1 Solubility

Solubility of the ligands was studied in various organic solvents. They were found to be soluble in ethanol, methanol, chloroform, ether, acetone DMSO and DMF and insoluble in water.

3.1.1.2 Electronic absorption spectra

The electronic absorption bands of the isolated ligands under investigation are listed in the Table 6.

Table 6: Physical and UV-Vis spectra of the ligands

Sr. No	Name of the reactants	Name of the ligands	Mol. Formula / Mol. Mass	Color	Melting/ decomp. Points	Absorbance maxima cm^{-1}
1	6,8-Dichloro-3-formylchromone + 4-Hydroxy benzoic hydrazide	(3,5-dichloro-2-hydroxyphenyl)[2-(4-hydroxyphenyl)-1,3,4-oxadiazepin-6-yl]methanone	$C_{17}H_{10}Cl_2N_2O_4$ (377.2)	Dark yellow	264-266 °C	38363
2	6,8-Dichloro-3-formylchromone + 4-Bromobenzoic hydrazide	[2-(4-bromophenyl)-1,3,4-oxadiazepin-6-yl](3,5-dichloro-2-hydroxyphenyl)methanone	$C_{17}H_9BrCl_2N_2O_3$ (440.07)	Light yellow	165-167 °C	33364
3	6,8-Dichloro-3-formylchromone + 4-Chlorobenzoic hydrazide	[2-(4-chlorophenyl)-1,3,4-oxadiazepin-6-yl](3,5-dichloro-2-hydroxyphenyl)methanone	$C_{17}H_9Cl_3N_2O_3$ (395.62)	Yellow	163-165 °C	28255
4	6,8-Dichloro-3-formylchromone + 4-Aminobenzoic hydrazide	[2-(4-aminophenyl)-1,3,4-oxadiazepin-6-yl](3,5-dichloro-2-hydroxyphenyl)methanone	$C_{17}H_{11}Cl_2N_3O_3$ (376.19)	Yellow	225-227 °C	33840
5	6,8-Dichloro-3-formylchromone + Isonicotinic hydrazide	(3,5-dichloro-2-hydroxyphenyl)(2-pyridin-4-yl-1,3,4-oxadiazepin-6-yl)methanone	$C_{16}H_9Cl_2N_3O_3$ (362.17)	Yellow	171-173 °C	28800
6	6,8-Dichloro-3-formylchromone + Nicotinic hydrazide	(3,5-dichloro-2-hydroxyphenyl)(2-pyridin-3-yl-1,3,4-oxadiazepin-6-yl)methanone	$C_{16}H_9Cl_2N_3O_3$ (362.17)	Creamish yellow	160-163 °C	36800
7	6,8-Dichloro-3-formylchromone + 2,3,5,6-Tetrafluorophenyl hydrazine	(3,5-dichloro-2-hydroxyphenyl)[1-(2,3,5,6-tetrafluorophenyl)-1*H*-pyrazol-4-yl]methanone	$C_{16}H_6Cl_2F_4N_2O_2$ (405.13)	Light yellow	91-93 °C	37350
8	6,8-Dichloro-3-formylchromone + 2,5-Difluorophenyl hydrazine	(3,5-dichloro-2-hydroxyphenyl)[1-(2,5-difluorophenyl)-1*H*-pyrazol-4-yl]methanone	$C_{16}H_8Cl_2F_2N_2O_2$ (369.15)	Yellowish	75-76 °C	30920
9	6,8-Dichloro-3-formylchromone + 2,5-Dichlorophenyl hydrazine	(3,5-dichloro-2-hydroxyphenyl)[1-(2,5-dichlorophenyl)-1*H*-pyrazol-4-yl]methanone	$C_{16}H_8Cl_4N_2O_2$ (402.06)	Yellowish	102-103 °C	36350
10	6,8-Dichloro-3-formylchromone + *m*-Metatolyl (methyl phenyl) hydrazine HCl	(3,5-dichloro-2-hydroxyphenyl)[1-(3-methylphenyl)-1*H*-pyrazol-4-yl]methanone	$C_{17}H_{12}Cl_2N_2O_2$ (347.19)	Bright-yellow	184-186 °C	35220
11	6,8-Dichloro-3-formylchromone + 4-Hydrazinobenzoic acid	4-[4-(3,5-dichloro-2-hydroxybenzoyl)-1*H*-pyrazol-1-yl]benzoic acid	$C_{17}H_{10}Cl_2N_2O_4$ (377.18)	Dull-yellow	253-255 °C	32560
12	6,8-Dichloro-3-formylchromone + 4-Methoxyphenyl hydrazine HCl	(3,5-dichloro-2-hydroxyphenyl)[1-(4-methoxyphenyl)-1*H*-pyrazol-4-yl]methanone	$C_{17}H_{12}Cl_2N_2O_3$ (363.19)	Yellowish	160-162 °C	31230

13	6,8-Dichloro-3-formylchromone + Thiosemicarbazide	(2-amino-1,3,4-thiadiazepin-6-yl)(3,5-dichloro-2-hydroxyphenyl)methanone	$C_{11}H_7Cl_2N_3O_2S$ (316.16)	Light-yellow	236-238 °C	29580
14	6,8-Dichloro-3-formylchromone + 4-Phenyl-3-thiosemicarbazide	(2-anilino-1,3,4-thiadiazepin-6-yl)(3,5-dichloro-2-hydroxyphenyl)methanone	$C_{17}H_{11}Cl_2N_3O_2S$ (392.26)	Creamish-yellow	222-224 °C	33570
15	6,8-Dichloro-3-formylchromone + 4-Phenylsemicarbazide	(2-anilino-1,3,4-oxadiazepin-6-yl)(3,5-dichloro-2-hydroxyphenyl)methanone	$C_{17}H_{11}Cl_2N_3O_3$ (376.20)	Creamish-yellow	220-222 °C	34230
16	6,8-Dichloro-3-formylchromone + 4,4-Diphenylsemicarbazide	(3,5-dichloro-2-hydroxyphenyl)[2-(diphenylamino)-1,3,4-oxadiazepin-6-yl]methanone	$C_{23}H_{15}Cl_2N_3O_3$ (452.29)	Bright-yellow	186-188 °C	35410
17	6,8-Dichloro-3-formylchromone + Semicarbazide	(2-amino-1,3,4-oxadiazepin-6-yl)(3,5-dichloro-2-hydroxyphenyl)methanone	$C_{11}H_7Cl_2N_3O_3$ (300.10)	Yellowish	228-230 °C	36540

The spectra showed that when a chromone and an aldehyde were mixed together, a bright yellow intermediate was first formed which converted to a pale yellow final product. In these spectra, an intense band between 240-294 nm was observed which is assigned to a π-π* transition originating in the phenyl rings *[156]*. The bands in the 325-370 nm range *[157]*, are attributed to π-π* transition originating in the –CH=N– chromophore.

3.1.1.3 Infrared spectra

In the IR spectra of the ligands all the absorption bands representing the functional groups characteristic of the ligands were observed. The characteristic absorption bands along with their assignments are listed in Table 7, 8. The IR spectra of all the ligands (1-17) exhibited the azomethine (CH=N) [Oxadazepin $\nu(C_7H{=}N_6)$] and Oxadiazepin $\nu(C_4H{=}N_5)$ strechings in the regions 1600-1650 and 1650-1670 cm^{-1}, respectively. In addition, the spectra of ligands showed a band resulting from the amide NH and C=O strechings at 3420-3442 and 1660-1685 cm^{-1}, respectively. The IR spectrum of ligand (1-17) displayed dichlorohydroxyphenyl OH strechings at 3450-3455 cm^{-1}. In the case of ligand (4) the amino NH_2 asymmetric and symmetric stretchings appeared at 3380 and 3320 cm^{-1}, respectively. The IR spectra of ligand (5) and (6) exhibited the isonicotinic and nicotinic C=N stretchings in the regions 1505 and 1510 cm^{-1}, respectively. In the case of ligands (7-12), the amino -NH strechings appeared in the 3400-3450 cm^{-1}, region and in the case of ligand (13) exhibited a band resulting from the amide $–NH_2$ asymetric and symmetric strechings appeared at 3350-3220 cm^{-1} respectively. In addition, the spectrum of ligand (12) showed bands resulting from the OH and C=O strechings of the carboxylic functions at 3520 and 1710 cm^{-1}, respectively. In case of ligands (16) and (17) the thioamide C=S stretchings appeared at 156 and 1570 cm^{-1}. In the spectra of ligands (16) and (17) bands resulting from amide $–NH_2$ asymetric and symmetric strechings also appeared at 3320-3180 cm^{-1} respectively.

Table 7: Observed infrared frequencies (cm^{-1}) and assignments of ligands (1 – 12)

Sr. No	Name of ligands	ν(carboxlic - OH)	Dichlorohydroxy phenyl ν(OH)	ν(amide NH)	ν(amino – NH/ NH_2)	ν(carboxlic - CO)	ν(amide – C=O)	Oxadazepin ν ($C_7H{=}N_6$)	Oxadiazepin ν ($C_4H{=}N_5$)
1	(3,5-dichloro-2-hydroxyphenyl)[2-(4-hydroxyphenyl)-1,3,4-oxadiazepin-6-yl]methanone	-	3450	3420	-	-	1670	1655	1630
2	[2-(4-bromophenyl)-1,3,4-oxadiazepin-6-yl](3,5-dichloro-2-hydroxyphenyl)methanone	-	3452	3430	-	-	1675	1655	1625
3	[2-(4-chlorophenyl)-1,3,4-oxadiazepin-6-yl](3,5-dichloro-2-hydroxyphenyl)methanone	-	3455	3425	-	-	1665		1622
4	[2-(4-aminophenyl)-1,3,4-oxadiazepin-6-yl](3,5-dichloro-2-hydroxyphenyl)methanone	-	3450	3418	3380_{asy} 3320_{sym}	-	1660	1655	1620
5	(3,5-dichloro-2-hydroxyphenyl)(2-pyridin-4-yl-1,3,4-oxadiazepin-6-yl)methanone	-	3452	3440	-	-	1680	1665	1632 1505 isonicoti
6	(3,5-dichloro-2-hydroxyphenyl)(2-pyridin-3-yl-1,3,4-oxadiazepin-6-yl)methanone	-	3450	3442	-	-	1685	1665	1632 1510 nicotino
7	(3,5-dichloro-2-hydroxyphenyl)[1-(2,3,5,6-tetrafluorophenyl)-1*H*-pyrazol-4-yl]methanone	-	3450	3440	-	-	1685	1665	1640
8	(3,5-dichloro-2-hydroxyphenyl)[1-(2,5-difluorophenyl)-1*H*-pyrazol-4-yl]methanone	-	3455	3420	-	-	1675	1655	1635
9	(3,5-dichloro-2-hydroxyphenyl)[1-(2,5-dichlorophenyl)-1*H*-pyrazol-4-yl]methanone	-	3455	3400	-	-	1670	1660	1634
10	(3,5-dichloro-2-hydroxyphenyl)[1-(3-methylphenyl)-1*H*-pyrazol-4-yl]methanone	-	3452	3410	-	-	1665	1650	1625
11	4-[4-(3,5-dichloro-2-hydroxybenzoyl)-1*H*-pyrazol-1-yl]benzoic acid	3520	3450		3450	1710	-	1665	1638
12	(3,5-dichloro-2-hydroxyphenyl)[1-(4-methoxyphenyl)-1*H*-pyrazol-4-yl]methanone	3520	3455		3425	1690	-	1652	1624

Table 8: Observed infrared frequencies (cm^{-1}) and assignments of ligands (13 – 17)

Sr. No	Name of ligands	ν(thioamide NH)	ν(thioamide NH_2)	ν(thioamide C-S)	ν(amide – NH)	ν(amide – NH_2)	ν(amide – C=O)	Oxadazepin ν ($C_7H=N_6$)	Oxadiazepin ν ($C_4H=N_5$)
13	(2-amino-1,3,4-thiadiazepin-6-yl)(3,5-dichloro-2-hydroxyphenyl)methanone	3425	3320_{asy} 3180_{sym}	1560	-	-	-	1655	1620
14	(2-anilino-1,3,4-thiadiazepin-6-yl)(3,5-dichloro-2-hydroxyphenyl)methanone	3435	-	1570	-	-	-	1665	1625
15	(2-anilino-1,3,4-oxadiazepin-6-yl)(3,5-dichloro-2-hydroxyphenyl)methanone	-	-	-	3440	-	1680	1650	1625
16	(3,5-dichloro-2-hydroxyphenyl)[2-(diphenylamino)-1,3,4-oxadiazepin-6-yl]methanone	-	-	-	3450	-	1690	1670	1630
17	(2-amino-1,3,4-oxadiazepin-6-yl)(3,5-dichloro-2-hydroxyphenyl)methanone	-	-	-	3440	3350_{asy} 3320_{sym}	1685	1660	1620

3.1.1.4 ^{1}H-NMR spectra

The numbering of atoms is shown in Fig. 4-20 and the ^{1}H-NMR spectral data listed in the Table 9. ^{1}H-NMR spectra of all the ligands (1–17) displayed the azomethine (CH=N) and chromene OH at δ 8.62-8.99 and δ 12.00-12.70 respectively as singlets. The C_7-H of the oxadiazepin moiety of the ligands (1–6) also appeared as singlets at δ 8.02-8.39 respectively. The 1H-NMR spectra of the ligands (1–5) demonstrated the phenyl $C_{12,10}$-H and $C_{13,9}$-H as doublets at δ 7.75-7.91 and δ 7.75-7.93 respectively. In addition, in the spectra of ligands (1) and (4), the protons of the hydroxyl (OH) and amino (NH_2) moieties appeared as singlets at δ 8.54 and δ 6.14. The ^{1}H-NMR spectrrum of the ligand (5) displayed pyridino $C_{17,19}$-H and $C_{16,20}$-H protons as separate doublets at δ 8.14 and δ 8.87 respectively. In the case of ligand (6), the nicotino C_{12}-H, C_{13}-H and C_{11}-H appeared as doublet doublet at δ 8.14, δ 8.66 and δ 8.70, however, C_9-H appeared as singlet at δ 8.90. The ^{1}H-NMR spectra of the ligands (7–12) demonstrated the pyrazole C_5-H appears as a singlet δ 7.09, δ 7.83. However the phenyl C_9-H appeared as singlet at δ 8.00 and δ 7.33 in the ligands (8-10). In case of ligand (7), the phenyl C_9-H experiencing a de-shielding effect due to inductive effect of floro atom, resonated as singlet at δ 8.00. The ^{1}H-NMR spectrum of the ligand (10) displayed phenyl C_9-H, C_{11}-H and C_{10}-H as doublet doublet at δ 6.85, δ 7.06 and δ 7.08 respectively. However C_7-H resonated as singlet at δ 6.19. In the ^{1}H-NMR spectrum of the ligand (11), the phenyl $C_{8,10}$-H and $C_{7,11}$-H experiencing a de-shielding effect due to inductive and resonance effect of carboxy function (COOH), resonated as doublets at δ 7.78 and δ 7.81 respectively. In addition, the carboxylic acid (COOH) proton appeared as singlet at δ 10.98. In case of ligand (12), the phenyl $C_{8,10}$-H and $C_{7,11}$-H appeared as doublet at δ 7.58 and δ 7.59 respectively. In addition, methoxy protons (OCH_3) appeared as singlet at δ 3.30.

Table 9: ^{1}H NMR spectra and assignment of ligands

Sr. No	Name of ligands	(DMSO-d_6, δ, ppm)
1	(3,5-dichloro-2-hydroxyphenyl)[2-(4-hydroxyphenyl)-1,3,4-oxadiazepin-6-yl]methanone	7.79 (*d*, 2H, *J*=8.22 Hz, hydroxyphenyl $C_{12,10}$-H), 7.81 (d, 2H, *J*=8.21 Hz, hydroxyphenyl $C_{13,9}$-H), 8.02 (s, 1H, oxadiazepin C_7-H), 8.22 (s, 1H, chromen C_{22}-H), 8.23 (s, 1H, chromen C_{20}-H), 8.54 (s, 1H, OH, hydroxyphenyl). 8.86 (s, 1H, CH=N), 12.02 (s, 1H, OH, chromene)
2	[2-(4-bromophenyl)-1,3,4-oxadiazepin-6-yl](3,5-dichloro-2-hydroxyphenyl)methanone	7.75 (*d*, 2H, *J*=8.22 Hz, bromophenyl $C_{12,10}$-H), 7.76 (d, 2H, *J*=8.21 Hz, bromophenyl $C_{13,9}$-H), 8.03 (s, 1H, oxadiazepin C_7-H), 8.24 (s, 1H, chromen C_{22}-H), 8.25 (s, 1H, chromen C_{20}-H), 8.91 (s, 1H, CH=N), 12.03 (s, 1H, OH, chromene)
3	[2-(4-chlorophenyl)-1,3,4-oxadiazepin-6-yl](3,5-dichloro-2-hydroxyphenyl)methanone	7.91 (*d*, 2H, *J*=8.22 Hz, chlorophenyl $C_{12,10}$-H), 7.93 (d, 2H, *J*=8.21 Hz, chlorophenyl $C_{13,9}$-H), 8.02 (s, 1H, oxadiazepin C_7-H), 8.23 (s, 1H, chromen C_{22}-H), 8.24 (s, 1H, chromen C_{20}-H), 8.91 (s, 1H, CH=N), 12.11 (s, 1H, OH, chromene)
4	[2-(4-aminophenyl)-1,3,4-oxadiazepin-6-yl](3,5-dichloro-2-hydroxyphenyl)methanone	6.14 (*d*, *J*=8.10 Hz 2H, NH_2) 7.79 (*d*, 2H, *J*=8.22 Hz, aminophenyl $C_{12,10}$-H), 7.80 (d, 2H, *J*=8.21 Hz, aminophenyl $C_{13,9}$-H), 8.15 (s, 1H, oxadiazepin C_7-H), 8.30 (s, 1H, chromen C_{22}-H), 8.34 (s, 1H, chromen C_{20}-H), 8.97 (s, 1H, CH=N), 12.04 (s, 1H, OH, chromene)
5	(3,5-dichloro-2-hydroxyphenyl)(2-pyridin-4-yl-1,3,4-oxadiazepin-6-yl)methanone	8.14 (d, 2H, J = 5.72 Hz, pyridine $C_{17,19}$ –H), 8.15 (s, 1H, oxadiazepin C_2 –H), 8.18 (s, 1 H, dichlorohydroxyphenyl C_{12} –H), 8.36 (s, 1 H, dichlorohydroxyphenyl C_{14} –H), 8.74 (s, 1 H, CH N), 8.87 (d, 2 H, J = 5.72 Hz, pyridine $C_{16,20}$ –H), 12.04 (s, 1 H, OH, dichlorohydroxyphenyl)
6	(3,5-dichloro-2-hydroxyphenyl)(2-pyridin-3-yl-1,3,4-oxadiazepin-6-yl)methanone	8.14 (dd, 1H, *J*=7.10, 5.36 Hz, nicotino C_{12}-H), 8.19 (s, 1H, chromen C_{21}-H), 8.38 (s, 1H, chromen C_{19}-H), 8.39 (s, 1H, oxadiazepin C_7-H), 8.62 (dd, 1H, *J*=5.30, 2.10 Hz, nicotino C_{13}-H), 8.70 (dd, 1H, J=5.32, 2.10 Hz, nicotino C_{11}-H), 8.77 (s, 1H, CH=N), 8.90 (s, 1H, nicotino C_9-H), 12.30 (s, 1H, OH, dichlorohydroxyphenyl)
7	(3,5-dichloro-2-hydroxyphenyl)[1-(2,3,5,6-tetrafluorophenyl)-1*H*-pyrazol-4-yl]methanone	7.25 (s, 1H, pyrazole C_5-H), 8.00 (s, 1H, phenyl C_9-H), 8.22 (s, 1H, chromene C_{23}-H), 8.23 (s, 1H, chromene C_{21}-H), 8.89 (s, 1H, CH=N), 12.06 (s, 1H, OH, chromene)

