SYNTHESIS AND CHARACTERIZATION OF METAL COMPLEXES WITH CHELATING LIGANDS

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Doctor of Philosophy
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by

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CERTIFICATE

This is to certify that the thesis entitled, **"SYNTHESIS AND CHARACTERIZATION OF METAL COMPLEXES WITH CHELATING LIGANDS"** is an authentic record of the research work carried out by **Mrs. PriyaVarma C**, under our supervision and guidance in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Chemistry under the faculty of Sciences, University of Calicut, Kerala and further that no part thereof has been presented before for any other degree.

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DECLARATION

I, PriyaVarma C., hereby declare that this thesis entitled, **"SYNTHESIS AND CHARACTERIZATION OF METAL COMPLEXES WITH CHELATING LIGANDS ",**submitted to the University of calicut in partial fulfilment of the requirements for the award of Doctor of Philosophy in Chemistry, is a bonafide research work done by me under the supervision and guidance of Dr. K. K. Aravindakshan(Guide) and Dr. N. K. Renuka (Co-guide).

I further declare that this thesis has not previously formed the basis for the award of any other degree, diploma or other similar title.

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PREFACE

During the last two decades, coordination chemistry has been the subject of great concern due to the extensively studied thiosemicarbazones, which is an important class of sulfur donor ligands for the transition metal ions. Thiosemicarbazide was first reported in the literature in the late 1800s and thiosemicarbazones were described as potential derivatisation agents for ketones and aldehydes in the 1900s. The basis of the coordination chemistry of these ligands was established in 1930s and this made the realization of their potentiality as ligands for a wide range of metals. It was then suggested that coordination occurred *via* sulfur and azomethine nitrogen with the formation of a five membered ring. The driving force for the study of coordination chemistry of thiosemicarbazones has definitely been due to their wide range of biological applications and also their use in analytical chemistry. Because of the interesting structural characteristics of thiosemicarbazone complexes, they have become the subject of intense research interests of the coordination chemist. Literature survey revealed that the alkyl substituents on the terminal nitrogen atom of thiosemicarbazones increase their *in vitro* cytotoxic activity. Several valuable reviews have also appeared on the chemistry and biological applications of thiosemicarbazones and hydrazones due to their flexible and diverse donor behaviours.

Earlier reports have summarized on the various aspects of the chemistry of these compounds, such as their synthesis, spectral, magnetic, stereochemical and other characteristics. An enhanced

interest in these compounds has provoked the discovery of their valuable pharmacological properties including antiviral-, antibacterial- and antitumoral activities. Hence it was considered worthy and interesting to synthesize and characterize N(4)-methyl(phenyl) thiosemicarbazones and hydrazone of several carbonyl compounds and their complexes

In the present research programme, four different ligands i.e., crotonaldehyde N(4)-methyl(phenyl)thiosemicarbazone, chalcone N(4)-methyl(phenyl)thiosemicarbazone, isatin N(4)-methyl(phenyl) thiosemicarbazone and isatin 2-methoxyisonicotinoylhydrazone were synthesized and characterized. Their reactions with several transition metal ions like Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) were studied and new solid complexes were isolated. These complexes were characterized using various physico-chemical methods and spectral techniques. Based on these analyses, the tentative structures were assigned for these complexes.

For the sake of convenience, the matter in this thesis is divided into two parts. Part I of the thesis comprises of a collection of six chapters discussing 'Synthesis and Characterization' of the ligands and their complexes. Chapter I of Part I give a brief introduction about the history of coordination chemistry and transition metal ion complexes. A review on the complexes of thiosemicarbazones and hydrazones and the significance and scope of the present investigation are also included in this chapter.

The reagents used, the general methods for the preparation of ligands and the physico-chemical techniques employed for the characterization of the compounds are given in Chapter II.

The synthesis and characterization of thiosemicarbazone ligands, *viz*, crotonaldehyde N(4)-methyl(phenyl)thiosemicarbazone, chalcone N(4)-methyl(phenyl)thiosemicarbazone, isatin N(4)-methyl (phenyl)thiosemicarbazone and isatin 2-methoxyisonicotinoyl hydrazone and their transition metal complexes are described in Chapters III, IV and V, respectively. Chapter VI deals with the synthesis and characterization of Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) complexes of isonicotinoylhydrazone of isatin.

Apart from the synthesis and structural studies of the compounds, the biotoxic evaluation of some of the compounds has been carried out. We have conducted the antibacterial activities of crotonaldehyde N(4)-methyl(phenyl)thiosemicarbazone, isatin N(4)-methyl(phenyl)thiosemicarbazone and their Ni(II) and Cu(II) complexes. The antifungal activities of crotonaldehyde N(4)-methyl(phenyl)thiosemicarbazone and their Ni(II), Cu(II) and Zn(II) complexes were also carried out. The results obtained from these studies are presented as two chapters, in Section A of Part II in the thesis.

In addition to the antimicrobial studies, the cytotoxic- and antitumour studies of isatin N(4)-methyl(phenyl)thiosemicarbazone and its complexes were conducted. The details of these investigations are presented in Section B of Part II in the thesis. It is divided into three chapters. The first chapter in Section B contains an introduction to cancer and a review on the thiosemicarbazone complexes as antitumour agents. The materials used and the experimental details are presented in the next chapter. The results of cytotoxic studies of isatin N(4)-methyl(phenyl)thiosemicarbazone and its complexes are

described in the third chapter of Section B. Copper complex of isatin N(4)-methyl(phenyl)thiosemicarbazone was selected for *in vivo* antitumour studies. The references cited in the text are arranged in serial order at the end of each chapter/section.

The work incorporated in this thesis has been partly published/presented as indicated below:

1. Synthesis and Biochemical studies of Transition metal complexes of Isatin N(4)-methyl(phenyl)thiosemicarbazone, C.Priya Varma, K.Subin Kumar, K. K. Aravindakshan, Int. Res. J. Pharm., 8 (10), 109 (2017)

2. Synthesis, Characterization and Pharmacological activity of complexes of Cu(II), Ni(II), Mn(II) and Co(II) from Chalcone N(4)-methyl(phenyl)thiosemicarbazone, C. Priya Verma, K. Subin Kumar, K.K. Aravindhakshan, J. Pharm. Sci. & Res., 9(9), 1444 (2017)

3. An oral presentation on "Synthesis and Characterization of Complexes of Co(II), Ni(II), Cu(II) and Zn(II) with crotonaldehyde thiosemicarbazone" at UGC sponsored National Seminar on Emerging Trends in Chemical Research, Research & Post Graduate Department of Chemistry, Christ College (Autonomous), Irinjalakuda, Thrissur on 28th Feb & 1st March 2017, Abstract No: 24, Page No: 33.

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PART I

SYNTHESIS AND CHARACTERIZATION

CHAPTER I

INTRODUCTION

The life has evolved from the metal ion interaction with organic molecules and life has depended on the complexes for its development and survival. The eagerness to understand the metal binding property of electron donating molecules dates from the progress of analytical procedure for metals by Berzelius and his contemporaries[1]. In the major fields of Science, there is generally a lag of theory behind practice due to inadequate experimental data, but attempts were usually made to explain these experimental facts and predict new phenomena prior to sufficient data. Because of the incredible importance of coordination compounds to the general question of chemical bonding, the lag of theory behind practice was not immense in the field of coordination chemistry. During the first half of nineteenth century, discoveries of coordination compounds were a few, erratic and often inadvertent, and it was not until behind Gibbs and Genth's typical chronicle that chemists dedicated themselves to an efficient study of this field[2]. As the number of known coordination compounds increased, theories to explain their formation and structure were devised[3]. The recognition for the first theory of metal ammines, the so-called 'ammonium theory' goes to Thomas Graham, who explained the formation of coordinate covalent bonds, which showed a remarkably close resemblance to the modern Lewis acid-base approach[4,5]. The conjugate theory of Jöns Jacob Berzelius viewed metal ammines as conjugated compounds, which was later

revived and tailored by Claus in 1854 and Blomstrand in 1869[6]. Claus' propositions, summarized as three postulates in his widely read paper of 1856, were attacked strongly by Karl Weltzien and Hugo Schiff[7-9].

By the late nineteenth century, chemists like S. M. Jorgensen rapidly developed the syntheses of a great variety of metal complexes. Any atom with a vacant orbital has in effect a positive charge and it attaches to any other atom that can provide its available electron pair, to form a sufficiently stable aggregate that can be studied experimentally as one molecule. Thus, the great events for the understanding of the residual affinity of the metal ions for other molecules and anions stemed from the classic work of a Swiss Chemist, Alfred Werner, who pioneered with outstanding success this vast area of chemistry, the chemistry of coordination compounds. It is the chemistry of 'compounds of higher order' or 'compound compounds', formed by the coordination of two or more substances through electron donor-acceptor interaction. Its formation demonstrates comprehensively the universal tendency for chemical combination to persist in the direction of maximum utilization of available orbitals.

The obvious feature of the transition metals is in the enormity of the number of compounds that they form. The transition metal compounds have always fascinated and held a special interest for inorganic chemists, because they do come in every color of the rainbow and it was sometimes possible to make compounds of the same formula in different colours. For example, $CrCl_3.6H_2O$ can be synthesized in purple, pale green and dark green colours. The early explanation for this crowd of compounds was that, like organic

compounds, the components of the transition metal compounds were strung-out in chains. The novel theory of transition metal compound formation, commonly referred to as 'Werner's Coordination Theory', devised in 1893 by Werner was awarded the Nobel Prize in 1913 as the recognition of his contribution. Consequently, explosion in the studies of metal complex chemistry was not only because of the economic importance but also of the inherent interest and intellectual challenges encountered with their structural problems.

Flexibility of metal ions in an environment of a nucleophilic molecule or anions delivered an innumerable amount and varieties of complexes. The formation, properties and uses of metal complexes are dependent both on the nature of the metal ion and the type of the ligand coordinated. Several new ligand systems have been developed in recent years because of the advancements in modern synthetic organic chemistry. In the early hours, coordination chemistry centered mainly on the theoretical aspects. However, in recent times they find applications in a much wider area. This is mainly because of the ability of coordinated metal atom or ion to influence the configuration, properties and applications of the organic compound. Hence, several highly efficient catalytic systems based on metal complexes for the synthesis and manufactures of a number of industrial chemicals are available. Likewise, a number of synthetic metal complexes that mimic the behavior of complex biomolecules are identified and at present the study of such compounds are receiving much consideration[10,11].

Biochemists have become greatly concerned with the metal complexes in biological process. The vital role played by many metals in living systems is mainly because of their ability to form positive

ions, which tend to be soluble in biological fluids[10,12,13]. Majority of the biological molecules such as proteins, DNA, etc. possess electron rich sites. Therefore, these positive metal ions can effectively bind to such biological molecules. Taking into account of the wide possibility of the interaction of metals in biological systems, it is not unexpected that natural evolution has incorporated many metals into essential biological functions. Metal containing compounds are also significant in the process of chemical- and energy transfer reactions that involve the transport of oxygen to the oxidation site and the various redox reactions resulting from its use. Thus, the interest in the role of metal ions in biological process has inspired the work in bioinorganic chemistry. Now, coordination chemistry has drawn attention as a supplementary channel because of the usage of metals in chemotherapy and chelation therapy. The importance of metal complexes is clearly understood by the incorporation of a wide variety of metal complexes, especially of organic ligands, commercially into different types of medicinal formulations and various biochemical fields.

1.1 Transition metal ions and their complexes

The d-block metals or transition metals are the workhorse elements of the periodic table. In general, a transition metal may be defined as an element that has atleast one simple ion with an incomplete outer set of d-electrons. A ‘naked transition metal ion’ is very rarely encountered because it is usually covalently bonded to other ions or molecules. These groups are called metal complexes, and

it is the number and variety of the metal complexes that provide wealth to transition metal chemistry. Werner proposed that these metal ions had some characteristic "combining power" and the complex formation is the result of coordinate covalent bond acting between the Lewis acid, metal ion and the Lewis bases, ligands. The physico-chemical properties of these metal complexes depend on the oxidation state of the metal and its coordination number.

Transition series comprises of d-block elements, lanthanides and actinides. In the case of d-block elements, the partly filled 3d, 4d and 5d orbitals project well out of the periphery of the atoms. Hence, the electrons occupying these orbitals are strongly influenced by the surroundings and are in turn able to influence the environments significantly. This makes the position of d-block metals in coordination chemistry more important than those of f-block elements, as the latter have intensely buried orbitals, which are mostly screened from the surroundings.

Scandium can be described really as a little-studied element. In their detailed review, 'The Coordination Chemistry of Scandium', Melson and Stotz showed that although many of the essential topics relating to this element had been to some extent investigated, a good deal of careful work is still to be done, to bring our knowledge of scandium chemistry to the level of that of its neighbours such as titanium or vanadium[14]. Regarding ligand preferences, Sc(III) readily coordinates with ligands containing O and N donor atoms or halides, but virtually no complexes are reported with S or P-based ligands.

Titanium is a reasonably common element that has been identified before 170 years. Hunter first obtained the pure element in

1910 *via* the reduction of its tetrachloride with sodium[15]. In 1925, van Arkel and de Boer obtained a very pure form of the metal by dissociation of the tetrachloride[16]. However, the titanium metal industry really dates from the publication of the Kroll process in 1940, which involves the tetrachloride reduction with magnesium[17]. Titanium is the first member of the 3d-transition series with four valence electrons, $3d^2$, $4s^2$. The most stable and most common oxidation state of +4 is obtained by the loss of all these electrons. Nevertheless, titanium compounds in the +3 or lower oxidation states are oxidized readily to the +4 state. Even though titanium compounds with 4, 5, 7 and 8 coordination numbers are known, the most common coordination number of titanium is six.

The striking colors of vanadium compounds had been observed by A. M. del Rio in 1801. In his experiments with a new element, Rio got red colour on treatment with an acid and therefore, he called it as Erythronium[18]. There are vanadium compounds with formal oxidation states from -3 up to +5 with the exception of -2. Under common circumstances, the most stable oxidation states are +4 and +5. Several V(V) compounds are oxo complexes with oxidizing properties and are helpful in many preparative reactions, namely for the catalysis of oxidation reactions, *viz*, catalysts for the oxidation of SO_2 to SO_3 in the industrial production of H_2SO_4.

The transition metal member of group VI is chromium with $3d^5\ 4s^1$ outer electronic configuration. Chromium forms compounds in oxidation states ranging from –2 to +4. Chromium(II) usually forms high-spin or low-spin 6-coordinate complexes, although it exhibits coordination numbers from 3 to 7. Oxidation states of chromium in

organometallic compounds are 0 and +1, which are stabilized by the π- acceptor C, N-heterocyclic and P-donor ligands[19]. The most stable and important oxidation state of chromium is +3 with a coordination number of 6, although a very few examples with coordination number greater than six are also known.

Manganese has been a metal much ignored by the coordination chemists in the early times. The negligence for manganese coordination chemistry certainly relate to the dominance of the Mn(II) state with its half filled d^5 configuration. Yet interest aroused by the recognition of its biological role, especially in photosynthesis[20]. For manganese, +2 is the dominant oxidation state and Mn(II) compounds are quite labile[21].

Iron is found most commonly in +2 and +3 oxidation states. Most Fe(III) complexes are octahedrally coordinated, with a few of them with 4, 5 and even 8 coordination number. Fe(III) is high-spin (S=5/2) in most of the complexes with a d^5 configuration and is isoelectronic with Mn(II). The high-spin Fe(III) is octahedral with a magnetic moment (spin-only value) of 5.92 B.M, which is temperature independent. However, there is a possibility of the stabilization of low-spin (S=1/2) ground states in the presence of strong octahedral fields, such as those generated by cyanide ion[22]. The most important cyano complex of Fe(III) is the red coloured hexacyanoferrate(III) anion, $[Fe(CN)_6]^{3-}$ with a magnetic moment of 2.25 B.M at 300K in accordance with one unpaired electron and considerable orbital contribution[23].

Cobalt is an element found in vitamin B_{12}. For cobalt, the lower oxidation state of +2 is more stable than +3. Divalent cobalt forms

many complexes of various stereochemical types. Octahedral and tetrahedral complexes are the most common, but there are a few square planar and 5-coordinate ones. The olive green pentacyanocobaltate(II) ion, $[Co(CN)_5]^{3-}$ is the predominant cobalt(II) cyanide species in aqueous solution, which is parmagnetic[24]. All cobalt(II) complexes are typically high-spin octahedral ones with weak field ligands. However, they become unstable in +2 oxidized state in the presence of ligands of strong field type.

A. F. Cronsted, in 1751, isolated a metallic species and proposed the name 'nickel' which was derived from 'kupfer nickel'. He verified that it contained the new element instead of copper as previously believed[25]. The oxidation states of nickel in its compounds range from –1 to +4[26]. However, relatively a very few compounds correspond to the lowest (-1) and to the highest (+3 and +4) oxidation states. A good number of the nickel compounds in the solid state and nearly all in aqueous solution contain the metal in +2 oxidation state, which in effect, can be considered the common oxidation state for nickel in its compounds. The electronic structure and stereochemistry of Ni(II) were reviewed in1968[27]. The great majority of Ni(II) complexes have coordination numbers of 4, 5 and 6. Nearly all 6-coordinate Ni(II) complexes have a pseudo octahedral stereochemistry with the metal ion in a high-spin configuration. The magnetic moments of Ni(II) ions in tetrahedral geometry are greater than those in an octahedral environment.

Among the first row transition metals, copper is one of the most abundant- and naturally occurring element[28-31]. Copper is a representative transition element, which covers a range of oxidation

states, starting from 0 to +4. The most abundant oxidation states of copper are (I) and (II) and the more stable being Cu(II) under normal conditions, forms a wealth of simple compounds and coordination compounds[32]. Several of the Cu(II) complexes are good crystals[33]. The probable coordination numbers in Cu(II) complexes are 4, 5 and 6 in the tetrahedral or square planar, trigonal bipyramidal and octahedral geometries, respectively. The Cu(I) state is less widespread and is readily oxidized to the Cu(II) state.

The general chemistry of the two elements, zinc and cadmium, has been surveyed in detail by Aylett[34]. Zinc belongs to group IIB with an outer electronic configuration of nd^{10} ns^2 and a common oxidation state of +2. The coordination numbers of elements of this group are 4, 5 and 6. One of the most important and outstanding features is their stereochemical flexibility; each is willing to agree readily to the structural demands of the ligand.

1.2 Thiosemicarbazones and hydrazones as ligands- a brief review

1.2.1 Introduction

Thiosemicarbazones and hydrazones are Schiff base ligands formed by the condensation of primary amino group of thiosemicarbazide or hydrazide with carbonyl compounds. They are represented by the general formula, RCH=NR', where, R and R' represent the alkyl and/or aryl substituent. They form an important class of N-donor Schiff base ligands and occupy an important position amongst the recent achievements in the field of coordination

chemistry. These N-substituted imines, often referred to as azomethines or anils, form an important class of compounds with various applications[35]. Hydrazones are azomethines characterized by the presence of a triatomic group, >C=N-N<, (shown in Fig 1) which is typical for a large number of organic compounds of the type,

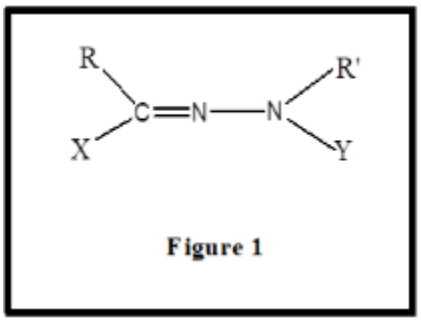


Figure 1

Generally, the compounds having the above structure (Figure 1) is termed as "hydrazones". When R'=Y=H, it is an unsubstituted hydrazone and if R'=NH_2CS and Y=H, it is a thiosemicarbazone. Substituted thiosemicarbazones are formed when one or both the hydrogens in R'=NH_2CS are replaced by alkyl/aryl groups.

Acylhydrazones are characterized by the presence of >C=O donor site, in addition to >C=N-NH- group which decides their versatility and flexibility. Generally, by the reaction with transition metals, acylhydrazones display their bidentate metal coordination, bonding through carbonyl oxygen and azomethine nitrogen atoms. During the binding of the ligand to the metal, there occurs structural

changes of the fragment attached to the hydrazone and the complex exhibits attractive coordination geometries: octahedral or tetrahedral or square-planar[36,37].

1.2.2 Thiosemicarbazones

Thiosemicarbazones are versatile ligands as they possess a number of donor atoms that may coordinate in various ways. In addition to this, thiosemicarbazones possess a variety of biological properties including antiproliferative activity[38,39]. Studies have demonstrated that thiosemicarbazones are potent inhibitors of the enzyme ribonucleotide reductase and are capable of interrupting DNA synthesis and repair. Incorporation of metals with thiosemicarbazone ligands can result in the alteration or enhancement of their biological activities[40,41]. For pharmaceutical uses, the binding capacity of thiosemicarbazones is increased further by the condensation of thiosemicarbazide with an aldehyde containing heteroatoms[42,43].

Thiosemicarbazone exists as an equilibrium mixture of keto- and enol tautomers (as in Fig 2) in solution.

Figure 2

It acts as neutral bidentate ligand in the keto form and by the loss of a proton yields a singly charged bidentate ligand. In most of the metal complexes, thiosemicarbazone coordinates in the keto form. Therefore, depending upon the preparative conditions, the complexes will be cationic, neutral or anionic.

Thiosemicarbazone molecule has been shown to exist itself in the *trans*-configuration (Figure 3). It binds through sulphur atom in the metal complexes. However, the coordination takes place through either hydrazine nitrogen or amide nitrogen atom, if the sulphur centre is substituted[44] (as shown in Figure 4).

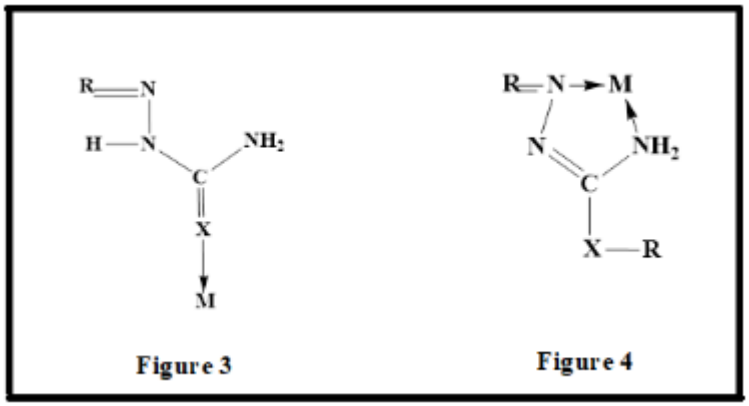


Figure 3 Figure 4

The coordination of thiosemicarbazone to most of the metals takes place in the *cis*-configuration, as shown in Figure 5, with the bonding through thiol/thione sulphur and hydrazine nitrogen in a bidentate manner.

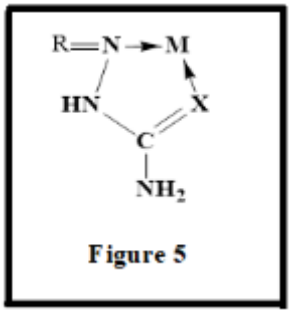


Figure 5

The presence of an additional coordinating atom in the proximity of S and N donating centres makes thiosemicarbazone to behave as a tridentate ligand (Figure 6).

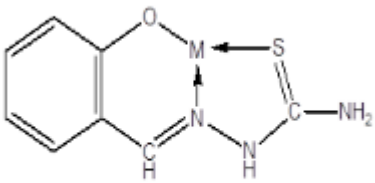


Figure 6

Thiosemicarbazones are the main class of sulphur donor ligands, particularly for transition metal ions, forming a variety of metal complexes, because of the high polarizability of electrons on the sulphur atom and the availability of unoccupied 3d orbitals for back bonding. A variety of coordination modes with potential donor atoms render flexibility to the ligand and it coordinates to the metal ion in a neutral or deprotonated form, yielding mono or polynuclear

complexes[45-48]. In addition to enhancing the biological activity of thiosemicarbazones and substituted thiosemicarbazones, through synergic effects they lead to reduced toxicities of metal ions and have become a reliable source of novel biologically active compounds[49,50].

1.2.3 Metal complexes of thiosemicarbazone

The synthesis and characterization of nitrogen and sulfur donor ligands have evolved during the last few years as one of the main research areas in coordination chemistry. Among the nitrogen/sulfur compounds, thiosemicarbazones have received considerable attention. They are an important class of organic ligands ($R^1R^2C^2=N^3-N^2(H)-C(=S)N^1R^3R^4$), with the affinity to coordinate a metal through its different donor atoms which has led to the formation of a variety of coordination compounds[51-54]. These metal complexes exhibit different binding modes and structures[55-58]. A number of reasons can be mentioned to be responsible for their versatility in coordination, such as intramolecular hydrogen bonding, steric crowding on the azomethine carbon atom and p–p stacking interactions[59–62]. The chemistry of thiosemicarbazones and their metal complexes is also important due to their potential and promising biological applications[58,63-65], catalytic-[58,66-68] and metal sensing properties[69-71].

The synthesis and characterization of 3-bromo-5-chloro-2-hydroxyacetophenone-N(4)-hexyl (L^1H_2), -cyclohexyl (L^2H_2) and -phenyl (L^3H_2) thiosemicarbazone ligands and their three nickel(II) complexes were reported by Güveli and co-workers[72]. The single crystal

X-ray diffraction studies of the complexes established the dibasic forms (L^{2-}) of hexyl and phenyl substituted and monobasic form (LH^{-}) of cyclohexyl substituted thiosemicarbazones in the nickel chelates. The electrochemical parameters indicated a relatively high negative potential values for their irreversible redox processes, when compared with similar thiosemicarbazone ligands[73-75]. It could be due to the relatively high carbon number (6) of the substituent present on the N(4) atom.

A new series of Ni(II) complexes containing 4-chromone-N(4)-substituted thiosemicarbazones was reported by Selvamurugan *et al*[76]. They were synthesized and characterized by analytical and various spectroscopic techniques (FT-IR, electronic, ^{1}H, ^{13}C, ^{31}P NMR and ESI-Mass). The ligands were found to act as tridentate ONS donors towards the metal. Drug-like properties of the new complexes were analyzed by a series of biological investigations such as DNA/protein interaction studies and *in vitro* cytotoxic study against human breast cancer cell line (MCF-7). The DNA binding studies revealed that the complexes bind to DNA *via* intercalative mode. However, the complex with the phenyl substituent on the ligand moiety showed considerably good DNA and protein binding interaction. In addition, the evaluation of cytotoxicity showed that the complexes have good anticancer activity against human breast cancer cell line (MCF-7) due to the electron withdrawing nature of the phenyl group on the terminal nitrogen atom of the thiosemicarbazone.

Ferraz and co-workers reported N(3)-meta-chlorophenyl- and N(3)-meta-fluorphenyl thiosemicarbazones derived from acetophenone and benzophenone as well as their Zn(II) complexes[77]. These bidentate

thiosemicarbazones coordinate to Zn(II) forming [Zn(L_2)] (L = anionic thiosemicarbazone). The presence of triethylamine in the reaction mixture and the excess of Zn(II) probably favored the formation of tetrahedral [Zn(L_2)] instead of octahedral [Zn($L)_3$] complexes, owing to induced deprotonation at N(2). Furthermore, the rigidity of the thiosemicarbazone's N-S chelating system is accountable for the highly distorted tetrahedral geometry of the complexes.

Six copper(II) complexes of 2-benzoylpyridine-N^4,N^4-dimethyl-3-thiosemicarbazone (HL) were reported by Jayakumar et *al*[78]. Various physico-chemical techniques such as partial elemental analysis, magnetic susceptibility and conductivity measurements, FT-IR, UV–Vis and EPR studies were used to characterize them. From the single crystal X-ray diffraction studies, it was found that each copper center has a distorted square pyramidal geometry with the basal plane occupied by NNS donor atoms of thiosemicarbazone and the acetate oxygen. From the physico-chemical studies, it was found that thiosemicarbazone coordinated through one of the pyridyl nitrogen, azomethine nitrogen and thioiminolate sulfur atoms. Binuclear natures of some of the complexes were confirmed from EPR parameters and magnetic moment values.

Ilies and co-workers[79] reported the synthesis of six new copper(II) complexes with 3-formyl-6-methylchromone-4-phenylthiosemicarbazone ligand (HL). The thiosemicarbazone was characterized by ^{1}H- and ^{13}C NMR spectroscopy. The complexes were characterized by electronic- and IR spectroscopy, EPR studies, molar conductivity and magnetic susceptibility measurements and elemental analysis. The antibacterial and antifungal activities of the copper(II)

complexes and the free ligand were determined and found that the metal complexes generally have a better activity than the free ligand. It was also found that the antimicrobial activity depended on the structures of the compounds tested.

Oliveira *et al*[80] synthesized a series of manganese complexes derived from 2- acetylpyridine-N(4)-R-thiosemicarbazones (Hatc-R), through a systematic variation of the substituents at N(4) as H, methyl, ethyl, cyclohexyl, phenyl and morpholinyl, in satisfactory yields. They were fully characterized by various analytical methods and single crystal X-ray diffraction studies. The results obtained were consistent with the monoanionic N, N, S-tridentate donor nature of the thiosemicarbazone ligands, resulting in octahedral complexes. The biological assays of these compounds showed better results and described these compounds as promising anti M.Tuberculosis agents, similar to or better than some commercial drugs available for the tuberculosis treatment. The coordination of thiosemicarbazones to the Mn(II) center may result in the improvement of their anti M.Tuberculosis activities.