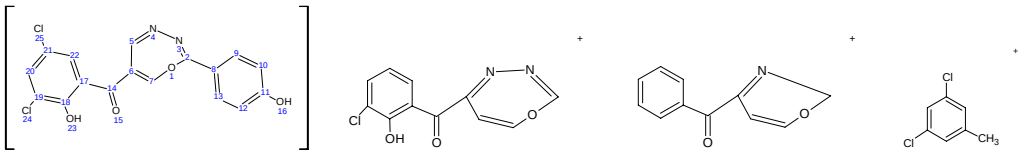
8	(3,5-dichloro-2-hydroxyphenyl)[1-(2,5-difluorophenyl)-1*H*-pyrazol-4-yl]methanone	7.19 (s, 1H, pyrazole C_5-H), 7.36 (d, 1H, *J*= 8.12 Hz, phenyl C_9-H), 7.37 (d, 1H, *J*= 8.12 Hz, phenyl C_8-H), 7.39 (s, 1H, phenyl C_{11}-H), 8.20 (s, 1H, chromene C_{21}-H), 8.21 (s, 1H, chromene C_{19}-H), 8.85 (s, 1H, CH=N), 12.12 (s, 1H, OH, chromene)
9	(3,5-dichloro-2-hydroxyphenyl)[1-(2,5-dichlorophenyl)-1*H*-pyrazol-4-yl]methanone	7.21 (s, 1H, pyrazole C_5-H), 7.33 (d, 1H, *J*= 8.31 Hz, phenyl C_9-H), 7.35 (d, 1H, *J*= 8.30 Hz, phenyl C_8-H), 7.38 (s, 1H, phenyl C_{11}-H), 8.21 (s, 1H, chromene C_{21}-H), 8.22 (s, 1H, chromene C_{19}-H), 8.79 (s, 1H, CH=N), 12.06 (s, 1H, OH, chromene)
10	(3,5-dichloro-2-hydroxyphenyl)[1-(3-methylphenyl)-1*H*-pyrazol-4-yl]methanone	2.12 (s, 3H, CH_3), 6.83 (s, 1H, pyrazole C_5-H), 6.85 (dd, 1H, *J*=7.52, 1.87 Hz, phenyl C_9-H), 6.19 (s, 1H, phenyl C_7-H), 7.06 (dd, 1H, *J*=7.75, 1.88 Hz, phenyl C_{11}-H), 7.08 (dd, 1H, *J*=7.75, 7.53 Hz, phenyl C_{10}-H), 8.19, (s, 1H, chromene C_{20}-H), 8.20 (s, 1H, chromene C_{18}-H), 8.92 (s, 1H, CH=N), 12.11 (s, 1H, OH, chromene)
11	4-[4-(3,5-dichloro-2-hydroxybenzoyl)-1*H*-pyrazol-1-yl]benzoic acid	7.10 (s, 1H, pyrazole C_5-H), 7.78 (d, 2H, *J*=8.90, Hz, phenyl $C_{7,11}$-H), 7.81 (d, 2H, *J*=8.90, Hz, phenyl $C_{8,10}$-H), 8.21 (s, 1H, chromene C_{20}-H), 8.22 (s, 1H, chromene C_{18}-H), 8.99 (s, 1H, CH=N), 10.98 (s, 1H, COOH), 12.30 (s, 1H, OH, chromene)
12	(3,5-dichloro-2-hydroxyphenyl)[1-(4-methoxyphenyl)-1*H*-pyrazol-4-yl]methanone	3.30 (s, 3H, OCH_3), 7.09 (s, 1H, pyrazole C_5-H), 7.58 (d, 2H, *J*=8.95, Hz, phenyl $C_{8,10}$-H), 7.59 (d, 2H, *J*=8.95, Hz, phenyl $C_{7,77}$-H), 8.13 (s, 1H, chromene C_{20}-H), 8.16 (s, 1H, chromene C_{18}-H), 8.87 (s, 1H, CH=N), 12.30 (s, 1H, OH, chromene)
13	(2-amino-1,3,4-thiadiazepin-6-yl)(3,5-dichloro-2-hydroxyphenyl)methanone	7.98 (s, 2H, NH_2), 8.09 (s, 1H, thiadiazepinio C_7-H), 8.21 (s, 1H, chromen C_{16}-H), 8.22 (s, 1H, chromen C_{14}-H), 8.78 (s, 1H, CH=N), 12.09 (s, 1H, OH, chromene)
14	(2-anilino-1,3,4-thiadiazepin-6-yl)(3,5-dichloro-2-hydroxyphenyl)methanone	7.92 (s, 1H, thiadiazepinio C_7-H), 8.00 (dd, 1H, *J*=7.70, *J*=1.91 Hz, phenyl C_{12}-H), 8.01 (dd, 2H, *J*=7.98, *J*=1.92 Hz, phenyl $C_{10,14}$-H), 8.02 (dd, 2H, *J*=7.98, *J*=7.70 Hz, phenyl $C_{11,13}$-H), 8.15 (s, 1H, NH-ph), 8.23 (s, 1H, chromen C_{22}-H), 8.24 (s, 1H, chromen C_{20}-H), 8.84 (s, 1H, CH=N), 12.00 (s, 1H, OH, chromene)

15	(2-anilino-1,3,4-oxadiazepin-6-yl)(3,5-dichloro-2-hydroxyphenyl)methanone	7.94 (s, 1H, oxadiazepinio C_7-H), 8.01 (dd, 1H, *J*=7.63, *J*=1.87 Hz, phenyl C_{12}-H), 8.02 (dd, 2H, *J*=7.94, *J*=1.87 Hz, phenyl $C_{10,14}$-H), 8.03 (dd, 2H, *J*=7.94, *J*=7.64 Hz, phenyl $C_{11,13}$-H), 8.18 (s, 1H, NH-ph), 8.23 (s, 1H, chromen C_{22}-H), 8.24 (s, 1H, chromen C_{20}-H), 8.88 (s, 1H, CH=N), 12.70 (s, 1H, OH, chromene)
16	(3,5-dichloro-2-hydroxyphenyl)[2-(diphenylamino)-1,3,4-oxadiazepin-6-yl]methanone	7.93 (s, 1H, oxadiazepinio C_7-H), 7.25 (dd, 2H, *J*=7.75, *J*=1.93 Hz, phenyl $C_{20,12'}$-H), 7.34 (dd, 4H, *J*=8.14, *J*=1.93 Hz, phenyl $C_{18,14,22,10'}$-H), 7.42 (dd, 4H, *J*=8.15, *J*=7.75 Hz, phenyl $C_{'19,13,21,11}$-H), 8.14 (s, 1H, chromen C_{28}-H), 8.16 (s, 1H, chromen C_{26}-H), 8.89 (s, 1H, CH=N). 12.17 (s, 1H, OH, chromene)
17	(2-amino-1,3,4-oxadiazepin-6-yl)(3,5-dichloro-2-hydroxyphenyl)methanone	7.99 (s, 2H, NH_2), 8.10 (s, 1H, oxadiazepinio C_7-H), 8.24 (s, 1H, chromen C_{16}-H), 8.25 (s, 1H, chromen C_{14}-H), 8.62 (s, 1H, CH=N), 12.19 (s, 1H, OH, chromene)

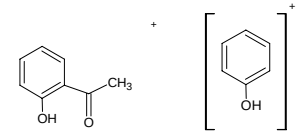
Similarly the ^{1}H-NMR spectra of the ligands (13 and 17) displayed the chromen C_{16}-H, and C_{14}-H protons at δ 8.21-8.24 and δ 8.22-8.25 as a singlets. In addition, in the spectra of ligands (13), (14) and (15), (17), the NH_2 and NH-Ph protons of amide function appeared as singlets at δ 7.98, δ 7.92 and δ 8.18, δ 7.99 respectively. The ^{1}H-NMR spectrum of ligand (14) demonstrated the phenyl $C_{11,13}$-H and $C_{10,14}$-H protons and C_{12}-H as doublet doublet at δ 8.02, δ 8.01 and δ 8.00. The ^{1}H-NMR spectrum of ligand (15), also displayed the $C_{11,13}$ -H and $C_{10,14}$-H and C_{12}-H protons as doublet doublet at δ 8.03, δ 8.02 and δ 8.01 respectively. In the ^{1}H-NMR spectrum of ligand (16), the phenyl $C_{19,13,21,11}$-H and $C_{18,14,22,10}$-H and $C_{20,12}$-H protons also appeared as doublet doublet at δ 7.42, δ 7.34 and δ 7.25.

3.1.1.5 Mass spectra

Mass spectral data along with the fragments of the ligands under study are given in Fig 4–20.The molecular ion peaks (M^{+}) were visible in all the spectra. These data clearly indicate the formation of the ligands having the proposed structures.

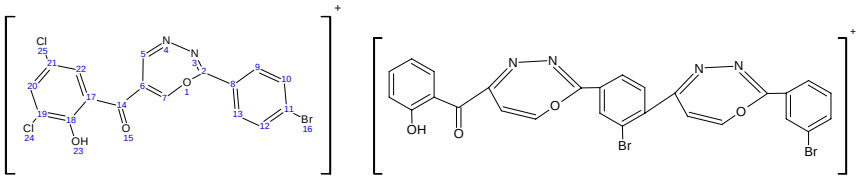


Molecular ions, m/z = 377.17 m/z = 250.63 m/z = 187.19 m/z = 161.03

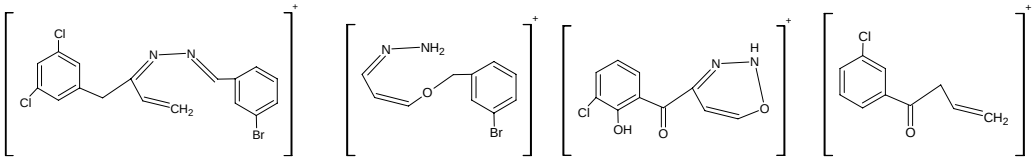


m/z = 136.14 m/z = 94.11

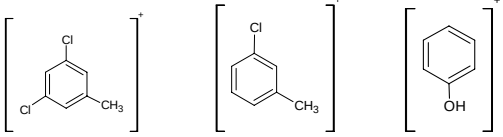
Fig. 4: The the fragmentation pattern of ligand 1



Molecular ions m/z = 440.07 m/z = 620.24

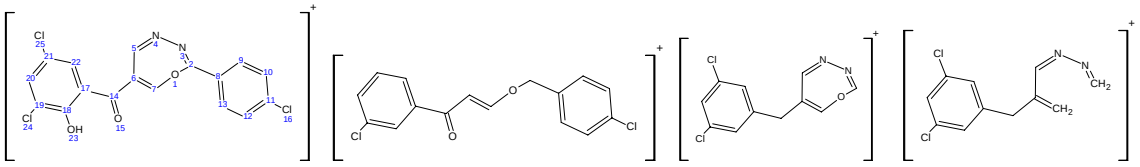


m/z = 396.10 m/z = 255.14 m/z = 238.62 m/z = 180.63

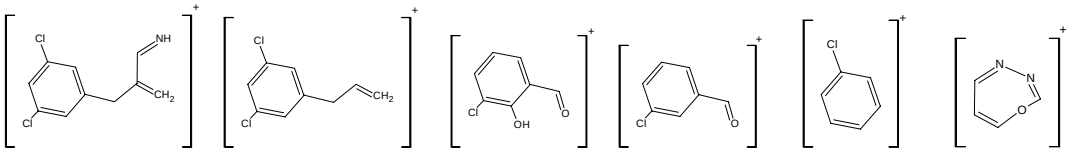


m/z = 161.02 m/z = 136.14 m/z = 94.11

Fig. 5: The fragmentation pattern of ligand 2

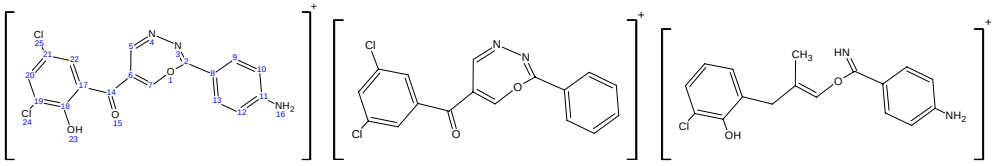


Molecular ions m/z = 395.62 m/z = 307.17 m/z = 255.10 m/z = 241.11

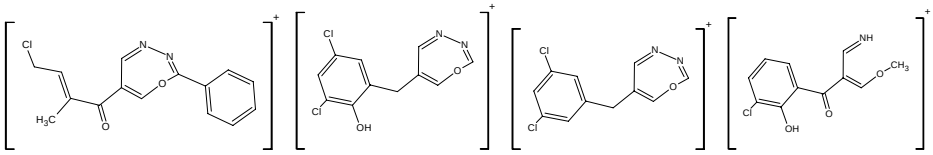


m/z = 214.09 m/z = 187.06 m/z = 156.56 m/z = 140.56 m/z = 112.55 m/z = 96.08

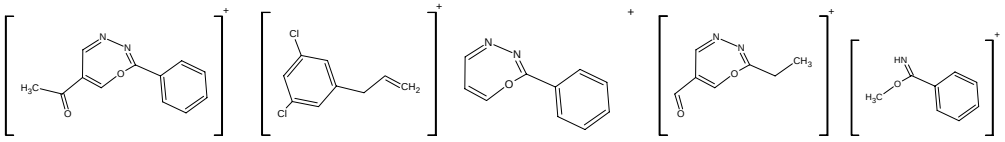
Fig. 6: The fragmentation pattern of ligand 3



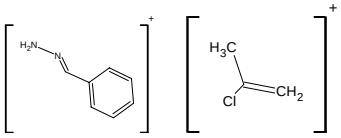
Molecular ions m/z = 361.17 m/z = 345.17 m/z = 316.78



m/z = 288.72 m/z = 271.09 m/z = 255.10 m/z = 239.65

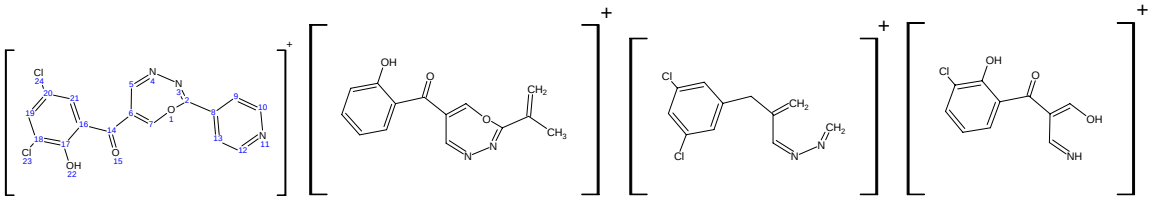


m/z = 214.22 m/z = 187.06 m/z = 172.18 m/z = 152.15 m/z = 135.16

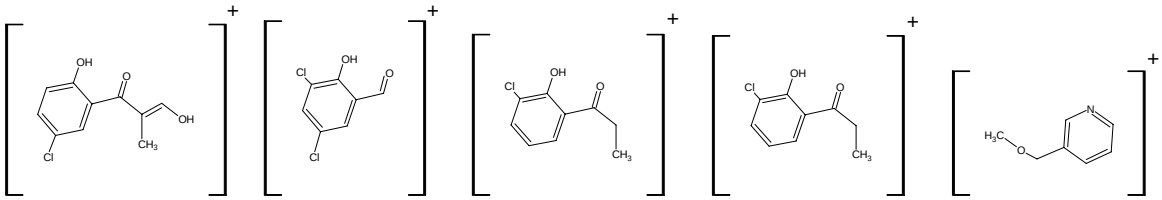


m/z = 120.15 m/z = 76.50

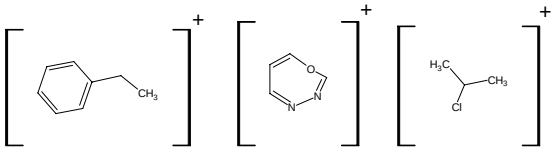
Fig. 7: The fragmentation pattern of ligand 4



Molecular ions m/z = 362.16 m/z = 256.2 m/z = 241.11 m/z = 225.62

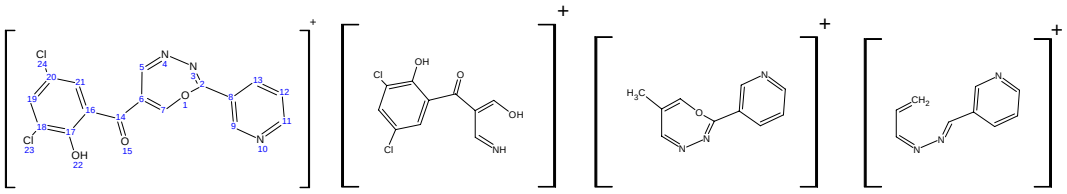


m/z = 212.62 m/z = 191.01 m/z = 186.61 m/z = 140.56 m/z = 123.15

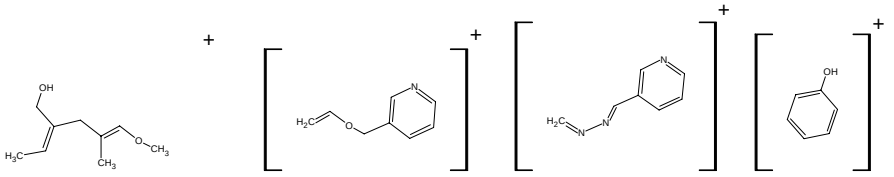


m/z = 106.16 m/z = 96.08 m/z = 78.54

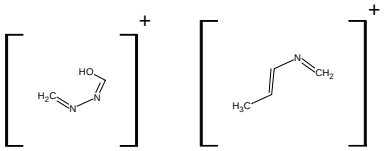
Fig. 8: The fragmentation pattern of ligand 5



Molecular ions m/z = 362.17 m/z = 260.07 m/z = 187.98 m/z = 159.18

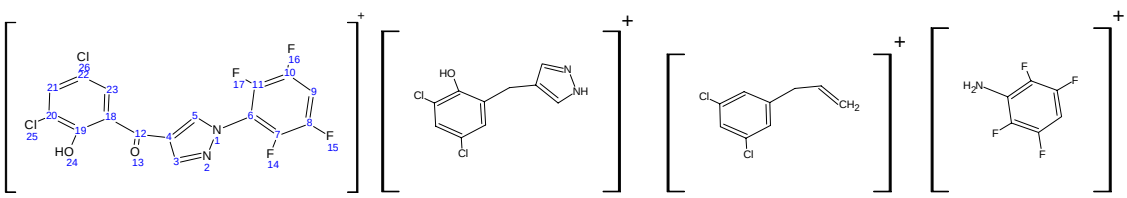


m/z = 156.22 m/z = 135.63 m/z = 133.15 m/z = 184.61

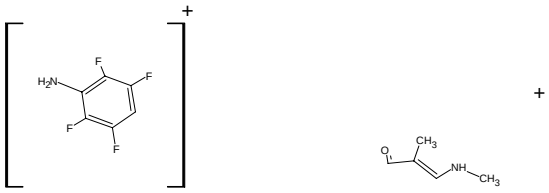


m/z = 94.11 m/z = 69.10

Fig. 9: The fragmentation pattern of ligand 6

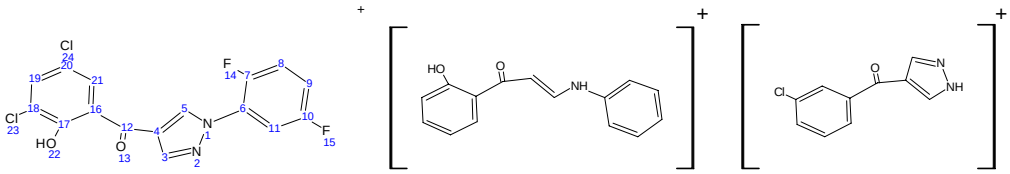


Molecular ions, m/z = 405.13 m/z = 243.08 m/z = 187.06 m/z = 165.08

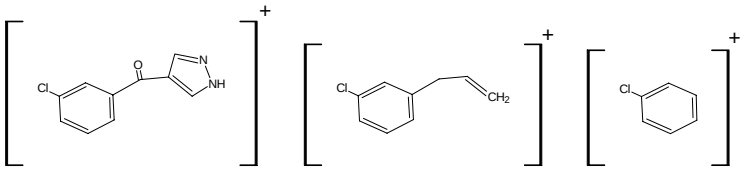


m/z = 137.15 m/z = 99.13

Fig. 10: The fragmentation pattern of ligand 7

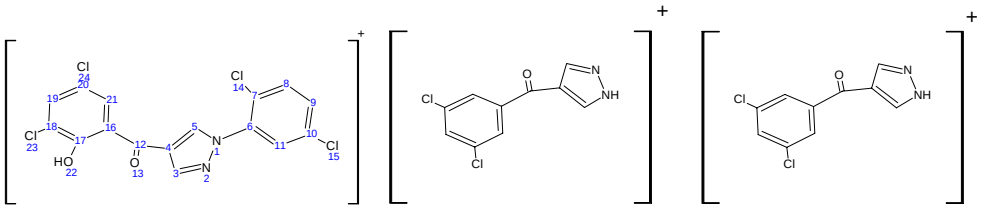


Molecular ions, m/z = 369.14 m/z = 239.26 m/z = 206.62

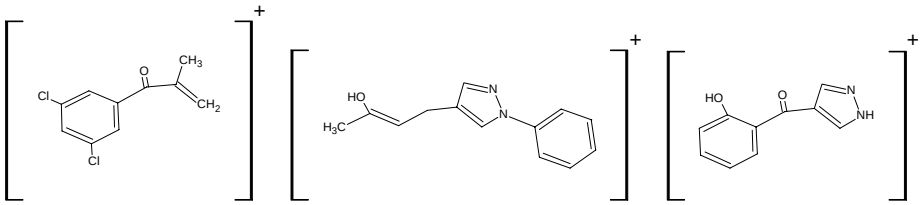


m/z = 188.18 m/z = 152.62 m/z = 112.55

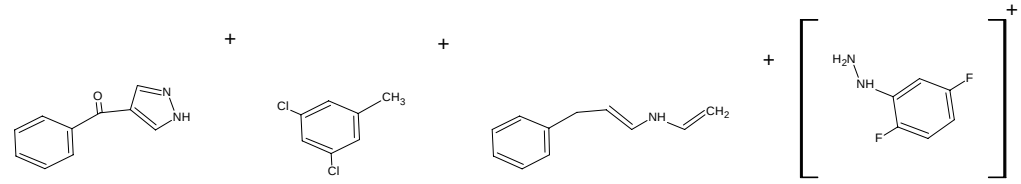
Fig. 11: The fragmentation pattern of ligand 8