The synthesis, characterization and biological studies of 2-acetylpyridine N-substituted thiosemicarbazones and their Co(III) complexes were reported by Manikandan and co-workers[81]. Their characterization by elemental analysis and various spectral studies supported the NNS-tridentate donor nature of the ligands in the Co(III) complexes. The two ligand molecules are deprotonated at the imino nitrogen resulting in two tridendate monoanionic species coordinating to the central metal *via* the thiolato sulfur, imine nitrogen and pyridine nitrogen, resulting in a distorted octahedral moiety. *In vitro* DNA

binding studies of the complexes revealed that their binding to DNA took place through intercalative mode. The free radical scavenging ability of the complexes was analysed for the antioxidant property evaluation, which revealed that all the complexes exhibited higher activities compared to the standards. Moreover, *in vitro* cytotoxicity of all the three complexes was assayed and found that they efficiently vanished the cancer cells even at low concentrations.

Li *et al*[82] reported the synthesis, characterization, crystal structures, biological activities and fluorescence studies of complexes of Cu(II), Zn(II) and Ni(II) with 3-carbaldehyde chromone thiosemicarbazone. Crystal structures of Zn(II) and Ni(II) complexes were confirmed from single crystal X-ray diffraction analysis. Interactions of the ligand and Cu(II), Zn(II) and Ni(II) complexes with DNA were investigated and found that the compounds bound to DNA *via* intercalative mode, especially, the Zn(II) complex bound to DNA very strongly than the free ligand, Cu(II)- and Ni(II) complexes. The metal complexes can stack between the DNA base pairs more easily and deeply than the free ligand. *In vitro* antioxidant activities of the compounds were studied and found that they possessed significant activity against superoxide- and hydroxyl radicals, and the scavenging property of Cu(II) complex were better than the ligand, Zn(II) and Ni(II) complexes and some standard antioxidants, such as mannitol and vitamin C.

Sampath and co-workers[83] prepared four new Ru(II) complexes of benzaldehyde 4-methyl-3-thiosemicarbazones as ligands and the PPh_3/$AsPh_3$ as co-ligands. They were fully characterized by various spectro-analytical techniques. The Schiff base ligands acted as

bidentate, monobasic chelating ones with N and S as the donor sites and preferably they existed in the thiol form in all the studied complexes. The complexes were tentatively assigned an octahedral geometry. All the newly synthesized compounds had been examined for their biological properties, like DNA binding, DNA cleavage, antioxidant and cytotoxicity under *in vitro* experimental conditions. The DNA binding abilities of the ligands and complexes were investigated and found that the complexes bound to DNA *via* an intercalative mode. Further, *in vitro* antioxidant, anticancer and cytotoxic studies carried out on the ligands and Ru(II) complexes showed that all the Ru(II) complexes served as potential antioxidants and good cytotoxic agents than the ligands. The significant result of this investigation was that the thiosemicarbazone moiety containing triphenylphosphine as co-ligand, led to an improved interaction with DNA, free radical and tumor cell line than the rest of the complexes.

Isatin (1H-indole-2, 3- dione) was first discovered by Erdmann and Laurent in 1840 as a product of indigo oxidation[84,85]. Isatin is an indole derivative generally formed endogenously both in human and other mammalian tissues and fluids. Isatin has also been recognized in natural products, such as in human body as a metabolic derivative of adrenaline, marine molluscs, fungal metabolites and in the fruits of the cannon ball tree[86–88]. The synthetic creativity of isatin has led to an extensive use of this compound in organic synthesis. The isatin ring is a major structural motif found in several pharmacologically active compounds by virtue of its typical size and privileged electronic properties[89-91].

Rodriguez-Argiielles *et al*[92] reported the synthesis and characterization of manganese, iron, cobalt, nickel, copper and zinc complexes of isatin-β-thiosernicarbazone (LH_2). The X-ray crystal structure studies of two nickel complexes, namely $[Ni(HL)_2]$.EtOH and $[Ni(HL)_2]$.2DMF revealed a distorted octahedral coordination with the mono deprotonated ligand that behaved as an O,N,S terdentate donor. ^{1}H- and ^{13}C NMR studies of the ligand and its zinc complexes in solution were carried out. Biological studies, carried out *in vitro* on human leukaemic cells have shown that the free ligand and the copper complex were more active in the inhibition of cell proliferation than the nickel complexes, and none of these was able to induce apoptosis.

N(4)-methyl- and N(4)-dimethyl-1-methylisatin thiosemicarbazones, (HMIs4M and HMIs4DM) and their Co(II), Ni(II) and Cu(II) complexes have been prepared by West and co-workers[93]. They were characterized by elemental analyses, molar conductivity- and magnetic susceptibility measurements and spectral techniques. The two thiosemicarbazones coordinated as monoanions with the loss of N(2)H, *via* the azomethine nitrogen, thiolato sulfur atoms and the isatin oxygen {except for $[Cu(MIs4M)_2]$ and $[Cu(MIs4DM)_2]$} to give complexes of the general formulae $[M(MIs)_2]$ and [M(MIs)Cl], where M = Co(II), Ni(II) or Cu(II) and MIs = HMIs4M or HMIs4DM. Binuclear $[Cu(MIs4M)Br]_2$ and $[Cu(MIs4DM)Br]_2$ complexes with low magnetic susceptibilities were formed with Cu(II) bromide.

High levels of reactive oxygen species (ROS), such as hydrogen peroxide, superoxide anion and hydroxyl radical, formed during the normal functions of organs or excessive oxidative stress, can harm various biomolecules such as nucleic acids, lipids and

proteins in cells and tissues[94]. The detection of a variety of diseases by free radicals has led to new medical perceptions, thereby leading to the exploration of compounds that can prevent the formation of free radicals. These compounds that can act as scavenger of oxygen radicals are known as antioxidants[95]. Thiosemicarbazone derivatives of chitosan have considerable antioxidant- and oxygen free-radical scavenging activities. The –NH- and C=S groups in the thiosemicarbazone react with free radicals, thereby increasing the antioxidant activity of these compounds[96,97]. The biological activities of certain thiosemicarbazones, such as isatin thiosemicarbazone, are correlated to their ability to form chelates with metal ions through O, N, and S atoms[98]. Among the various chelates, copper compounds are important ones that inhibit cell proliferation[99–102]. Several copper complexes have also been described to cleave DNA through oxidative mechanism which requires oxygen[103] Copper compounds of thiosemicarbazones can induce ROS production *in vitro* with significant DNA degradation[104] through the redox cycling between the Cu(II) and Cu(I).

A promising anti TB lead compound, Schiff base of isatin derivative and nalidixic acid carbohydrazide has been reported by Aboul-Fadl *et al*[105]. In recent years, Schiff bases of 1H-indole-2, 3-diones were reported to exhibit anti-TB activity. Consequently, this work involved the design and synthesis of Schiff bases of nalidixic acid carbohydrazide and isatin derivatives. Their structures were confirmed from the spectral analyses. They showed modest anti TB activity. However, Schiff base compound derived from nalidixic acid

carbohydrazide and N-benzylisatin showed high anti TB activity, which was 20 times greater than the reference drug, isoniazid.

Vine and co-workers[106] synthesized a variety of substituted 1H-indole-2,3-diones (isatins) using standard procedures and they were characterized by various spectral techniques. *In vitro* cytotoxicity was evaluated against the human lymphoma cell line. Of the 23 compounds tested, four were selected for further screening against a panel of five human cancer cell lines. These compounds, in general, showed better selectivity toward leukemia and lymphoma cells over carcinoma cell lines. The most active compound, 5,6,7-tribromoisatin was found to be antiproliferative. This indicated that di- and tri-substituted isatins may lead to new anticancer drug in the future with enhanced selective activity.

Natural products have been reported to exhibit promising antiinfective activity. Among them, flavonoid frameworks are the principle candidates. Flavonols, flavones, flavanones, isoflavones, aurones and chalcones are well known for their remarkable antiinfective activities. Kostanecki and Tambor were the first to synthesize a series of natural chromophoric products comprising of α, β unsaturated carbonyl bridge and termed them "chalcones"[107]. Chalcones or 1, 3-diphenyl-2E-propene-1-one, constitute an important class of natural products across the plant kingdom and have recently been of great importance for their interesting pharmacological activities[108]. The chemistry of chalcone remained as a fascination among researchers in the 21st century due to its simple chemistry, ease of synthesis and a large number of replaceable hydrogen to yield great number of derivatives.

Chalcones are structurally related to curcumin, a known antioxidant[109] that is being studied widely for its activity as a chemopreventive agent[110]. Hence, they are effective as *in vivo* cell proliferating inhibitors, antitumor promoting and chemo preventing agents. Cytotoxic effects of antioxidant flavonoids (including chalcones) are associated with their pro-oxidant effects[111]. The antioxidant properties of chalcones are identified to be influenced largely by the two aryl structures, joined by a three carbon α, β-unsaturated carbonyl bridge. The presence of double bond in conjugation with the carbonyl functionality is thought to be responsible for the biological activities of chalcones, as their removal will make them inactive.

A series of novel chalcone thiosemicarbazone derivatives have been designed and synthesized by Zhang and co-workers[112]. They were structurally characterized and their biological activities were evaluated as potential anticancer- and antiproliferative agents against human liver carcinoma cell. Among these compounds, methyl substituted compound showed the most potent biological activity, which was comparable to the positive controls. In order to achieve more information of the structure-activity relationships, molecular docking of the most potent methyl substituted inhibitor was performed on the binding model. Antiproliferative assay results demonstrated that some of these compounds possessed good antiproliferative activity. Methyl substituted compound with potent inhibitory activity in tumor growth inhibition may be a potential anticancer agent. Analysis of the binding conformation of the compound demonstrated that it was stabilized by

hydrogen bonding interaction and a cation-pi interaction between benzene ring and nitrogen atom of the amino acid residue.

The synthesis of several new hexa-coordinated Ru(II) chalcone thiosemicarbazone complexes of the type, [Ru(CO)(EPh_3)(B)(L)] (E = P or As; B = PPh_3, $AsPh_3$ or Py; L= chalcone thiosemicarbazone) were reported by Muthukumar and Viswanathamurthi[113]. They have been prepared by reacting [RuHCl(CO)(EPh_3)$_2$(B)] (E = P or As; B = PPh_3, $AsPh_3$ or Py) with chalcone thiosemicarbazones in benzene under reflux. The chalcone thiosemicarbazones were derived from 2-hydroxychalcone and thiosemicarbazide. The new complexes have been characterized by various analytical and spectroscopic (IR, UV-Vis, ^{1}H, ^{31}P and ^{13}C NMR) methods. Based on the data, an octahedral structure was proposed for all the complexes. The chalcone thiosemicarbazones behaved as dianionic tridentate O, N, S donors coordinating ruthenium *via* the phenolic oxygen, the imine nitrogen and thiol sulfur. The new complexes displayed efficient catalytic activity with higher conversion rate and excellent yields in short reaction time.

Thiosemicarbazones of cinnamaldehyde and cuminaldehyde and their Ni(II) and Cu(II) complexes were synthesized and characterized by Bisceglie *et al*[114]. The compounds were tested *in vitro* for their antileukemic activity. The results showed that cinnamaldehyde thiosemicarbazone (Htcin) was unable to exert any effect even at high concentrations while cuminaldehyde thiosemicarbazone (Htcum) inhibited cell proliferation. All the complexes had very low IC_{50} values when compared with the most famous metal-based drug, *cis*-platin. It was striking that in the case of

cinnamaldehyde derivatives, upon coordination to metal ions, the bioactivity considerably increased confirming that complexation can be an interesting strategy for dose reduction. It was confirmed that the copper complexes, [Cu(tcum)(H_2O)Cl] in particular was found to be more active.

Datta and co-workers[115] reported the reactions of salicylaldehyde thiosemicarbazone, 2-hydroxyacetophenone thiosemicarbazone and 2-hydroxynaphthaldehyde thiosemicarbazone with $Ni(ClO_4)_2.6H_2O$, which afforded dimeric complexes of type, $[\{Ni(L)\}_2]$. In all the complexes, thiosemicarbazones coordinated to nickel as dinegative tridentate ONS-donors. The mixed-ligand nickel complexes were recognized as successful catalysts for Heck type C–C coupling reactions. *In vitro* cytotoxicity screening studies of the six mononuclear nickel complexes have been also carried out in human tumour cell lines.

Two new ligands derived from coumarin and thiosemicarbazide were reported by Mosa *et al*[116]. They were used for the preparation of a series of transition metal complexes. Elemental analyses, IR, UV–Vis, and electrical conductivity, magnetic susceptibility measurements and thermal analyses confirmed the structures of these complexes. IR spectral data indicated that in all the complexes, the ligands acted as monobasic tridentate, coordinating through keto oxygen or sulfur, azomethine nitrogen and deprotonated phenolic oxygen atoms. Based on other physico-chemical investigations, tetrahedral- or square planar geometries were assigned for Cu(II) complexes. In the case of the Co(II), Ni(II) and Fe(III) complexes, octahedral structures were suggested.

1.2.4 Hydrazones

Hydrazones are a group of azomethine compounds with >C=N-N< group and are distinguished by the presence of two interlinked nitrogen atoms. Ever since Emil Fischer established the use of hydrazones in chemistry in 1884, a wide range of applications have been reported[117]. Hydrazones have attracted considerable attention due to their striking chemical and physical properties[118,119], biological activities[120,121] and their analytical applications[122]. The Wolff–Kishner reduction or aza-Nenitzescu indole cyclisation are examples for the significance of hydrazones in chemical synthesis[123,124]. They play important roles in pharmacology and as biological materials, due to their sensitivity and selectivity to metal ions[125-128]. They have also gained importance as chromogenic reagents for the spectrophotometric determination of transition metal ions, metal extractants and biologically active compounds[129]. They are also useful in the field of molecular recognition, as selective sensors[130–132]. The metal complexes of hydrazones with heterocyclic moieties containing nitrogen, oxygen and sulfur as heteroatoms have been studied widely in order to ascertain a probable relationship between the chemical structure and biological activity[133–136]. The presence of >C=O donor site gives flexibility to acylhydrazones and their metal complexes [i.e, Cu(II), Co(II) and Pt(II) complexes] are known for their broad spectrum of biological and pharmaceutical activities, such as inhibition of tumor growth, antioxidative effect, antimicrobial and antiviral activities[137-140].

1.2.5 Metal complexes of Hydrazones

Pyridoxal isonicotinoyl hydrazone and its copper(II) complexes were synthesized and characterized by Mezey *et al*[141]. The molecular structure of the ligand was confirmed from the physico-chemical data, while the stereochemistries of the complexes were proposed based on IR, UV–VIS, EPR spectroscopy and magnetic moment values. In all the chelate compounds, the ligand acted as a tridentate monoanionic one retaining their keto tautomeric form. The microbiological effects of the ligand and all the five complexes against both Gram positive and Gram negative strains were tested. It was found that the ligand had a low effect on the tested pathogens and the MIC values of the complexes indicated an improved antibacterial activity after coordination. This behaviour underlines the structural influence of the Cu(II) complex on the microbiological activity.

Complexes of the chloride, nitrate and acetate salts of Co(II), Ni(II) Cu(II), Zn(II), Cd(II) and Hg(II) with 2,3-butanedione bis(isonicotinylhydrazone) [BBINH] have been synthesized and structurally characterized by El-Sayed *et al*[142]. The complexes were assigned structures based on the elemental analysis and mass spectra. BBINH behaved as a neutral tetradentate (N_2O_2) in the chloride complexes of Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II). In the acetate complexes of nickel and copper, BBINH acted as a dinegative-tetradentate with the two metal ions. In the nitrate complexes, the ligand acted as a neutral bidentate using the two azomethine units. The data were supported by ^{1}H and ^{13}C NMR spectra. The magnetic moments and electronic spectra of all complexes indicated tetrahedral,

square planar and/or octahedral structures. The complexes were active against certain bacteria and fungi. The ligand was inactive against all the tested organisms. The improved biological activities of Cd(II) and Hg(II) complexes may be related to their geometry.

Angelusiu *et al*[143] reported a new aroylhydrazone ligand, formed by the condensation of 4-(4-chloro-phenylsulfonyl)benzoylhydrazide with 2-pyridinecarbaldehyde and their Cu(II), Co(II) and Ni(II) complexes. The structures of these compounds have been confirmed from various analytical methods and spectral techniques. The semi-empirical method and the IR spectra signified that the ligand behaved as mononegative bidentate/tridentate with NO/NON donor sequence in E-isomeric form towards the metal ions. The magnetic and spectral data proposed a square-planar geometry for Ni(II) complex and an octahedral or pseudo-tetrahedral geometry for Co(II) and Cu(II) complexes. Antibacterial activity of acyl hydrazone and its complexes were studied against Gram positive bacteria: Staphylococcus aureus, Bacillus subtilis and Gram negative bacteria: Pseudomonas aeruginosa, Escherichia coli by minimum inhibitory concentration (MIC) method. A comparative study of MIC values indicated that the complexes displayed higher antibacterial activity against Gram negative bacteria than the free ligand, but it was weak compared to the standard drug.

Jeragh and El-Asmy[144] reported the reaction between 2,5-hexanedione and salicylic acid hydrazide. The complexes of 2,5-hexanedione bis(salicyloylhydrazone) with Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Hg(II) and Pd(II) were characterized by elemental analyses, spectral studies (IR, ^{1}H NMR, ESR and MS), thermal- and magnetic measurements. The magnetic moments and electronic spectra

of the complexes indicated their geometries. The ESR spectra agreed with the mononuclear structure for the copper(II) complex. The thermal decomposition of the complexes revealed the coordinated nature of the water molecules in them. Another organic compound isolated during the preparation of the hydrazone was N-(2,5-dimethyl-1H-pyrrol-1-yl)-2-hydroxybenzamide, and its structure was solved crystallographically.

Singh and co-workers[145] synthesized a series of complexes of Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) with a novel Schiff base ligand, o-hydroxyacetophenone-2-thiophenoylhydrazone. All the compounds were characterized by elemental analyses, magnetic susceptibility measurements, electronic, IR, NMR and ESR spectral techniques. The structures of the ligand and its Mn(II) complex have also been determined from X-ray diffraction studies. The hydrazone ligand displayed E-configuration about the >C=N– bond. In all the complexes, the hydrazone behaved as monoanionic tridentate ligand that coordinates to the metal ions through deprotonated phenolate oxygen, carbonyl oxygen and azomethine nitrogen. Magnetic moments and electronic spectral studies proposed octahedral geometries around metal ions in all the complexes. The ligand and its metal complexes showed significant corrosion inhibition activity against mild steel in 1M HCl solution. The metal complexes showed better inhibition efficiency than the free ligand.

Hydrazones are present in many of the heterocyclic compounds that are of broad interest due to their biological applications. The work reported by El-Wahab[146] focused on the synthesis of the hydrazone and its metal complexes and their potential applications as antimicrobial,

antifouling and flame retardant additives in epoxy formulations for surface coating application. Co(II), Cr(III) and Fe(III) complexes of arylhydrazones, namely o-hydroxyacetophenone benzoylhydrazone have been isolated and characterized by elemental analysis and spectral techniques. The results of non-flammability of the epoxy resin containing the ligand and its metal complexes as additives, indicated a very good flame retardancy effect. They also showed exceptional flame retardancy properties when compared to the blank epoxy resin. The results of the biological activity indicated that the ligand and its metal complexes exhibited a very good antimicrobial- and antifouling effects, with the metal complexes showing better activities than the ligand. Thus, the incorporation of these additives into an epoxy paint results in enhanced antimicrobial and antifouling properties.

Mandewale and co-workers[147] synthesized some new hydrazone derivatives with quinoline core (quinoline hydrazone ligands) by the condensation of 2-hydroxy-3-formylquinoline with hydrazide derivatives. Among the ligand systems, quinoline hydrazone derivatives were highly significant due to their diverse chelating ability, structural flexibility and a large number of pharmacological activities. The preliminary results of antituberculosis study showed that most of the Zn(II) complexes demonstrated very good activity while the ligands showed only moderate activity. The inclusion of Zn(II) effectively increased the conformational rigidity of the hydrazones and increased the fluorescence intensities of complexes.

Ferreira *et al*[148] obtained the complexes of bismuth with 2-acetylpyridine phenylhydrazone, as well as with the 2-benzoylpyridine phenylhydrazone analogues. The antibacterial

activities of these hydrazones against Gram positive and Gram negative bacterial strains was improved significantly upon their coordination to Bi(III). The cytotoxic effects of the compounds under study were evaluated on solid tumor cells as well as on non-malignant cells. In general, 2-acetylpyridine derived hydrazones proved to be more effective and more selective as cytotoxic agents than the corresponding 2-benzoylpyridine derived counter parts. Exposure of solid tumour cells to 2-acetylpyridine phenylhydrazone and its Bi(III) complex led to 99% decrease of the clonogenic survival.

1.**3 Conclusions**

Schiff bases continue to exist as a vital class of organic ligands for metal complexation, due to their ready availability and facile structural alteration[149,150]. In recent years, transition metal complexes of Schiff base ligands have attracted attention of chemists due to their extensive applications. Among them, thiosemicarbazones are recognized as an important class of versatile multi-donor ligands in coordination chemistry. They are considered as mixed hard–soft chelating agents since they contain both N and S donor atoms.

Aroylhydrazones as well constitute a versatile type of Schiff base compounds that contain amide oxygen and the imine nitrogen as donor sites. Interest in coordination chemistry of hydrazones has paid attention on the introduction of additional coordination sites by using both aldehyde and hydrazide precursors, which bring probable expansion of the structures of their coordination complexes. These ligands as well as their metal complexes have aroused great interest

because of their analytical applications and attractive chemical and structural properties. They possess a wide variety of biological activities such as antitumoral, fungicidal, bactericidal and antiviral activities. Metal complexes derived from such ligands encapsulated by N, O and S donor atoms, appear as particularly attractive, owing to the stability that they confer to their complexes by chelation.

In this brief literature survey, it has been found that there are only a few reports on the synthesis, characterization and application of N-disubstituted thiosemicarbazones and hydrazones from isatin, unsaturated aldehydes and chalcones. Therefore, the present research programme has been focused on the synthesis, characterization and application of metal chelates derived from N(4)-methyl(phenyl)thiosemicarbazones and hydrazones.

1.4 Significance and scope of the present investigation

The preparation of a novel ligand is the most important step in the development of metal complexes that exhibit unique properties and reactivity. This may lead to the formation of structurally diverse coordination compounds capable of potential applications. The synthesis of nitrogen and sulphur donor ligands, particularly, thiosemicarbazones has received great attention due to their wide range of pharmacological- and biological applications. The structural diversity of such compounds can be considerably increased by the use of the different carbonyl compounds and by alkyl/aryl substitution on the thiosemicarbazide moiety[151]. Both the approaches reveal an incredible structural flexibility to this class of compounds.

Incorporation of metals in these thiosemicarbazone ligands can result in a remarkably stable and attractive metal chelates with modified or improved biological activities[152,153]. In addition to these, metal complexes of thiosemicarbazones and substituted thiosemicarbazones have lead to reduced toxicities through synergic effects.They have also turned out to be reliable sources for discovering novel biologically active compounds[154,155].

The probability is that structural variation by substitution on the hydrazone or thiosemicarbazone moiety as well as incorporation of the heterocyclic ring might prove beneficial and lead to spectacular improvement in the properties of such novel compounds. All these factors promoted us to carry out the synthesis of N(4)-methyl(phenyl)thiosemicarbazones and hydrazones and several complexes of these ligands.

In the present investigation, we studied the interactions of N(4)-methyl(phenyl)thiosemicarbazide and isonicotinic acid hydrazide with several carbonyl compounds such as isatin, crotonaldehyde and chalcone. The following were the ligands prepared and characterized during these studies.

1.Crotonaldehyde N(4)-methyl(phenyl)thiosemicarbazone (CRTSC)
2.Chalcone N(4)-methyl(phenyl)thiosemicarbazone (CHTSC)
3.Isatin N(4)-methyl(phenyl)thiosemicarbazone (ISTSC)
4.Isatin 2-methoxyisonicotinoylhydrazone (ISISNHY)

The complexes of Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) with these ligands were prepared. Various physico-chemical methods like elemental analysis, magnetic susceptibility measurement and IR-, UV-Vis- and ^{1}H NMR spectral techniques were employed for

the characterization of these compounds. They were assigned the tentative structures. The synthesis and characterization of the ligands and the complexes are included in the Part I of the thesis.

Besides the structural characterization of the ligands and the complexes, we have conducted the antibacterial studies of isatin N(4)-methyl(phenyl)thiosemicarbazone, crotonaldehyde N(4)-methyl (phenyl)thiosemicarbazone and their Ni(II) and Cu(II) complexes. The antifungal studies of crotonaldehyde N(4)-methyl (phenyl)thiosemicarbazone and their Ni(II), Cu(II) and Zn(II) complexes were also carried out. The results of antimicrobial studies are included in the Part II of the thesis (Section A).

Literature survey on the antitumour activities of thiosemicarbazones persuaded us to conduct the cytotoxic- and antitumour studies of isatin N(4)-methyl(phenyl)thiosemicarbazone and its complexes. Copper(II) complex of isatin N(4)-methyl(phenyl)thiosemicarbazone was selected for conducting the *in vivo* antitumour studies. The results of the antitumour studies are included in the Part II of the thesis (Section B).

1.5 References

[1] G. Wilkinson, R. D. Gillard, A. McCleverty Comprehensive Coordination Chemistry The Synthesis, Reactions, Properties & Applications of Coordination Compounds. Vol 1 Pergamon Press, Oxford (1987)

[2] F. W. Clarke, J. Chem. Soc., Trans., 95, 1299 (1909)

[3] J. D. Corbett, Acc. Chem. Res., 14, 239 (1981)

[4] W. B. Schapp, A. E. Messner, F. C. Schmidt, J. Am. Chem. Soc., 77, 2683 (1955)

[5] G. F. Reynolds, 'Progress in Polarography', Wiley, New York, 243 (1962)

[6] M. Maskovits, G. Ozm, 'Cryochemistry' ed., Wiley, New York (1976)

[7] Anon, Chem. Eng. News., 27, 21 (1984)

[8] R. E. Sievers, R. W. Moshier, M. L. Morris, Inorg. Chem., 1, 966 (1962)

[9] R. E. Sievers, B. W. Ponder, M. L. Morris, R. W. Moshier, Inorg. Chem., 2, 693 (1963)

[10] S. J. Lippard, J. M. Berg, 'Principles of Bioinorganic Chemistry', University Science Books, California (1994)

[11] A.E. Martell, R. D. Hancock, 'Coordination Chemistry: A century of progress' ACS Symp. Ser., 565 (1994)

[12] L. O. Tiffen, 'Biological implications of metals in environment' Proceedings of the 15th Annual Hanford Life Sciences Symposium, Richland, USA (1975)

[13] G. Wilkinson, 'Comprehensive Coordination Chemistry' 1sted, Peragmon, New York, 6 (1987)

[14] G. A. Melson, R. W. Stotz, Coord. Chem. Rev., 7, 133 (1971)

[15] M. A. Hunter, J. Am. Chem. Soc., 32, 330 (1910)

[16] A.E. Van Arkel, J. H. de Boer, Z. Anorg. Allg. Chem., 148, 345 (1925)

[17] W. Kroll, Trans. Electrochem. Soc., 78, 35 (1940), J. Less. Common. Met., 8, 361 (1965)

[18] M. E. Weeks, H. M. Leicester, 'Discovery of the Elements' 7th ed, Chemical Education Publishing, Easton, PA, 351 (1968)

[19] G. Wilkinson, F. G. A. Stone, E. W. Abel (eds), 'Comprehensive Organometallic Chemistry' Pergamon, Oxford III (1982)

[20] G. D. Lawrance, D.T. Sawyer, Coord. Chem. Rev., 27, 173 (1978)

[21] S. Arhland, J. Chatt, N. R. Davies, Q. Rev., Chem. Soc., 12, 265 (1958)

[22] B. N. Figgis, M. Gerloch, R. Mason, Proc. R. Soc., (London), Ser. A, 91, 309 (1969)

[23] G. M. Bancroft, M. J. Mays, B. E. Prater, J. Chem. Soc., (A), 956 (1970)

[24] A.W. Adamson, J. Am. Chem. Soc., 73, 5710 (1951)

[25] J. W. Mellor, 'A Comprehensive Treatise on Inorganic and Theoretical Chemistry' Longmans, London XV (1961)

[26] F. A. Cotton, G. Wilkinson, 'Advanced Inorganic Chemistry' 4th ed, Wiley Interscience, New York 785 (1980)

[27] L. Sacconi, 'Transition Metal Chemistry' ed, R. L. Carlin, Dekker, New York IV, 199 (1968)

[28] Gmelin, 'Handbook of Inorganic Chemistry' Springer Verlag, Berlin (1955); V. M. Goldschmidt, J. Chem. Soc., Dalton Trans., 656 (1975)

[29] D. Mendeleev, J. Russ. Phys. Chem. Soc., 1, 60 (1869); L. S. Foster, J. Chem. Educ., 16, 409 (1939)

[30] J. W. van Spronsen, 'The Periodic System of the Elements' Elsevier, Amsterdam (1969)

[31] A. Massey, 'Comprehensive Inorganic Chemistry' ed J. C. Bailar, H. J. Emeleus, R. S. Nyholm, A. F. Trotman Dickinson, Pergamon, Oxford III, 1 (1973)

[32] N. V. Sidgwick, 'Chemical Elements and their compounds' Clarendon, Oxford I, 103 (1949)

[33] C. K. Jorgenson, 'Adsorption spectra and Chemical bonding' Academic, London (1962); 'Inorganic complexes' Academic, London (1963)

[34] B. J. Aylett, 'The Chemistry of Zinc, Cadmium and Mercury' Pergamon, New York (1975)