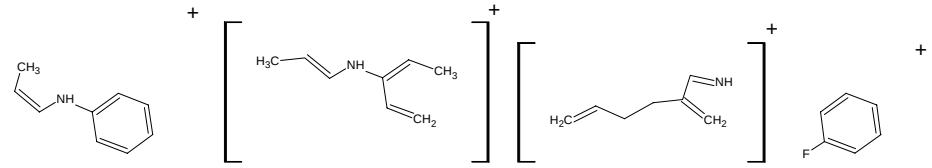
Molecular ions, m/z = 402.06 m/z = 241.07 m/z = 228.07



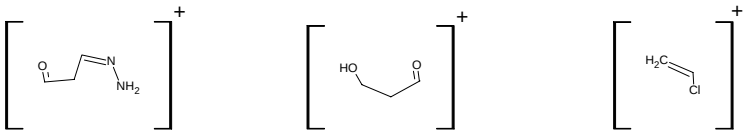
m/z = 215.07 m/z = 214.06 m/z = 188.18



m/z = 172.18 m/z = 161.02 m/z = 159.22 m/z = 144.12

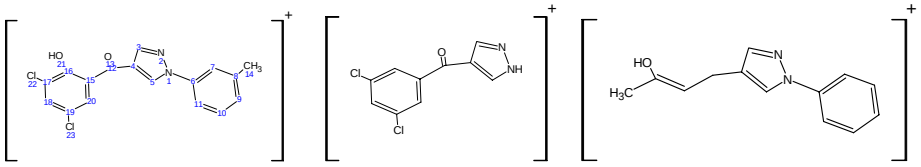


m/z = 133.19 m/z = 123.19 m/z = 109.16 m/z = 96.10

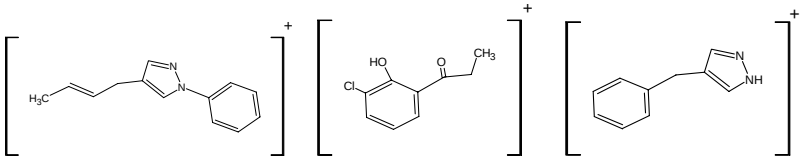


m/z = 86.09 m/z = 74.04 m/z = 62.49

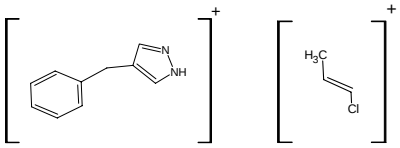
Fig. 12: The fragmentation pattern of the ligand 9



Molecular ions, m/z = 347.19 m/z = 241.07 m/z = 214.26

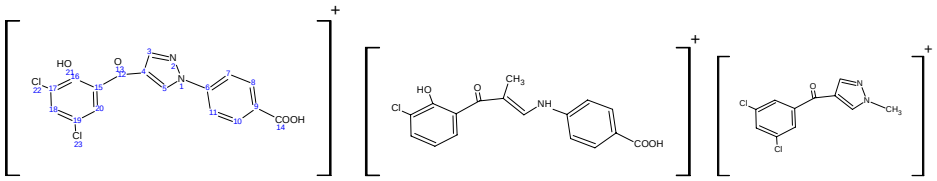


m/z = 198.26 m/z = 184.61 m/z = 158.20

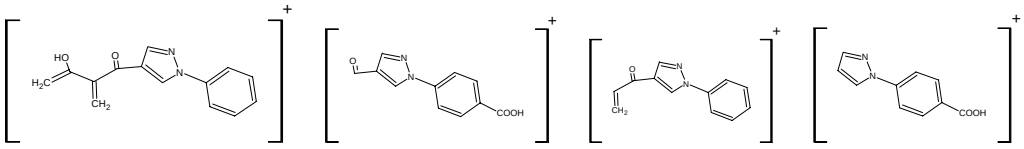


m/z = 149.19 m/z = 76.59

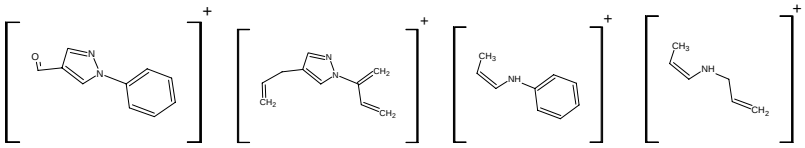
Fig. 13: The fragmentation pattern of the ligand 10



Molecular ions, m/z = 377.17 m/z = 331.75 m/z = 225.10

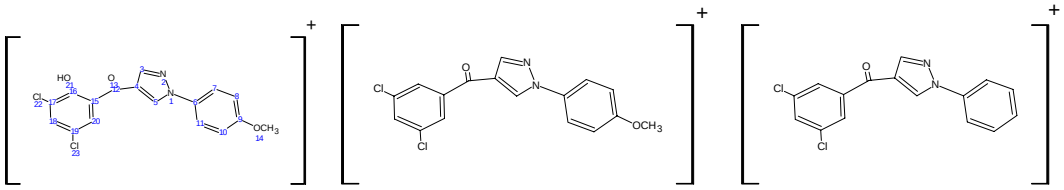


m/z = 240.25 m/z = 216.19 m/z = 198.22 m/z = 188.18

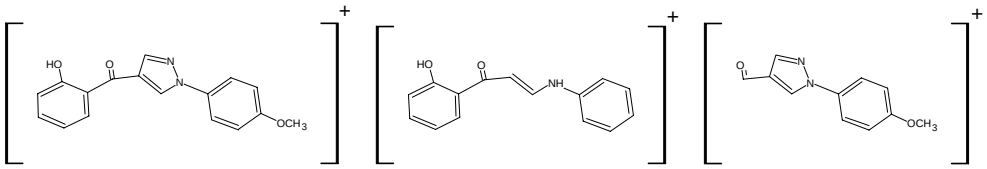


m/z =172.18 m/z = 160.21 m/z = 133.19 m/z = 97.15

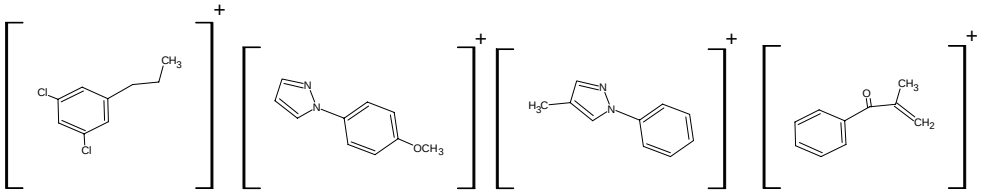
Fig. 14: The fragmentation pattern of the ligand 11



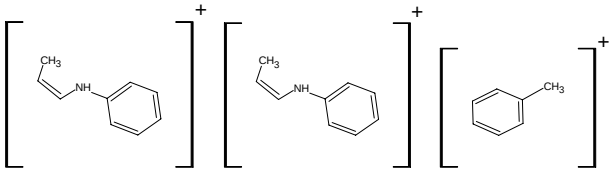
Molecular ions, m/z = 363.19 m/z = 347.19 m/z = 317.16



m/z = 294.30 m/z = 239.26 m/z = 202.20

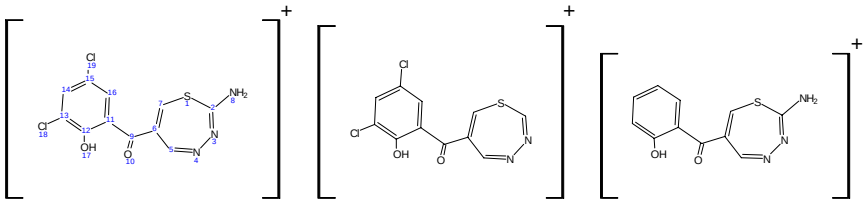


m/z = 189.08 m/z = 174.19 m/z = 158.19 m/z = 146.18

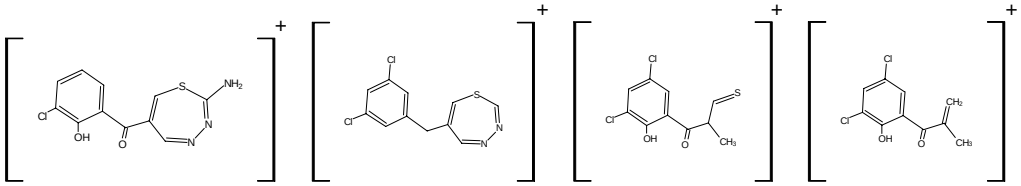


m/z = 133.19 m/z = 107.15 m/z = 92.13

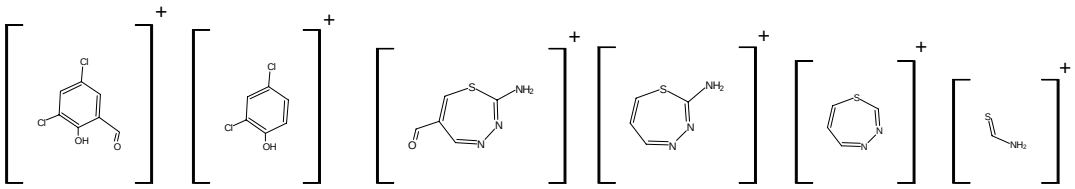
Fig. 15: The fragmentation pattern of the ligand 12



Molecular ions, m/z = 316.16 m/z = 301.14 m/z =247.27

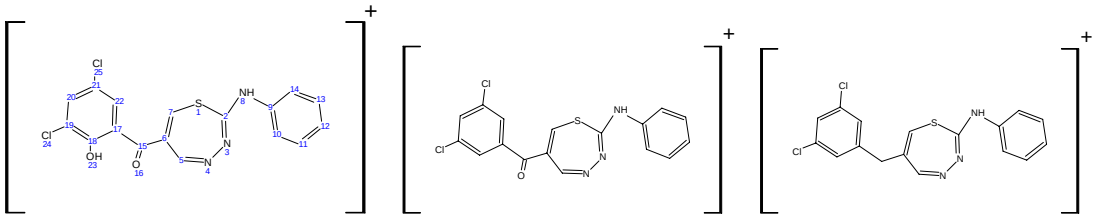


m/z = 281.72 m/z = 271.16 m/z = 263.14 m/z = 231.07

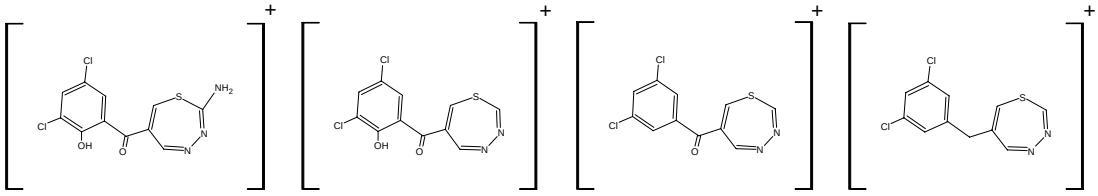


m/z = 191.01 m/z = 163.00 m/z = 155.18 m/z = 127.17 m/z = 112.15 m/z = 61.10

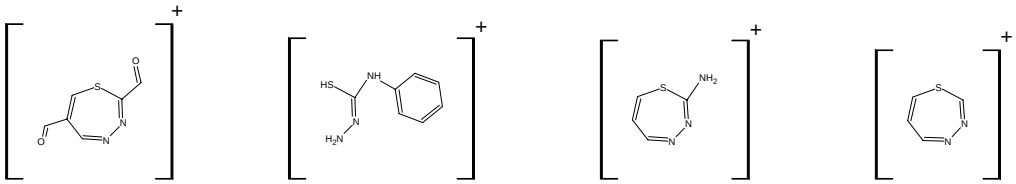
Fig. 16: The fragmentation pattern of the ligand 13



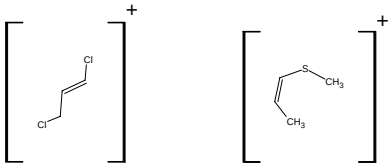
Molecular ions, m/z = 392.26 m/z = 376.26 m/z = 362.27



m/z = 316.16 m/z = 301.15 m/z = 285.15 m/z = 271.16

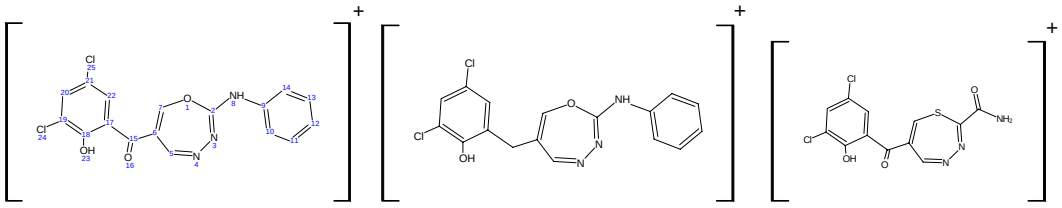


m/z = 191.01 m/z = 167.23 m/z = 127.17 m/z = 112.15

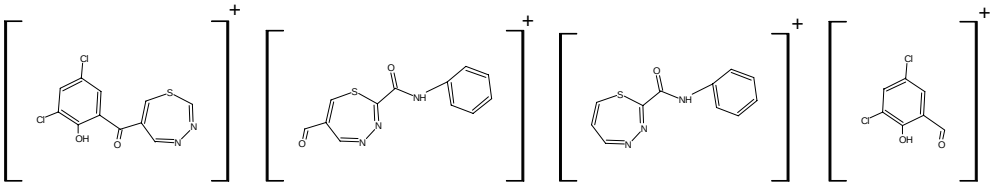


m/z = 110.96 m/z = 75.13

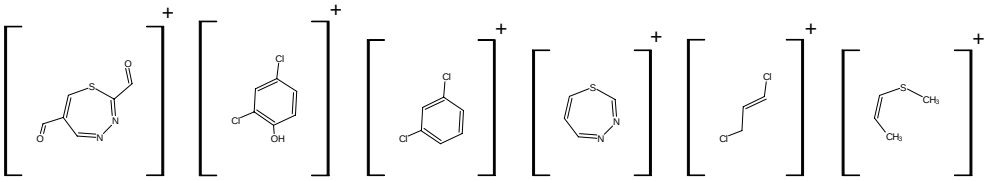
Fig. 17: The fragmentation pattern of the ligand 14



Molecular ions, m/z = 376.19 m/z = 362.21 m/z = 344.17

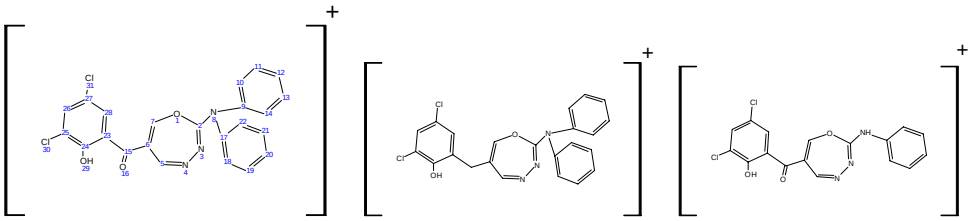


m/z = 301.14 m/z = 259.28 m/z = 231.27 m/z = 191.01

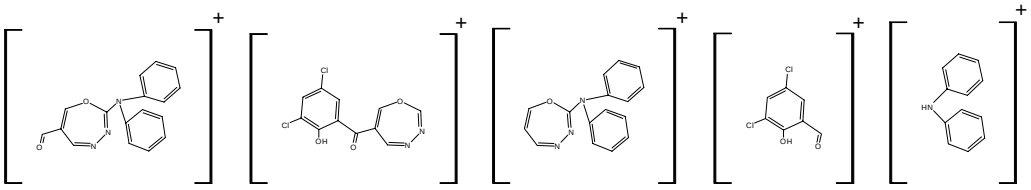


m/z = 168.17 m/z = 163.00 m/z = 147.00 m/z = 112.15 m/z = 110.96 m/z = 88.17

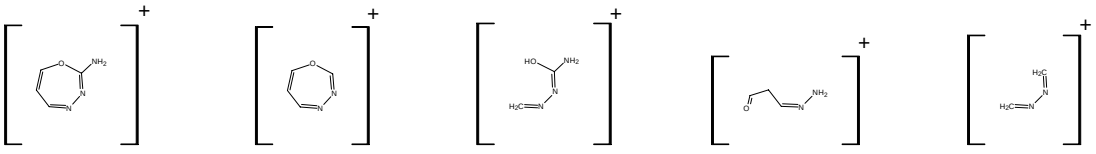
Fig. 18: The fragmentation pattern of the ligand 15



Molecular ions, m/z = 452.28 m/z = 438.30 m/z = 376.19

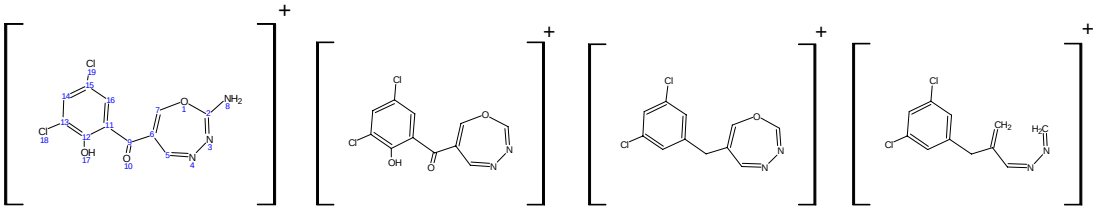


m/z = 291.30 m/z = 285.08 m/z = 263.29 m/z = 191.01 m/z = 169.22



m/z = 111.10 m/z = 96.08 m/z = 87.08 m/z = 86.09 m/z = 56.06

Fig. 19: The fragmentation pattern of the ligand 16



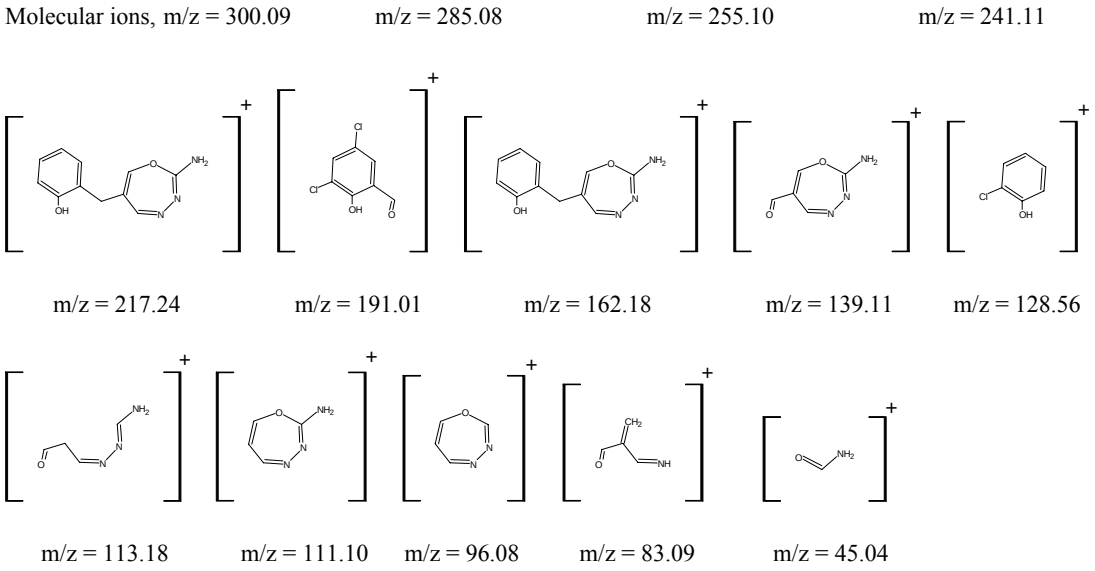


Fig. 20: The fragmentation pattern of the ligand 17

3.2 Preparation of Cu (II) complexes (18 – 29)

Cu (II) complexes (18–29) of the synthesized ligands were easily obtained as per the experimental procedure and characterized by their physical solubility, melting/decomposition points, micro-analytical, magnetic moments, conductivity measurements, water contents, thermal (TGA, DSC, DTA), and spectral UV-Visible as well as FT-IR data. The physical data of Cu (II) complexes are given in the Table 10. Cu (II) complexes thus prepared are given in the Table 11

3.2.1 Characterization of Cu (II) complexes (18–29)

In the present study seventeen Cu (II) complexes were prepared and studied. All the metal complexes (18–29) of the ligands (1–17), were air stable and prepared by the stoichiometric reaction of $CuCl._2H_2O$ with the prepared ligands in a metal-ligand ratio of 1:2. These complexes were intensely colored and amorphous solids, which decomposed without melting. They were insoluble in common organic solvents such as ethanol, methanol, chloroform or acetone but are soluble in DMSO and DMF. The lower molar conductance values (7.4–9.0 μS cm^{-1}) of the complexes indicated that they are non-electrolytic in nature *[158,159]*. The elemental analyses data agree well with the proposed composition of the metal (II) complexes. All of the complexes were found to be anhydrous.