[35] K. Dey, J. Scient. Ind. Res., 33, 76 (1974)

[36] H. S. Seleem, G. A. El-Inany, M. Mousa, F. I. Hanafy, Spectrochim. Acta Part A.,74, 869 (2009)

[37] V. P. Singh, Spectrochim. Acta Part A., 71, 17 (2008)

[38] A. I. Matesanz, C.Hernandez, A. Rodriguez, P. Souza, J. Inorg. Biochem., 105, 1613 (2011)

[39] H. Beraldo, D. Gambino, Mini Rev. Med. Chem., 4, 31 (2004)

[40] D. K. Dermertzi, M. A. Demertzis, J. R. Miller, C. S. Frampton, J. P. Jasinski, D. X. West, J. Inorg. Biochem., 92, 137 (2002)

[41] J. G. Tojal, A. G. Orad, J. L. Serra, J. L. Pizarro, L. Lezamma, M. I. Arriortua, T. Rojo, J. Inorg. Biochem., 75, 45 (1999)

[42] R. N. Prabhu, D. Pandiarajan, R. Ramesh, J. Organomet. Chem., 694, 4170 (2009)

[43] J. S. Casas, M. S. Garcia-Tasende, J. Sordo, Coord. Chem. Rev., 209, 197 (2000)

[44] N. V. Gerbeleu, M. D. Revenko, V. M. Leovats, Russ. J. Inorg. Chem., 22, 1009 (1977)

[45] L. S. Shebaldina, O.V. Kovalchukova, S. B. Strashnova, B. E. Zaitsev, T. M. Ivanova, Russ. J. Coord. Chem., 30, 38 (2004)

[46] R. Prabhakaran, K. Palaniappan, R. Huang, M. Sieger, W. Kaim, P. Viswanathamurthi, F. Dallemer, K. Natarajan, Inorg. Chim. Acta., 376, 317 (2011)

[47] H. B. Shawish, M. Paydar, C.Y. Looi, Y. L. Wong, E. Movahed, S. N. A. Halim, W. F. Wong, M. Mustafa, M. J. Maah, Transition Met. Chem., 39, 81 (2014)

[48] R. Prabhakaran, P. Kalaivani, P. Poornima, F. Dallemer, G. Paramaguru, V. Vijaya Padma, R. Renganathan, R. Huange, K. Natarajan, Dalton Trans., 41, 9323 (2012)

[49] Z. Iakovidou, E. Mioglou, D. Mourelatos, A. Kotsis, M. A. Demertzis, A. Papagoergiou, J. R. Miller, D. Kovala-Demertzi, Anticancer Drugs, 12, 65 (2001)

[50] D. Kovala-Demertzi, M. A. Demertzis, J. R. Miller, C. Papadopoulou, C. Dodorou, G. Filousis, J. Inorg. Biochem., 86, 555 (2001)

[51] M. A. Ali, S. E. Livingstone, Coord. Chem. Rev., 13, 101 (1974)

[52] S. Padhye, G. B. Kauffman, Coord. Chem. Rev., 63, 127 (1985)

[53] D. X. West, S. Padhye, P. B. Sonawane, Struct. Bonding (Berlin), 76, 4 (1991)

[54] D. X. West, A. E. Liberta, S. Padhye, R. C. Chilkate, P. B. Sonawane, A. S. Kumbhar, R. G. Yerande, Coord. Chem. Rev., 123, 49 (1993)

[55] A. E. Liberta, D. X. West, Biometals, 5, 121 (1992)

[56] J. S. Casas, M. S. Garcia-Tasende, J. Sordo, Coord. Chem. Rev., 193, 283 (1999)

[57] J. S. Casas, M. S. Garcia-Tasende, J. Sordo, Coord. Chem. Rev., 209, 197 (2000)

[58] T. S. Lobana, R. Sharma, G. Bawa, S. Khanna, Coord. Chem. Rev., 253, 977 (2009)

[59] R. Prabhakaran, P. Kalaivani, S.V. Renukadevi, R. Huang, K. Senthilkumar, R. Karvembu, K. Natarajan, Inorg. Chem., 51, 3525 (2012)

[60] L. Ze-hua, D. C.-Ying, L. Ji-hui, L. Young-jiang, M. Yu-hua, Y. X-Zeng, New J. Chem., 24, 1057 (2000)

[61] F. Basuli, S. M. Peng, S. Bhattacharya, Inorg. Chem., 36, 5645 (1997)

[62] C. Metcalfe, J. A. Thomas, Chem. Soc. Rev., 32, 215 (2003)

[63] J. S. Casas, M. S. Garcia-Tasende, J. Sordo, Coord. Chem. Rev., 197, 209 (2000)

[64] P. P. Netalkar, S. P. Netalkar, V. K. Revankar, Transition Met. Chem., 39, 519 (2014)

[65] D. Mishra, S. Naskar, M. G. B. Drew, S. K. Chattopadhyay, Inorg. Chim. Acta., 359, 585 (2006)

[66] J. Dutta, S. Datta, D. K. Seth, S. Bhattacharya, RSC Adv., 11751 (2012)

[67] J. Dutta, S. Bhattacharya, RSC Adv., 10707 (2013)

[68] S. Datta, D. K. Seth, R. J. Butcher, S. Bhattacharya, Inorg. Chim. Acta., 377, 120 (2011)

[69] R. K. Mahajan, T. P. S. Walia, Sumanjit, T. S. Lobana, Anal. Sci., 22, 389 (2006)

[70] R. K. Mahajan, I. Kaur, T. S. Lobana, Talanta., 59, 101 (2003)

[71] R. K. Mahajan, T. P. S. Walia, Sumanjit, T. S. Lobana, Talanta., 67, 755 (2005)

[72] S. Güveli, N. Özdemir, A. Koca, T. Bal-Demirci, B.Ülküseven, Inorg. Chim. Acta., 443, 7 (2016)

[73] S. Güveli, A. Koca, N. Özdemir, T. Bal-Demirci, B. Ülküseven, New J. Chem., 38, 5582 (2014)

[74] S. Duman, I. Kızılcıklı, A. Koca, M. Akkurt, B. Ülküseven, Polyhedron, 29, 2924 (2010)

[75] Y. Kurt, A. Koca, M. Akkurt, B. Ülküseven, Inorg. Chim. Acta., 388, 148 (2012)

[76] S. Selvamurugan, R. Ramachandran, P. Vijayan, R. Manikandan, G. Prakash, P. Viswanathamurthi,

K. Velmurugan, R. Nandhakumar, A. Endo, Polyhedron, 107, 57 (2016)

[77] K. S. O. Ferraz, N. F. Silva, J. G. Da Silva, N. L. Speziali, I. C. Mendes, H. Beraldo, J. Mol. Str.,1008, 102 (2012)

[78] K. Jayakumar, M. Sithambaresan, A. Ambili Aravindakshan, M. R. Prathapachandra Kurup, Polyhedron, 75, 50 (2014)

[79] D. C. Ilies, E. Pahontu, S. Shova, R. Georgescu, N. Stanica, R. Olar, A. Gulea, T. Rosu, Polyhedron, 81, 123 (2014)

[80] G. Oliveira, P. Ivo da S. Maia, P. C. Souza, F. R. Pavan, C. Q. F. Leite, R. B. Viana, A. A. Batista, O. R. Nascimento, V. M. Deflon, J. Inorg. Biochem., 132, 21 (2014)

[81] R. Manikandan, P. Viswanathamurthi, K.Velmurugan, R. Nandhakumar, T. Hashimoto, A. Endo, J. Photochem. Photobiol B., 130, 205 (2014)

[82] Y. Li, Z.Y. Yang, J. C. Wu, Eur. J. Med. Chem., 45, 5692 (2010)

[83] K. Sampath, S. Sathiyaraj, C. Jayabalakrishnan, Spectrochim. Acta, A: Mol. Biomol. Spectrosc., 105, 582 (2013)

[84] J. F. M. Silva, S. J. Garden, A. C. Pinto, Braz. Chem. Soc., 12, 3 (2001)

[85] T. O. Olomola, D. A. Bada, C. A. Obafem, Toxicol. Environ. Chem., 91, 5 (2009)

[86] B. Bhrigu, D. Pathak, N. Siddiqui, M.S. Alam, W. Ahsan, Int. J. Pharm. Sci. Drug Res., 2, 4 (2010)

[87] O. Bekircan, H. Bektas, Molecules, 13, 2126 (2008)

[88] A. Jarrahpour, D. Khalili, E. De Clercq, C. Salmi, J.M. Brunel, Molecules, 12, 1720 (2007)

[89] L. Zhou, Y. Liu, W. Zhang, P. Wei, C. Huang, J. Pei, Y. Yuan, L. Lai, J. Med. Chem.,49, 3440 (2006)

[90] R. Ghahremanzadeh, M. Sayyafi, S. Ahadi, A. Bazgir, J. Comb. Chem.,11, 393 (2009)

[91] S. K. Sridhar, S. N. Pandeya, J. P. Stables, A. Ramesh, Eur. J. Pharm. Sci., 16, 129 (2002)

[92] M. C. Rodriguez-Argiielles, A. Sanchez, M. B. Fermi, G. G. Fava, C. Pelizzi, G. Pelosi, R. Albertini, P. Lunghi, S. Pinelli,J.Inorg. Biochem., 73, 7 (1999)

[93] D. X. West, A. K. El-Sawaf, G. A. Bain, Transition Met. Chem., 23, 1 (1998)

[94] N. Karalı, Ö. Güzel, N. Özsoy, S. Özbey, A. Salman, Eur. J. Med. Chem., 45, 1068 (2010)

[95] S. Agrawal, G.T. Kulkarni, V.N. Sharma, Free Radical Antioxid., 1, 4 (2011)

[96] Z. Zhimei, Z. Zhiguo, X. Ronge, L. Pengcheng, L. M. Guo, Int. J. Biol. Macromol., 47, 93(2010)

[97] S. Ghosh, A. K. Misra, G. Bhatia, M. M. Khan, A. K. Khanna, Chem. Lett., 19, 386 (2009)

[98] T. S. Lobana, Rekha, B. S. Sidhu, A. Castineiras, E. Bermejo, T. Nishioka, J. Coord. Chem., 58, 9 (2005)

[99] G. Cerchiaro, K. Aquilano, G. Filomeni, G. Rotilio, M. R. Ciriolo, A. M. D. C.Ferreira, J. Inorg. Biochem., 99, 1433 (2005)

[100] C. N. Hancock, L. H. Stockwin, B. Han, R. D. Divelbiss, J. H. Jun, S. V. Malhotra, M. G. Hollingshead, D. L. Newton, Free Radical Biol. Med., 50, 110 (2011)

[101] C. Marzano, M. Pellei, F. Tisato, C. Santini, Anti-Cancer Agents Med. Chem., 9, 185 (2009)

[102] M. P. Sathisha, V. K. Revankar, K. S. R. Pai, Metal-Based Drugs., 1 (2008)

[103] T. S. Lobana, Rekha, A. P. S. Pannu, G. Hundal, R. J. Butcher, A. Castineiras, Polyhedron, 26, 2621 (2007)

[104] S. Tardito, L. Marchiò, Curr. Med. Chem., 16, 1325 (2009)

[105] T. Aboul-Fadl, F. A. S. Bin-Jubair, O. Aboul-Wafa, Eur. J. Med. Chem., 45, 4578 (2010)

[106] K. L. Vine, J. M. Locke, M. Ranson, S. G. Pyneb, J. B. Bremner, Bioorg. Med. Chem., 15, 931 (2007)

[107] S.V. Kostanecki, J. Tambor, Chem. Ber., 32, 1921 (1899)

[108] G. Di Carlo, N. Mascolo, A.A. Izzo, F. Capasso, Life Sci., 65, 337 (1999)

[109] K. Elizabeth, M. N. A. Rao, Int. J. Pharmacol., 58, 237, (1990)

[110] G. J. Kelloff, C. W. Boone, J. A. Crowelt, V. E. Steele, R. Luber, C. C. Sigman, Cancer Epidemiol. Biomarkers Prev., 3, 85 (1994)

[111] Z. Rozmer, T. Berki, P. Perjési, Toxicol. Vitro., 20, 1354 (2006)

[112] H.-J. Zhang, Y. Qian, D. –D. Zhu, X.-G. Yang, H.- L. Zhu, Eur. J. Med. Chem., 46, 4702 (2011)

[113] M. Muthukumar, P. Viswanathamurthi, Cent. Eur. J. Chem., 8, 1 (2010)

[114] F. Bisceglie, S. Pinelli, R. Alinovi, M. Goldoni, A. Mutti, A. Camerini, L. Piola, P.Tarasconi, G. Pelosi, J. Inorg. Biochem., 140, 111 (2014)

[115] S. Datta, D. K. Seth, S. Gangopadhyay, P. Karmakar, S. Bhattacharya, Inorg. Chim. Acta., 392, 118 (2012)

[116] A. I. Mosa, M. M. Ibrahim, S. A. Aldhlmani, J. Solu. Chem., 42, 2364 (2013)

[117] E. Fischer, Dtsch. Chem. Ges., 17, 579 (1884)

[118] C. M. Armstrong, P. V. Bernhardt, P. Chin, D. R. Richardson, Eur. J. Inorg. Chem.,1145 (2003)

[119] E. Mezzina, D. Spinelli, L. Lamartina, S. Buscemi, V. Frenna, G. Macaluso, Eur. J. Org. Chem., 203 (2002)

[120] Y. L. Sang, X. S. Lin, J. Coord. Chem., 63, 315 (2010)

[121] G. Pastorin, S. Federico, S. Paoletta, M. Corradino, F. Cateni, B. Cacciari, K. Klotz, Z. Gao, K. A. Jacobson, G. Spalluto, S. Moro, Bioorg. Med. Chem., 18, 2524 (2010)

[122] M. Y. Wani, A. R. Bhat, A. Azam, I. Choi, F. Athar, Eur. J. Med. Chem., 48, 313 (2012)

[123] V. R. Avupati, R. P. Yejella, V. R. Parala, K. N. Killari, V. M. R. Papasani, P. Cheepurupalli, V. R. Gavalapu, B. Boddeda, Bioorg. Med. Chem. Lett., 23, 5968 (2013)

[124] N. Nawar, M. A. Khattab, N. H. Hosny, Synth. React. Inorg. Met. Org. Chem., 29, 1365 (1999)

[125] H. H. Szmant, Angew. Chem., Int. ed, 7, 120 (1968)

[126] V. M. Lyubchanskaya, L. M. Alekseeva, V. G. Granik, Chem. Heterocycl. Compd., 35, 570 (1999)

[127] D. G. Themelis, P. D. Tzanavaras, A. A. Liakou, H. D. Tzanavaras, J. K. Papadimitriou, Analyst 125, 2106 (2000)

[128] D. G. Themelis, P. D. Tzanavaras, F. S. Kika, Talanta 55, 127 (2001)

[129] D. van Reyk, S. Sarel, N. Hunt, Biochem. Pharmacol. 60, 581 (2000)

[130] J. T. Edward, Biometals, 11, 203 (1998)

[131] H. Hoshino, Y. Saitoh, K. Nakano, K. Takahashi, T. Yotsuuyanagi, Bull. Chem. Soc. Jpn., 74, 1279 (2001) and references therein.

[132] M. B. Ferrari, S. Capacchi, G. Pelosi, G. Reffo, P. Tarasconi, R. Albertini, S. Pinelli, P. Lunghi, Inorg. Chim. Acta., 134, 286 (1999)

[133] M. Ruben, J. Rojo, F. J. Romero-Salguero, L. H. Uppadine, J. M. Lehn, Angew. Chem., Int. ed, 43, 3644 (2004)

[134] C. Lodeiro, F. Pina, Coord. Chem. Rev., 253, 1353 (2009)

[135] S. B. Padhy, G. B. Kauffman, Coord. Chem. Rev., 63, 127 (1985)

[136] D. X. West, S. B. Padhy, P. B. Sonawane, R. C. Chikte, Struct. Bond., 76, 1 (1991)

[137] R. Manikandan, P. Viswanathamurthi, M. Muthukumar, Spectrochim. Acta A., 83, 297 (2011)

[138] C. M. Sharaby, Spectrochim. Acta A., 66, 1271 (2007)

[139] P. V. Bernhardt, J. Mattson, D. R. Richardson, Inorg. Chem., 45, 752 (2006)

[140] M. F. Iskander, L. El-Sayed, N. M. H. Salem, W. Haase, H. J. Linder, S. Foro, Polyhedron, 23, 23 (2004)

[141] R.-Ș. Mezey, I. Máthé, S. Shova, M.-N. Grecu, T. Roșu, Polyhedron, 102, 684 (2015)

[142] A. E. M. E1-Sayed, O. A. A1-Fulaij A. A. Elaasar, M. M. El-Defrawy, A. A. El-Asmy, Spectrochim. Acta Part A Mol. Biomol. Spectrosc., 135, 211 (2015)

[143] M. V. Angelusiu, S.-F. Barbuceanu, C. Draghici, G. L. Almajan, Eur. J. Med. Chem., 45, 2055 (2010)

[144] B. Jeragh, A. A. El-Asmy, Spectrochim. Acta, A: Mol. Biomol. Spectrosc., 129, 307 (2014)

[145] P. Singh, A. K. Singh, V. P. Singh, Polyhedron, 65, 73 (2013)

[146] H. Abd El-Wahab, Prog. Org. Coat., 89, 106 (2015)

[147] M. C. Mandewale, B. Thorat, Y. Nivid, R. Jadhav, A. Nagarsekar, R.Yamgar,J.Saudi.Chem.Soc.,(2016) http://dx.doi.org/10.1016/j.jscs.2016.04.003

[148] A. P. Ferreira, E. D. L. Pilo, A. A. Recio-Despaigne, J. G. Da Silva, J. P. Ramos, L. B. Marques, P. H.D.M. Prazeres, J. A. Takahashia, E. M. Souza-Fagundes, W. Rocha, H. Beraldo, Bioorg. Med. Chem., 24, 2988 (2016)

[149] X. Yang, D. Schipper, R.A. Jones, L.A. Lytwak, B.J. Holliday, S. Huang, J. Am. Chem. Soc., 135, 8468 (2013)

[150] M. Orio, O. Jarjayes, H. Kanso, C. Philouze, F. Neese, F. Thomas, Angew. Chem. Int. Ed., 49, 4989 (2010)

[151] S. B. Novakovic, G. A. Bogdanovic, V. M. Leovac, Polyhedron, 25, 1096 (2006)

[152] D. K. Dermertzi, M. A. Demertzis, J. R. Miller, C. S. Frampton, J. P. Jasinski, D. X. West, J. Inorg. Biochem; 92: 137 (2002)

[153] K. Subin Kumar, C. PriyaVarma, V. N. Reena, K. K. Aravindakshan, J. Pharm. Sci. Res; 9: 1317 (2017)

[154] Z. Iakovidou, E. Mioglou, D. Mourelatos, A. Kotsis, M. A. Demertzis, Papagoergiou, J. R. Miller, D. Kovala-Demertzi, Anticancer Drugs; 12: 65 (2001)

[155] D. Kovala-Demertzi, M. A. Demertzis, J. R. Miller, C. Papadopoulou, C. Dodorou, G. Filousis, J. Inorg. Biochem; 86: 555 (2001)

CHAPTER II

REAGENTS, APPARATUS AND GENERAL METHODS

A brief description of the materials used, details of the experiments and adopted procedures for the characterization of the ligand and complexes are specified in this chapter. The details of the different types of instruments used for the physico-chemical investigation of the compounds with their operational characteristics and reference materials are also outlined here. However, other specific synthetic procedures and experimental details are given in the relevant chapters.

2.1 Metal salts

The metal salts used for the study were of E.Merck, AnalaR grade or equivalent grade. They include mainly the chlorides of Mn(II), Fe(III), Co(II), Ni(II), Cu(II) and acetates of Zn(II) and Cd(II). The anhydrous salt of Fe(III) and all other hydrated metal salts were used as such for the synthesis of complexes.

2.2 Solvents

The solvents such as methanol, ethanol, diethyl ether, petroleum ether, chloroform and dimethyl sulphoxide were used for the preparation, extraction and recrystallisation of ligands and their complexes. Commercially available solvents like ethanol were purified

according to the standard procedures[1]. Others were E. Merck reagents and used as such. The solvents used for the spectral studies were of spectroscopic grade.

2.3 Other reagents

Other reagents used in the present investigation were hydrochloric acid, nitric acid, sulphuric acid, glacial acetic acid, N-methylaniline, carbondisulphide, sodium chloroacetate, hydrazine hydrate, sodium hydroxide pellets, benzaldehyde, acetophenone, etc. All were E. Merck reagents. Precursors of ligands such as isatin, 2-methoxy isonicotinic acid hydrazide and crotonaldehyde were purchased from Aldrich and SRL chemicals. They were used as such without further purification.

2.4 Ligands

In the present investigation, four ligand systems were designed for the preparation of chelates of Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II) and Cd(II). These ligands were synthesized from diketo compound (isatin), α, β- unsaturated aldehyde (crotonaldehyde) and α, β-unsaturated ketone (chalcone) by condensing them with N(4)-methyl(phenyl)thiosemicarbazide. Another was a hydrazone derived from isatin and 2-methoxy isonicotinic acid hydrazide. The detailed procedures for the synthesis of the ligands and their complexes are given in the respective chapter. The structural assignments of all the compounds are included in the relevant chapters.

The synthetic procedures of N(4)-methyl(phenyl)thiosemicarbazide and chalcone are given below.

2.4.1 Synthesis of N(4)-methyl(phenyl)thiosemicarbazide

2.4.1.1 Preparation of carboxymethyl N(4)-methyl (phenyl) dithiocarbamate

To a mixture consisting 12 ml (15.2 g, 0.20 mol) of carbon disulphide and 21.6 mL (21.2 g, 0.20 mol) of N-methylaniline, an aqueous solution of 8.4 g (0.21 mol in 250 mL) of NaOH was added. This solution was stirred at room temperature for about 4 h till the organic layer disappeared. At this point, the resulting pale orange coloured solution was treated with about 23.2 g (0.20 mol) of sodium chloroacetate added in small portions with stirring. The solution was then allowed to stand overnight (for about 17 h). The resulting pale yellow solution was acidified with 25 mL of conc. HCl and the buff coloured solid thus separated was collected and dried. (Yield 80%, M.P 197-198^{0}C).

2.4.1.2 Preparation of N(4)-methyl(phenyl)thiosemicarbazide

A solution of 17.7 g (0.0733 mol) of carboxymethyl N(4)-methyl(phenyl)dithiocarbamate in 20 mL of 98% hydrazine hydrate and 10 mL of water was heated on a water bath for about 10 minutes when colourless crystals began to appear. Heating was continued for another 5 minutes. The cooled mixture was filtered,

washed with water and dried under lamp. The crude product was recrystallised from a mixture of ethanol and water. The colourless triclinic crystals formed were filtered and dried. (Yield 80%, M.P 125^{0}C).

2.4.2 Preparation of chalcone

The chalcone was synthesized by base catalyzed Claisen-Schmidt condensation reaction of acetophenone with benzaldehyde. A mixture of benzaldehyde (0.01 mol) and acetophenone (0.01 mol) was dissolved in 20 mL rectified spirit in a 250 mL round-bottomed flask equipped with a magnetic stirrer. Then added 10 mL of NaOH solution (1g in 10mL H_2O) drop wise to this reaction mixture with vigorous stirring which continued for another 30 minutes, when the solution became turbid. The reaction mixture was maintained at a temperature between 20-25^{0}C by stirring in an ice-cold water bath on the magnetic stirrer. Following a vigorous stirring for 4-5 h, the reaction mixture was neutralized by 0.2N HCl whereby the precipitation occurred giving the peach coloured compound. The crude chalcone was filtered, dried in air and recrystallized from rectified spirit. (Yield 83%, M.P 142-144^{0}C).

2.4.3. Preparation of thiosemicarbazones

The simple method of preparation of thiosemicarbazone by the condensation of carbonyl compound and a thiosemicarbazide has been known for more than 50 years[2,3]. A mixture of equimolar amounts of

isatin and N(4)-methyl(phenyl)thiosemicarbazide in ethanol with a catalytic amount of acetic acid was boiled. Isatin N(4)-methyl(phenyl)thiosemicarbazone separated out on cooling the resultant solution to room temperature. Chalcone N(4)-methyl(phenyl)thiosemicarbazone was obtained by refluxing an ethanolic solution of chalcone with a methanolic solution of N(4)-methyl(phenyl)thiosemicarbazide for 13 h. By refluxing the methanolic solutions of crotonaldehyde and N(4)-methyl(phenyl)thiosemicarbazide with a few drops of acetic acid for 4 h and on subsequent cooling of the reaction mixture, crotonaldehyde N(4)-methyl(phenyl)thiosemicarbazone precipitated.

A typical aroylhydrazone, isatin 2-methoxy isonicotinoylhydrazone was prepared by refluxing ethanolic solutions of 2-methoxyisonicotinoylhydrazine with isatin in the presence of glacial acetic acid. Cooling and concentration of the solutions, yielded the crystals of isatin 2-methoxyisonicotinoylhydrazone.

2.5 Analytical methods

Semi microanalysis was carried out by standard methods to check the purity of the compounds. Carbon, hydrogen and nitrogen were estimated by microanalysis using Vario EL III CHNS rapid analyser at Sophisticated Test and Instrumentation Centre, Kochi. For the estimation of metal, a known amount of the complex was digested with a mixture of conc.HNO_3 (10 mL) and 2 or 3 drops of perchloric acid. The digestion process was repeated twice or thrice by the addition of fresh conc.HNO_3 and finally with conc.HCl to ensure

complete decomposition. After cooling, the digested mass was extracted with distilled water and the solution was used for the estimation of the metal present in it. The metal percentages in the complexes were found out by the reported methods[4]. Manganese, iron and nickel were estimated gravimetrically. Cobalt and zinc were estimated by EDTA titrimetric method. Copper was estimated iodometrically using standard sodium thiosulphate solution[5]. Estimation of chloride was done gravimetrically as silver chloride.

2.6 Physico-chemical methods

The physico-chemical properties and structures of all the ligands and the complexes were established by magnetic susceptibility measurements and spectral techniques like IR, ^{1}H NMR and electronic spectral studies.

2.6.1 Magnetic measurements

The magnetic susceptibility measurements of the complexes were carried out at room temperature on a Gouy-type magnetic balance (Sherwood Scientific magnetic susceptibility balance), using $Hg[Co(NCS)_4]$ as calibrant[6]. Diamagnetic corrections, using Pascal's constants, were applied by adding the diamagnetic contributions of various atoms and structural units[7]. The effective magnetic moments of the complexes were calculated in Bohr Magneton (B.M) from the corrected molar susceptibilities obtained after the diamagnetic corrections.

2.6.2 Electronic spectra

The electronic spectra of the compounds were recorded on a Jasco UV-Visible spectrometer model V-550. Depending on the solubilities of complexes, their electronic spectra were recorded either in solution (10^{-3} M) or in the solid state.

2.6.3 Infrared spectra

The infrared spectra of the ligands and the complexes, in the range 4000-400 cm^{-1} were recorded using KBr pellets on a Jasco FTIR 4100 spectrometer.

2.6.4 ^{1}H NMR spectra

The ^{1}H NMR spectra of the ligands were recorded in $CDCl_3$ or DMSO-d_6 by using 400MHz, Bruker Advance III spectrometer. The methods, materials and instruments used for the biological studies are described in Part II of the thesis.

2.7 References

[1] A. Weissberger, E.S. Proskauer, J. A. Riddick, E. E. Toops, Organic solvents, Interscience, New York, VII (1956)

[2] J. F. M. da Silva, S. J. Garden, A. C. Pinto, J. Braz. Chem. Soc., 12, 273 (2001)

[3] F. D. Popp, Adv. Hetercycl. Chem., 18, 1 (1975)

[4] A. I. Vogel, 'Quantitative Inorganic Analysis' 7th Ed. Longman, London (2010)

[5] A. I. Vogel, 'Practical Organic Chemistry' Longman, London (1973)

[6] B. N. Figgis, R. S. Nyholm, J. Chem. Soc., 4150 (1958)

[7] A. Eanshaw, 'Introduction to Magnetochemistry' Academic Press, London and New York (1978)

CHAPTER III

SYNTHESIS AND CHARACTERIZATION OF TRANSITION METAL COMPLEXES OF CROTONALDEHYDE N(4)-METHYL(PHENYL)THIOSEMICARBAZONE

3.1 Introduction

Schiff base compounds with conjugated –C=C– and –C=N– bonds in their systems are of great importance due to their nonlinear optical properties[1] and their use as solid stationary phase in gas chromatography[2]. The treatment of α, β-unsaturated carbonyl compounds with suitable amines having additional donor atoms make them potential ligands for metal ions[3,4]. Thiosemicarbazones are interesting class of compounds due to their diverse coordination sites with potential to act as bi- or polydentate ligands. Mono- or polynuclear complexes derived from such ligands are highly attractive owing to the stability of their complexes[5-8]. The structural diversity of thiosemicarbazones can be increased by condensing different types of carbonyl compounds with N-substituted thiosemicarbazide. The presence of a lone pair of electrons in either a π or sp^2 hybridised orbital or trigonally hybridized orbital on nitrogen atom of the >C=N– group gives the basic requirement for their important chemical and biological properties.

A thorough literature survey revealed that many studies on thiosemicarbazones of aromatic aldehydes are centered on

cinnamaldehyde[9], cuminaldehyde, salicylaldehyde[10] and coumarin derivatives[11]. However, reports on thiosemicarbazones of aliphatic unsaturated aldehydes are scandy. Furthermore, no studies have been made so far on the coordination behaviour of N-disubstituted thiosemicarbazone of crotonaldehyde. Therefore, in the present work, a new ligand, crotonaldehyde N(4)-methyl(phenyl)thiosemicarbazone (CRTSC), formed by the condensation of crotonaldehyde and N(4)-methyl(phenyl)thiosemicarbazide has been reported and used as a chelating ligand for the preparation of Mn(II), Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) complexes. The characterization of the ligand and its complexes are also discussed in this chapter. The structure of the ligand is shown in Figure 1.