All of the complexes were also found to be dimeric with two hydrazine molecules bridiging the ttwo copper atoms through coordination. Efforts to grow good crystals of the compounds and their metal complexes for X-ray diffraction studies were unsuccessful due to their poor solubility in common organic solvents. Complexes under study did not possess sharp melting points and decomposed above 312°C. The decomposition points are listed in Table11.

Table 10: Physical data of Copper (II) complexes

Sr. No.	Mol. Formula	Mol. Mass	Melting / Decomp. Points	Colors
18	$C_{68}H_{44}Cl_8Cu_2N_{12}O_{16}$	1695.86	310-312 °C	Bluish-green
19	$C_{68}H_{40}Br_4Cl_8Cu_2N_{12}O_{12}$	1947.45	216-218 °C	Green
20	$C_{68}H_{40}Cl_{12}Cu_2N_{12}O_{12}$	1769.64	200-202 °C	Brown
21	$C_{68}H_{48}Cl_8Cu_2N_{16}O_{12}$	1691.92	264-266 °C	Brown
22	$C_{64}H_{40}Cl_8Cu_2N_{16}O_{12}$	1635.82	260-262 °C	Dull-green
23	$C_{64}H_{40}Cl_8Cu_2N_{16}O_{12}$	1635.82	230-232 °C	Dark-brown
24	$C_{64}H_{28}Cl_8Cu_2F_{16}N_{12}O_8$	1807.67	194-196 °C	Brownish
25	$C_{64}H_{36}Cl_8Cu_2F_8N_{12}O_8$	1663.75	188-190 °C	Dark green
26	$C_{64}H_{36}Cl_{16}Cu_2N_{12}O_8$	1795.38	214-217 °C	Reddish brown
27	$C_{68}H_{52}Cl_8Cu_2N_{12}O_8$	1575.93	310-312 °C	Brown
28	$C_{68}H_{44}Cl_8Cu_2N_{12}O_{16}$	1695.86	240-243 °C	Brownish
29	$C_{68}H_{52}Cl_8Cu_2N_{12}O_{12}$	1639.93	309-311 °C	Dark green
30	$C_{44}H_{32}Cl_8Cu_2N_{16}O_8S_4$	1451.81	216-218 °C	Greenish
31	$C_{68}H_{48}Cl_8Cu_2N_{16}O_8S_4$	1756.19	305-307 °C	Green
32	$C_{68}H_{48}Cl_8Cu_2N_{16}O_{12}$	1691.92	294-296 °C	Green
33	$C_{92}H_{66}Cl_8Cu_2N_{16}O_{12}$	1998.32	290-293 °C	Brown
34	$C_{44}H_{32}Cl_8Cu_2N_{16}O_{12}$	1387.54	311-313 °C	Brownish-green

Table 11: Microanalytical and UV-visible data of Copper (II) complexes

Metal II Complexes	CHN, Found (Calculated) %			Absorbance maxima cm^{-1}
	C	H	N	
18	48.25 (48.16)	2.50 (2.62)	9.84 (9.9)	14080
19	41.81 (41.98)	2.22 (2.07)	8.71 (8.63)	13680
20	46.22 (46.15)	2.36 (2.28)	9.58 (9.50)	14735
21	48.35 (48.27)	2.66 (2.86)	13.34 (13.25)	16670
22	46.82 (46.99)	2.37 (2.46)	13.53 (13.70)	12230
23	46.78 (46.99)	2.24 (2.46)	12.59 (13.70)	14810
24	42.63 (42.52)	1.65 (1.56)	9.43 (9.30)	12985
25	46.41 (46.20)	2.16 (2.18)	11.24 (10.10)	14150
26	42.78 (42.81)	2.00 (2.02)	9.35 (9.36)	16810
27	51.80 (51.83)	3.30 (3.33)	10.66 (10.67)	11760
28	48.14 (48.16)	2.59 (2.62)	9.91 (9.91)	14620
29	49.68 (49.80)	3.27 (3.20)	10.34 (10.25)	12700
30	36.58 (36.40)	2.40 (2.22)	15.23 (15.44)	13850
31	46.39 (46.51)	2.88 (2.75)	12.59 (12.76)	14700
32	48.15 (48.27)	2.63 (2.86)	13.14 (13.25)	13280
33	55.18 (55.30)	3.20 (3.33)	11.08 (11.21)	12950
34	38.28 (38.09)	2.15 (2.32)	16.24 (16.15)	12565

3.2.1.1 Solubility

Solubility of the Cu (II) complexes was studied in different organic solvents. They were found to be soluble only in DMSO and DMF.

3.2.1.2 Micro-analytical data

Table 11 shows the data of elemental analysis of the Cu (II) complexes which is in agreement with the proposed composition.

3.2.1.3 Magnetic moments

Magnetic moments of the complexes under study were as given in Table 12. The values of the magnetic moments of the complexes (18-33) were higher than normal values of 1.73 BM for Cu (II) ion suggesting that these complexes are not mononuclear. Thus the magnetic moment values support the molecular structure of the complexes as proposed.

3.2.1.4 Conductivity measurements

The results are given in the Table 12. Conductance values in DMF at the concentration 10^{-3} mole/dm^3 fall in the range 7.4-9.0 μS cm^{-1}, this indicate the non-electrolytic nature of the complexes.

3.2.1.5 Water content

Water content of the Cu (II) complexes as determined by the Karl-Fischer titration method and is given in Table 12. The values do not equate with any whole number of

Table 12: Magnetic moment, Conductivity and Water content data of Cu (II) complexes

Sr. No.	μ .effective (B.M)	Conductance Ohm^{-1} cm^2 mol^{-1}	%age of water contents	Absorbance spectrum cm^{-1}
18	1.97	36	0.76	14080
19	2.35	38	3.16	13680
20	2.25	34	0.97	14735
21	2.18	36	4.26	16670
22	2.50	38	3.92	12230
23	2.08	38	3.54	14810
24	2.36	39	5.92	12985
25	2.42	34	0.49	14150
26	1.92	36	0.53	16810
27	2.05	34	0.98	11760
28	1.95	32	0.01	14620
29	1.98	37	0.01	12700
30	1.99	33	0.82	13850
31	2.37	38	0.65	14700
32	2.42	39	0.34	13280
33	2.31	38	0.92	12950
34	1.90	33	2.21	12565

water molecules suggesting that the water content is just the lattice water and the complexes are not completely dry.

3.2.1.6 Thermal analysis

The TG, DSC and DTA thermograms are shown in the Fig.s 21-37 and the relevant data obtained from the thermograms are given in the Table13. The complexes begin to lose weight around 200 to 500°C a sharp decrease in weight shows the loss of one of the ligands from the complexes. The DTA curves show different peaks in the range of 210–390°C. The endothermic peaks in these complexes in the range of 210–375°C is assigned to the loss of the ligands.

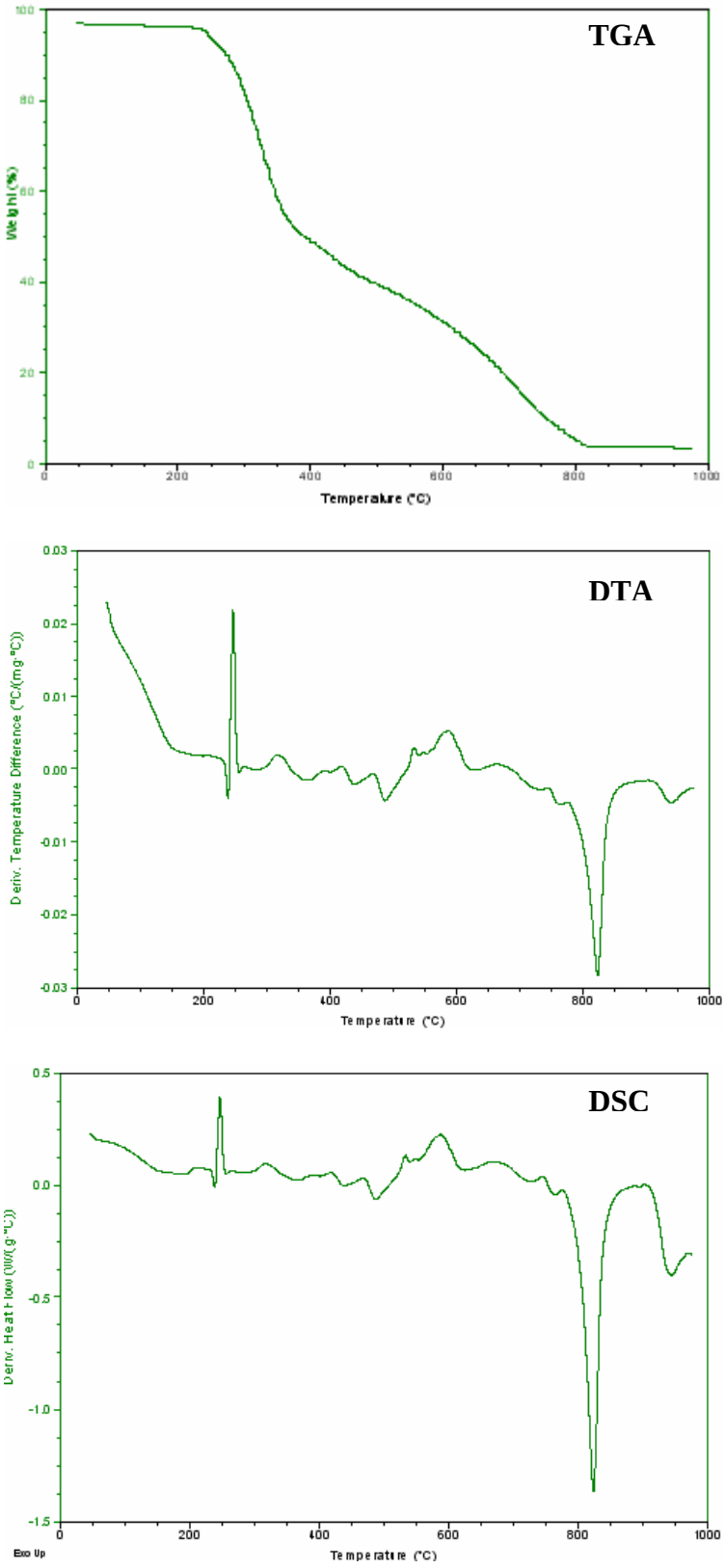


Fig. 21: TGA, DTA and DSC curves of Complex 18.

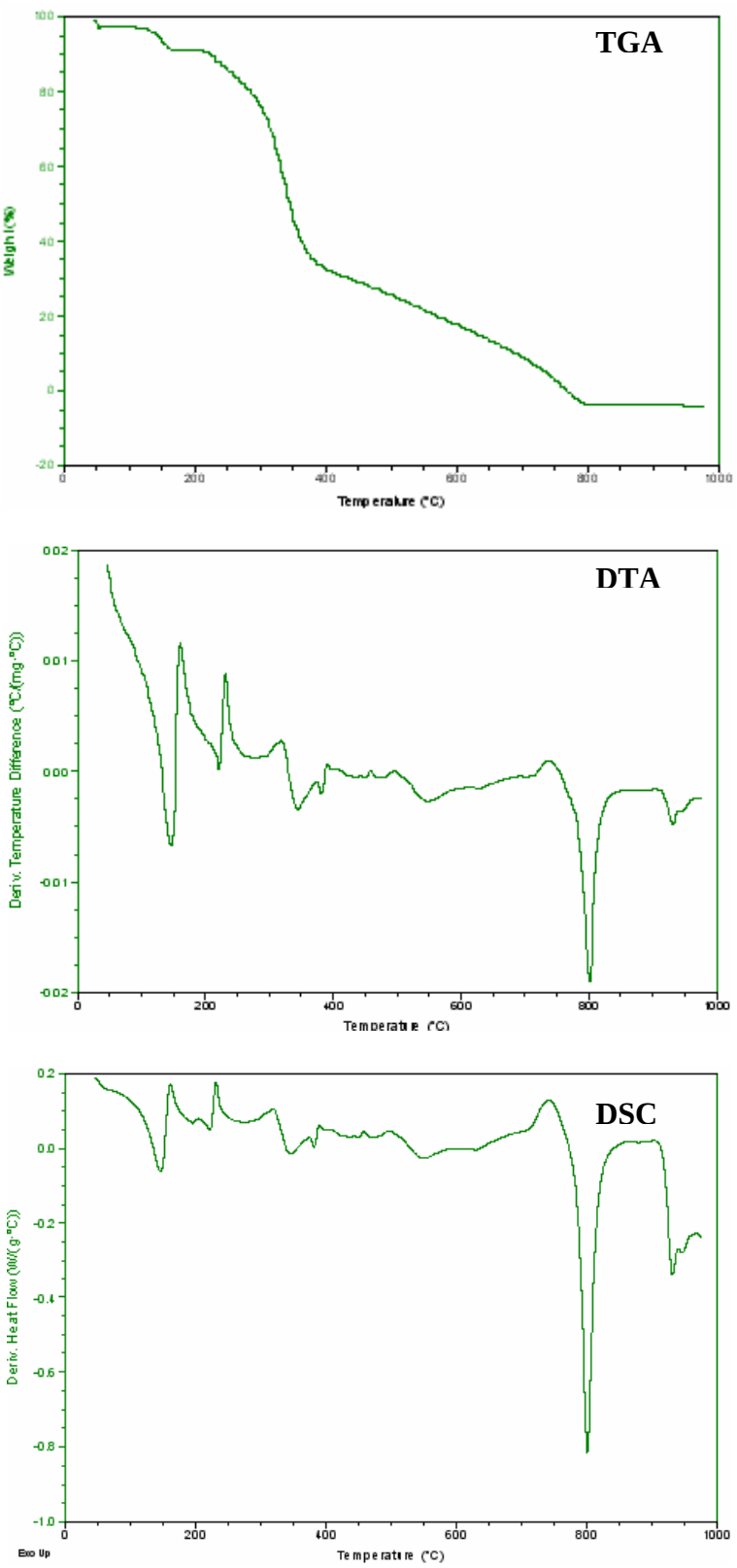


Fig. 22: TGA, DTA and DSC curves of Complex 19.

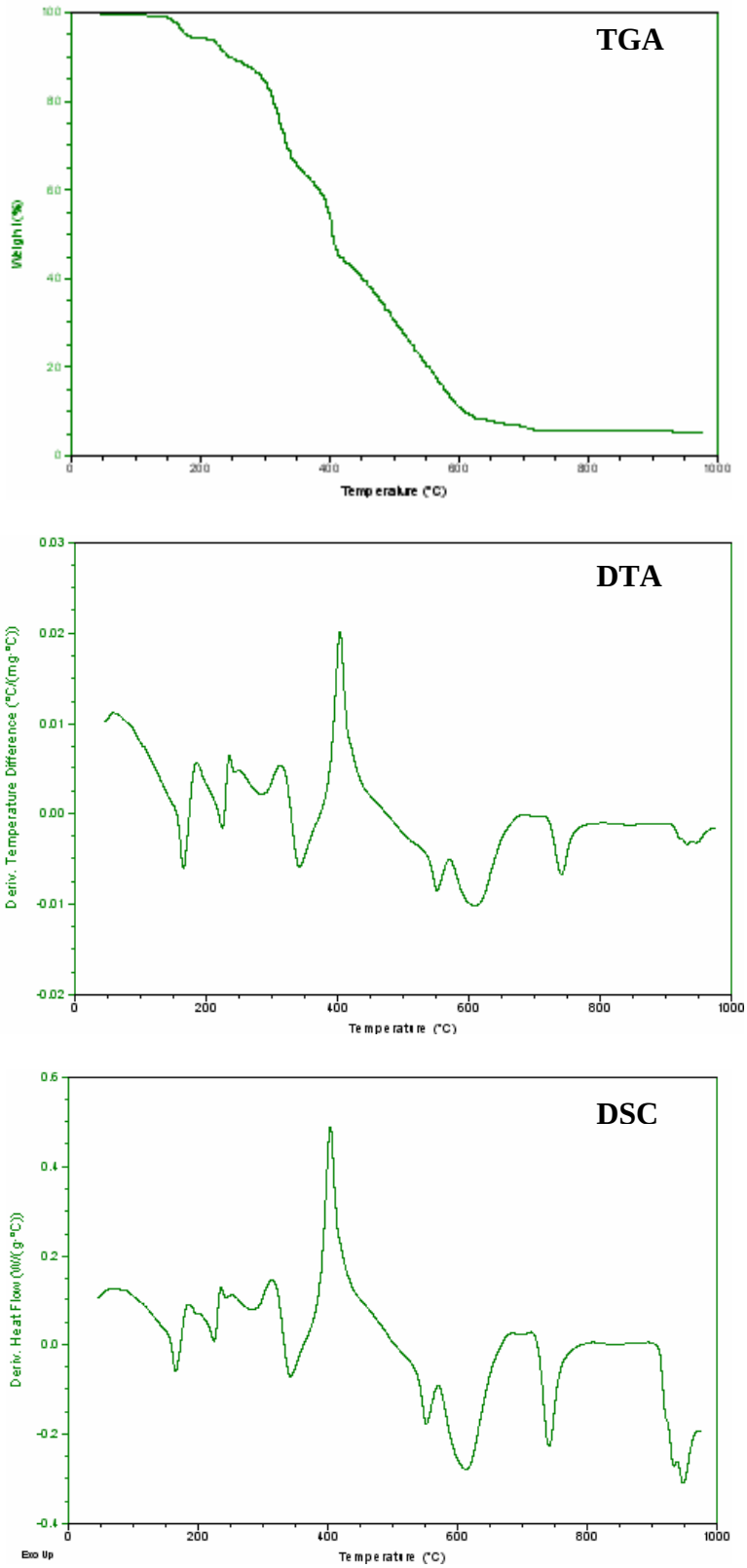


Fig. 23: TGA, DTA and DSC curves of Complex 20.

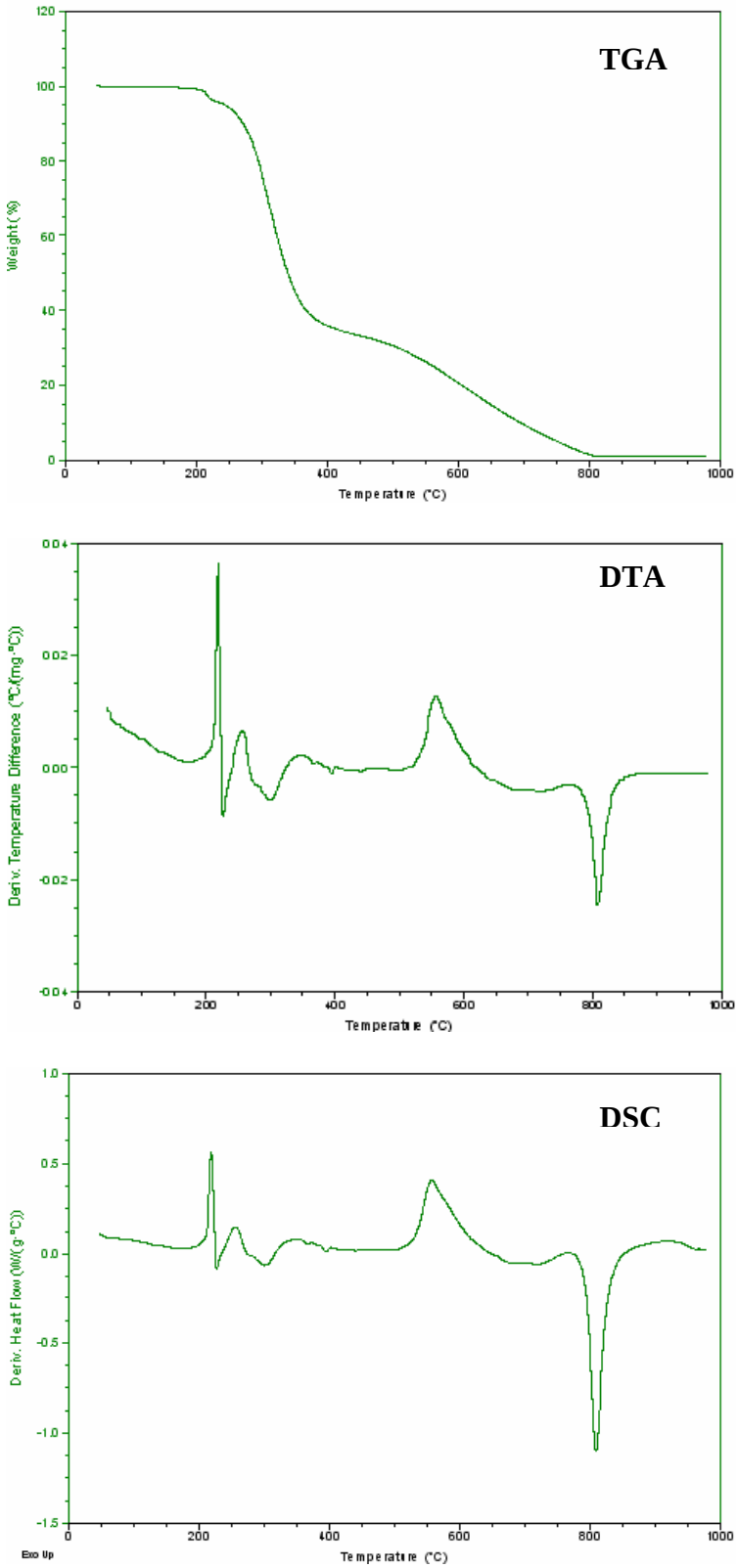


Fig. 24: TGA, DTA and DSC curves of Complex 21.