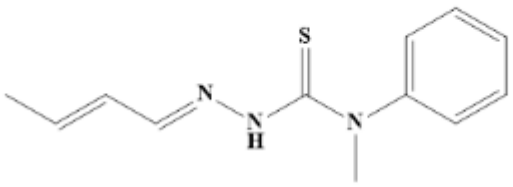


Figure 1

Crotonaldehyde N(4)-methyl(phenyl)thiosemicarbazone (CRTSC) (LH)*

*IUPAC name

(1E)-1-((E)-but-2-enylidene)-4-methyl-4-phenylthiosemicarbazide

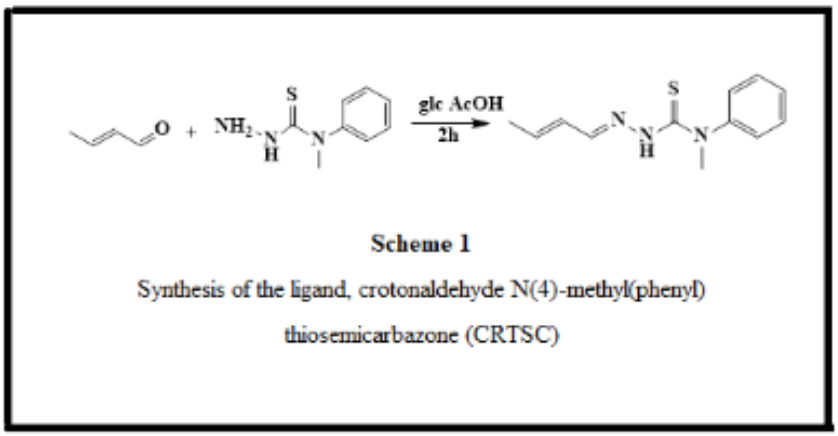
3.2 Experimental

3.2.1 Materials and methods

The details about the chemicals used and the adopted scheme for the synthesis of precursor, N(4)-methyl(phenyl)thiosemicarbazide and the characterization methods used are described in Chapter II.

3.2.2 Synthesis of ligand

N(4)-methyl(phenyl)thiosemicarbazide (0.005 mol) dissolved in hot methanol was added to a hot ethanolic solution of crotonaldehyde (0.005 mol) and refluxed for 2 h in the presence of a catalytic amount of glacial acetic acid. The colour of the solution changed from yellow to brown. It was then concentrated to half the volume. The sticky mass obtained was washed several times with petroleum ether. The cream coloured solid thus separated out was suction filtered, washed with methanol and dried under vacuum (Yield 65%, M.P 148^0C). The synthetic procedure of the ligand, crotonaldehyde N(4)-methyl(phenyl)thiosemicarbazone is shown below in Scheme 1.



Scheme 1

Synthesis of the ligand, crotonaldehyde N(4)-methyl(phenyl) thiosemicarbazone (CRTSC)

3.2.3 Synthesis of metal complexes

The complexes of Mn(II), Co(II), Ni(II) and Cu(II) with CRTSC were prepared by refluxing a methanolic solution of the respective metal salt (0.005 mol in 20 mL) with an ethanolic solution of the ligand (0.005 mol in 30 mL) for 4 h. The complexes of Zn(II) and Cd(II) were prepared by taking ligand and metal acetate in 2:1 molar ratio. The resulting solution was concentrated and cooled. The solid complexes thus formed were filtered off, washed several times with petroleum benzene and finally with methanol. They were then dried under vacuum. The physical constants and yields are presented in Table 5.

3.3 Results and discussion

The data obtained from the elemental analysis, physico-chemical- and spectral investigations have been used to elucidate the structure and bonding of the compounds.

3.3.1 Characterization of the ligand

The ligand, crotonaldehyde N(4)-methyl(phenyl)thiosemi carbazone was a cream coloured solid, soluble in methanol, ethanol, etc. The homogeneity and purity of the ligand were checked by thin-layer chromatographic (TLC) method. It was characterized by elemental analysis (Table 1), and further by UV-Vis- (Table 2), IR- (Table 3) and ^{1}H NMR (Table 4) spectral techniques. The data obtained from these were correlated to explain the structure of the ligand.

3.3.1.1 Elemental analysis

The elemental analysis of the ligand was carried out on a Vario EL III CHNS instrument. The results of the analysis confirmed the molecular formula of the ligand (LH) as $C_{12}H_{15}N_3S$ (Table 1).

Table 1 Physico-chemical and analytical data of ligand							
Compound (Empirical Formula)	% of Yield	Melting Point (^{0}C)	Color of the Compound	CHNS Analysis Found % (Calculated)%			
				C	H	N	S
$C_{12}H_{15}N_3S$ (LH)	65	148	Cream	62.31	6.74	19.01	14.03
				(61.93)	(6.42)	(18.52)	(13.72)

3.3.1.2 Electronic spectrum

The electronic spectrum of the ligand showed an intense band at 282 nm which can be attributed to π-π* transition. Similarly, a band at 364 nm in the ligand spectrum may be due to n –π* transition of the azomethine group. The data are given in Table 2.

Table 2 UV-Vis spectral data of the CRTSC ligand		
Compound	Spectral Bands λmax	Assignment
	(nm)	
$C_{12}H_{15}N_3S$ (LH)	282	π→π* transition
	364	n→π* transition

3.3.1.3 Infrared spectrum

The IR spectroscopy is a powerful tool that provides a spectrum of large number of vibrational frequencies or absorption bands from which considerable information can be derived about the different functional groups present in a compound. Hence, vibrational spectroscopy is principally an important technique that gives a dynamic picture of the molecule or helps in the molecular structural elucidation. The absorption of infrared radiation in the range, 4000-400 cm^{-1} gives rise to molecular vibrational spectral bands that are important in the case of a ligand molecule.

The prominent IR spectral bands of the ligand and their assignments are given in Table 3. The presence of a thioamide (–NH–C=S–) group in the ligand indicated the possibility of its existence as thione-thiol tautomers[12]. However, the IR spectrum of the ligand showed a broad band of medium intensity at 3392 cm^{-1} due to asymmetric stretching of the secondary –NH group of the thioamide part and this ruled out the existence of the ligand as a thiol tautomer[13,14]. In the spectrum of thiosemicarbazone, the band in the range, 1260-1420 cm^{-1} due to –N–C=S– group suggested its existence in the thione form[15]. There was no characteristic band due to $\nu_{(S-H)}$ in the range 2700-2500 cm^{-1}. Moreover, the presence of a strong band at 862 cm^{-1} due to C=S group, confirmed the existence of the free ligand as the thione tautomer in solid state[16]. The medium intensity band at 2965 cm^{-1} may be assigned to $\nu_{(=CH)}$ vibration[17]. The presence of a strong band at 1633 cm^{-1} in the ligand spectrum was characteristic of

the azomethine group[18]. The band at 1023 cm^{-1} in the spectrum of ligand can be assigned to $\nu_{(N-N)}$[19].

Table 3 Significant IR spectral bands (cm^{-1}) of the ligand	
Spectral Bands	Assignments
3392	$\nu_{(N-H)}$ (asymmetric stretching)
2965	$\nu_{(=CH)}$
1633	$\nu_{(C=N)}$
1023	$\nu_{(N-N)}$
862	$\nu_{(C=S)}$

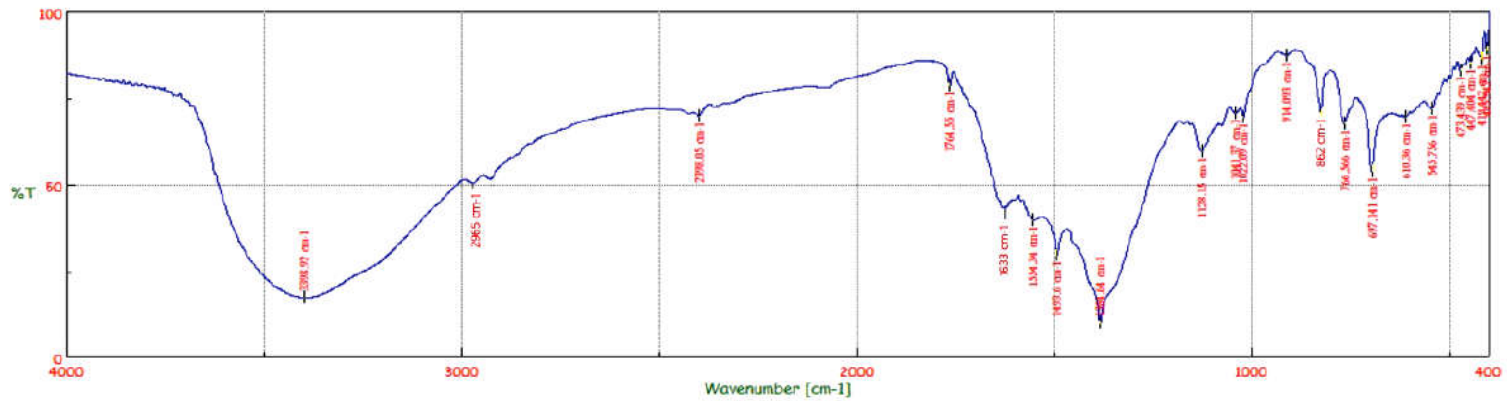


Figure 2

IR spectrum of crotonaldehyde N(4)-methyl(phenyl)thiosemicarbazone

3.3.1.4 ^{1}H NMR spectrum

The formation of the crotonaldehyde N(4)-methyl (phenyl)thiosemicarbazone was confirmed by analysing its ^{1}H NMR spectrum recorded in DMSO-d_6 using TMS as internal standard. The ^{1}H NMR spectrum of the ligand showed a multiplet between 7.63-7.12 ppm, which was due to C_6H_5 protons[20]. There was a sharp singlet at 6.52 ppm that was attributed to the -NH proton[21,22]. The quartet that appeared at 2.6 ppm was due to olefinic =CH protons adjacent to the -CH_3 group. A sharp signal at 3.24 ppm was assignable to -CH_3 protons on the terminal N-atom of the thiosemicarbazone moeity. The signals that appeared at 1.34 and 1.83 ppm were due to the olefinic =CH proton near the imine bond and of the -CH_3 group adjacent to the olefinic =CH proton, respectively. (Table 4)

Table 4 ^{1}H NMR data of the ligand

Sl No.	δ (ppm)	Assignment
1	7.12-7.63 (m)	Aromatic (phenyl group)
2	6.52 (s)	–NH group
3	3.24 (s)	–CH_3 adjacent to the phenyl group
4	2.60 (q)	olefinic =C–H adjacent to –CH_3 group
5	1.83 (d)	–CH_3 adjacent to the olefinic =CH
6	1.34 (d)	=CH near to the imine bond

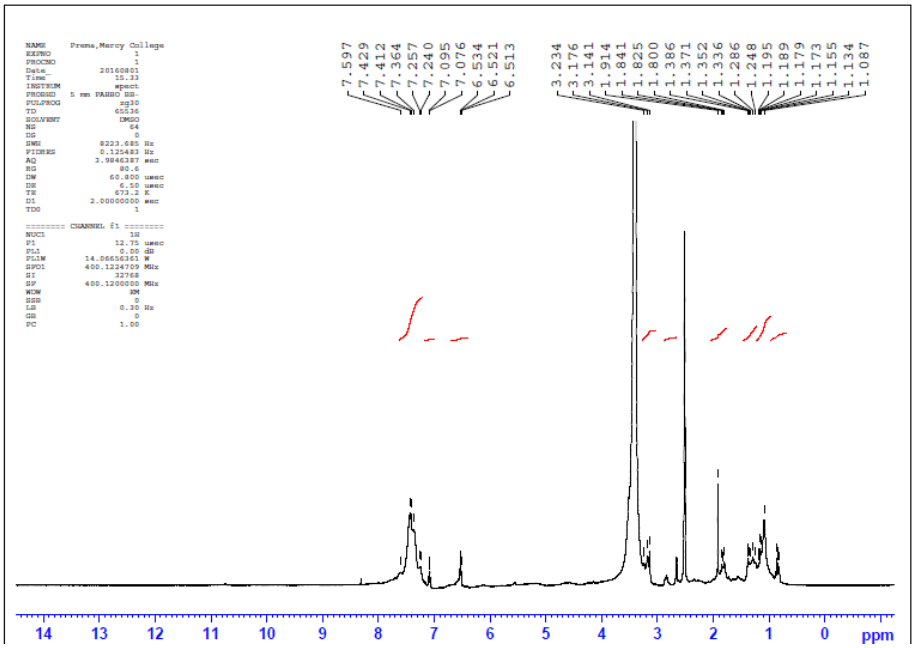


Figure 3

^{1}H NMR spectrum of crotonaldehyde N(4)-methyl(phenyl)thiosemicarbazone

3.3.2 Characterization of the complexes

All the complexes of crotonaldehyde N(4)-methyl(phenyl) thiosemicarbazone were stable under normal atmospheric conditions. They were non-hygroscopic and soluble in chloroform and DMSO, but less soluble in other common organic solvents like methanol and ethanol. Elemental analysis, magnetic moment measurements, IR- and electronic spectral studies were employed to characterize them.

3.3.2.1 Analytical data

The analytical data of all the complexes agreed very well with their proposed molecular formulae and are shown in Table 5. The complexes corresponded to the general formulae: $[ML_2(H_2O)_2]$, where M=Mn(II), Co(II) or Ni(II), $[ML_2]$, M=Zn(II) or Cd(II), and $[CuL(H_2O)Cl]$. The ligand was present in deprotonated monoanionic form, L^- in all the complexes. The electrical conductance values of approximately 10^{-3} M solutions of the complexes in DMSO indicated their non-electrolytic nature[23].

Table 5 Physico-chemical and analytical data of the complexes

Compound (Empirical formula)	% of Yield	Melting point (^{0}C)	Colour of the Compound	Anal. Found % (Calculated %)					
				M	C	H	N	S	Cl
$[MnL_2(H_2O)_2]$	58	248	Pale Brown	9.79 (9.93)	52.48 (51.88)	6.43 (5.76)	14.73 (15.13)	10.83 (10.53)	
$[CoL_2(H_2O)_2]$	72	>300	Pale Maroon	10.92 (10.64)	50.13 (50.52)	5.2 (5.07)	13.92 (14.10)	12.04 (12.32)	
$[NiL_2(H_2O)_2]$	78	>300	Pale Brown	11.82 (11.54)	52.13 (51.57)	5.44 (5.05)	16.86 (16.03)	10.97 (11.51)	
$[CuL(H_2O)Cl]$	65	288	Brown	16.97 (17.13)	42.52 (41.83)	5.2 (4.88)	10.93 (11.20)	9.72 (9.24)	10.24 (10.18)
$[ZnL_2]$	75	275	Offwhite	14.21 (13.46)	55.37 (54.43)	4.49 (5.13)	17.1 (16.78)	11.65 (12.10)	
$[CdL_2]$	73	278	Offwhite	18.65 (19.53)	48.79 (49.30)	5.07 (4.72)	15.26 (15.46)	11.15 (10.72)	

3.3.2.2 Magnetic moments

The magnetic susceptibility measurements of the complexes were carried out at room temperature on a Gouy balance using

$Hg[Co(NCS)_4]$ as a calibrant[24]. The effective magnetic moments of the complexes were calculated in Bohr Magneton (B.M) from the corrected molar susceptibilities that were obtained after applying the diamagnetic corrections. These corrections were done for the atoms and structural units using Pascal's constants. The transition metal complexes usually give spin-only magnetic moment values depending to the number of unpaired electrons present in them. But in some cases, they show deviations from these spin-only values which can be attributed to the contributions from orbital magnetic moment or spin-orbit coupling. Thus, the magnetic moment value of a metal complex not only provides information about the number of unpaired electrons and the orbitals in which they are residing, but also helps us to predict the oxidation state of the metal and thereby, to establish the possible geometry and structure of the complex.

The calculated magnetic moment values of the complexes of Mn(II), Co(II), Ni(II) and Cu(II) are given in Table 6. Mn(II) forms both high-spin (S =5/2) and low-spin (S = 1/2) octahedral complexes, but majority of them are high-spin type[25]. It also forms high-spin complexes in tetrahedral stereochemistry[26]. In the case of high-spin octahedral complexes of Mn(II) with a d^5 configuration, the observed spin-only magnetic moment values are in the range of 5.65-6.10 B.M[27,28]. A low-spin Mn(II) octahedral complex has the expected spin-only magnetic moment value of 1.73 B.M with $^2T_{2g}$ as the ground term. However, due to orbital contribution, the experimental values are found close to 2.50 B.M[29]. In the present case, an observed magnetic moment value of 5.87 B.M suggested a high-spin octahedral geometry for $[MnL_2(H_2O)_2]$.

The complexes of Co(II) usually exhibit octahedral, tetrahedral and square planar geometries. Even five coordinate geometries are possible, if the ligand has several donor atoms[30]. The high-spin octahedral Co(II) ion has $^4T_{1g}$ as the ground state term with considerable orbital contribution and has an observed magnetic moment value of 5.20 B.M, which is higher than the expected spin-only value of 3.87 B.M (for three unpaired electrons)[31,27]. This could be due to the effect of spin-orbit coupling. Low-spin octahedral Co(II) complexes have ^2E_g ground term with no orbital contribution. The reported magnetic moments of such complexes are in the range of 1.80-1.90 B.M, close to the spin-only value for one unpaired electron (1.72 B.M)[32,33]. Tetrahedral high-spin Co(II) complexes have $^4A_2$ ground term with no orbital contribution. The expected magnetic moment value is 3.87 B.M, which is close to the spin-only value for three unpaired electrons. However, the observed magnetic moment values will be higher and usually lie in the range, 4.40-4.80 B.M. This is due to the spin-orbit coupling perturbations[27,34]. Square planar complexes of Co(II) are always low-spin (S=1/2) with the observed magnetic moments in the range of 2.20-2.70 B.M which is rather higher than the expected spin-only value for one unpaired electron (1.73 B.M)[35,27]. This could be due to spin-orbit coupling. The Co(II) complex of CRTSC registered a magnetic moment value of 4.82 B.M. This value, together with the colour of the complex corresponded to a high-spin octahedral geometry around Co(II) ion.

The bivalent nickel complexes can exist in paramagnetic six-coordinate octahedral geometry with $^3A_{2g}$ ground term for both the high-spin and low-spin type. Octahedral Ni(II) complexes register

magnetic moment values in the range 2.90-3.30 B.M which are slightly higher than the spin-only value for two unpaired electrons. Since the ground term is $^3A_{2g}$ that lacks orbital contribution, the slightly higher value of the moment is due to the spin-orbit coupling or higher state mixing with the ground state term. For tetrahedral Ni(II) complexes, the experimental values of magnetic moments fall in the range of 3.60-4.10 B.M[34,36,37]. These observed higher values of the moment than the spin-only value for the two unpaired electrons are due to the orbital contribution of the spin-triplet ($^3T_1$) ground term. If the distortions in the field of coordinated ligands are large, then the magnetic moment with small orbital contributions may result and gives rise to an observed moment value as low as 3.20 B.M[38]. Four-coordinate square planar Ni(II) complexes have spin-singlet ground term and are diamagnetic. The Ni(II) complex, $[NiL_2(H_2O)_2]$ in the present case exhibited a magnetic moment value of 2.84 B.M indicating its octahedral geometry.

The Cu(II) complexes usually exhibit distorted octahedral geometry and their observed magnetic moment values are slightly above the spin-only values for one unpaired electron[27]. Octahedral Cu(II) complex has a ground state of $^2T_{2g}$ with no orbital contribution. The observed magnetic moment values corresponding to one unpaired electron fall in the range of 1.80-2.10 B.M. This is slightly higher than the expected spin-only value of 1.73 B.M, due to the spin-orbit coupling. For regular tetrahedral Cu(II) complex, a magnetic moment of 2.20 B.M is predicted at room temperature[39]. However, the observed moment falls in the range 1.95-2.00 B.M[40]. The Cu(II) complex of

CRTSC registered a magnetic moment of 1.98 B.M, indicating a four-coordinate tetrahedral geometry.

Table 6 Magnetic moments of the complexes	
Complex	μ_{eff} (B.M)
$[MnL_2(H_2O)_2]$	5.87
$[CoL_2(H_2O)_2]$	4.82
$[NiL_2(H_2O)_2]$	2.84
$[CuL(H_2O)Cl]$	1.98

3.3.2.3 Electronic spectra

The electronic spectra of transition metal complexes mainly include metal d-d transition bands and charge-transfer bands of ligands[41]. The d-d transition bands are of great importance in the electronic spectral studies of metal complexes, because they give an idea about the geometry around the metal ion. According to the selection rules, the d-d spectra of transition metal complexes contain weak absorption bands due to doubly forbidden (Laporte- and spin-forbidden) transitions[42]. The electronic spectral bands along with their band assignments of the complexes of Mn(II), Co(II), Ni(II) and Cu(II) of CRTSC are presented in Table 7.

Majority of the octahedral Mn(II) complexes are high-spin with a d^5 configuration. According to Laporte selection rule, all the d-d transitions are spin-forbidden as well as parity-forbidden for high-spin

Mn(II) octahedral complexes[43,44,45]. Therefore, the high-spin octahedral complexes of Mn(II) are commonly pale coloured. However, the tetrahedral complexes of Mn(II) are intensely coloured because their d-d transitions are not Laporte forbidden. The Mn(II) complex in the present case, $[MnL_2(H_2O)_2]$ registered a magnetic moment of 5.87 B.M at room temperature which indicates an octahedral geometry with 5 unpaired electrons. The extremely pale brown colour of the Mn(II) complex is due to the multiplicity- and Laporte-forbidden transitions. Thus the Mn(II) complex showed a weak d-d band in the region around 480 nm which may be assigned to $^6A_{1g} \rightarrow {}^4T_{2g}$ transition and is an evidence for the proposed octahedral geometry[46,47].

Though octahedral geometry is usually common for Co(II) complexes, tetrahedral complexes are also found. The octahedral complexes of Co(II) has $^4T_{1g}$ as the ground term with three spin-allowed transitions to the excited quartet state. Due to spin-orbit coupling, the spectrum is usually complicated and results in the poor resolution of these bands. The electronic spectra of octahedral Co(II) complexes show absorption bands in the range, 1040, 620 and 413 nm. They are assigned, respectively, to the $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$, $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ and $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ transitions[43,48,49]. In the present case, the spectrum of the Co(II) complex was characterized by the presence of three spin-allowed bands at 1048, 621 and 407 nm corresponding to the above transitions of an octahedral complex.

For octahedral Ni(II) complexes with d^8 configuration, the spectra is characterized by the presence of three spin-allowed transitions[44]. The electronic spectrum of Ni(II) complex investigated

here registered three bands characteristic of octahedral geometry and are assigned as:

$^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ ν_1 1034 nm

$^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ ν_2 715 nm

$^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ ν_3 410 nm

The electronic spectrum and the magnetic moment supported an octahedral structure for the complex[50].

Table 7 Electronic spectral band assignments of the complexes of CRTSC

Complex	Spectral Bands λ_{max} (nm)	Assignment
$[MnL_2(H_2O)_2]$	432	$^6A_{1g} \rightarrow {}^4E_g$
	492	$^6A_{1g} \rightarrow {}^4T_{2g}$
	623	$^6A_{1g} \rightarrow {}^4T_{1g}$
$[CoL_2(H_2O)_2]$	407	$^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$
	621	$^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$
	1048	$^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$
$[NiL_2(H_2O)_2]$	410	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$
	715	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$
	1034	$^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$
$[CuL(H_2O)Cl]$	558	$^2E \rightarrow {}^2T_2$

In the case of Cu(II) complexes, blue or green colours are observed usually for hexa-coordinated complexes that are characterized by the presence of a single absorption band corresponding to $^2T_{2g} \rightarrow {}^2E_g$ transition[51,52]. This observed band will be a broad one due to Jahn Teller effect[44]. In the electronic spectrum of

[CuL(H_2O)Cl], a band at 558 nm due to $^2E \rightarrow {}^2T_2$ transition indicated a tetrahedral geometry for the complex. A magnetic moment value of 1.98 B.M also supported this. The complexes of Zn(II) and Cd(II) were found to be diamagnetic.

3.3.2.4 Infrared spectra

The IR spectra of the complexes were compared with that of the ligand in order to infer the bonding sites of the ligand in metal complexes. The significant IR spectral bands in the ligand spectrum generally undergo shifts from their positions upon coordination to metal ions in the complexes. The IR bands of the complexes along with their probable assignments are listed in Table 8.

The medium intensity band at 3392 cm^{-1} in the ligand spectrum, due to the secondary –NH of the thioamide group was not found in the spectra of all the complexes, indicating the loss of H-atom *via* enolisation of –NH-C=S to –N=C-SH and subsequent coordination through the thiolate sulphur atom to the metal ion. This in turn was established by the disappearance of a strong band at 862 cm^{-1}due to -C=S group in the spectra of all the complexes. Therefore, the appearance of new bands in the region of 754-795 cm^{-1} in the spectra of the complexes due to –C-S, indicated the coordination of thioamide to the metal through the deprotonated thiolate sulphur atom[53]. The presence of a band at 1633 cm^{-1} in the ligand spectrum, which was characteristic of azomethine group, got shifted to lower frequency region, ~1625-1575 cm^{-1} in the spectra of all the metal complexes, indicating the coordination of azomethine nitrogen to the metal ion[54,55].

The band observed in the region of 1025cm^{-1} in the ligand spectrum due to $\nu_{(N-N)}$ stretching, has been found to be shifted to higher wave numbers ~1028-1042 cm^{-1} in the spectra of all the complexes. This further supported the coordination of the ligand through azomethine nitrogen and deprotonated thiolate sulphur atom in these complexes[56]. These observations were confirmed by the appearance of new bands in the low frequency regions, ~543-690 cm^{-1} and ~440 cm^{-1} in the spectra of all the metal complexes which can be assigned to $\nu_{(M-N)}$ and $\nu_{(M-S)}$, respectively[57].

The broad bands observed in the range, 3425-3484 cm^{-1} in the spectra of the complexes of Mn(II), Co(II), Ni(II) and Cu(II) were attributed to O-H stretching modes of the coordinated water molecules in them[58]. The supportive data for the presence of coordinated water molecules in these complexes were the appearance of new bands in the region ~463-485cm^{-1} due to $\nu_{(M-O)}$[57]. The absorption band at 423 cm^{-1}in the spectrum of Cu(II) complex may be due to $\nu_{(M-Cl)}$[59]. All these explain the mode of coordination of the ligand through the thiolate sulphur and azomethine nitrogen atom in all the complexes. Therefore, the ligand acted as a monoanionic bidentate one in all these complexes.

Table 8 Characteristic IR spectral bands (in cm^{-1}) of the CRTSC ligand and its complexes							
Compound	$C_{12}H_{15}N_3S$ (LH)	$[MnL_2(H_2O)_2]$	$[CoL_2(H_2O)_2]$	$[NiL_2(H_2O)_2]$	$[CuL(H_2O)Cl]$	$[ZnL_2]$	$[CdL_2]$
$\nu_{(N-H)}$	3392						
$\nu_{(O-H)}$		3462	3485	3427	3452	3443	3458
$\nu_{(C=N)}$	1633	1586	1622	1627	1585	1619	1589
$\nu_{(N-N)}$	1023	1044	1032	1041	1022	1055	1033
$\nu_{(C=S)}$	862	792	766	753	765	753	764
$\nu_{(M-N)}$		697	645	626	698	526	638
$\nu_{(M-S)}$		437	437	425	437	428	437
$\nu_{(M-O)}$		476	473	462	471		
$\nu_{(M-Cl)}$					423		

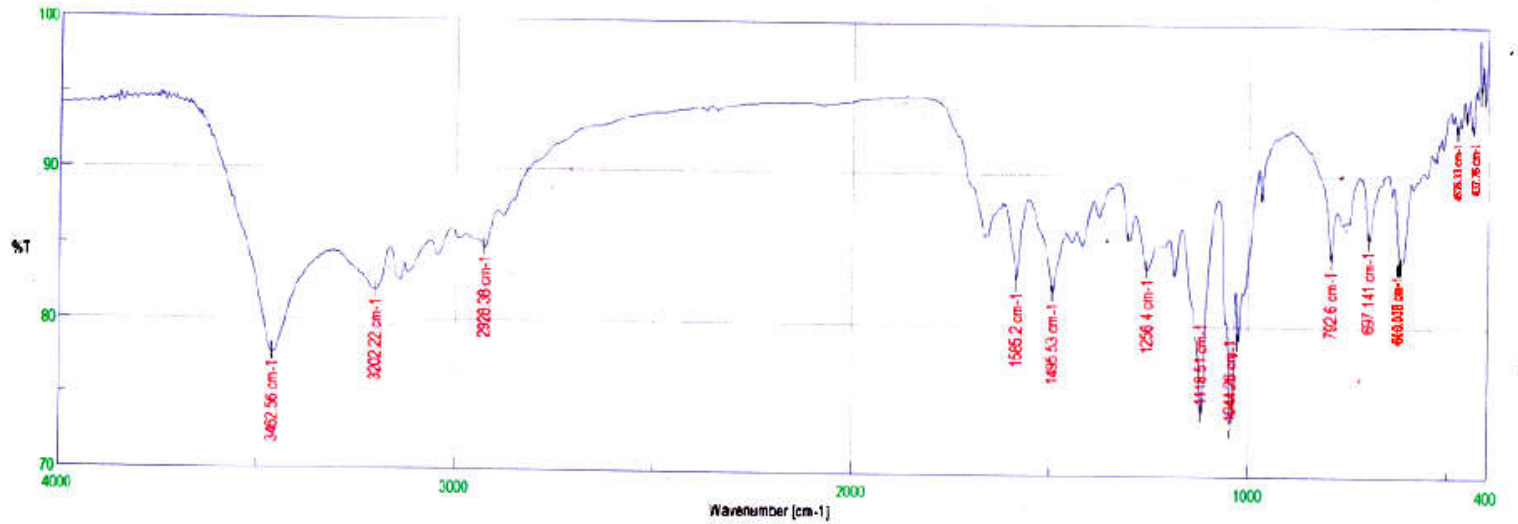


Figure 4

IR spectrum of Mn(II) complex of crotonaldehyde N(4)-methyl(phenyl) thiosemicarbazone $[MnL_2(H_2O)_2]$

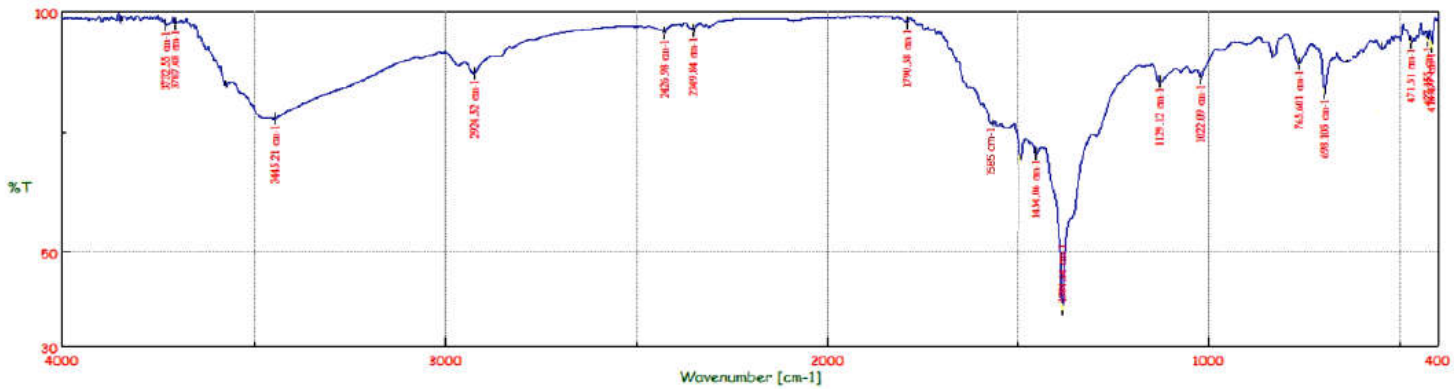


Figure 5

IR spectrum of Cu(II) complex of crotonaldehyde N(4)-methyl(phenyl)thiosemicarbazone

[CuL(H_2O)Cl]

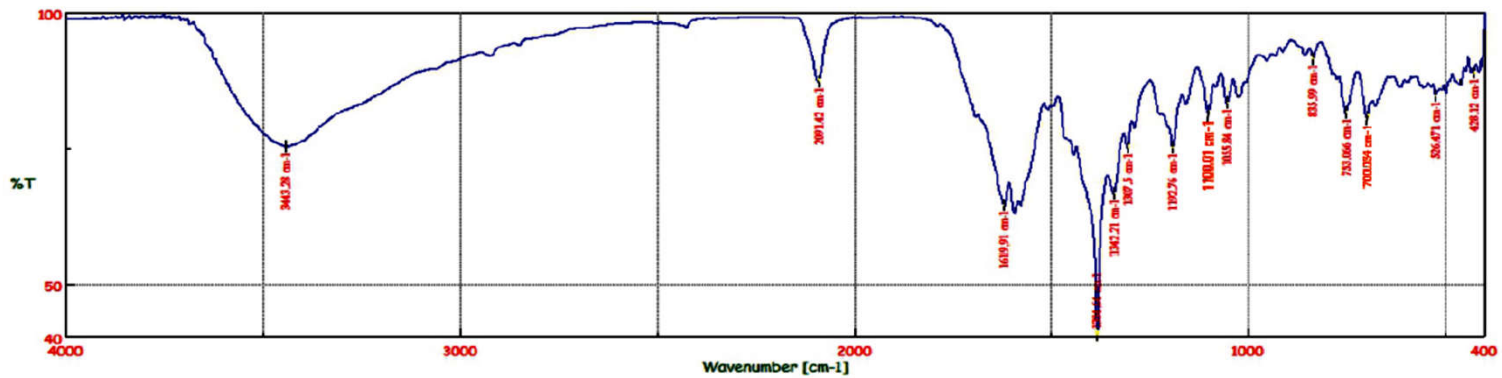


Figure 6

IR spectrum of Zn(II) complex of crotonaldehyde N(4)-methyl(phenyl)thiosemicarbazone [ZnL_2]

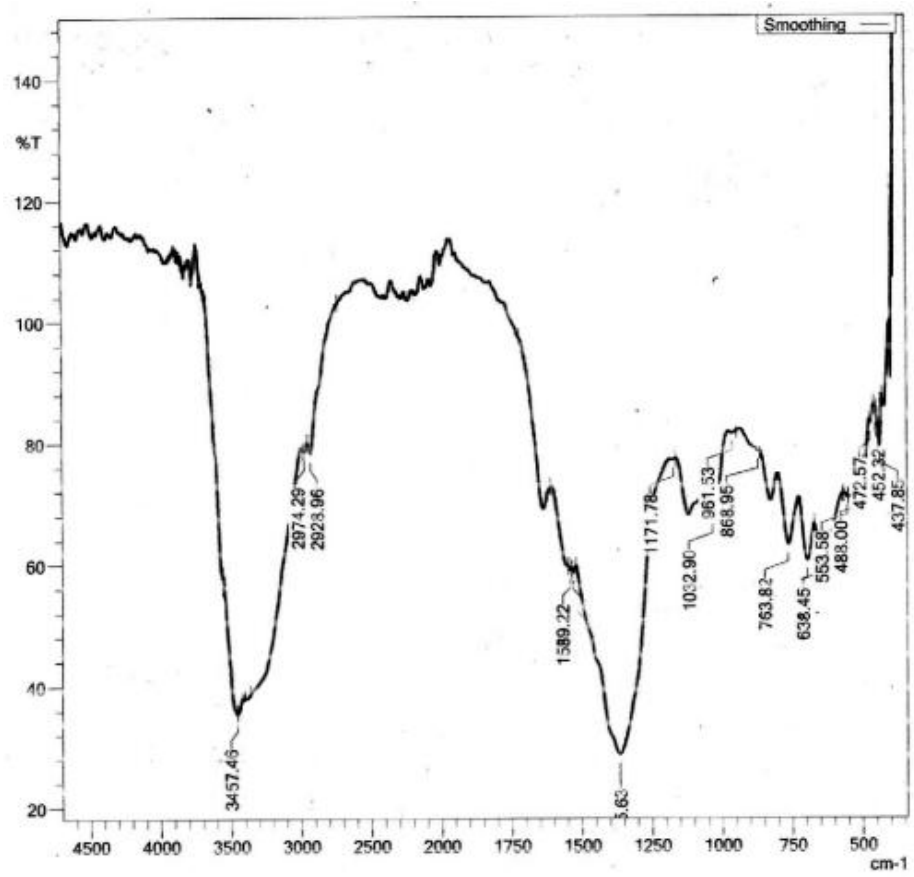


Figure 7

IR spectrum of Cd(II) complex of crotonaldehyde N(4)-methyl(phenyl)thiosemicarbazone [CdL_2]

3.4 Conclusions

The complexes of Mn(II), Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) with a bidentate ligand, crotonaldehyde N(4)-methyl(phenyl)thiosemicarbazone (LH) were prepared and their physico-chemical properties have been studied. The ligand behaved as a monoanionic bidentate chelating agent, coordinating through azomethine nitrogen and thiolate sulfur in all the complexes. Based on the magnetic moment measurements and various spectral studies, the complexes of Mn(II), Co(II) and Ni(II), were assigned octahedral geometry with a general molecular formula $[ML_2(H_2O)_2]$, which is given in Figure 8. $[CuL(Cl)H_2O]$, $[ZnL_2]$ and $[CdL_2]$ complexes were found to be tetrahedral and their structures are shown in Figures 9 and 10.

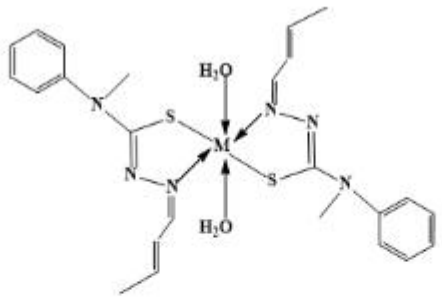


Figure 8

Suggested structure of the complexes of the formula $[ML_2(H_2O)_2]$, where M = Mn(II), Co(II) or Ni(II)

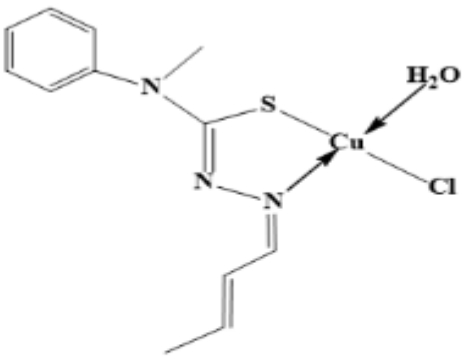


Figure 9

Suggested structure of $[CuL(H_2O)Cl]$

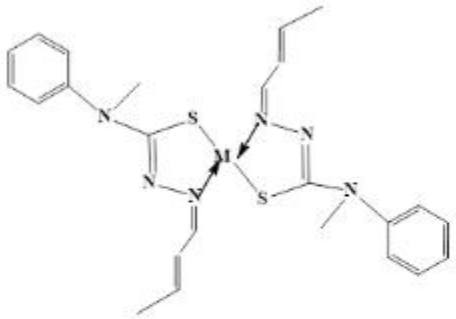


Figure 10

Suggested structure of the complexes of the formula $[ML_2]$,

where M = Zn(II) or Cd(II)

3.5 References

[1] L. Orazzhanova, M. G. Yashkarova, L. A. Bimendina, S. E. Kudaibergenov, J. Appl. Polym. Sci; 5, 87 (2003)

[2] R. Grunes, W. Sawondy, J. Chromatogr; 122, 63 (1985)

[3] P. Pattanayak, J. L. Pratihar, D. Patra, P. Brandao, V. Felix, Inorg. Chim. Acta., 418, 171 (2014)

[4] L. J. Li, L. K. Yang, Z. K. Chen, Y. Y. Huang, F. Bin, D. J. Long, Inorg. Chem. Commun., 50, 62 (2014)

[5] T. S. Lobana, R. Rekha, R. J. Butcher, A. Castineiras, E. Bermejo, P. V. Bharatam, Inorg. Chem., 45, 1535 (2006)

[6] D. X. West, I. S. Billeh, J. P. Jasinski, J. M. Jasinski, R. J. Butcher, Transit. Metal Chem., 23, 209 (1998)

[7] W. Kaminsky, J. P. Jasinski, R. Noudenberg, K. I. Goldberg, D. X. West, J. Mol. Struct., 608, 135 (2002)

[8] M. Baldini, M. B. Ferrari, F. Bisceglie, G. Pelosi, S. Pinelli, P. Tarasconi, Inorg. Chem., 42, 2049 (2003)

[9] F. Bisceglie, S. Pinelli, R. Alinovi, M. Goldoni, A. Mutti, A. Camerini, L. Piola, P. Tarasconi, G. Pelosi, J. Inorg. Biochem., 140, 111 (2014)

[10] S. Datta, D. K. Seth, S. Gangopadhyay, P. Karmakar, S. Bhattacharya, Inorg. Chim. Acta., 392, 118 (2012)

[11] A. I. Mosa, M. M. Ibrahim, S. A. Aldhlmani, J. Solu. Chem., 42, 2364 (2013)

[12] Y. P. Tian, C.-Y. Duan, C.-Y. Zhao, X.-Z. You, T.C.W. Mak, Z.-Y. Zhang, Inorg. Chem., 36, 1247 (1997)

[13] B. D. Sarma, J. C. Bailer Jr., J. Am. Chem. Soc., 77, 5476 (1955)

[14] L. J. Bellamy, 'The Infrared Spectra of Complex Molecules' Vol 2, Chapman and Hall, London (1980)

[15] C. N. R. Rao, R. Venkataraghavan, Specrochim. Acta., 18, 541 (1962)

[16] R. H. U.Borges, H. Beraldo, A. Abras, Braz. J. Chem. Soc., 8, 33 (1997)

[17] S. M. Silverstein, G. C. Basssler, T. C. Morrill, 'Spectrophotometric Identification of Organic Compounds' 5th Ed., John Wiley, New York (1991)

[18] K. Alomar, M.A. Khan, M. Allain, G. Bouet, Polyhedron., 28, 1273 (2009)

[19] P. S. Kalsi, 'Spectroscopy of Organic Compounds' 3rd Ed., New Age International (P) Ltd (1998)

[20] A. W. Coats, J. P. Redfern, Nature., 20, 68 (1964)

[21] L. Sacconi, G. Speroni, J. Am. Chem. Soc., 87, 3102 (1965)

[22] M. E. Khalifa, A. A. El-Asmy, K. M. Ibrahim, M. M. Mostafa, Synth. React. Inorg. Met-Org. Chem., 16, 1319 (1986)

[23] W. J. Geary, J. Coord. Chem. Rev., 7, 81 (1971)

[24] A. Earnshaw, 'Indroduction to Magnetochemistry' Acadamic Press, London, New York (1968).

[25] A. P. Ginseberg, M. B. Robin, Inorg. Chem., 2, 817 (1963)

[26] A. K. Mukherjee, P. Ray, Ind. J. Chem. Soc., 32, 633 (1955)

[27] B. N. Figgis, J. Lewis, Prog. Inorg. Chem., Ed. F. A. Cotton, 6, 126 (1964)

[28] L. Sacconi, R. Ginni, Ann. Chim., 42, 723 (1952)

[29] F. A. Cotton, G. Wilkinson, 'Advanced Inorganic Chemistry' 5th Ed, Wiley Interscience (1988)

[30] R. L. Carlin, 'Transition Metal Chemistry' Ed. R. L. Carlin, Marcel Dekker, Inc., New York (1965)

[31] B. N. Figgis, R. S. Nyholm, J. Chem. Soc., 338 (1959)

[32] A. Sacconi, F. A. Cotton, J. Am. Chem. Soc., 84, 2043 (1962)

[33] B. Chiswell, S. E. Livingstone, J. Chem. Soc., 84, 20432 (1962)

[34] N. S. Gill, R. S. Nyholm, J. Chem. Soc., 3997 (1959)

[35] B. N. Figgis, R. S. Nyholm, J. Chem. Soc., 4190 (1958)

[36] L. Sacconi, 'Transition Metal Chemistry' Ed. R. L. Carlin, Marcel Dekker, Inc.,New York (1968)

[37] B. N. Figgis, J. Lewis, F. E. Mabbs, G. A. Weff, Nature., 203, 1138 (1964)

[38] G. Bullock, F. W. Hartstock, L. K. Thompson, Can. J. Chem., 61, 57 (1983)

[39] E. Ruiz, A. R. Fortea, J. Cano, S. Alvarez, P. Alemany, J. Comput. Chem., 24, 982 (2003)

[40] A. Earnshaw, J. Lewis, J. Chem. Soc., 36 (1961)

[41] J. E. Huheey, E. A. Keiter, R. L. Keiter, 'Inorganic Chemistry, Principles of Structure and Reactivity' 4th ed, Pearson Education India (2001)

[42] R. L. Dutta, A. Syamal, 'Elements of Magnetochemistry' 2nd Ed, New Delhi (1992)

[43] N. F. Curtis, Y. M. Curtis, Inorg. Chem., 4, 804 (1965)

[44] D. Sutton, 'Electronic spectra of Metal Complexes' McGraw Hill, London (1968)

[45] A. B. P. Lever, 'Inorganic Electronic Spectroscopy' 2nd Ed, Elsevier, New York (1968)

[46] R. N. Prasad, N. Gupta, J. Ser. Chem. Soc., 68, 455 (2003)

[47] N. I. Dodoff, M. Kubiak, J. K. Jaworska, Z. Naturforsch., 57b, 1174 (2002)

[48] F. A. El-Saied, M. I. Ayad, R. M. Issa, S. A. Aly, Polish J. Chem., 75, 773 (2001)

[49] F. H. Barstall, R. S. Nyholm, J. Chem. Soc., 3570 (1952)

[50] F.A Cotton, G. Wilkinson, 'Advanced Inorganic Chemistry' Wiley Interscience, New York (1980)

[51] T. N. Waters, D. Hall, J. Chem. Soc., 1200 (1959)

[52] J. M. Waters, T. N. Waters, J. Chem. Soc., 2489 (1964)

[53] D. X. West, R. M. Makeever, G. Ertem, Transit. Metal Chem., 11, 132 (1986)

[54] I. M. Issa, R. M. Issa, M. R. Mahmoud, Y. M. Temerk, Z. Phys. Chem., 254, 314 (1973)

[55] N. Raman, S. Sobha, A. Thamaraichelvan, Spectrochim Acta A., 78, 888 (2011)

[56] T. A. Yousef, G.M. Abu El-Reash, O.A. El-Gammal, R.A. Bedier, J. Mol. Struct., 1029, 149 (2012)

[57] T. A. Yousef, T. H. Rakha, U. El-Ayaan, G. M. Abu El-Reash, J. Mol. Struct., 1007, 146 (2012)

[58] M. Wang, L.-F. Wang, Y.-Z. Li, Q.-X. Li, Z.-D. Xu, D.-M. Qu, Transition Met. Chem., 26, 307 (2001)

[59] P. M. Reddy, K. Shanker, R. Rohini, V. Ravinder, Intern. J. Chem.Tech Res., 1, 367 (2009)

CHAPTER IV

SYNTHESIS AND CHARACTERIZATION OF TRANSITION METAL COMPLEXES OF CHALCONE N(4)-METHYL(PHENYL)THIOSEMICARBAZONE

4.1 Introduction

The synthesis and characterization of thiosemicarbazones have received a great attention in the main research areas of coordination chemistry during the last few years. This is owing to their ability to coordinate a metal through different donor atoms that has led to the formation of a variety of coordination compounds[1-4]. Thiosemicarbazones are, therefore, the main class of sulphur donor ligands, which possess a variety of coordination modes through their potential donor atoms. They give rise to complexes of transition metal ions, either in the neutral or deprotonated form. The coordination chemistry of thiosemicarbazones has also gained immense attention because of their varied physiological activities and extensive applications in synthetic chemistry[5]. The biological activities of these compounds are due to their ability to chelate with trace metals.

Among the natural products that have been reported to show promising antiinfective activity, flavonoid frameworks remained to be the principle candidates. Thus, chalcones or 1, 3-diphenyl-2E-propene-1-one, an important class of natural products across the plant kingdom, are well correlated for their significant antiinfective activities. They

have also been the subject of great concern in the recent times, for their attractive pharmacological activities[6]. Chalcone is a typical model that displays several interesting and promising biological applications which include anticancer, antioxidant, cytotoxic, antimicrobial, analgesic and antipyretic applications.

During the recent time, a great number of chalcone derived thiosemicarbazones have been found to possess potential therapeutic applications such as antiinflammatory, antimicrobial and antimalarial applications[7]. A detailed literature survey showed that no work has been done on the metal complexes of N-disubstituted thiosemicarbazone of chalcone[8-10]. Hence, in the present work, the complexes of Mn(II), Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) with chalcone N(4)-methyl(phenyl)thiosemicarbazone were prepared and characterized. The structure of the ligand, chalcone N(4)-methyl(phenyl)thiosemicarbazone is shown in Figure 1.

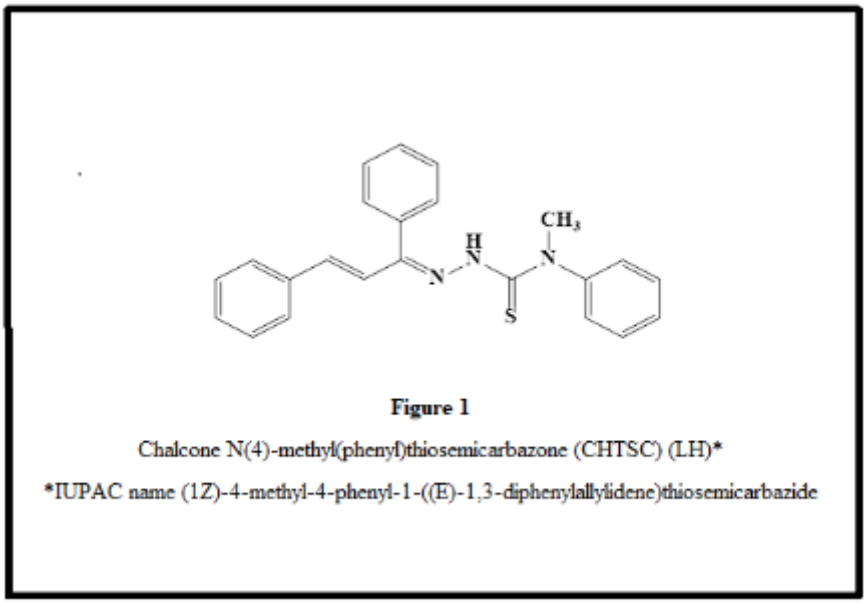


Figure 1

Chalcone N(4)-methyl(phenyl)thiosemicarbazone (CHTSC) (LH)*

*IUPAC name (1Z)-4-methyl-4-phenyl-1-((E)-1,3-diphenylallylidene)thiosemicarbazide

4.2 Experimental

4.2.1 Materials and methods

A meticulous discussion about the chemicals used and the scheme adopted for the synthesis and the characterization methods used are presented in Chapter II.

4.2.2 Synthesis of ligand

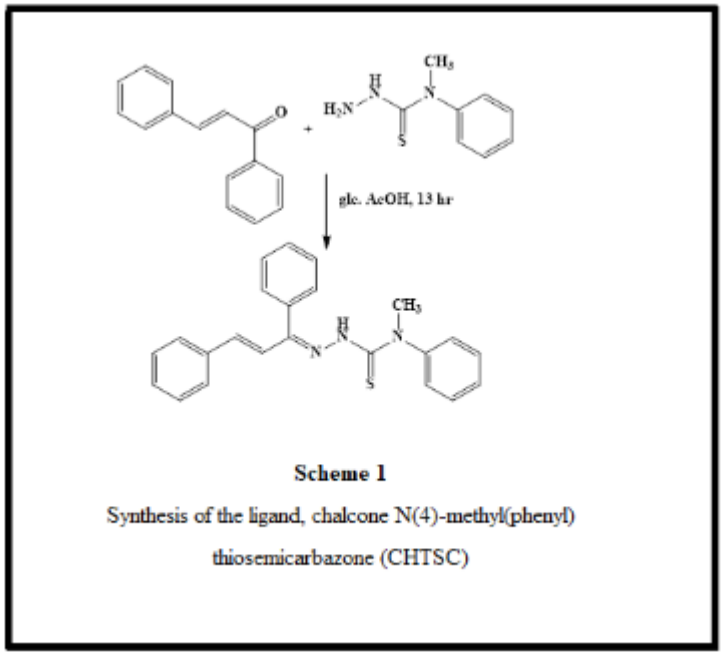
Preparation of the precursor of the ligand, N(4)-methyl(phenyl)thiosemicarbazide was done as described in Chapter II.

4.2.2.1 Synthesis of chalcone

The Claisen-Schmidt base catalyzed condensation reaction was used for the synthesis of chalcone from acetophenone and benzaldehyde. Ethanolic solutions of benzaldehyde (0.01 mol) and acetophenone (0.01 mol) were taken in a 250 mL round bottomed flask equipped with a magnetic stirrer. By keeping the reaction mixture in an ice bath, 20 mL of 25% NaOH solution was added in drops to it. The stirring was continued for another 40 minutes, until the solution became turbid. After constant stirring for about 4-5 h, the reaction mixture was neutralized by 1M HCl, whereby the peach coloured compound (M.P 142^0C) precipitated out (Yield 83%). It was filtered off, dried in air and recrystallized from rectified spirit[11].

4.2.2.2 Synthesis of chalcone N(4)-methyl(phenyl)thiosemi carbazone

An ethanolic solution of chalcone (0.01 mol) was added to a refluxing methanolic solution of N(4)-methyl(phenyl) thiosemicarbazide (0.01mol), followed by 2 drops of glacial acetic acid. The mixture was refluxed for 13 h. The resulting solution was refrigerated for 3 h, stirred well and filtered. The deep yellow solid isolated was washed with 50% ethanol and dried under vacuum (Yield 85%, M.P 193^{0}C). The synthetic procedure of the ligand, chalcone N(4)-methyl(phenyl)thiosemicarbazone is shown in Scheme 1.



Scheme 1

Synthesis of the ligand, chalcone N(4)-methyl(phenyl) thiosemicarbazone (CHTSC)

4.2.3 Synthesis of metal complexes

The complexes were prepared by following a general procedure. A hot methanolic solution of the hydrated metal chloride (0.01mol in 20 mL) [chlorides of Mn(II), Co(II), Ni(II) orCu(II)] was added to a hot ethanolic solution of thiosemicarbazone (0.01mol in 20 mL) and the reaction mixture was refluxed for about 4-5 h. The complexes of Zn(II) and Cd(II) were prepared by taking the metal acetate and ligand in 1:2 molar ratio. The complexes thus obtained were filtered off, washed several times with methanol and dried in vacuum.Their physical- and analytical data are presented in Table 5.

4.3 Results and discussion

The data obtained from the analytical and physico-chemical methods have been used to assign the structures and geometries of the compounds.

4.3.1 Characterization of the ligand

The deep yellow coloured chalcone N(4)-methyl(phenyl)thiosemicarbazone was soluble in chloroform, ethanol, etc. It was characterized analytically and by IR, UV-Vis and ^{1}H NMR spectral techniques. The data obtained from these were used to explain the structure of the ligand.

4.3.1.1 Elemental analysis

The elemental analysis of the ligand was carried out on a Vario ELIII CHNS instrument. The experimentally found out and calculated percentages of C, H, N and S were in good agreement, confirming the molecular formula, $C_{23}H_{21}N_3S$ (LH) of the ligand (Table 1).

Table 1 Physico-chemical and analytical data of CHTSC ligand

Compound (Empirical Formula)	% of Yield	Melting Point (^{0}C)	Color of the Compound	CHNS Analysis Found % (Calculated) %			
				C	H	N	S
$C_{23}H_{21}N_3S$ (LH)	85	193	Deep Yellow	75.21 (74.69)	5.72 (5.66)	11.53 (11.32)	8.58 (8.63)

4.3.1.2 Electronic spectrum

The electronic spectrum of the ligand showed an intense band at 289 nm which can be attributed to π-π* transition. Similarly, a band at 358 nm in the ligand spectrum may be due to the n –π* transition. The data are given in Table 2.

Table 2 UV-Vis spectral data of the ligand		
Compound	Spectral Bands	Assignment
	λ_{max} (nm)	
$C_{23}H_{21}N_3S$ (LH)	289	$\pi \rightarrow \pi^*$ transition
	358	$n \rightarrow \pi^*$ transition

4.3.1.3 Infrared spectrum

The IR spectral bands and assignments of the ligand are given in Table 3. The IR spectrum of the ligand showed a broad band at 3187 cm^{-1}, which may be due to $\nu_{(N-H)}$ of the secondary –NH group. The band at 3125 cm^{-1} may be attributed to the stretching vibration of the =C-H. A medium intensity absorption band at 1621 cm^{-1} may be due to $\nu_{(C=C)}$ of the olefinic bond in the chalcone moiety. The presence of a band at 1638 cm^{-1} was characteristic of an azomethine (>C=N) group. In the spectrum of the ligand, the stretching vibration of the C=S group may be assigned for a band at 856 cm^{-1}. The medium intensity band at 1048 cm^{-1} in the spectrum can be assigned to $\nu_{(N-N)}$.