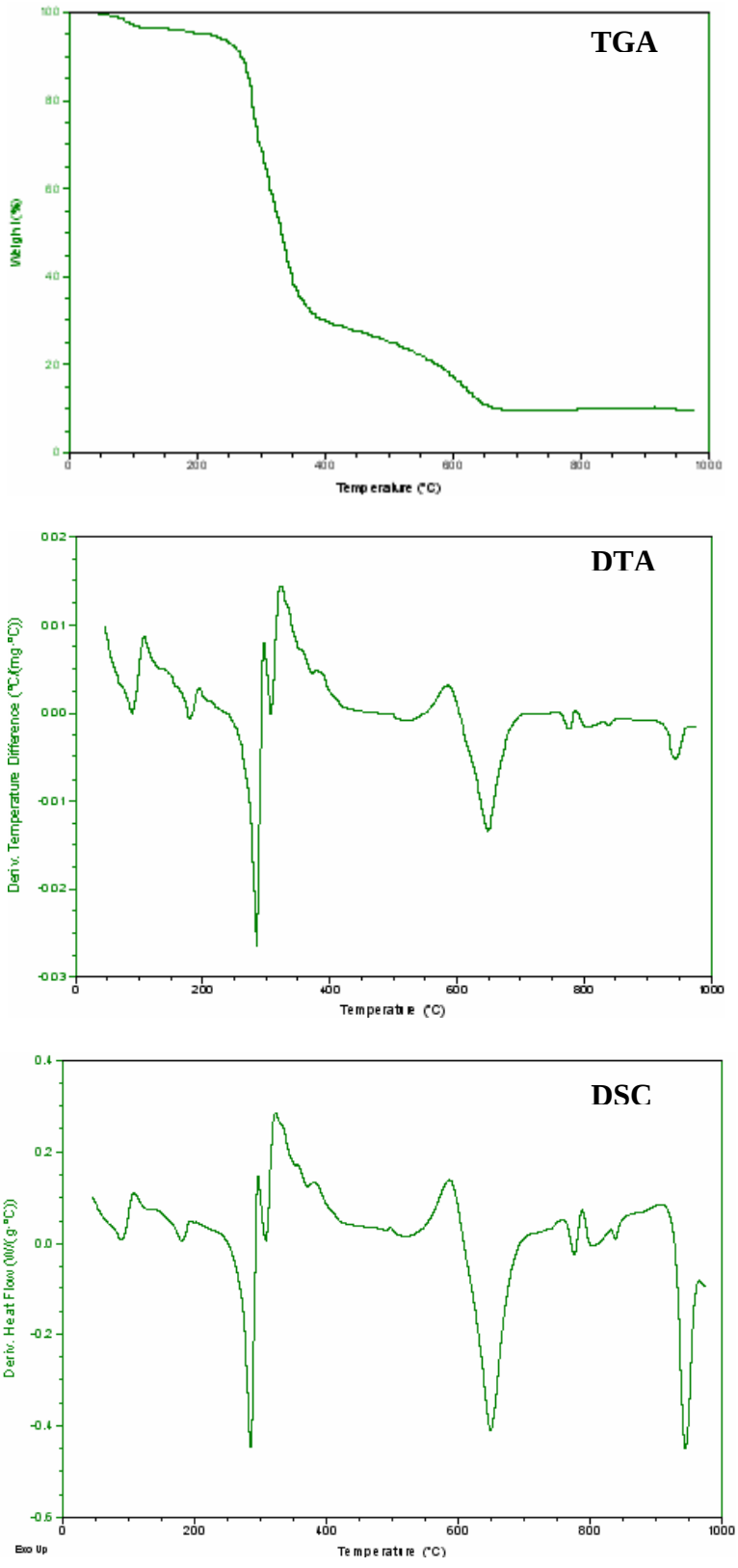


Fig. 25: TGA, DTA and DSC curves of Complex 22.

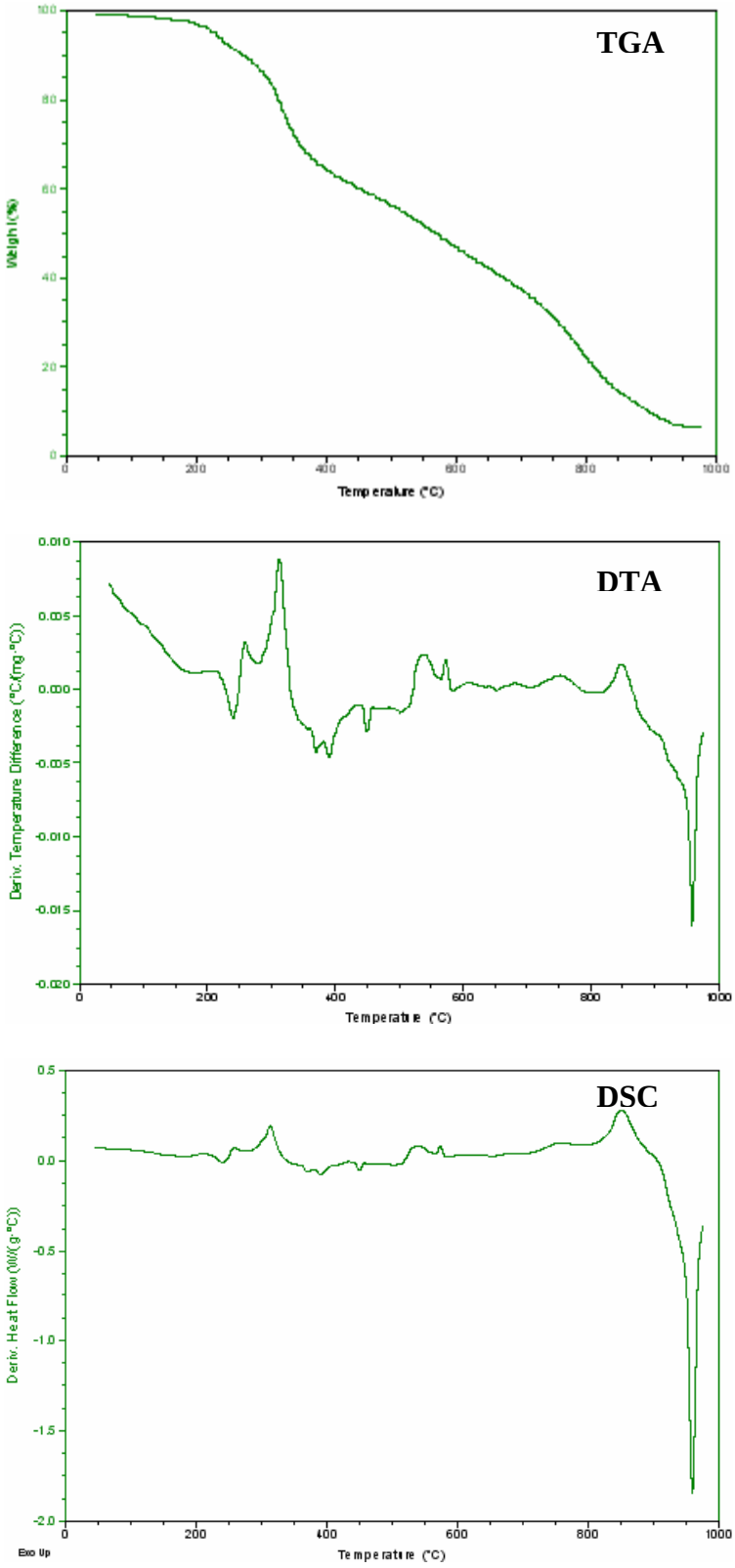


Fig. 26: TGA, DTA and DSC curves of Complex 23.

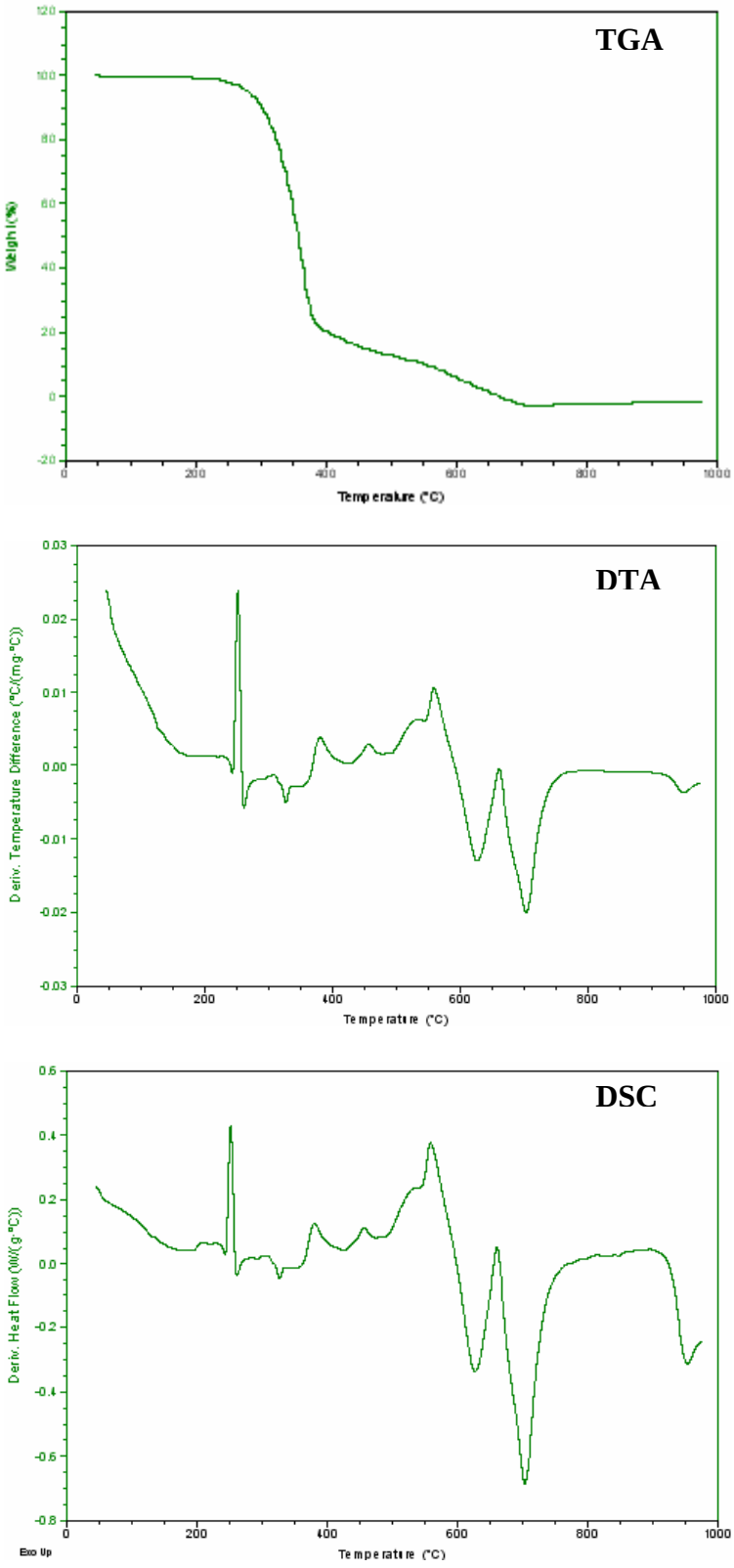


Fig. 27: TGA, DTA and DSC curves of Complex 24.

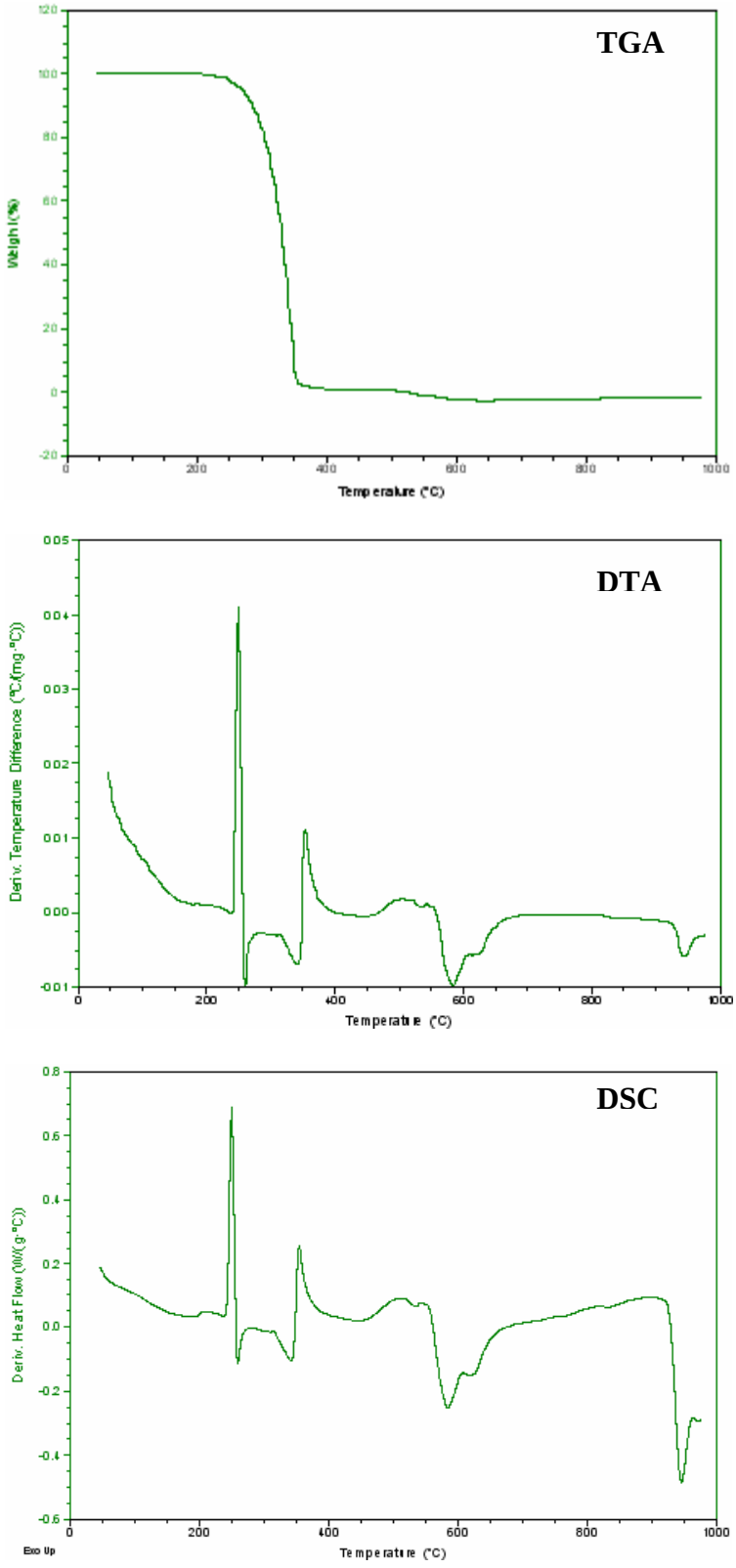


Fig. 28: TGA, DTA and DSC curves of Complex 25.

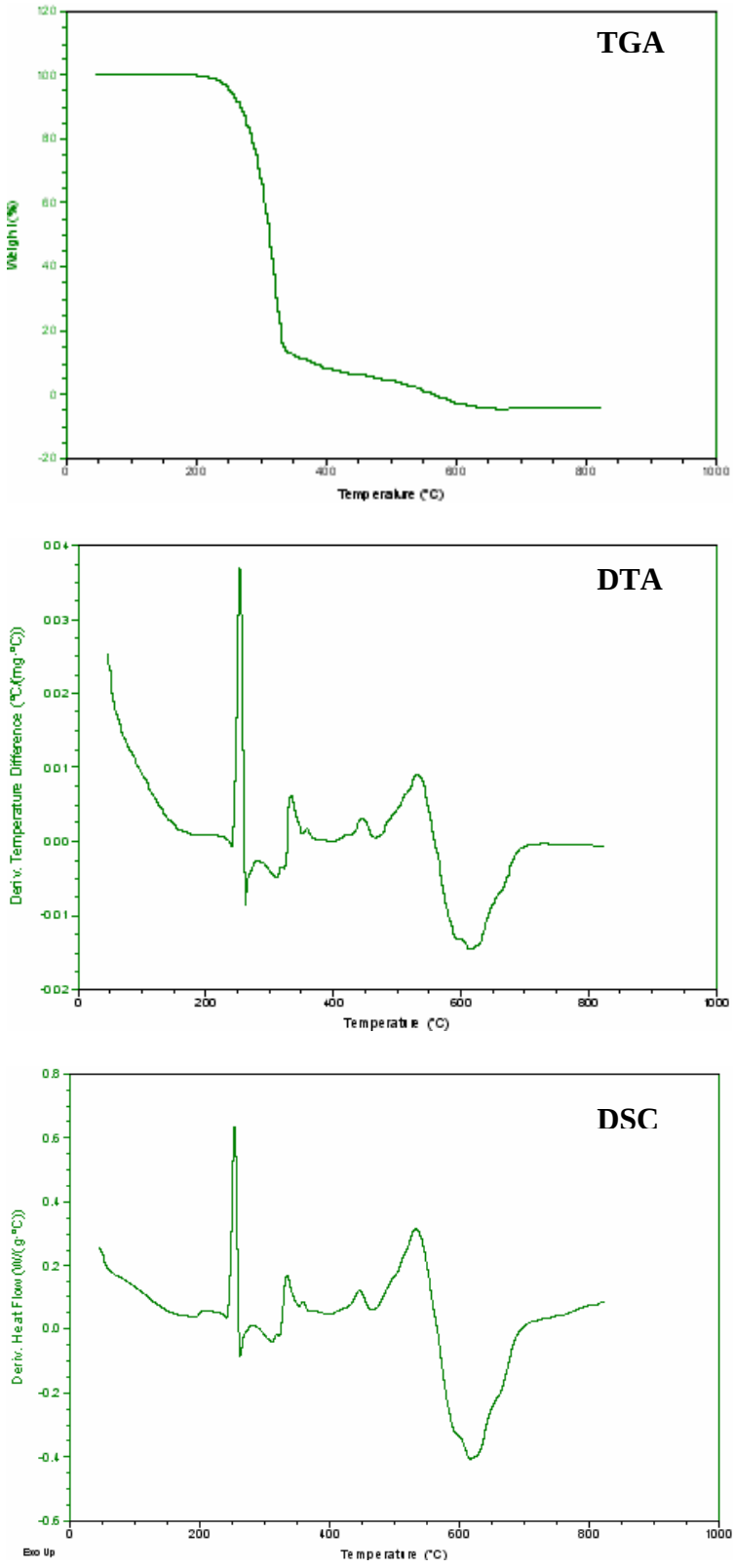


Fig. 29: TGA, DTA and DSC curves of Complex 26.