Table 3 Significant IR spectral bands (cm^{-1}) of the ligand	
Spectral Bands	Assignments
3187	$\nu_{(N-H)}$
3125	$\nu_{(=C-H)}$
1621	$\nu_{(C=C)}$
1638	$\nu_{(C=N)}$
1048	$\nu_{(N-N)}$
856	$\nu_{(C=S)}$

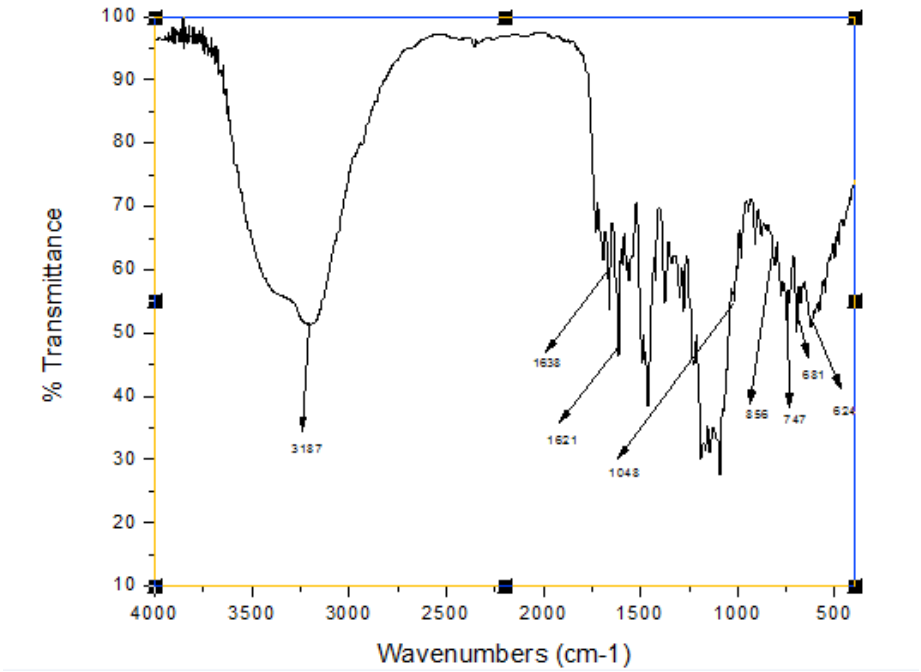


Figure 2

IR spectrum of chalcone N(4)-methyl(phenyl)thiosemicarbazone

4.3.1.4 ^{1}H NMR spectrum

The ^{1}H NMR spectrum of the ligand was recorded in DMSO-d_6. The multiplet in the spectrum observed between 7.04-7.82 ppm can be assigned to the protons of phenyl groups present on the terminal nitrogen atom, on the olefinic –CH and on the imine nitrogen. There was a sharp singlet at 12.43 ppm due to -NH proton. A sharp signal at 3.4 ppm was assignable to CH_3 protons on the terminal N-atom. The signals at 5.7 and 6.8 ppm were due to the olefinic -CH protons adjacent to the phenyl group- and to the imine bond, respectively (Table 4).

Table 4 ^{1}H NMR data of the ligand

SL No.	δ (ppm)	Assignment
1	7.04-7.82 (m)	Aromatic (phenyl group)
2	12.43 (s)	–NH group
3	3.4 (s)	$-CH_3$ present on the terminal N-atom
4	6.8 (d)	olefinic -CH adjacent to the imine bond
5	5.7 (d)	olefinic -CH adjacent to the phenyl group

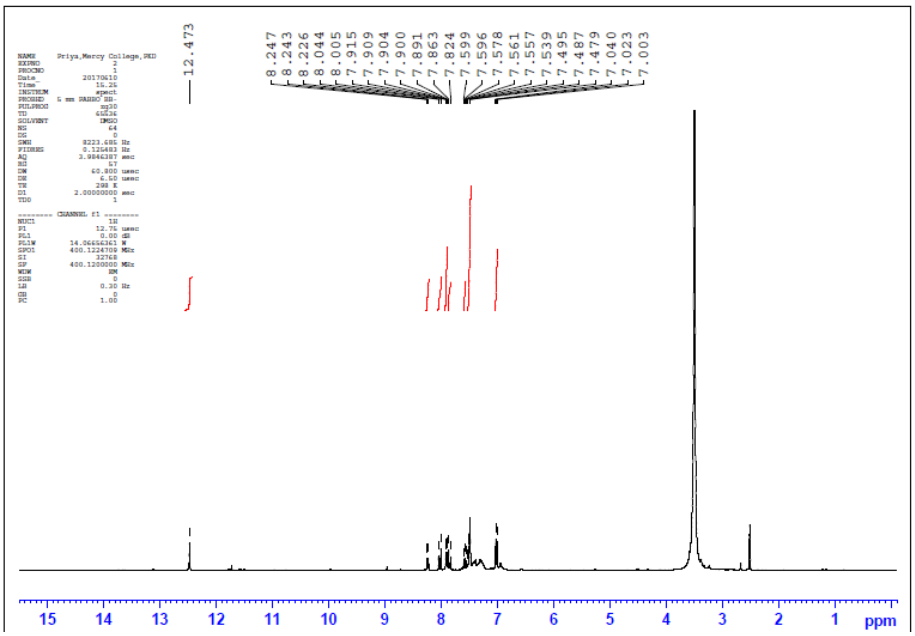


Figure 3

^{1}H NMR spectrum of chalcone N(4)-methyl(phenyl)thiosemicarbazone

4.3.2 Characterization of the complexes

All the complexes of chalcone N(4)-methyl(phenyl)thio semicarbazone were obtained in reasonable yields. They were non-hygroscopic and soluble in chloroform and DMSO, but less soluble in the common organic solvents like methanol and ethanol. Physical characterization (Yield, M.P, Colour and CHNS microanalysis) of the ligand and all the complexes were done, and they were further characterized by IR, UV-Vis, ^{1}H NMR and magnetic susceptibility measurements.

4.3.2.1 Analytical data

The physical properties and the elemental analytical data of the ligand and its complexes are given in Table 5. The analytical data of all the complexes agreed very well with their proposed molecular formulae, $[M(LH)_2Cl_2]$, where M=Mn(II) or Cu(II) and $[ML_2(H_2O)_2]$, where M=Co(II), Ni(II), Zn(II) or Cd(II). The very low electrical conductance values of approximately 10^{-3} M DMSO solutions of the complexes indicated their non-electrolytic nature[12].

Table 5 Physico-chemical and analytical data of the complexes

Compound (Empirical formula)	% of Yield	Melting point (^{0}C)	Colourof the Compound	Anal. Found % (Calculated %)					
				M	C	H	N	S	Cl
$[Mn(LH)_2Cl_2]$	74	262	Pale Yellow	6.38 (6.35)	62.85 (63.44)	4.56 (4.62)	9.62 (9.70)	7.33 (7.39)	8.16 (8.18)
$[CoL_2(H_2O)_2]$	65	254	Brown	7.08 (7.07)	65.91 (66.10)	5.24 (5.27)	10.09 (10.06)	7.67 (7.66)	
$[NiL_2(H_2O)_2]$	65	258	Maroon	7.07 (7.05)	66.16 (66.12)	5.23 (5.27)	10.04 (10.06)	7.65 (7.67)	
$[Cu(LH)_2Cl_2]$	74	270	Dull Brown	6.79 (6.78)	59.47 (59.42)	4.56 (4.52)	12.04 (12.06)	6.87 (6.89)	8.13 (8.11)
$[ZnL_2(H_2O)_2]$	75	249	Pale Yellow	7.69 (7.78)	55.37 (54.53)	4.49 (5.01)	17.1 (16.78)	11.65 (12.10)	
$[CdL_2(H_2O)_2]$	73	252	Pale Yellow	12.35 (12.66)	48.79 (48.94)	5.07 (4.72)	15.26 (14.73)	11.15 (10.25)	

4.3.2.2 Magnetic moments

The magnetic measurements of the complexes at room temperature are highly useful for ascertaining their geometries. The

effective magnetic moment values obtained for the complexes of Mn(II), Co(II), Ni(II) and Cu(II) are given in Table 6.

The high-spin octahedral Mn(II) complex has an expected spin-only magnetic moment value of 5.92 B.M at room temperature. However, the observed magnetic moment values are in the range of 5.65-6.10 B.M. The pale yellow coloured Mn(II) complex in the present case showed a magnetic moment of 5.82 B.M,. which suggested its high-spin octahedral structure. A magnetic moment value of 5.20 B.M is shown by high-spin octahedral Co(II) complex which is higher than the expected spin-only value of 3.87 B.M. This is due to the spin-orbit coupling that leads to magnetic moment values between 4.70-5.20 B.M. In the present case, the Co(II) complex showed a room temperature magnetic moment of 4.89 B.M, which indicated a distorted octahedral environment around the metal ion.

The expected magnetic moment values of octahedral Ni(II) complexes fall in the range 2.90-3.30 B.M. This is slightly higher than the spin-only value. A magnetic moment value of 2.78 B.M for $[NiL_2(H_2O)_2]$ complex was very near to the observed value for an octahedral Ni(II) complex. Distorted octahedral Cu(II) complexes have magnetic moment values in the range 1.80-2.10 B.M. This is slightly higher than the expected spin-only value of 1.73 B.M corresponding to one unpaired electron. An observed magnetic moment of 1.92 B.M. for $[Cu(LH)_2Cl_2]$ indicated the presence of one unpaired electron with a very small orbital contribution and thereby suggested its octahedral geometry[13]. As expected, the Zn(II) and Cd(II) complexes were found to be diamagnetic.

Table 6 Magnetic moments of the complexes	
Complex	μ_{eff} (B.M)
$[Mn(LH)_2Cl_2]$	5.82
$[CoL_2(H_2O)_2]$	4.89
$[NiL_2(H_2O)_2]$	2.78
$[Cu(LH)_2Cl_2]$	1.92

4.3.2.3 Electronic spectra

The electronic spectra of the complexes were recorded in the solid state. The electronic spectral bands of Mn(II), Co(II), Ni(II) and Cu(II) complexes of CHTSC along with their band assignments are presented in Table 7.

High-spin octahedral Mn(II) complexes have forbidden d-d transitions. Therefore, they do not register characteristic bands in the visible region. The bands at 672 and 468 nm in the electronic spectrum of $[Mn(LH)_2Cl_2]$ may be assigned to $^6A_{1g}\rightarrow{}^4T_{1g}$ and $^6A_{1g}\rightarrow{}^4T_{2g}$ transitions, characteristic of its octahedral geometry[14]. There are three spin-allowed transitions for an octahedral Co(II) complex with $^4T_{1g}(F)$ as the ground term. The electronic spectrum of $[CoL_2(H_2O)_2]$ showed three spin-allowed bands at 1036, 592 and 427 nm that may be assigned to $^4T_{1g}(F)\rightarrow{}^4T_{2g}(F)$, $^4T_{1g}(F)\rightarrow{}^4A_{2g}(F)$ and $^4T_{1g}(F)\rightarrow{}^4T_{1g}(P)$ transitions, respectively[15,16].

The spectra of octahedral Ni(II) complexes are characterized by the presence of three spin-allowed transitions. Therefore, the electronic

spectrum with bands at 1053, 682 and 435 nm corresponding to the $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$, $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ and $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ transitions was characteristic of octahedral structure for $[NiL_2(H_2O)_2]$[17,18]. The d-d absorption bands observed for an octahedral Cu(II) complex are in the range 1000-500 nm. The Cu(II) complex prepared here showed a d-d band at 542 nm which may be assigned to the $^2T_{2g} \rightarrow {}^2E_g$ transition, usually observed for an octahedral Cu(II) ion[19].

Table 7 Electronic spectral band assignments of the complexes of CHTSC ligand

Complex	Spectral Bands λmax (nm)	Assignment
$[Mn(LH)_2Cl_2]$	418	$^6A_{1g} \rightarrow {}^4E_g$
	468	$^6A_{1g} \rightarrow {}^4T_{2g}$
	672	$^6A_{1g} \rightarrow {}^4T_{1g}$
$[CoL_2(H_2O)_2]$	427	$^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$
	592	$^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$
	1036	$^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$
$[NiL_2(H_2O)_2]$	437	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$
	682	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$
	1053	$^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$
$[Cu(LH)_2Cl_2]$	542	$^2T_{2g} \rightarrow {}^2E_g$

4.3.2.4 Infrared spectra

The prominent IR spectral bands of the ligand and the complexes are given in Table 8. The binding sites of the ligand in metal complexes are assigned based on the following assumptions. By comparing the spectrum of the ligand with that of the corresponding metal complexes, it can be inferred that the absorption bands of the ligand generally undergo shifts from their positions upon coordination to metal ions in the complexes.

The presence of a band at 1638 cm^{-1} attributed to the azomethine group in the ligand spectrum, was shifted towards lower frequency, ~ 1597 cm^{-1} in the spectra of all the complexes, indicating the involvement of the azomethine nitrogen atom in coordination[20,21]. The medium intensity band at 3187 cm^{-1}in the ligand spectrum, due to the secondary –NH group of the thiosemicarbazone moiety, has been retained nearly at the same region in the spectra of the complexes of Mn(II) and Cu(II). This suggested the coordination of thioamide in the thione form itself. However, this band was not found in the spectra of Co(II), Ni(II), Zn(II) and Cd(II) complexes. This indicated the loss of H atom of –NH, *via* the enolization of –NH-C=S to N=C-SH in the presence of metal ions and subsequent coordination through the thiolate sulphur. This in turn was confirmed by the appearance of new bands in the region 790-796 cm^{-1} due to $\nu_{(C-S)}$.

The presence of broad bands in the regions of 3300-3450 cm^{-1} in the spectra of the complexes of Co(II), Ni(II), Zn(II) and Cd(II) were due to O-H stretching modes of water molecules in them. The presence of coordinated water molecules in these complexes was

supported by the bands in the low frequency region, 465–475 cm^{-1}due to $\nu_{(M-O)}$. The bands that appeared at the very low frequency region, ~420 cm^{-1} in the spectra of the complexes of Mn(II) and Cu(II) may be attributed to $\nu_{(M-Cl)}$[22]. The $\nu_{(C=S)}$ in the ligand spectrum got shifted to lower frequency region by a few cm^{-1} upon the coordination through thione sulphur in the complexes of Mn(II) and Cu(II). The bands at 1079-1058 cm^{-1} in the spectra of ligand and complexes can be assigned to $\nu_{(N-N)}$. The new bands in the spectra of all the metal complexes in the low frequency regions, 535–695 and ~ 440 cm^{-1} were the characteristic of M–N and M–S stretching vibrations, respectively. These observations indicated the involvement of thiolato/thione sulfur and the azomethine nitrogen in bonding during the complex formation. Therefore, in the complexes of Mn(II) and Cu(II), the ligand acted as a monoanionic bidentate one (L^-) and as a neutral bidentate one (LH) in Co(II), Ni(II), Zn(II) and Cd(II) complexes.

Table 8 Characteristic IR spectral bands (in cm^{-1})of the CHTSC ligand and its complexes

Compound	$C_{23}H_{21}N_3S$ (LH)	$[Mn(LH)_2Cl_2]$	$[CoL_2(H_2O)_2]$	$[NiL_2(H_2O)_2]$	$[Cu(LH)_2Cl_2]$	$[ZnL_2(H_2O)_2]$	$[CdL_2(H_2O)_2]$
$\nu_{(N-H)}$	3187	3159			3242		
$\nu_{(O-H)}$			3392	3446		3452	3430
$\nu_{(C=N)}$	1638	1618	1577	1598	1586	1583	1617
$\nu_{(N-N)}$	1048	1060	1077	1054	1081	1038	1048
$\nu_{(C=S)}$	856	821	766	789	766	783	752
$\nu_{(M-N)}$		544	697	528	630	526	548
$\nu_{(M-S)}$		450	471	424	439	429	431
$\nu_{(M-O)}$			437	472		459	468
$\nu_{(M-Cl)}$		421			428		

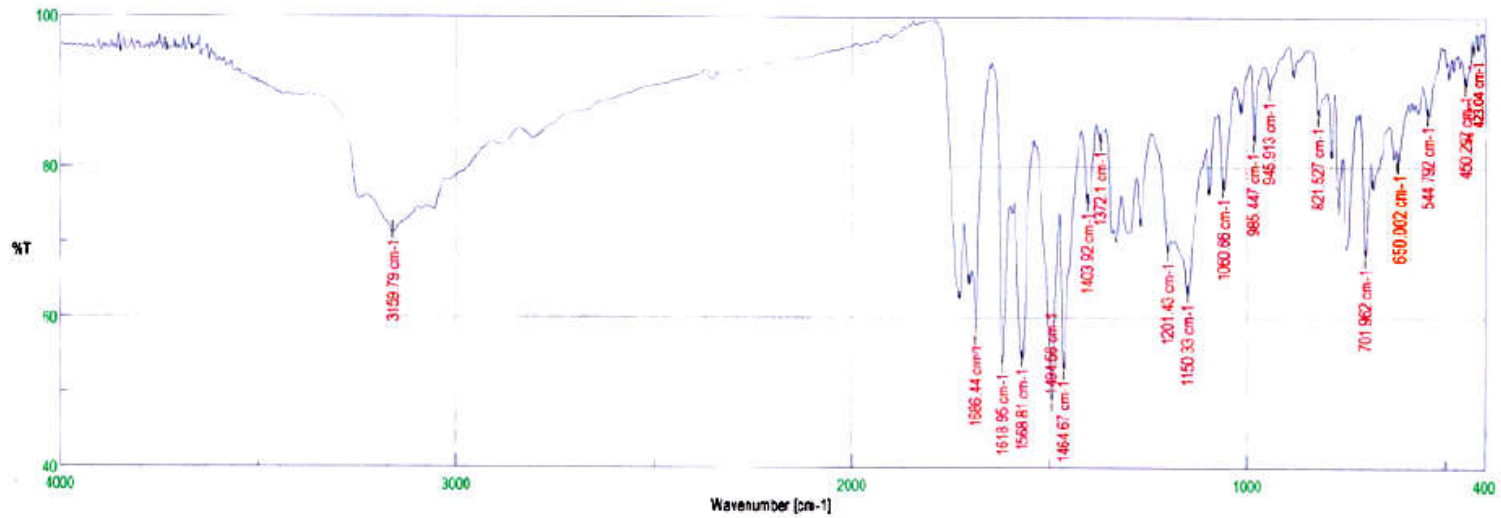


Figure 4

IR spectrum of Mn(II) complex of chalcone N(4)-methyl(phenyl)thiosemicarbazone [$Mn(LH)_2Cl_2$]

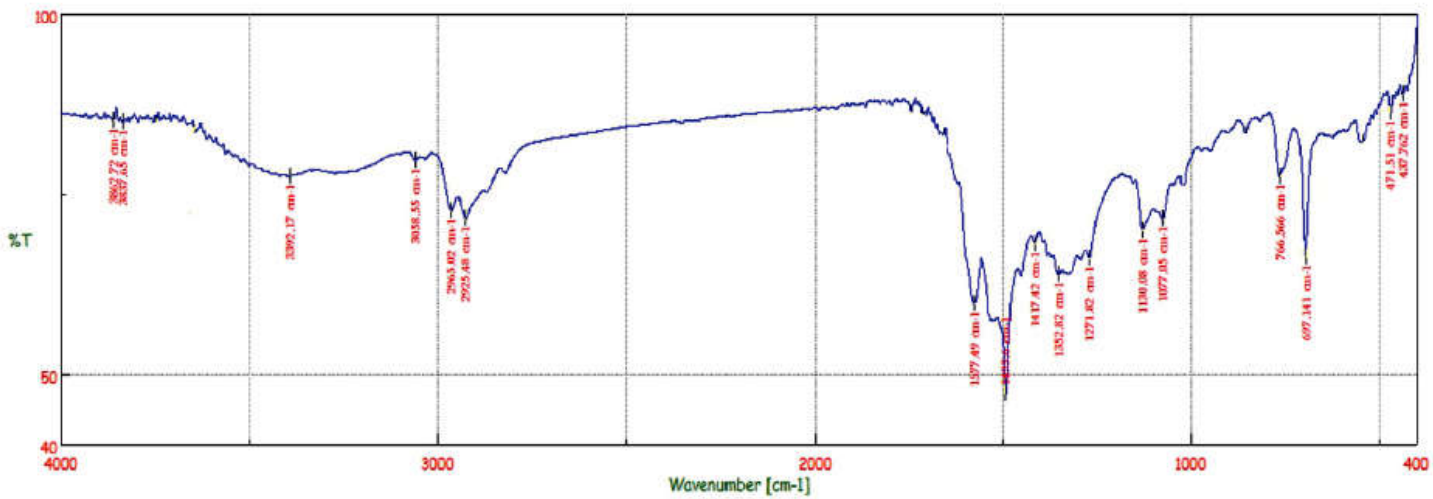


Figure 5

IR spectrum of Co(II) complex of chalcone N(4)-methyl(phenyl)thiosemicarbazone [$CoL_2(H_2O)_2$]

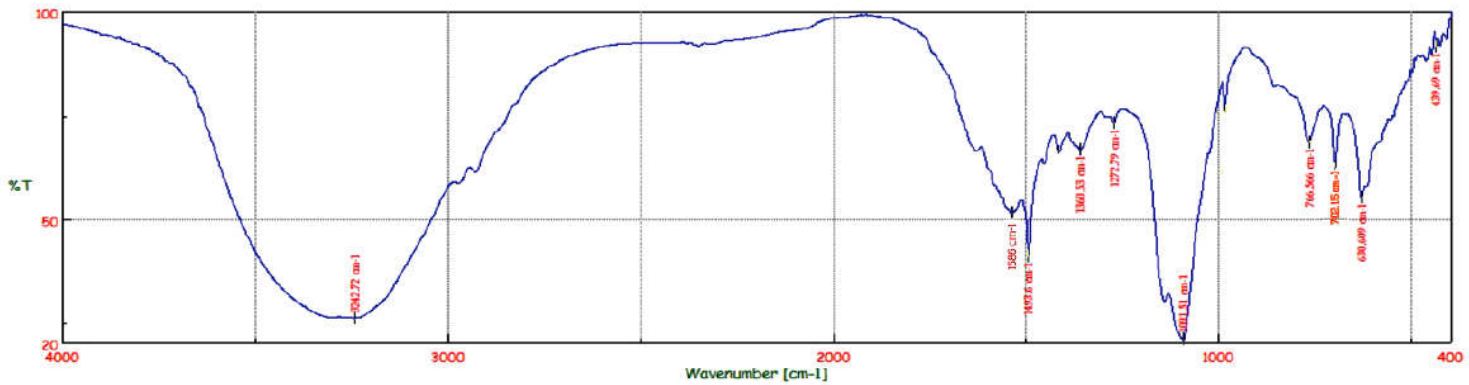


Figure 6

IR spectrum of Cu(II) complex of chalcone N(4)-methyl(phenyl)thiosemicarbazone [$Cu(LH)_2Cl_2$]

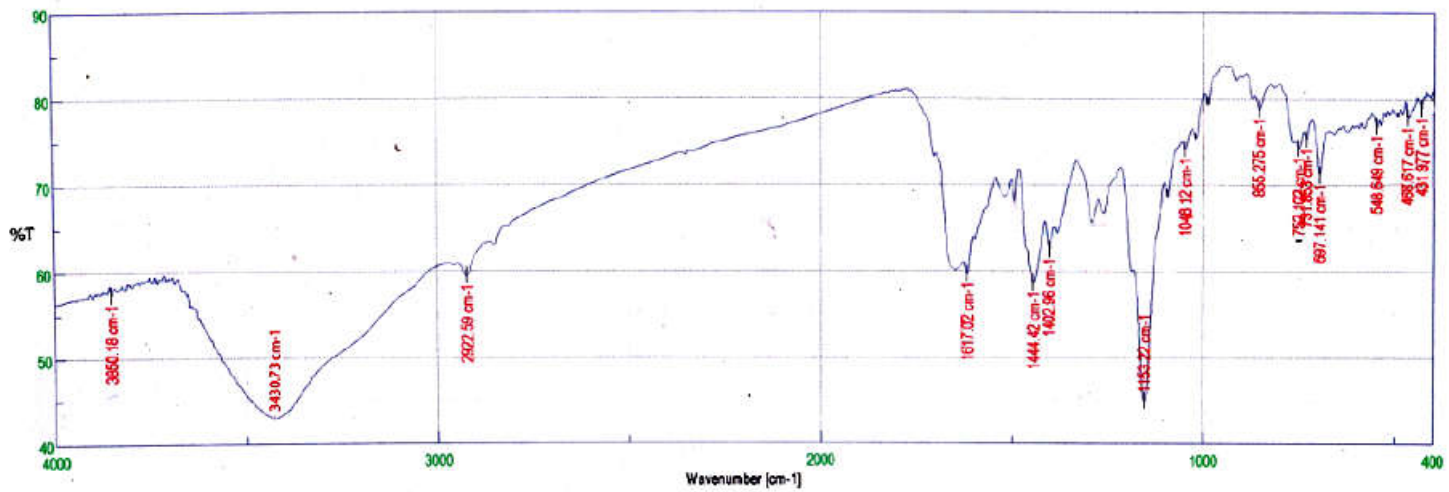


Figure 7

IR spectrum of Cd(II) complex of chalcone N(4)-methyl(phenyl)thiosemicarbazone [$CdL_2(H_2O)_2$]

4.4 Conclusions

In the present investigation, synthesis and characterization of the complexes of Mn(II), Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) with a bidentate ligand, chalcone N(4)-methyl(phenyl)thiosemicarbazone (LH) have been carried out. The elemental analysis and the spectral data revealed the monoanionic/neutral bidentate chelating nature of the ligand. The ligand coordinated through azomethine nitrogen and thiolato/thione sulfur in all the complexes. Based on the magnetic moment measurements and electronic spectral studies, the formation of mononuclear octahedral complexes with the general formulae, $[M(LH)_2Cl_2]$ [M: Mn(II) or Cu(II)] and $[ML_2(H_2O)_2]$ [M:Co(II), Ni(II), Zn(II) or Cd(II)] has been confirmed. The structures of the complexes are shown in Figures 8 and 9.

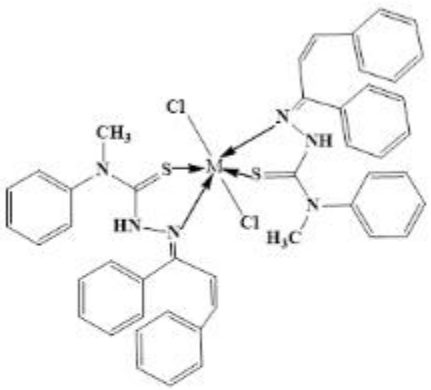


Figure 8

Suggested octahedral structure of the complexes of the formula

$[M(LH)_2Cl_2]$,

where M = Mn(II) or Cu(II)

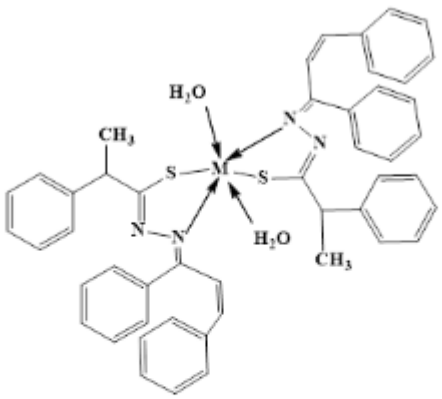


Figure 9

Suggested structure of the complexes of the formula

$[ML_2(H_2O)_2]$,

where M = Co(II), Ni(II), Zn(II) or Cd(II)

4.5 References

[1] M. A. Ali, S. E. Livingstone, Coord. Chem. Rev., 13, 101 (1974)

[2] S. Padhye, G. B. Kauffman, Coord. Chem. Rev., 63, 127 (1985)

[3] D. X. West, A. E. Liberta, S. Padhye, R.C. Chilkate, P.B. Sonawane, A.S. Kumbhar, R. G. Yerande, Coord. Chem. Rev., 123, 49 (1993)

[4] H. Beraldo, D. Gambino, Mini-Rev. Med. Chem., 4, 31 (2004)

[5] A. A. Hassan, A. M. Shawky, H. S. Shehatta, J. Heterocycl. Chem., 1, 49 (2012)

[6] G. Di Carlo, N. Mascolo, A.A. Izzo, F. Capasso, Life Sci., 65, 337 (1999)

[7] J. G. Da Silva, C. C. H. Perdigao, N. L. Speziali, H. Beraldo, J. Coord. Chem., 3, 66 (2013)

[8] M. Muthukumar, P. Viswanathamurthi, Cent. Eur. J. Chem., 8, 229 (2010)

[9] H. J. Zhang, Y. Qian, D. D. Zhu, X. G. Yang, H. L. Zhu, Eur. J. Med. Chem., 46, 4702 (2011)

[10] J. G. Da Silva, C.C.H. Perdigao, N.L. Speziali, H. Beraldo, J. Coord. Chem., 66, 385 (2013)

[11] A. N. Choudhary, V. Juyal, Int. Pharm. Pharm. Sci., 3, 125 (2011)

[12] W. J. Geary, J. Coord. Chem. Rev., 7, 81(1971)

[13] K. Patel, M. M. Patel, Ind. J. Chem., 29, 90 (1990)

[14] B. P. Lever, Inorganic Electronic Spectroscopy, Elsevier, Amsterdam, The Netherlands (1984)

[15] F. A. Cotton, G. Wilkinson, C. A. Murillo, M. Bochmann, Advanced Inorganic Chemistry, Wiley, New York (1999)

[16] G. G. Mohamed, N. E. A. El-Gamel, F. Teixidor, Polyhedron., 20, 2689 (2001)

[17] R. K. Agarwal, P. Garg, H. Agarwal, S. Chandra, Synth. React. Inorg. Met.-Org. Chem., 27, 251 (1997)

[18] L. Sacconi, M. Ciampolini, U. Campigli, Inorg. Chem., 4, 407 (1965)

[19] P. S. Patel, R. M. Ray, M. M. Patel, Ind. J. Chem. Soc., 32, 597 (1993)

[20] K.Shanker, R. Rohini, V. Ravinder, P. Muralidhar Reddy, Y. P. Ho, Spectrochim. Acta A.,73,205 (2009)

[21] N. Raman, S. Sobha, A. Thamaraichelvan, Spectrochim. Acta Mol. Biomol. Spectrosc.,78, 888 (2011)

[22] P. M. Reddy, K. Shanker, R. Rohini, V. Ravinder, Int. J. ChemTech Res., 1, 367 (2009)

CHAPTER V

SYNTHESIS AND CHARACTERIZATION OF TRANSITION METAL COMPLEXES OF ISATIN N(4)-METHYL(PHENYL)THIOSEMICARBAZONE

5.1 Introduction

Thiosemicarbazones are considered as mixed hard-soft N, S chelating agents with variable binding modes. By virtue of their versatile NS donor systems, thiosemicarbazones give rise to exceptionally stable metal chelates with enhanced biological properties[1,2]. The structural variations of thiosemicarbazone based compounds can be increased considerably by using different carbonyl compounds and by changing the substituents present at N(4) position of the thiosemicarbazide moiety[3]. Among the thiosemicarbazones, those with electron withdrawing substituents at N(4)-position have been studied widely due to their exceptional biological- and cytotoxic activities[4-7].

The first-row transition metals are important in human life. Many indole derivatives usually present endogenously in both human- and other mammalian tissues, are the main structural motifs found in many pharmacologically active compounds. Isatin, an indole derivative possesses considerable biological activity by virtue of its characteristic size and restricted electronic properties[8-10]. Therefore, the transition metal complexes of isatin thiosemicarbazone are expected to give rise to biologically active drugs.

Increasing number of researchers, both from industrial and academic fields, are involved in the expansion of isatin-based anticancer drugs[11-14]. This is due to the susceptibility of isatin's molecular structure for further modification and derivatization. This is evidenced by the fact that numerous isatin derivatives show broad ranges of biological activities such as anticancer[15,16] antidepressant[17], anticonvulsant[18], antifungal[19], antiHIV[20] and antiinflammatory activities[21]. During the last few decades, there has been considerable interest on the synthesis of thiosemicarbazones derived from isatin and their metal complexes[22-25]. Haribabu and co-workers[26] reported the syntheses of a new series of isatin based thiosemicarbazone from benzylisatin and unsubstituted/substituted thiosemicarbazides. *In vitro* antioxidant and antihaemolytic studies of these thiosemicarbazone derivatives revealed their excellent effects.

Ali *et al*[27-29] reported the synthesis of substituted thiosemicarbazones of isatin and their Pt(II) and Cu(II) complexes. The tridentate thiosemicarbazone derived from methylisatin and N-ethylthiosemicarbazide and their complexes were evaluated for their DNA binding, cleavage and *in vitro* antiproliferative activities against human colon cancer cell line. The overall positive results can be attributed to the presence of an extended aromatic phenyl ring that allows the intercalating ligand to deeply penetrate into the DNA base pairs.

Though other N-substituted thiosemicarbazones of isatin are known[27-29] a thorough literature exploration revealed that no work has been reported on isatin N(4)-disubstituted thiosemicarbazone,. Therefore, in the current work, a new ligand system, isatin N(4)-methyl(phenyl)thio semicarbazone (ISTSC), is reported and used as a chelating ligand for the preparation of Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II) and Cd(II)

complexes. The characterization of the ligand and its complexes are discussed in this chapter. The structure of the ligand is shown in Figure 1.

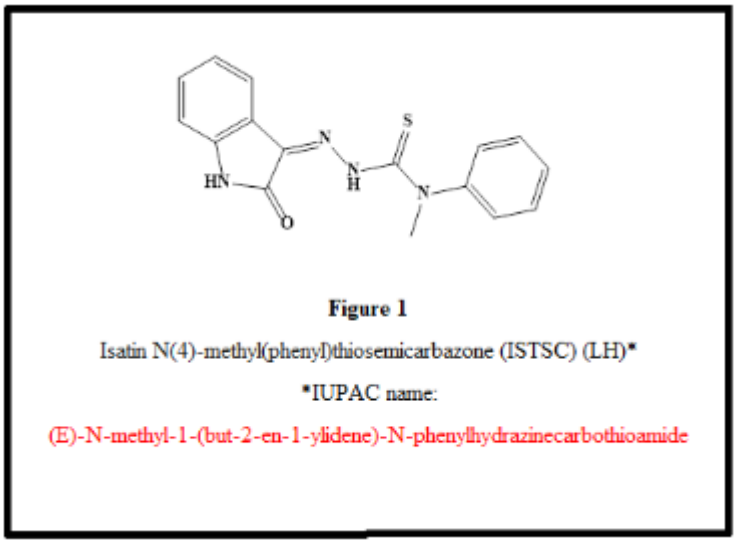


Figure 1

Isatin N(4)-methyl(phenyl)thiosemicarbazone (ISTSC) (LH)*

*IUPAC name:

(E)-N-methyl-1-(but-2-en-1-ylidene)-N-phenylhydrazinecarbothioamide

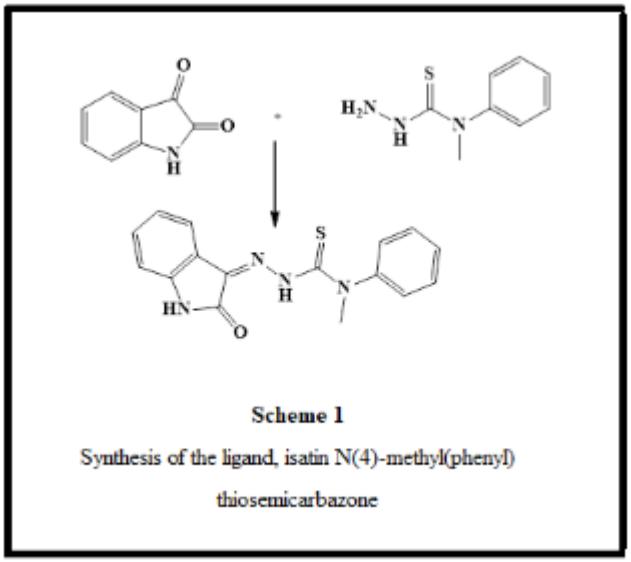
5.2 Experimental

5.2.1 Materials and methods

The details about the chemicals used and the adopted scheme for the synthesis of precursor and the characterization methods used are described in Chapter II.

5.2.2 Synthesis of ligand

Isatin N(4)-methyl(phenyl)thiosemicarbazone was synthesized by refluxing ethanolic solutions of isatin (0.05 mol) and N(4)-methyl(phenyl)thiosemicarbazide (0.05 mol) in the presence of a catalytic amount of glacial acetic acid on a water bath for 2 h. A dark orange solid separated at room temperature was filtered and dried under vacuum (Yield 76%, M.P 183^{0}C). The synthetic procedure of the ligand, isatin N(4)-methyl(phenyl)thiosemicarbazone is shown in Scheme 1.



Scheme 1

Synthesis of the ligand, isatin N(4)-methyl(phenyl) thiosemicarbazone

5.2.3 Synthesis of metal complexes

The complexes of Mn(II), Fe(III), Co(II), Ni(II) and Cu(II) with ISTSC were prepared by refluxing a methanolic solution of the respective metal salt (0.005 mol) with an ethanolic solution of the ligand (0.005 mol)

for 4 h. The resulting solution was concentrated and cooled. The complexes of Zn(II) and Cd(II) were prepared using the same procedure by taking the ligand and metal acetates in 2:1 molar ratio. The solid complexes thus obtained were filtered off, washed several times with methanol and then dried under vacuum. The physico-chemical and analytical data obtained for the ligand and its complexes are presented in Table 5.

5.3 Results and discussion

The physical properties and elemental analytical data have been used to explain the structure and bonding of the compounds.

5.3.1 Characterization of the ligand

The dark orange coloured ligand, isatin N(4)-methyl(phenyl)thiosemicarbazone was soluble in chloroform, ethanol, etc. The compound was non-hygroscopic and stable under atmospheric conditions. It was characterized analytically and further by IR-, UV-Vis- and ^{1}H NMR spectral techniques. The data obtained from these were used to elucidate its structure.

5.3.1.1 Elemental analysis

The elemental analysis of the ligand was carried out on a Vario EL III CHNS instrument. The experimentally found out and calculated percentages of C, H, N and S were in good agreement, confirming the molecular formula, $C_{16}H_{14}N_4OS$ (LH) for the ligand (Table 1).

Table 1 Physico-chemical and analytical data of the ligand							
Compound (Empirical formula)	% of Yield	Melting point (^{0}C)	Color of the Compound	Anal. Found (Calcu) %			
				C	H	N	S
$C_{16}H_{14}N_4OS$ (LH)	76	183	Dark Orange	61.81 (61.91)	4.58 (4.51)	18.17 (18.07)	10.43 (10.31)

5.3.1.2 Electronic spectrum

The electronic spectrum of the ligand showed a strong band at 278 nm which can be attributed to π-π* transition. Similarly, a band at 362 nm in the ligand spectrum may be due to n –π* transition of the azomethine group and thioamide part of the thiosemicarbazone moeity. The data are given in Table 2.

Table 2 UV-Vis spectral data of the ligand		
Compound	Spectral Bands	Assignment
	λ_{max}	
	(nm)	
$C_{16}H_{14}N_4OS$ (LH)	278	π→π* transition
	362	n→π* transition

5.3.1.3 Infrared spectrum

The observed IR spectral bands of the ligand and their assignments are given in Table 3. The IR spectrum of the ligand showed bands of medium intensities in the region 3500-3000 cm^{-1}. They may be attributed to the stretching vibrations of the secondary –NH group of the thiosemicarbazone moiety[30-32]. The other medium intensity bands at 2898 and 2814 cm^{-1} may be assigned, respectively, to asymmetric- and symmetric stretching of =CH. The presence of C=N bond was confirmed by the presence of an absorption band at 1620 cm^{-1}. The $\nu_{(C=O)}$ absorption band of the aromatic ketones usually appears in the range, 1700 cm^{-1} [33]. A sharp peak at 1698 cm^{-1} in the spectrum of the ligand can be assigned to the $\nu_{(C=O)}$ of the isatin moeity. The identification of the absorption band due to C=S stretching vibration is a very difficult, because it usually results an extremely broad band in the 1420–700 cm^{-1} region[34] and is mixed possibly with several other bands. In the spectrum of the ligand, the vibrational mode of C=S group was accounted for a band at 792 cm^{-1} and this band was affected during the coordination of sulphur to the metal atom in the complexes. The presence of a band at 984 cm^{-1} in the spectrum of ligand can be assigned to $\nu_{(N-N).}$

Table 3 Significant IR spectral bands (cm^{-1}) of the ligand	
Spectral bands	Assignments
3448	$\nu_{(N-H)}$
3161	$\nu_{(N-H)}$
3056	$\nu_{(N-H)}$
2898	$\nu_{=CH}$ (asymmetric stretching)
2814	$\nu_{=CH}$ (symmetric stretching)
1698	$\nu_{(C=O)}$
1620	$\nu_{(C=N)}$
984	$\nu_{(N-N)}$
792	$\nu_{(C=S)}$

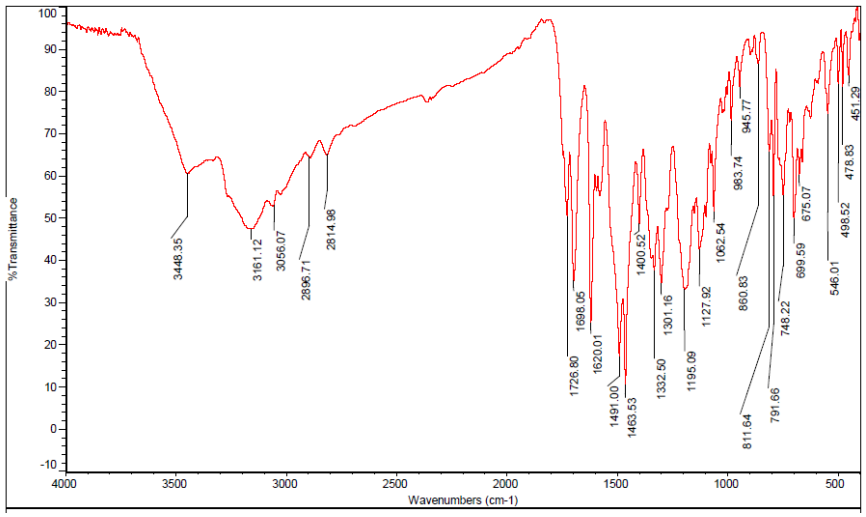


Figure 2

IR spectrum of isatin N(4)-methyl(phenyl)thiosemicarbazone

5.3.1.4 ^{1}H NMR spectrum

The ^{1}H NMR spectrum of the ligand has a multiplet between 6.8-7.2 ppm due to the protons of C_6H_5 group present on the terminal nitrogen atom. Another multiplet in the range of 7.24-7.75 ppm was due to the aromatic protons of isatin moiety. A singlet observed at 9.4 ppm was attributed to the –NH proton. A sharp signal appeared at 3.4 ppm was assignable to $–CH_3$ protons on the terminal N-atom. The signal that appeared at 10.8 ppm was due to the –NH proton of the isatin ring. (Table 4)

Table 4 ^{1}H NMR data of the ligand

Sl.No.	δ (ppm)	Assignment
1	3.4 (s)	$–CH_3$ on terminal N atom
2	6.8-7.2 (m)	Aromatic (phenyl group)
3	7.24-7.75 (m)	aromatic (isatin moiety)
4	9.4 (s)	–NH group
5	10.8 (s)	–NH of isatin ring

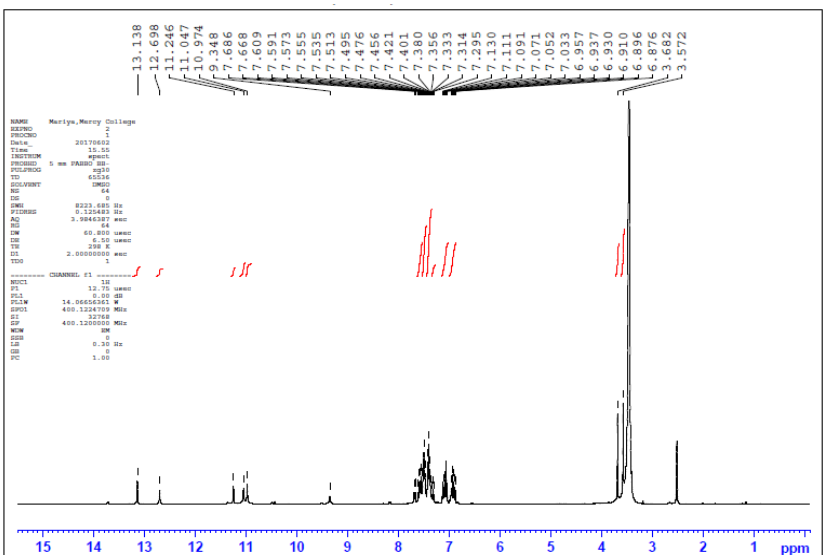


Figure 3

^{1}H NMR spectrum of isatin N(4)-methyl(phenyl)thiosemicarbazone

5.3.2 Characterization of the complexes

All the complexes of isatin N(4)-methyl(phenyl)thiosemicarbazone were stable under normal atmospheric conditions. They were non-hygroscopic and soluble in chloroform and DMSO, but less soluble in other common organic solvents like methanol and ethanol. The elemental analytical data of the complexes were obtained on the CHNS rapid analyzer. Magnetic measurements, IR- and electronic spectral studies were employed for the characterization and structural elucidation of the complexes.

5.3.2.1 Analytical data

The analytical data of all the complexes agreed well with their proposed molecular formulae. The Mn(II), Ni(II), Zn(II) and Cd(II) complexes have a general formula, $[ML_2(H_2O)_2]$. The complexes of Fe(III) and Co(II) were found to be octahedral and they corresponded to the formulae of $[FeL_2Cl(H_2O)]$ and $[Co(LH)_2Cl_2]$, respectively. $[CuL(H_2O)Cl]$ was found to possess a tetrahedral geometry. The determination of metal and chloride contents in the complexes was done by the standard procedures[35]. The electrical conductance of 10^{-3} M DMSO solutions of the complexes were measured and the molar conductance values indicated the non-electrolytic nature of the complexes.

Table 5 Physico-chemical and analytical data of the complexes

Compound (Empirical formula)	% of Yield	Melting point (^{0}C)	Colour of the Compound	Anal. Found % (Calculated %)					
				M	C	H	N	S	Cl
$[MnL_2(H_2O)_2]$	58	214	Pale Brown	7.69 (7.76)	54.24 (54.16)	4.34 (4.23)	15.73 (15.80)	9.1 (9.03)	
$[FeL_2Cl(H_2O)]$	76	225	Brown	7.58 (7.68)	52.74 (52.70)	3.79 (3.84)	15.40 (15.37)	8.67 (8.78)	4.72 (4.88)
$[Co(LH)_2Cl_2]$	72	234	Pale Red	7.92 (7.88)	47.68 (47.92)	3.26 (3.24)	17.42 (17.47)	7.89 (7.99)	9.38 (9.47)
$[NiL_2(H_2O)_2]$	78	249	Brownish Red	8.18 (8.25)	53.89 (53.85)	4.17 (4.21)	15.76 (15.71)	8.86 (8.97)	
$[CuL(H_2O)Cl]$	65	255	Dark Brown	16.97 (17.13)	53.62 (53.54)	4.1 (4.18)	15.43 (15.61)	8.84 (8.92)	8.24 (8.34)
$[ZnL_2(H_2O)_2]$	75	218	Brown	14.21 (13.76)	55.86 (55.79)	4.58 (4.65)	16.21 (16.27)	9.21 (9.30)	
$[CdL_2(H_2O)_2]$	73	224	Brown	14.56 (14.68)	49.97 (50.10)	3.77 (3.91)	14.46 (14.61)	8.15 (8.35)	

5.3.2.2 Magnetic moments

The magnetic moments were calculated from the corrected molar susceptibilities. The magnetic moment values of the metal complexes usually depend upon the number of unpaired electrons present in them. The magnetic susceptibility measurement helps to determine the oxidation state of the metal ion and the probable geometry of the complex. The calculated magnetic moment values of the complexes of Mn(II), Fe(III), Co(II), Ni(II) and Cu(II) are given in Table 6.

Manganese(II) forms both high-spin (S =5/2) and low-spin (S = 1/2) octahedral as well as tetrahedral complexes. In the case of high-spin octahedral and tetrahedral complexes of Mn(II), the spin-only magnetic moment value of 5.92 B.M is expected at room temperature. However, the observed magnetic moments of high-spin octahedral Mn(II) complexes are found to be in the range of 5.65-6.10 B.M. The experimental magnetic moment values for a number of Mn(II) complexes are found to be in the range of 4.74-5.50 B.M. However, these complexes may not be magnetically dilute and there are no apparent explanations for these low magnetic moment values[36,37]. In the present case, the observed value of magnetic moment was 5.78 B.M for the $[MnL_2(H_2O)_2]$ complex which indicated an octahedral geometry around the central metal ion.

Iron(III) is usually high-spin in all its complexes. Fe(III) is isoelectronic with Mn(II). The high-spin octahedral Fe(III) complex with d^5 configuration, has a spin-only magnetic moment value of 5.90 B.M. In the present investigation, the Fe(III) complex showed a magnetic moment value of 5.63 B.M and it was assumed to have an octahedral geometry.

The high-spin octahedral Co(II) ion has an observed magnetic moment value of 5.20 B.M, which is greater than the spin-only value of 3.87 B.M. This could be due to the considerable orbital contribution that leads to magnetic moment values in the range of 4.70-5.20 B.M. The Co(II) complex reported here registered a magnetic moment value of 5.28 B.M. This value together with the colour of the complex clearly indicated a high-spin octahedral geometry around Co(II) ion.

The octahedral complexes of Ni(II) are paramagnetic and can exist as both the high-spin and low-spin type. The magnetic moment values for octahedral Ni(II) complexes are usually in the range of 2.90-3.30 B.M, which is slightly higher than the spin-only value. The slightly higher value of the moment is due to the spin-orbit coupling. A magnetic moment value of 3.13 B.M for $[NiL_2(H_2O)_2]$ indicated its octahedral geometry.

Generally the magnetic moment values of Cu(II) complexes are independent of their stereochemistry. Both octahedral and tetrahedral Cu(II) complexes have almost identical magnetic moments. For a regular tetrahedral Cu(II) complex, a room temperature magnetic moment of 2.20 B.M is expected. However, the observed moments are in the range of 1.95-2.00 B.M. The Cu(II) complexes exhibit distorted octahedral geometry with observed magnetic moment values in the range of 1.80-2.10 B.M which are slightly greater than the spin-only value of 1.73 B.M. This is due to the spin-orbit coupling in the ion. The Cu(II) complex obtained here showed a magnetic moment of 2.12 B.M, indicating a tetrahedral structure.

Table 6 Magnetic moments of the complexes	
Complex	μ_{eff} (B.M)
$[MnL_2(H_2O)_2]$	5.78
$[FeL_2Cl(H_2O)]$	5.63
$[Co(LH)_2Cl_2]$	5.28
$[NiL_2(H_2O)_2]$	3.13
$[CuL(H_2O)Cl]$	2.12

5.3.2.3 Electronic spectra

The electronic spectra of the complexes were recorded in the solid state. They provide detailed information about the electronic structures of the complexes. Main emphasis is given for the d-d transition bands, because they give an obvious idea about the environment around the metal ion. The electronic spectral data of the complexes, the spectral band positions and transition assignments are of particular significance, because they are highly dependent on the geometry of the complexes. The spectral bands of Mn(II), Fe(III), Co(II), Ni(II) and Cu(II) complexes of ISTSC are presented in Table 7, along with their band assignments.

The majority of Mn(II) complexes are high-spin type in an octahedral environment, with no spin-allowed d-d transitions. All are multiplicity and Laporte-forbidden transitions. The Mn(II) complexes do not register any characteristic bands in the visible region. The magnetic moment value of $[MnL_2(H_2O)_2]$ along with extremely pale brown colour indicated its high-spin octahedral geometry. The spectrum of the complex

in the present case, registered bands at 647 and 489 nm, which may be assigned to $^6A_{1g} \rightarrow {}^4T_{1g}$ and $^6A_{1g} \rightarrow {}^4T_{2g}$ transitions, typical of an octahedral Mn(II) complex.

Iron(III) is isoelectronic with Mn(II) and almost all the complexes of Fe(III) are of high-spin type with an octahedral geometry. The ground term of high-spin Fe(III) complex with d^5 configuration is $^6A_{1g}$. In the present investigation, the electronic spectrum of Fe(III) chelate registered two bands at 428 and 642 nm. These may be assigned to C.T and $^6A_{1g} \rightarrow {}^4T_{1g}$ transitions, respectively of Fe(III) in a high-spin d^5 configuration. The observed magnetic moment of 5.63 B.M for $[FeL_2Cl(H_2O)]$ also confirmed its octahedral geometry**.**

The octahedral complexes of Co(II) has $^4T_{1g}$ as the ground term with considerable orbital contribution and has an observed magnetic moment value around 5.20 B.M. The highest energy transition, $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ is the most prominent one among the three spin-allowed transitions of an octahedral Co(II) complex. The transition, $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ occurs usually in the near IR region. The electronic spectrum of the Co(II) complex investigated here, showed transitions at 1046, 628 and 403 nm corresponding, respectively, to $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$, $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ and $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ transitions, typically of an octahedral Co(II) complex. In the present investigation, the magnetic moment value of 5.28 B.M together with these bands indicate an octahedral structure for the Co(II) complex.

The electronic spectral bands corresponding to three spin-allowed transitions in the case of octahedral Ni(II) complexes are due to the expected transitions, $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$, $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ and $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$. The spectral bands corresponding to these transitions are

usually found in the regions around 1050, 650 and 450 nm, respectively. The electronic spectrum of the Ni(II) complex investigated here registered three bands at 1023, 658 and 423 nm, corresponding to these transitions. These bands together with a magnetic moment value of 3.13 B.M supported an octahedral geometry for $[NiL_2(H_2O)_2]$.

The energy bands of tetrahedral Cu(II) complexes are in the low energy region when compared to that of square planar ones. In the electronic spectrum of $[CuL(H_2O)Cl]$, the band found at 749 nm may be due to $^2E \rightarrow {}^2T_2$ transition. This band and a magnetic moment value of 2.12 B.M supported a tetrahedral structure for $[CuL(H_2O)Cl]$.

Table 7 Electronic Spectral Bands of the Complexes of ISTSC and their Assignments

Complex	Spectral Bands λ_{max} (nm)	Assignment
$[MnL_2(H_2O)_2]$	424	$^6A_{1g} \rightarrow {}^4E_g$
	489	$^6A_{1g} \rightarrow {}^4T_{2g}$
	647	$^6A_{1g} \rightarrow {}^4T_{1g}$
$[FeL_2Cl(H_2O)]$	428	C.T
	642	$^6A_{1g} \rightarrow {}^4T_{1g}$
$[Co(LH)_2Cl_2]$	403	$^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$
	628	$^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$
	1046	$^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$
$[NiL_2(H_2O)_2]$	423	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$
	658	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$
	1023	$^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$
$[CuL(H_2O)Cl]$	749	$^2E \rightarrow {}^2T_2$

5.3.2.4 Infrared spectra

The significant absorption bands in the ligand spectrum generally undergo shift from their positions upon coordination to metal ions. This in turn provides indication regarding the bonding sites of the ligands. The IR spectral bands of the ligand and its complexes along with their probable assignments are listed in Table 8.

The infrared spectrum of isatin thiosemicarbazone and its metal complexes are mainly characterized by the absorptions due to the –NH, –C=S, –C=N, –N-N and –C=O groups. The ligand spectrum showed bands in the range, 3400–3050 cm^{-1} which can be assigned to $\nu_{(N-H)}$ of the thiosemicarbazone moiety. The medium intensity band at 3161 cm^{-1} in the ligand spectrum can be assigned to the $\nu_{(N-H)}$ of the thioamide group. This was not found in the spectra of all the complexes, except that of the Co(II) complex. The band found at 792 cm^{-1} in the spectrum of the free ligand due to $\nu_{(C=S)}$, shifted to lower wave numbers during the complex formation. In the spectra of the complexes, except that of the Co(II), this band disappeared and a new band appeared in the region of 750-760 cm^{-1} due to $\nu_{(-C-S)}$. This is compatible with the enolisation of –NH-C=S to –N=C-SH, deprotonation of S-H and its subsequent coordination through the thiolate sulphur atom (S^-) to the metal ions. However, in the spectrum of Co(II) complex, the $\nu_{(C=S)}$ shifted slightly to lower wave number and found at 780 cm^{-1}. This suggested the coordination of thioamide in the thione form itself to Co(II).

The presence of a characteristic vibrational band at 1620 cm^{-1} in the ligand spectrum may be assigned to $\nu_{(C=N)}$. This band shifted to lower wave numbers in the spectra of all the complexes, indicating the participation of

nitrogen atom of the azomethine group in coordination. The band observed at 984 cm^{-1} in the ligand spectrum was due to $\nu_{(N-N)}$. This shifted to higher wave numbers of 1002-1045 cm^{-1} in all the complexes. In comparison with the spectrum of thiosemicarbazone, a strong band was observed in the range, 1700 cm^{-1} in the spectra of all the complexes, due to $\nu_{(C=O)}$. This indicated that the carbonyl oxygen was not involved in coordination with the metal ion. All these supported the coordination of the ligand through azomethine nitrogen and deprotonated thiolate-sulphur atom in the complexes of Mn(II), Fe(III), Ni(II), Cu(II), Zn(II) and Cd(II). However, in the Co(II) complex, the azomethine nitrogen and thione-sulphur atoms are involved in the coordination. The conclusive evidence for the bonding was the appearance of new bands in the low frequency regions ~553-690 cm^{-1} and ~470 cm^{-1} in the spectra of all the complexes which can be assigned to $\nu_{(M-N)}$ and $\nu_{(M-S)}$, respectively.

The broad bands observed in the range 3484 cm^{-1} in the spectra of the complexes of Mn(II), Fe(III), Ni(II), Cu(II), Zn(II) and Cd(II) were recognized as O-H stretching modes of the coordinated water molecules in them. The other evidence that supported the presence of coordinated water molecules in these complexes was the appearance of new bands in the region ~475-490cm^{-1} due to $\nu_{(M-O)}$. The absorption bands in the very low frequency region of ~420 cm^{-1}in the spectra of Fe(III), Co(II) and Cu(II) complexes may be due to $\nu_{(M-Cl)}$.

Taking into account of the assignments of all the vibrational absorptions, the donor behavior of the ligand in the metal complexes can be proposed as N, S/S^- bidentate one.