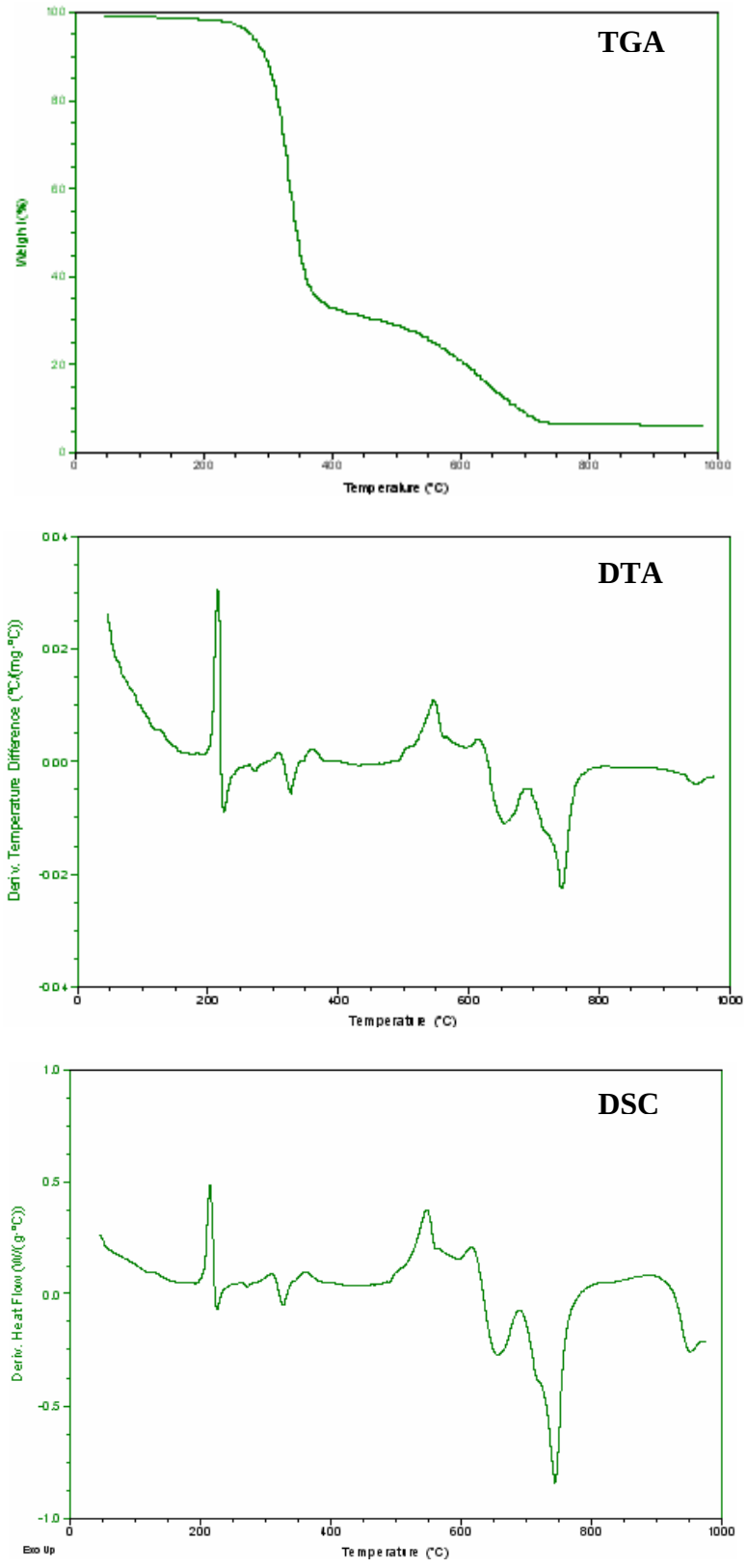


Fig. 30: TGA, DTA and DSC curves of Complex 27.

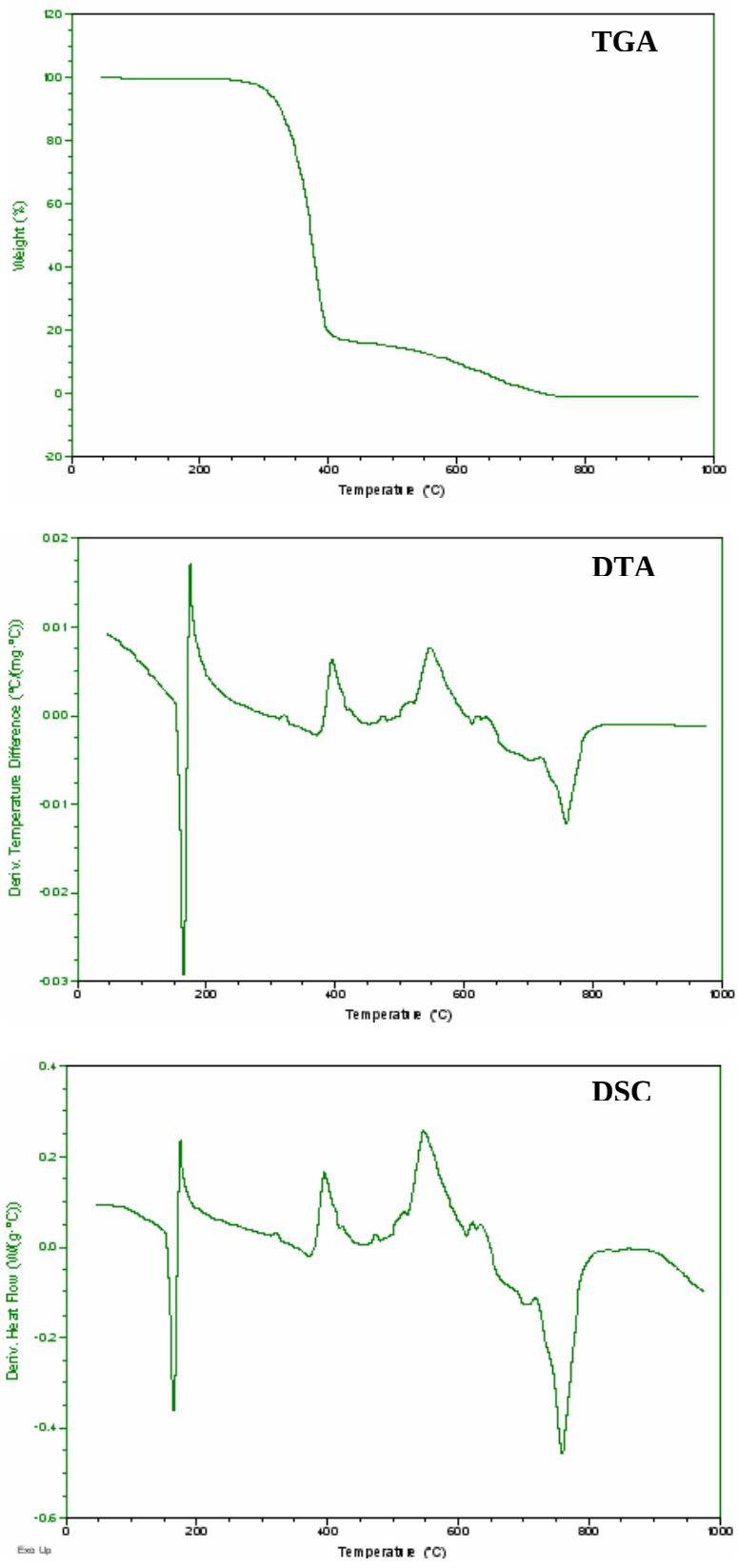


Fig. 31: TGA, DTA and DSC curves of Complex 28.

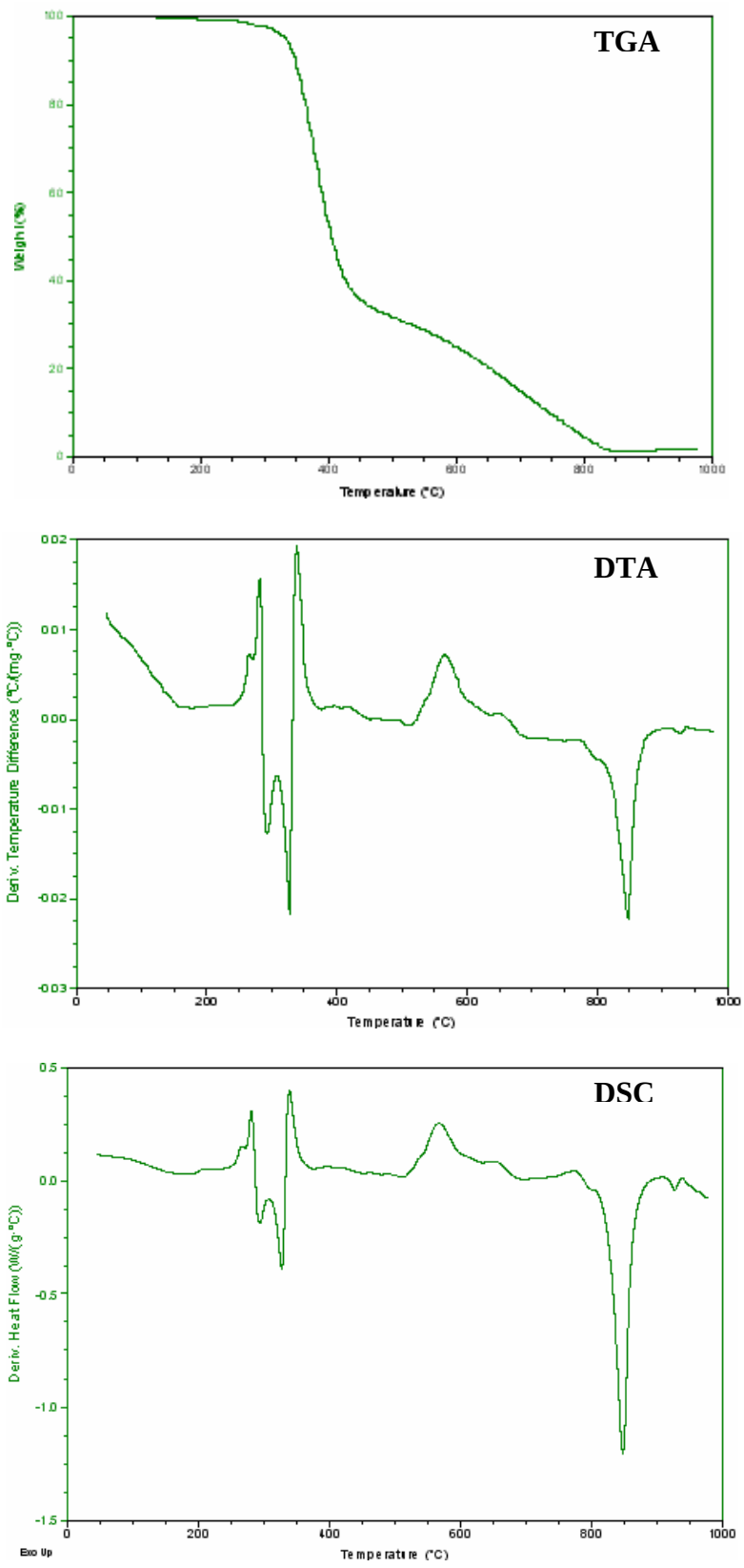


Fig. 32: TGA, DTA and DSC curves of Complex 29.

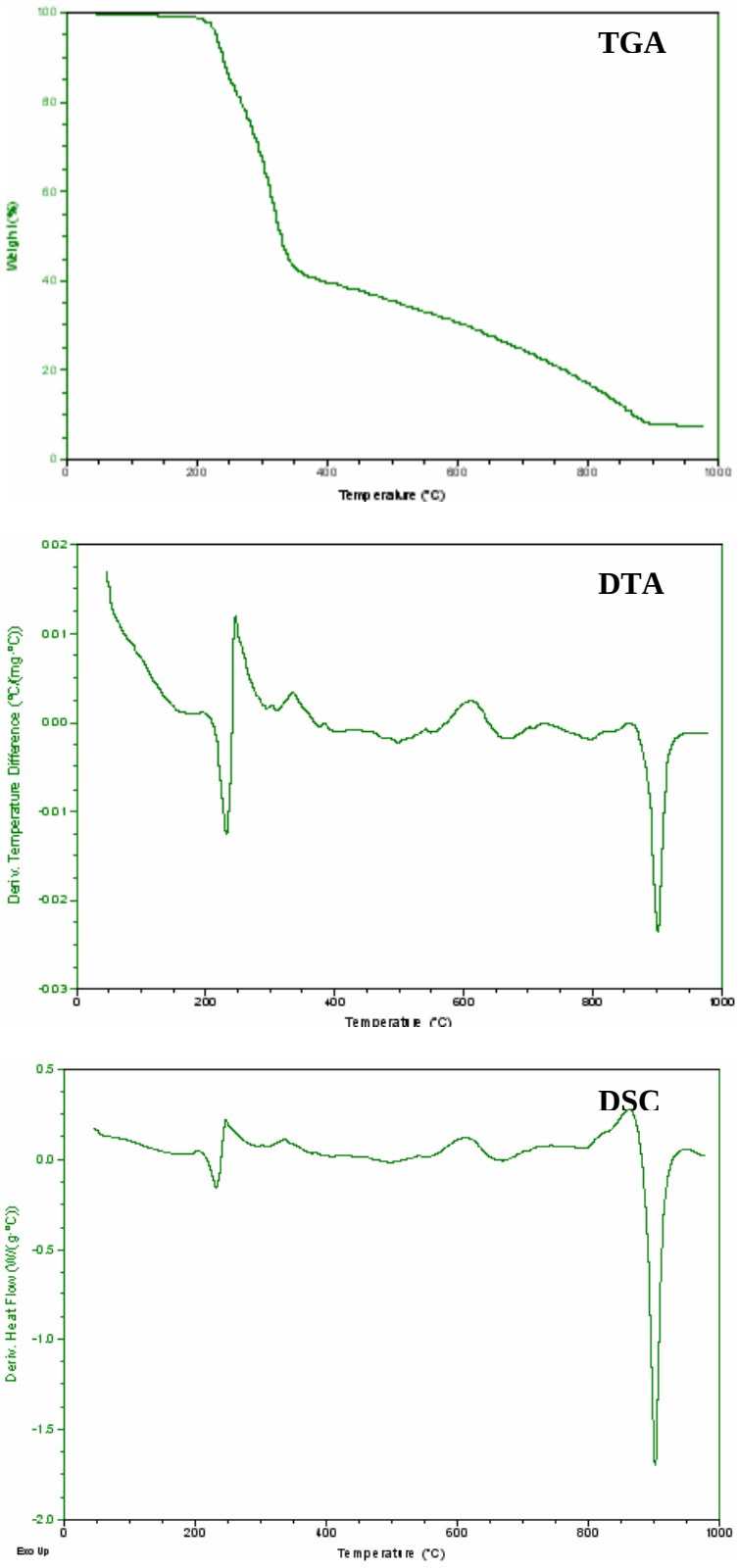
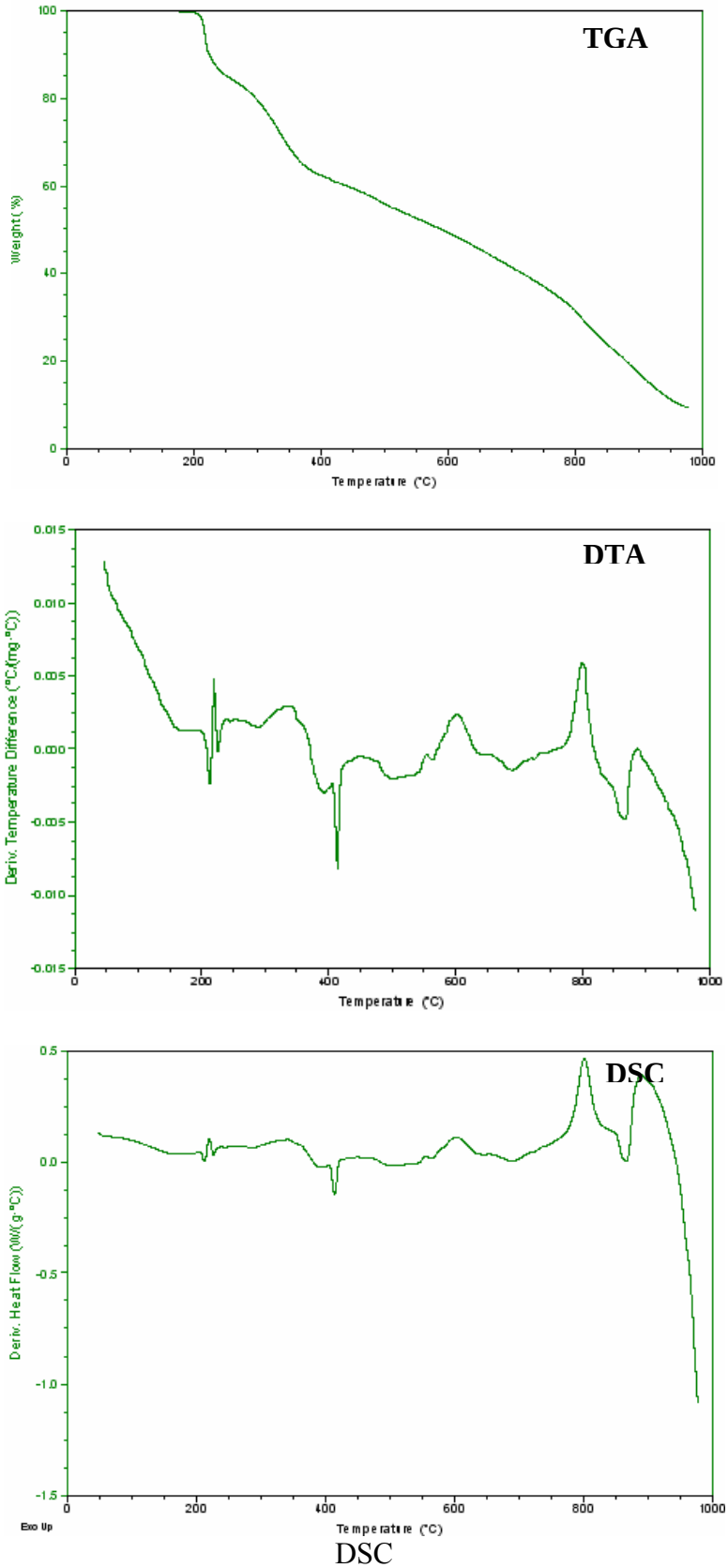


Fig. 33: TGA, DTA and DSC curves of Complex 30.



DSC

Fig. 34: TGA, DTA and DSC curves of Complex 31.

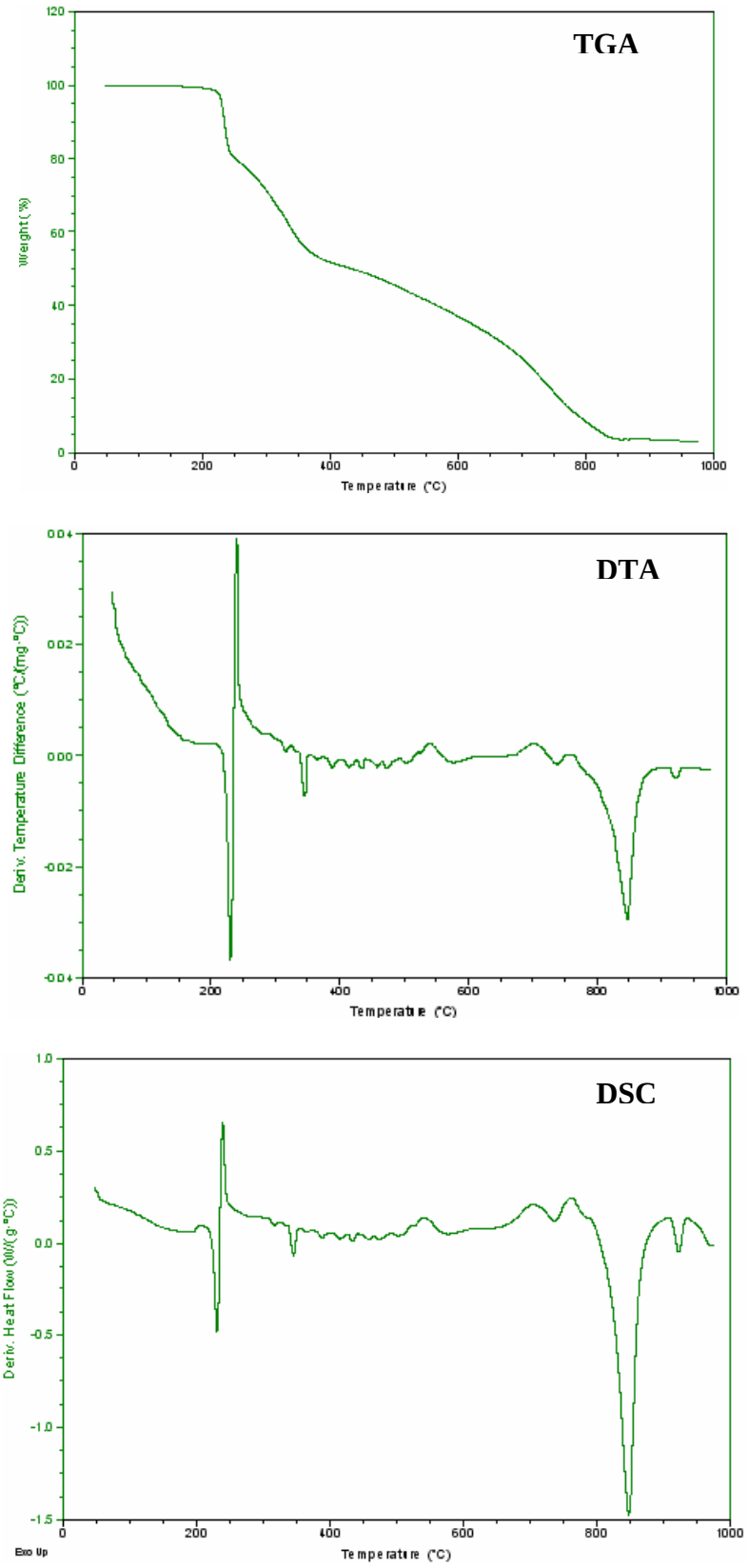


Fig. 35: TGA, DTA and DSC curves of Complex 32.

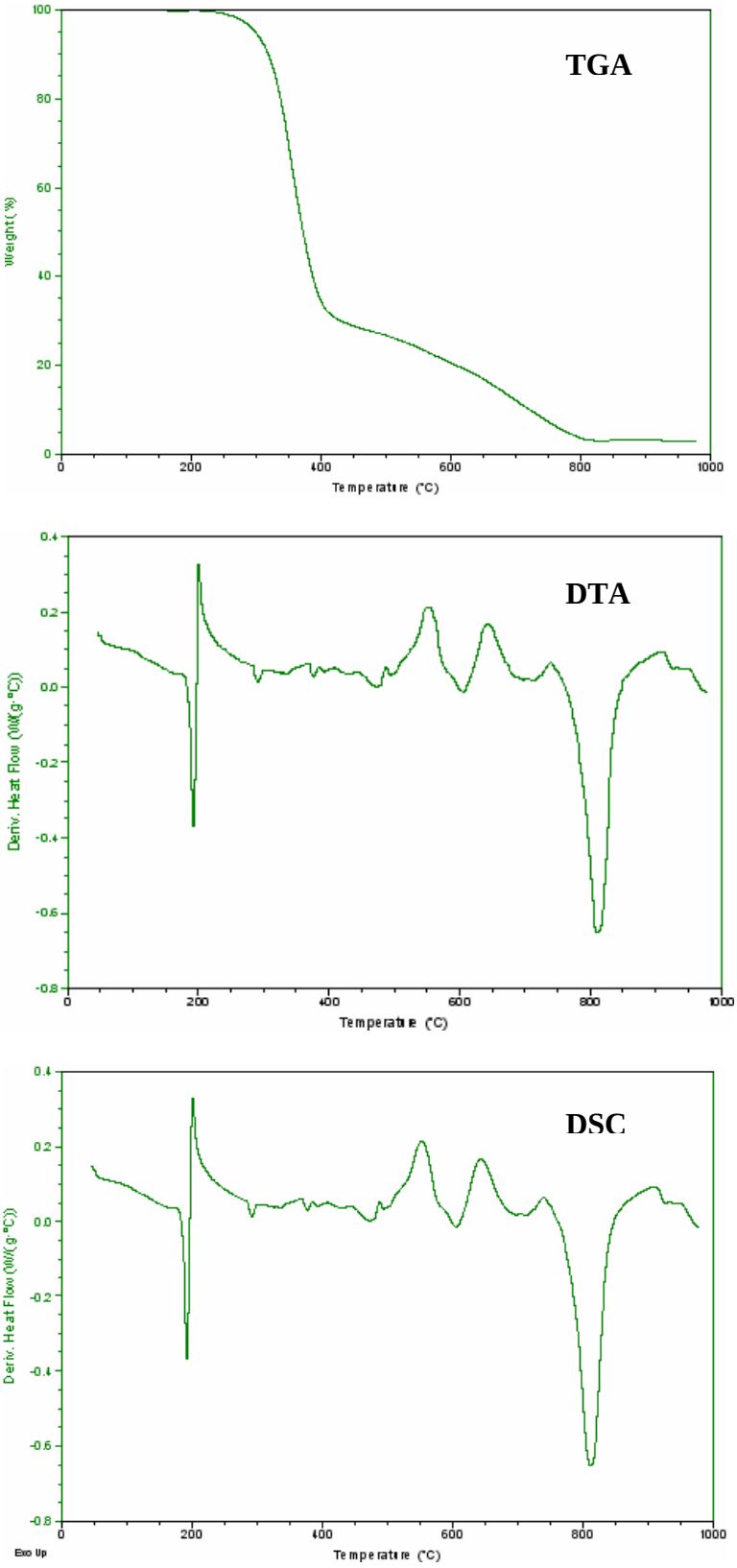


Fig. 36: TGA, DTA and DSC curves of Complex 33.

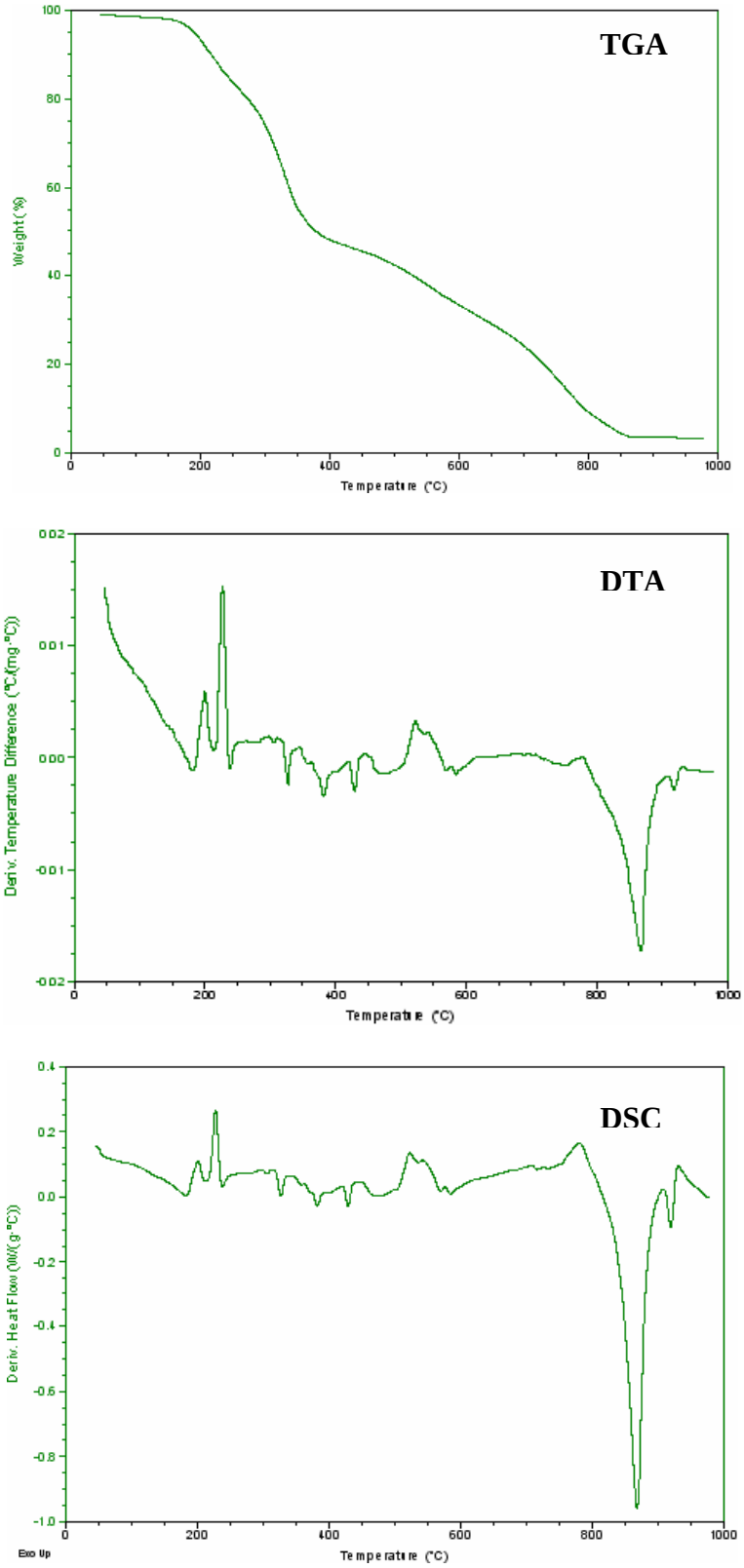


Fig. 37: TGA, DTA and DSC curves of Complex 34.

Table 13: Thermal analysis data of Cu (II) complexes

Sr. No.	Temp. range °C	Decomp. Stages	TGA Wt. loss, % found	DTA temp. peak °C / Thermal Effact (Exo/Endo)	Evolved moiety Formula	Mass Calcd. %
18	100-250	1	4.0	246.3 (Exo)	C_4H_2O	67.8
	250-300	2	11.0	531.9 (Exo)	$2C_{12}H_5O$	186.5
	300-400	3	48.5	585.2 (Exo)	$C_{34}H_{25}Cl_4CuN_4O_8$	822.5
19	100-133	1	3.87	145.64 (Endo)	C_6H_4	75.3
	133-222	2	9.94	161.0 (Exo)	$C_7H_6C_{l2}O_2$	193.5
	122-380	3	65.0	232.1 (Exo)	$C_{51}H_{24}Br_2Cl_5CuN_6O_9$	1265.6
20	100-156	1	1.66	163.4 (Endo)	H_4N_2	23.2
	156-217	2	6.13	341.1 (Endo)	$C_5H_4N_2O$	108.5
	217-295	3	14.82	402.7 (Exo)	$C_{10}H_9Cl_2NO_3$	262.2
	295-357	4	35.84	742.8 (Endo)	$C_{26}H_9Cl_4CuN_2O_5$	634.2
21	100-223	1	2.08	229.5 (Exo)	C_6H_4	75.2
	223-242	2	18.12	239.4 (Exo)	$C_{12}H_3Cl_2N_2O_3$	306.6
	242-371	3	45.99	345.6 (Endo)	$C_{34}H_{20}Cl_3CuN_6O_6$	778.1
	371-691	4	73.16	848.2 (Endo)	$C_{47}H_{37}Cl_3Cu_2N_9O_{11}$	1237.8
22	0.0-76.8	1	1.21	88.76 (Endo)	N_2H_4	32.0
	77-246	2	6.47	107.7 (Exo)	$C_5H_2N_2O$	105.8
	246-385	3	68.92	180.0 (Endo)	$C_{46}H_{27}Cl_3Cu_2N_{10}O_{11}$	1127.4
	385-586	4	81.11	285.4 (Endo)	$C_{54}H_{31}Cl_5Cu_2N_{13}O_{10}$	1326.8
	586-670	5	89.96	296.1 (Exo)	$C_{34}H_{20}Cl_3CuN_6O_6$ $C_{58}H_{41}Cl_4Cu_2N_{13}O_{11}$	1471.5
23	100-219	1	4.14	240.4 (Endo)	C_4H_2O	67.7
	219-313	2	16.21	257.0 (Exo)	$C_{10}H_{12}Cl_2NO_3$	265.3
	313-388	3	34.45	312.7 (Exo)	$C_{25}H_7Cl_2CuN_3O_5$	563.4
	388-836	4	83.8	959.6 (Endo)	$C_{56}H_{34}Cl_5Cu_2N_{13}O_{11}$	1370.8
24	100-219	1	0.94	251.1 (Exo)	NH_3	16.9
	219-384	2	77.42	379.1 (Exo)	$C_{48}H_{19}Cl_6Cu_2F_{12}N_{10}O_6$	1399.3
	384-582	3	92.42	557.9 (Exo)	$C_{56}H_{25}Cl_8Cu_2F_{14}N_{12}O_8$	1670.2
25	100-210	1	0.12	249.4 (Exo)	N_2H_4	32.6
	210-356	2	97.35	259.3 (Endo)	$C_{64}H_{34}Cl_8Cu_2F_8N_9O_8$	1619.3
26	100-200	1	0.09	252.3 (Exo)	C_6H_4	75.3
	200-338	2	86.32	262.9 (Endo)	$C_{53}H_{32}Cl_{13}Cu_2F_8N_{12}O_8$	1549.3
	338-412	3	92.4	334.3 (Exo)	$C_{53}H_{23}Cl_{13}Cu_2F_8N_{12}O_8$	1558.9
27	100-217	1	1.7	215.5 (Exo)	NH_3	16.2
	217-403	2	67.22	225.0 (Endo)	$C_{46}H_{38}Cl_6Cu_2F_8N_{10}O_5$	1059.4
	403-733	3	93.23	325.7 (Endo)	$C_{61}H_{44}Cl_8Cu_2N_{11}O_8$	1469.2
28	100-313	1	6.23	164.79 (Endo)	$C_6H_4O_2$	105.6
	313-407	2	81.43	173.1 (Exo)	$C_{54}H_{44}Cl_6Cu_2N_{11}O_{13}$	1380.9
29	100-338	1	6.35	279.2 (Exo)	$C_6H_3O_2$	104.3
	338-460	2	65.49	292.5 (Endo)	$C_{44}H_{38}Cl_5CuN_{11}O_7$	1073.9
	460-849	3	98.87	325.7 (Endo)	$C_{68}H_{50}Cl_8Cu_2N_{12}O_{11}$	1621.4
30	100-217	1	2.13	230.9 (Endo)	N_2H_2	32.9
	217-357	2	57.96	247.5 (Exo)	$C_{24}H_{24}Cl_5CuN_{10}O_4S_3$	841.3
	357-893	3	91.85	901.6 (Endo)	$C_{41}H_{27}Cl_7Cu_2N_{13}O_8S_4$	1333.2
31	100-206	1	0.81	211.9 (Endo)	NH_2	16.06
	206-231	2	12.0	217.9 (Exo)	$C_9H_8NO_3S$	210.7

	231-289	3	18.85	413.4 (Endo)	$C_{11}H_7Cl_2N_4O_2S$	331.4
	289-479	4	42.45	601.8 (Exo)	$C_{29}H_{15}Cl_3CuN_6O_4S_2$	745.5
	479-791	5	64.53	798.5 (Exo)	$C_{44}H_{36}Cl_5CuN_{12}O_4S_3$	1133.2
32	100-223	1	2.08	229.5 (Endo)	N_2H_4	32.4
	223-242	2	18.12	239.4 (Exo)	$C_{11}H_{13}Cl_2N_3O_3$	306.5
	242-371	3	45.99	345.6 (Endo)	$C_{34}H_{20}Cl_{13}CuN_6O_6$	778.4
	371-691	4	73.16	848.2 (Endo)	$C_{51}H_{38}Cl_6CuN_{13}O_8$	1237.8
33	100-241	1	0.68	191.3 (Endo)	C_6H_4	75.4
	241-420	2	69.32	201.3 (Exo)	$C_{66}H_{57}Cl_5CuN_{13}O_7$	1385.4
	420-627	3	81.4	551.3 (Endo)	$C_{60}H_{42}Cl_4CuN_{10}O_7$	1226.6
	627-811	4	96.79	642.5 (Exo)	$C_{92}H_{66}Cl_7Cu_2N_{14}O_{12}$	1934.5
34	100-164	1	2.55	198.9 (Endo)	N_2H_4	32.38
	164-277	2	20.81	226.2 (Exo)	$C_{10}H_7Cl_2N_3O_3$	288.74
	277-403	3	52.32	326.9 (Endo)	$C_{23}H_{18}Cl_4CuN_4O_7$	725.96
	403-864	4	96.35	381.4 (Endo)	$C_{41}H_{39}Cl_8Cu_2N_{16}O_{12}$	1336.9

3.2.1.7 Electronic absorption spectra

The electronic absorption bands of the complexes under investigation are listed in the Table 11. The d-d spectra are consistant with the distorted octahederal geometry of the complexes. In the absorption spectra of Cu (II) complexes (18–34) there is an intense broad band observed at 11,760–16,670 cm^{-1} which is assigned to a $^2T_2g \rightarrow {}^2E_g$ transition *[160]*. Although three transitions are expected in this case, but they are very close in energy and often appear in the form of one broad band envelop *[161]*. The values of the electronic transitions for the Cu (II) complexes are specific to an axially deformed octahedral geometry *[162]*.

3.2.1.8 FT-IR spectra

The comparison of the IR spectra of the ligands (1-17) with their metal complexes (18-34) principally revealed that the ligands are bidentately coordinated to the metal ions. In all the metal complexes the band appearing at 1650-1670 cm^{-1} assigned to the 4-oxo-*4H*-chromone C=O vibrations is shifted to lower frequency by 17-25 cm^{-1}, which is indicative of the involvement of the 4-oxo-*4H*-chromone C=O in chelation. In addition, a band at 1680-1690 cm^{-1} attributed to amide C=O strechings in the compounds (15-17) is shifted to lower frequency by 15-25 cm^{-1} in its metal complexes (32-34) which indicates that chelation takes place through the oxygen atom (O). These new bands were not present in the spectra of their corresponding ligands.

Further conclusive evidence of the coordination of compounds with the metal ion was established by the far IR spectra in which new bands at 455-470 cm^{-1} and 420-440 cm^{-1}, assigned to M-O (amide) and M-O (4-oxo-*4H*-chromone) in the spectra of the metal complexes were observed. The characteristic absorption bands along with their assignments are listed in the Table 14, 15.

Table 14: Observed infrared frequencies (cm^{-1}) and assignments of Cu (II) complexes (18 – 29)

Sr. No	ν(carboxlic - OH)	ν(OH)	ν(amide – NH)	ν(amino – NH/ NH_2)	ν(carboxlic - CO)	ν(amide – C=O)	ν (4H-chromene C=O)	ν (C=N)	ν (M-O amide) (C=O)	ν (M-O chromene) (OH)
18	-	3455	3422	-	-	1665	1648	1635	455	425
19	-		3433	-	-	1662	1641	1628	470	430
20	-	-	3427	-	-	1655		1625	465	430
21	-	-	3419	3386_{asy} 3326_{sym}	-	1651	1638	1627	480	455
22	-	-	3443	-	-	1678	1655	1636 1512 isonicoti	460	435
23	-	-	3444	-	-	1674	1652	1638 1516 nicotino	455	440
24	-	-	3446	-	-	1669	1660	1647	485	455
25	-	-	3424	-	-	1684	1651	1639	490	465
26	-	-	3409	-	-	1668	1653	1637	470	465
27	-	-	3417	-	-	1652	1641	1629	465	460
28	3525	-		3455	1714	-	1652	1646	460	445
29	3523	-		3429	1693	-	1647	1635	455	465

Table 15: Observed infrared frequencies (cm^{-1}) and assignments of Cu (II) complexes (30 – 34)

Sr. No	ν(thio amide NH)	ν(thio amide NH_2)	ν(thio amide C-S)	ν(amide – NH)	ν(amide – NH_2)	ν(amide – C=O)	ν (4H-chromene C=O)	ν (C=N)	ν (M-O amide) (C=O)	ν (M-O chromene) (OH)
30	3428	3326_{asy} 3188_{sym}	1568	-	-	-	1650	1625	450	420
31	3439	-	1576	-	-	-	1660	1635	465	425
32	-	-	-	3447	-	1670	1645	1630	460	435
33	-	-	-	3455	-	1686	1660	1638	470	450
34	-	-	-	3449	3355_{asy} 3326_{sym}	1680	1650	1635	455	445

3.2.1.9 Proposed structures

Based on the experimental evidence available through this study the complexes are proposed to have the structures as shown in Fig. 38-54. Construction of molecular models also reports the proposed structures.

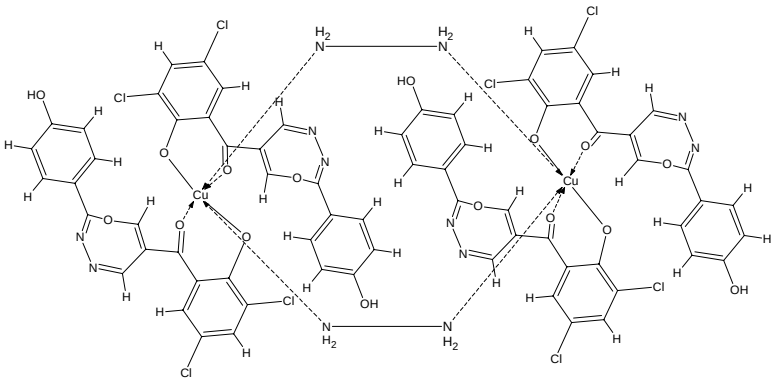


Fig. 38: Proposed structure of Cu (II) complex No. 18

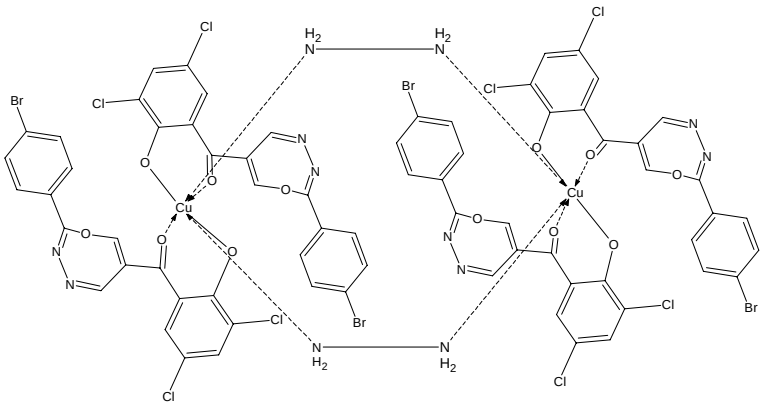


Fig. 39: Proposed structure of Cu (II) complex No. 19

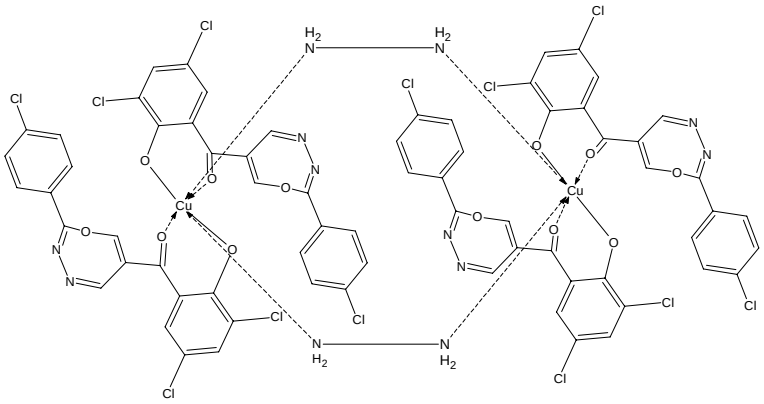


Fig. 40: Proposed structure of Cu (II) complex No. 20

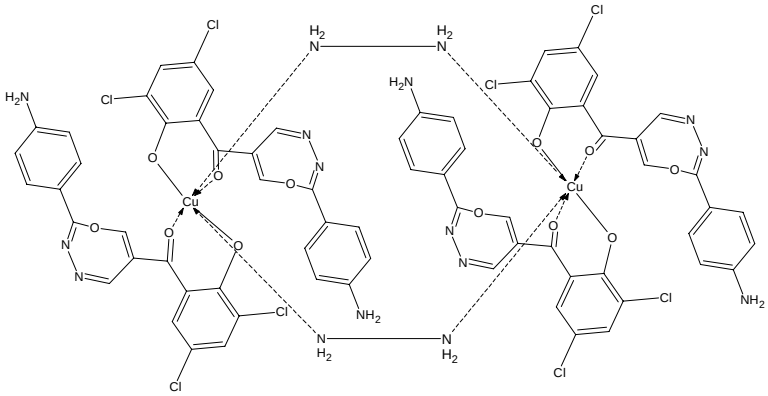


Fig. 41: Proposed structure of Cu (II) complex No. 21

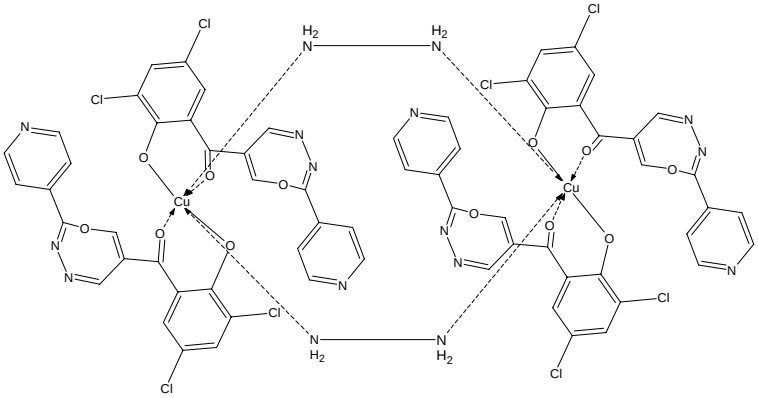


Fig. 42: Proposed structure of Cu (II) complex No. 22

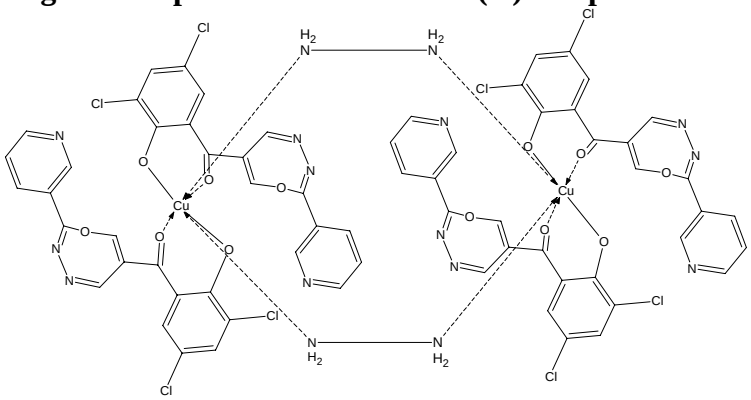


Fig. 43: Proposed structure of Cu (II) complex No. 23

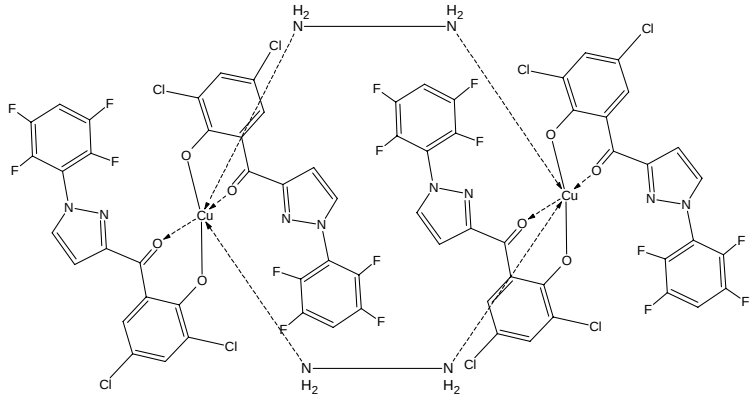


Fig. 44: Proposed structure of Cu (II) complex No. 24

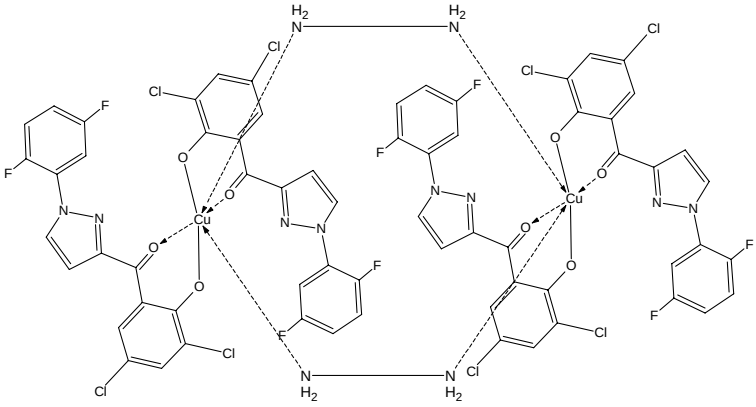


Fig. 45: Proposed structure of Cu (II) complex No. 25

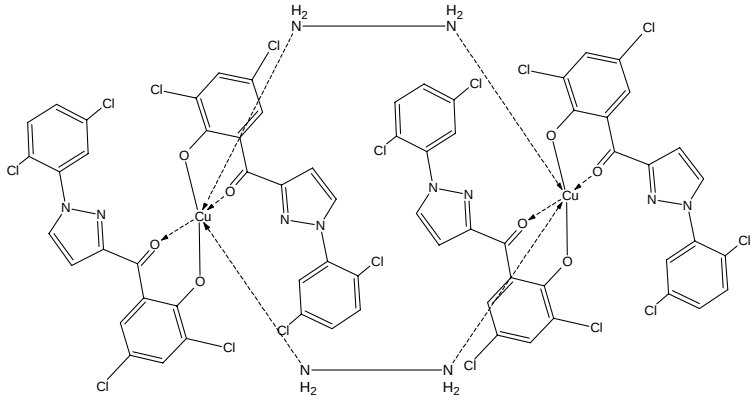


Fig. 46: Proposed structure of Cu (II) complex No. 26

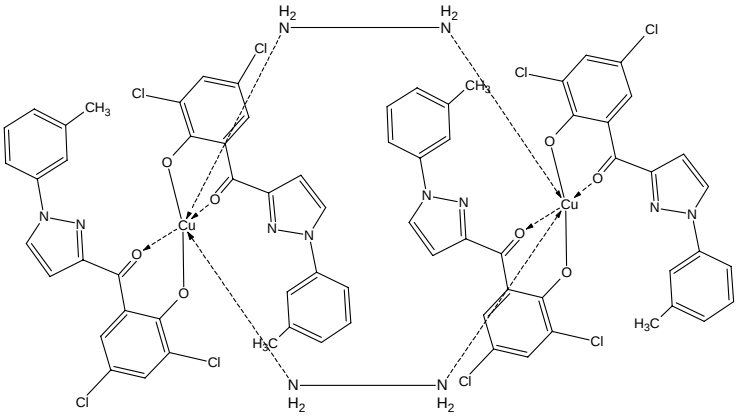


Fig. 47: Proposed structure of Cu (II) complex No. 27

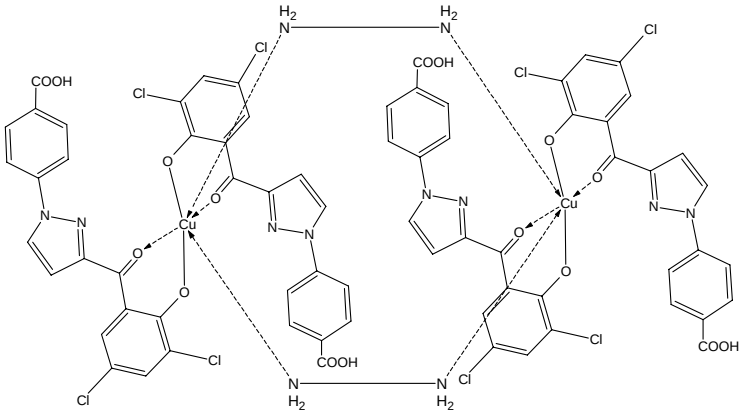


Fig. 48: Proposed structure of Cu (II) complex No. 28

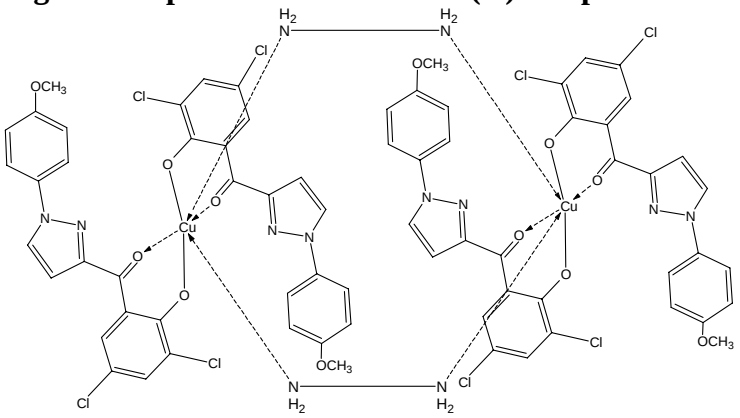


Fig. 49: Proposed structure of Cu (II) complex No. 29

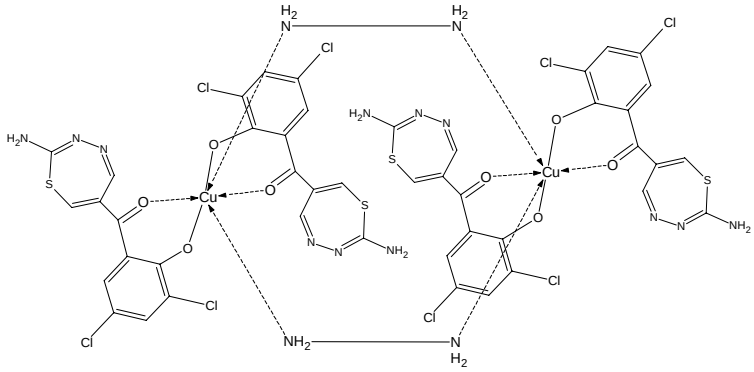


Fig. 5: Proposed structure of Cu (II) complex No. 30

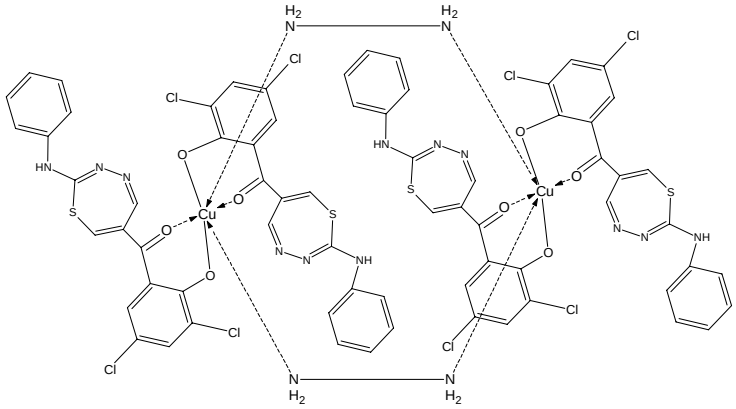


Fig. 51: Proposed structure of Cu (II) complex No. 31

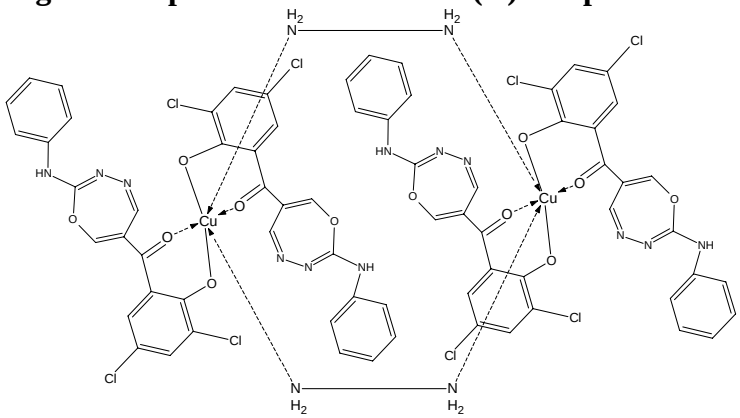


Fig. 52: Proposed structure of Cu (II) complex No. 32

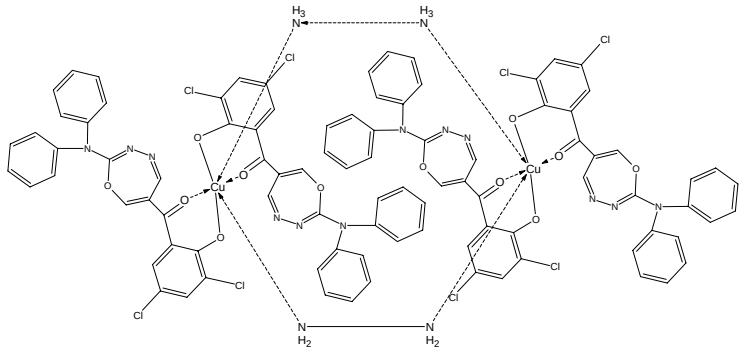


Fig. 53: Proposed structure of Cu (II) complex No. 33

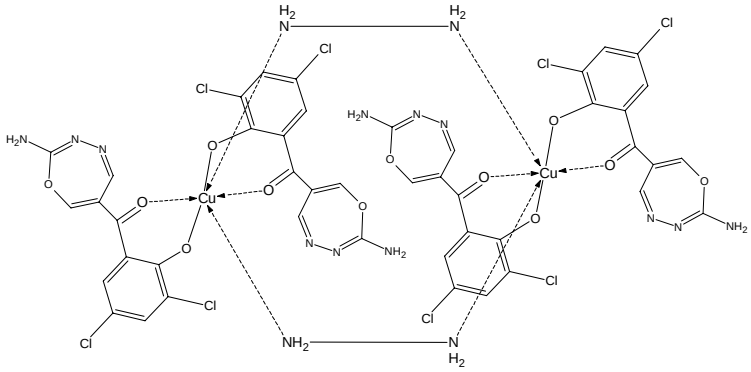


Fig. 54: Proposed structure of Cu (II) complex No. 34

3.3 Antibacterial studies of the ligands (1 – 17) and their Cu (II) complexes (18 – 34)

3.3.1 Preliminary Screening

Antibacterial activities of the synthesized ligands (1-17) and their corresponding metal (II) complexes (18-34) were determined against four Gram-negative (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and *Shigella flexneri)* and two Gram-positive (*Bacillus subtilis and Staphylococcus aureus)* bacterial strains. The ligands either exhibited no or varying degree of inhibitory effects (low to moderate) on the growth of different tested strains Table 16. The compounds **(2)**, **(6), (7)**, **(10), (11)**, **(13) (15)** and **(17)** were, however, found to be active against all the Gram-negative and Gram-positive bacteria.

In contrast, the growth of all the Gram-negative and Gram-positive species was inhibited by all the metal complexes under investigation except **(19)** and **(26)**. However, fifteen of them i.e. **(18), (20-25)**, and **(27-34)** were found to be comparatively much more active. Of these, **(28)** and **(32)** were found to be the most active one. These results substantiate our own findings *[163-166]* and the findings of some other workers *[167-170]* that biologically inactive compounds become active and less biologically active compounds become more active upon coordination. Such induction or enhancement in activity of the metal complexes can be explained on the basis of Overtone's concept and chelation theory. According to Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favours the passage of only lipid soluble materials due to which liposolubility is an important factor that controls antimicrobial activity. On chelation, the polarity of the metal ion is reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups.

Table 16: Antibacterial activity data of the prepared ligands and their Cu (II) complexe

Compounds	Inhibition zone (mm)					
	Gram-negative				Gram-positive	
	Escherichia coli	*Pseudomonas aeruginosa*	*Salmonella typhi*	*Shigella flexneri*	*Bacillus subtilis*	*Staphylococcus aureus*
1	0	0	6.33	0	0	0
2	6.33	6.33	6.66	0	6.66	6
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	7.33	6.66	7	6.33	7	6.33
7	12.66	12	11	9.33	10	11
8	0	0	0	0	0	0
9	0	0	0	0	0	0
10	6.33	6.33	7	6.66	7.33	7.33
11	6.66	7.33	6.66	8.33	8.33	7.66
12	0	0	0	0	0	0
13	7	6.66	0	6.33	7	6.33
14	0	0	0	0	0	0
15	11.66	10	8	11.66	8.33	8.33
16	0	0	0	0	6.66	0
17	8	8.66	7.33	8.66	9	8.66
18	25	22	21	23	28	23
19	0	0	0	0	0	0
20	21	12	19	13	29	21

21	21	11	16	19	22	19
22	14	13	17	18	22	24
23	21	12	16	19	22	23
24	12	9	12	17	29	19
25	22	16	18	13	22	22
26	0	0	0	0	0	0
27	22	19	12	17	24	19
28	26	14	14	16	26	18
29	22	17	15	14	22	16
30	24	15	17	18	24	19
31	22	13	16	20	24	21
32	26	16	15	19	26	22
33	22	18	17	17	23	25
34	24	20	18	15	21	20
Standard	30	24	25	27	33	33

0 : absence of measurable inhibitory action; 9 <: weak; 9-16: moderate; >16: significant.

No activity observed against negative control

Further, it increases the delocalization of π-electrons over the whole chelate ring and enhances the lipophilicity of the complex. This increased lipophilicity in turn enhances the penetration of the complexes into lipid membranes and blocking of metal binding sites on the enzymes of the microorganisms *[171]*. The metal complex may also be a vehicle for activation of the ligand as the cytotoxic agent. Moreover, coordination may lead to significant reduction of drug – resistance *[172]*. Apart from this, other factors such as solubility, conductivity and dipole moment as influenced by the presence of metal ions may also be amongst the possible reasons causing enhancement of the bactericidal activity of the metal complexes as compared to the uncomplexed compounds *[173]*.

3.3.2 Minimum Inhibitory Concentration (MIC)

The preliminary screening showed that the compounds **(2), (7), (15)** and **(21), (28), (32)** were significantly active against both Gram-negative and Gram-positive organisms. These compounds were selected for minimum inhibitory concentration (MIC) studies. The MICs of these compounds varies from 10-100 μg $mL^{-1.}$ The Cu (II) complex **(32)** again showed the highest activity. It inhibited the growth of *Pseudomonas aeruginosa, Shigella flexneri, Bacillus subtilis* and *Staphylococcus aureus* at 10 μg mL^{-1} concentration Table 17.

The present investigation suggests that of all the metal complexes, those derived from the compound, 6,8-dichloro-3-formylchromone and hydrazinobenzoic acid phenylsemicarbazide moieties are comparatively more active than others. These studies may serve as a basis for the chemical modifications directed towards the development of new classes of antibacterial agents.

Table 17: Minimum inhibitory concentration (μg/mL) of the compounds (2), (7), (15), (21), (28) and (32) against selected bacteria.

Compound No.	**2**	**7**	**15**	**21**	**28**	**32**
Gram-negative						
Pseudomonas aeruginosa	25	10	50	25	50	10
Shigella flexneri	25	10	10	25	25	10
Gram-positive						
Bacillus subtilis	>100	25	>100	>100	>100	10
Staphlococcus aureus	50	10	50	10	25	10

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