Table 8 Characteristic IR spectral bands (in cm^{-1})of the ISTSC ligand and its complexes									
Compound	$\nu_{(N-H)}$	$\nu_{(O-H)}$	$\nu_{(C=N)}$	$\nu_{(N-N)}$	$\nu_{(C=S)}$	$\nu_{(M-N)}$	$\nu_{(M-S)}$	$\nu_{(M-O)}$	$\nu_{(M-Cl)}$
$C_{16}H_{14}N_4OS$ (LH)	3160		1620	984	792				
$[MnL_2(H_2O)_2]$		3478	1586	1027	758	558	432	486	
$[FeL_2Cl(H_2O)]$		3485	1593	1023	765	673	450	483	426
$[Co(LH)_2Cl_2]$	3142		1614	1002	780	695	470		423
$[NiL_2(H_2O)_2]$		3472	1615	1041	750	585	445	489	
$[CuL(H_2O)Cl]$		3482	1617	1042	764	650	447	493	425
$[ZnL_2(H_2O)_2]$		3470	1586	1030	763	560	455	485	
$[CdL_2(H_2O)_2]$		3479	1597	1033	758	635	439	476	

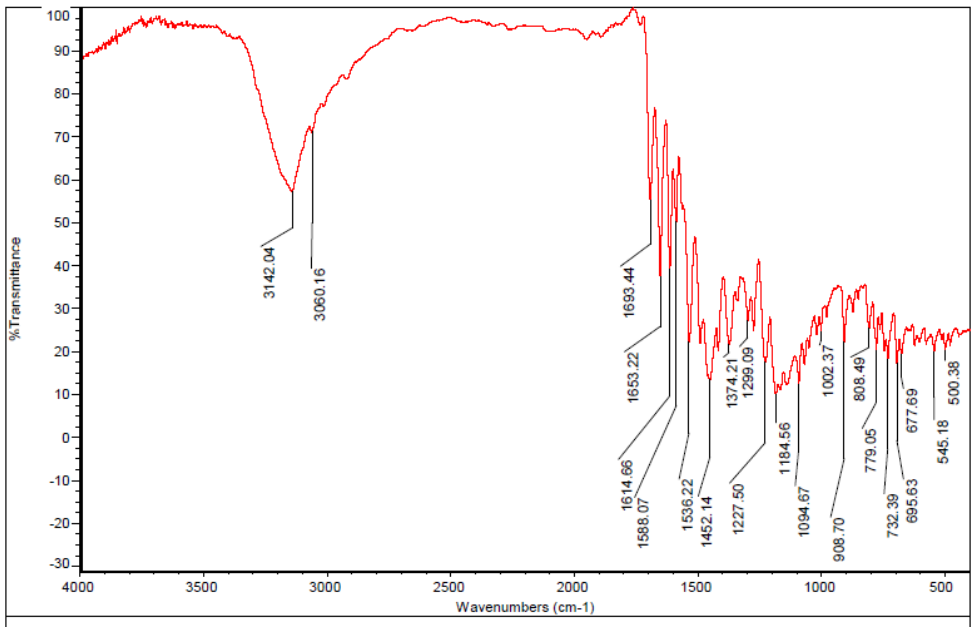


Figure 4

IR spectrum of Co(II) complex of isatinN(4)-methyl(phenyl)thiosemicarbazone $[Co(LH)_2Cl_2]$

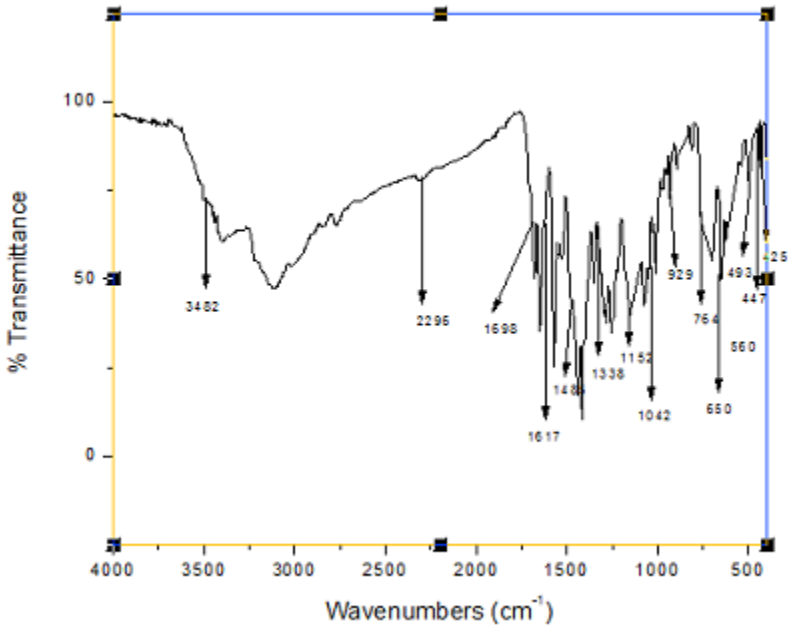


Figure 5

IR spectrum of Cu(II) complex of isatin N(4)-methyl(phenyl)thiosemicarbazone [$CuL(H_2O)Cl$]

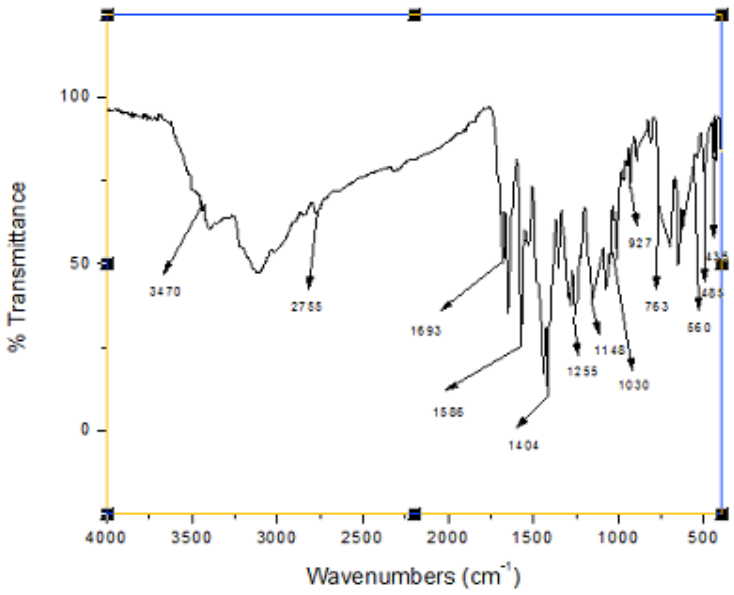


Figure 6

IR spectrum of Zn(II) complex of isatin N(4)-methyl(phenyl)thiosemicarbazone $[ZnL_2(H_2O)_2]$

5.4 Conclusions

The synthesis and characterization of a bidentate ligand, isatin N(4)-methyl(phenyl)thiosemicarbazone (LH) and its Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) complexes have been discussed. From the IR spectral data, the mode of coordination of the ligand was ascertained. The ligand was assumed to behave as a monoanionic/neutral bidentate chelating agent, coordinating through azomethine nitrogen and thiolate/thione sulfur in all the complexes. Based on the magnetic moment measurements and electronic spectral studies, the complexes of Mn(II), Fe(III),Co(II), Ni(II), Zn(II) and Cd(II) were assigned octahedral

geometries with general molecular formulae,. $[ML_2(H_2O)_2]$, where M = Mn(II), Ni(II), Zn(II), Cd(II), $[Co(LH)_2Cl_2]$ and $[FeL_2Cl(H_2O)]$ are shown in Figures 7, 8 and 9. A tetrahedral geometry was assigned to $[CuL(Cl)H_2O]$, which is given in Figure 10.

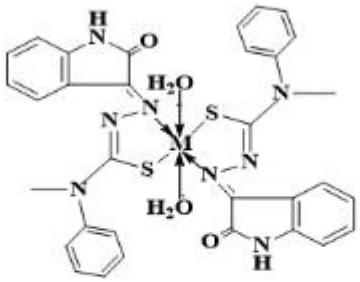


Figure 7

Suggested structure of the complexes of the formula

$[ML_2(H_2O)_2]$,

where M = Mn(II), Ni(II), Zn(II) or Cd(II)

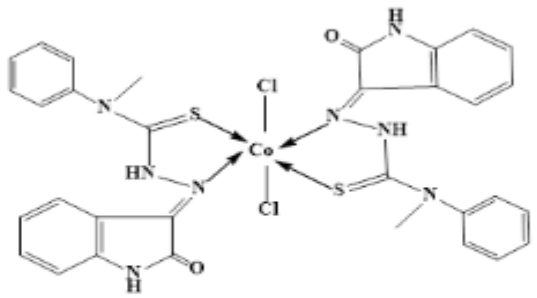


Figure 8

Suggested structure of $[Co(LH)_2Cl_2]$

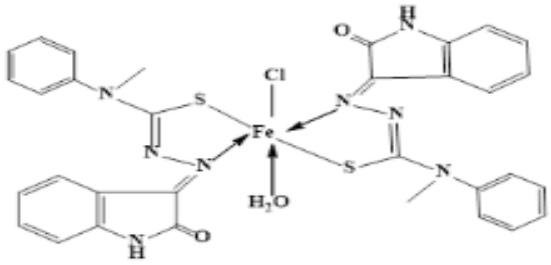


Figure 9

Suggested structure of $[FeL_2Cl(H_2O)]$

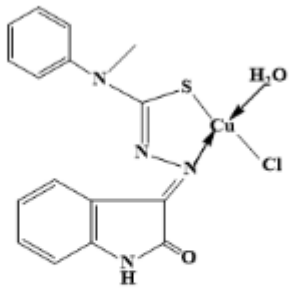


Figure 10

Suggested structure of [CuL(Cl)H_2O]

5.5 References

[1] D. K. Dermertzi, M. A. Demertzis, J. R. Miller, C. S. Frampton, J. P. Jasinski, D. X. West, J. Inorg. Biochem., 92, 137 (2002)

[2] J. G. Tojal, A. G. Orad, J. L. Serra, J. L. Pizarro, L. Lezamma, M. I. Arriortua, T. Rojo, J. Inorg. Biochem., 75, 45 (1999)

[3] S. B. Novakovic, G. A. Bogdanovic, V. M. Leovac, Polyhedron, 25, 1096 (2006)

[4] D. X. West, C. E. Ooms, J. S. Saleda, H. Gebremedhin, A. E. Liberta, Trans. Metal Chem., 19, 553 (1994)

[5] A. E. Liberta, D. X. West, Biometals, 5, 121(1992)

[6] D. X. West, J. S. Ives, J. Krejici, M. M. Salberg, T. L. Zumbahlen, G. A. Bain, A. E. Liberta, J. Valdes-Martinez, S. Hernandez Ortega, R.A. Toscano, Polyhedron, 14, 2189 (1995)

[7] D. X. West, H. Gebremedhin, R. J. Butcher, J. P. Jasinski, A. E. Liberta, Polyhedron, 12, 2489 (1993)

[8] L. Zhou, Y. Liu, W. Zhang, P. Wei, C. Huang, J. Pei, Y. Yuan , L. Lai, J. Med. Chem., 49, 3440 (2006)

[9] R. Ghahremanzadeh, M. Sayyafi, S. Ahadi, A. Bazgir, J. Comb. Chem., 11, 393 (2009)

[10] S. K. Sridhar, S. N. Pandeya, J. P. Stables, A. Ramesh, Eur. J.Pharm. Sci., 16, 129 (2002)

[11] L. Matesic, J. M. Locke, J. B. Bremner, S. G. Pyne, D. Skropeta, M. Ranson, K. L. Vine, Bioorg. Med. Chem., 16, 3118 (2008)

[12] M. M. Hossain, N. Islam, R. Khan, M. Islam, Bangladesh J. Pharmacol., 2, 66 (2008)

[13] H. Pervez, M. Ramzan, M. Yaqub, K.M. Khan, Lett. Drug Des. Discovery, 8, 452 (2011)

[14] K. L. Vine, L. Matesic, J. M. Locke, M. Ranson, D. Skropeta, Anticancer Agents Med. Chem., 9, 397 (2009)

[15] A. Cane, M. C. Tournaire, D. Barritault, M. C.-Arias, Biochem. Biophys. Res. Commun., 276, 379 (2000)

[16] K. L. Vine, J. M. Locke, M. Ranson, S. G. Pyne, J. B. Bremner, Bioorg. Med. Chem., 15, 931(2007)

[17] G. S. Singh, T. Singh, R. Lakhan, Indian J. Chem., 36B, 951(1997)

[18] M. Verma, S. N. Pandeya, K. N. Singh, P. S. James, Acta Pharm., 54, 49 (2004)

[19] S. N. Pandeya, P. Yogeeshwari, D. Sriram, G. Nath, Indian J. Pharm. Sci., 64, 209 (2002)

[20] P. Selvam, N. Murugesh, M. Chandramohan, Z. Debyser, M. Witvroum, Indian J. Pharm. Sci., 70, 779 (2008)

[21] K. S. Seshaiah, R. Atmakuru, Biol. Pharm. Bull., 24, 1149 (2001)

[22] D. X. West, S. B. Padhye, P. B. Sonwane, R. C. Chikate, Asian J. Chem. Rev., 1, 125 (1990)

[23] V. E. Ivanov, N. G. Tihomirova, A. B. Tomchin, Zh. Obshch. Khim., 58, 2737 (1988)

[24] Y. K. Bhoon, Indian J. Chem., 22A, 430 (1983)

[25] K. M. Ibrahim, A. A. El. Asmy, M. M. Bekheit, M. M. Mostafa, Synth. React. Inorg. Met.-Org. Chem., 15, 1247 (1985)

[26] J. Haribabu, G. R. Subhashree, S. Saranya, K. Gomathi, R. Karvembu, D. Gayathri, J. Mol. Struct., 1110, 185 (2016)

[27] A. Q. Ali, S. G. Teoh, A. Salhin, N. E. Eltayeb, M. B. K. Ahamed, A. M. S. Abdul Majid, Spectrochim. Acta Part A Mol. Biomol. Spectrosc., 125, 440 (2014)

[28] A. Q. Ali, S. G. Teoh, A. Salhin, N. E. Eltayeb, M. B. K. Ahamed, A. M. S. Abdul Majid, Inorg. Chim. Acta., 416, 235 (2014)

[29] A. Q. Ali, S. G. Teoh, A. Salhin, N. E. Eltayeb, M. B. K. Ahamed, A.M.S. Abdul Majid, Polyhedron, 74, 6 (2014)

[30] A. Rai, S. K. Sengupta, O. P. Pandey, Spectrochim. Acta A., 61, 2761 (2005)

[31] G. A. Bain, D. X. West, J. Krejci, J. Valdes-Martinez, S. Hernandez-Ortega, R. A. Toscano, Polyhedron, 16, 855 (1997)

[32] B. Smith, Infrared Spectral Interpretation, CRC Press, USA (1999)

[33] N. B. Colthup, L. H. Daly, S.E. Wieberly, Introduction to Infrared and Raman Spectroscopy, Academic Press, USA (1990)

[34] A. I. Vogel, 'Quantitative Inorganic Analysis' 7th Ed. Longman, London (2010)

[35] A. K. Mukherjee, P. Ray, J. Ind. Chem. Soc., 32, 633 (1955)

[36] L. Sacconi, R. Cini, Ann. Chim. (Italy), 42, 723 (1952)

[37] J. Lewis, G. Wilkinson, 'Modern Coordination Chemistry' Wiley Interscience, New York (1960)

CHAPTER VI

SYNTHESIS AND CHARACTERIZATION OF TRANSITION METAL COMPLEXES OF ISATIN 2-METHOXYISONICOTINOYLHYDRAZONE

6.1 Introduction

During the past few decades, considerable research on the metal complexes of hydrazone-type ligands has been undertaken. In particular, the hydrazones containing a pyridine ring moiety are of huge importance due to their extensive applications in biological- and pharmacological fields. Hydrazones act as an anticonvulsant, antimicrobial, antitubercular, antitumor and analgesic agents[1–5]. Due to their diverse donor behaviours with metal ions and significant flexibility, such compounds have fascinated the inorganic- and bioinorganic chemists. Metal complexes of hydrazones have a wide range of applications in catalysis, optic- luminescence and obviously in medicine[6–11]. The most striking class of metal complexes with hydrazone ligand is the one obtained from isonicotinic acid hydrazide. Isonicotinoyl hydrazide is also known as isoniazid. It is used as an antimicrobial agent and profoundly in the treatment of tuberculosis caused by the bacteria, Mycobacterium tuberculosis[12-14].

The Schiff bases obtained by the condensation of acid hydrazide (isoniazid) and carbonyl compounds are found to possess antimicrobial effect and in several cases it is superior than the original

hydrazide[15–17]. Thus, some Ni(II), Co(II) and Mn(II) complexes of these ligands have been found to exhibit enhanced microbiological activities[9,11]. These hydrazones are good ligands and the structures of the final coordination compounds depend largely on the number and position of the donor atoms in them.

Aroylhydrazones have a combination of donor sites, such as amide oxygen and the imine nitrogen of hydrazone moiety; very frequently, additional donor sites (usually N or O) are provided by the aldehydes or ketones forming the hydrazones. Aroylhydrazones have proved to be strong chelating agents for transition metals[18–20], lanthanides[21,22] and main group elements[23,24]. Interest in coordination chemistry of such ligands has been enhanced by the introduction of additional coordination sites by using substituted aldehyde and hydrazide precursors. The precursors in turn bring the probable expansion of structures from simple coordination complexes to complex supramolecules and polymeric networks. Thus, they have been found to be useful molecules for building up controllable supramolecular structures[**18**]. Hydrazones act as multidentate ligands through their nitrogen atom or in combination with other electronegative atoms such as oxygen, with metals forming colored chelates, which are used in the selective and sensitive determination of metals. Some 2-hydroxy acetophenone aroylhydrazones have been reported as successful corrosion inhibitors for copper metal in acid media[25].

A thorough literature investigation revealed that no work has been reported on 2-methoxy substituted isonicotinoylhydrazone of isatin, though the other isatin derived isonicotinoylhydazones are

known. Therefore, in the current work, a new ligand system, isatin 2-methoxyisonicotinoylhydrazone (ISISNHY), formed by the condensation of isatin and 2-methoxyisonicotinoyl hydrazine has been reported and used as a chelating ligand for the preparation of Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) complexes. The structure of the ligand is shown in Figure 1.

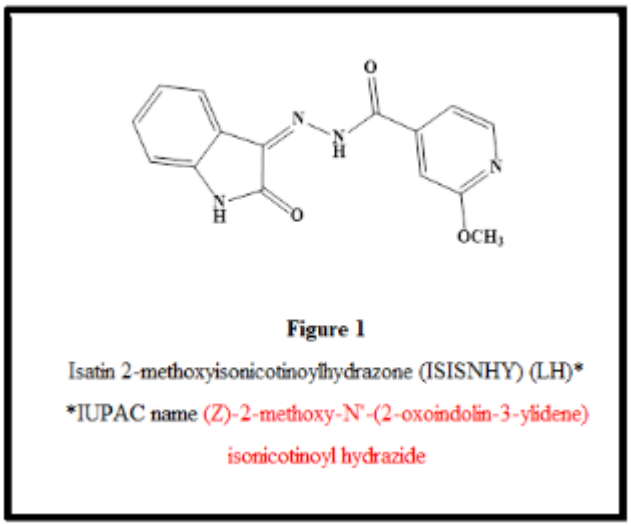


Figure 1

Isatin 2-methoxyisonicotinoylhydrazone (ISISNHY) (LH)*

*IUPAC name (Z)-2-methoxy-N'-(2-oxoindolin-3-ylidene) isonicotinoyl hydrazide

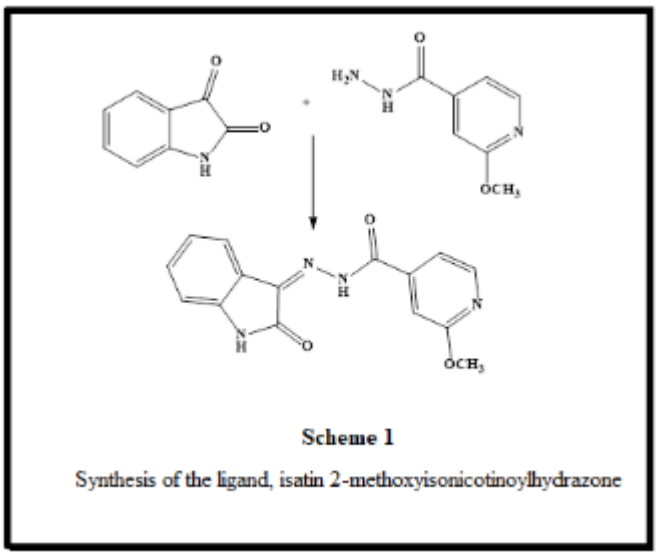
6.2 Experimental

6.2.1 Materials and methods

The specification regarding the chemicals used and the methods of characterization of the compounds adopted are described in Chapter II.

6.2.2 Synthesis of ligand

To a hot solution of 2-methoxyisonicotinoyl hydrazine in ethanol (0.05 mol), an ethanolic solution of isatin (0.05 mol) was added slowly. The reaction mixture was refluxed on a water bath for 4 h in the presence of catalytic amount of glacial acetic acid. The solid compound, isatin 2-methoxyisonicotinoylhydrazone formed was filtered and dried under vacuum (Yield 78%, M.P 179^{0}C). The synthetic procedure of the ligand is shown in Scheme 1.



Scheme 1

Synthesis of the ligand, isatin 2-methoxyisonicotinoylhydrazone

6.2.3 Synthesis of metal complexes

The complexes of Co(II), Ni(II) and Cu(II) with ISISNHY were prepared by adding a methanolic solution of the respective metal chlorides (0.005 mol) to an ethanolic solution of the ligand (0.005

mol). The mixture was refluxed for 6 h. It was cooled and evaporated to isolate the solid complex. In the case of Zn(II) and Cd(II) complexes, the ligand and metal acetates were taken in 2:1 molar ratio for better yield. The solid complexes thus separated were filtered off and washed several times with methanol. They were then dried under vacuum. The physico-chemical and analytical data obtained for the ligand and its complexes are presented in Table 5.

6.3 Results and discussion

The data obtained from the physico-chemical and spectral studies can be correlated to explain the structure, properties and geometries of the compounds.

6.3.1 Characterization of the ligand

The compound was soluble in chloroform, ethanol, etc. It was characterized by elemental analysis (Table 1) and further by UV-Vis- (Table 2), IR- (Table 3) and ^{1}H NMR (Table 4) spectral techniques.

6.3.1.1 Elemental analysis

The elemental analysis of the ligand was carried out on a Vario EL III CHN instrument. The analytical data were in agreement with the molecular formula, $C_{15}H_{12}N_4O_3$ (LH) for the ligand (Table 1).

Table 1 Physico-chemical and analytical data of the ligand						
Compound (Empirical formula)	% of Yield	Melting point (^{0}C)	Color of the Compound	Anal. Found (Calcu) %		
				C	H	N
$C_{15}H_{12}N_4O_3$ (LH)	78	179	Orange	61.81 (61.91)	4.58 (4.51)	18.17 (18.07)

6.3.1.2 Electronic spectrum

The electronic spectrum of the ligand showed a strong band at 272 nm which can be attributed to π-π* transition. Similarly, a band at 354 nm in the ligand spectrum may be due to n –π* transition from the azomethine group and amide part of the hydrazone moeity. The data are given in Table 2.

Table 2 UV-Vis spectral data of the ligand		
Compound	Spectral Bands λ_{max} (nm)	Assignment
$C_{15}H_{12}N_4O_3$ (LH)	272	π→π* transition
	354	n→π* transition

6.3.1.3 Infrared spectrum

IR spectroscopy is principally an important technique that provides a spectrum of large number of absorption bands and helps in the molecular structural elucidation. It gives considerable information about the different functional groups present in the compound. The IR spectral assignments of the ligand are listed in Table 3.

The IR spectrum of the ligand showed characteristic bands in the region 3400-3200 cm^{-1}. They may be assigned to the symmetric and asymmetric stretching vibrations of–NH group. The medium intensity bands found at 3023 and 2990 cm^{-1} may be assigned to asymmetric- and symmetric =CH stretching frequencies, respectively. The IR spectrum of the ligand showed a strong band at 1612 cm^{-1}, which was characteristic of azomethine (>C=N) group. In the spectrum of isatin 2-methoxyisonicotinoylhydrazone, a sharp peak at 1705 cm^{-1} can be assigned to the $\nu_{(C=O)}$. The presence of a band at 1018 cm^{-1} in the spectrum of the ligand can be assigned to $\nu_{(N-N)}$.

Table 3 Significant IR spectral bands (cm^{-1}) of the ligand	
Spectral bands	Assignments
3403	$\nu_{(N-H)}$
3329	$\nu_{(N-H)}$
3226	$\nu_{(N-H)}$
3023	$\nu_{=CH}$ (asymmetric stretching)
2990	$\nu_{=CH}$ (symmetric stretching)
1705	$\nu_{(C=O)}$
1612	$\nu_{(C=N)}$
1018	$\nu_{(N-N)}$

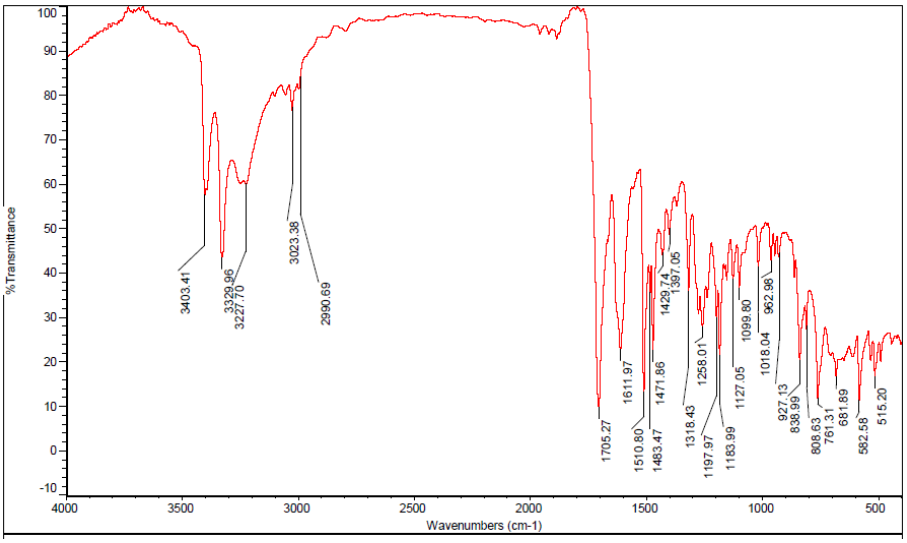


Figure 2

IR spectrum of isatin 2-methoxyisonicotinoylhydrazone

6.3.1.4 ^{1}H NMR spectrum

The ^{1}H NMR spectrum of the ligand showed two doublets and a multiplet in the range 7.82 and 7.14-7.44 ppm, which were due to C_6H_5 protons of the isatin moeity. Other doublets were observed at 6.96 and 8.85 ppm corresponding to the aromatic protons of isoniazid moiety. There was a singlet at 11.40 ppm that can be attributed to the –NH proton. A sharp signal appeared at 13.98 ppm was assignable to –NH proton of the isatin ring. A singlet that appeared at 7.60 ppm can be assigned to the $–OCH_3$ group present in the isonicotinoylhydrazine (Table 4).

Table 4 ^{1}H NMR data of the ligand

Sl.No.	δ (ppm)	Assignment
1	6.96, 8.85 (d)	Aromatic (isoniazid group)
2	7.14-7.44 (m)	aromatic (isatin moiety)
3	7.82 (d)	aromatic (isatin moiety)
3	7.60 (s)	$–OCH_3$ group
4	11.40 (s)	-NH group
5	13.98 (s)	-NH of isatin ring

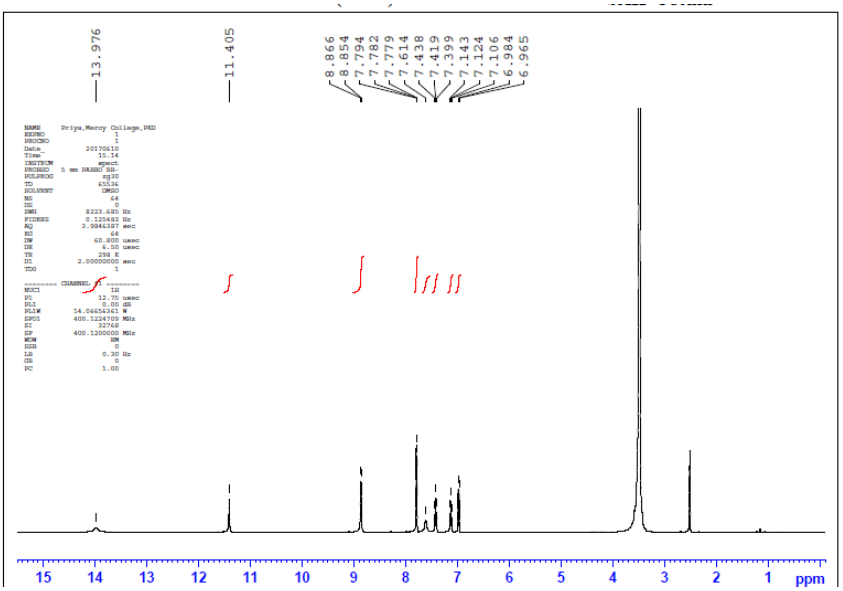


Figure 3

^{1}H NMR spectrum of isatin 2-methoxyisonicotinoylhydrazone

6.3.2 Characterization of the complexes

All the complexes of isatin 2-methoxyisonicotinoylhydrazone were coloured and stable under normal atmospheric conditions. They were non-hygroscopic and soluble in chloroform and DMSO, but less soluble in other common organic solvents like methanol and ethanol. The elemental analytical data of the complexes were obtained on a Vario ELIII CHN instrument. IR- and electronic spectral studies and magnetic moment measurements were employed as techniques for the structure elucidation of the complexes.

6.3.2.1 Analytical data

The analytical data of all the complexes agreed well with their proposed molecular formulae. The Co(II) and Cu(II) complexes were found to have the formula, $[M(LH)_2Cl_2]$, where M=Co(II) or Cu(II). The complex of Ni(II) corresponded to the formula, $[ML_2(H_2O)_2]$. All these complexes were found to be octahedral. The Zn(II) and Cd(II) complexes were found to possess a tetrahedral geometry with a general formula, $[ML_2]$. The determination of the metal and chloride contents in the complexes were done by the standard procedures. The electrical conductance measurements of the complexes indicated that they were non-electrolytic in nature.

Table 5. Physico-chemical and analytical data of the complexes

Table 5 Physico-chemical and analytical data of the complexes								
Compound (Empirical formula)	% of Yield	Melting point (^{0}C)	Colour of the Compound	Anal. Found % (Calculated %)				
				M	C	H	N	Cl
$[Co(LH)_2Cl_2]$	68	214	Maroon	7.93 (8.18)	49.78 (49.86)	3.24 (3.32)	15.42 (15.51)	9.78 (9.83)
$[NiL_2(H_2O)_2]$	70	226	Red	8.56 (8.62)	52.6 (52.72)	3.7 (3.81)	16.32 (16.40)	
$[Cu(LH)_2Cl_2]$	72	230	Brown	8.57 (8.68)	49.48 (49.59)	3.23 (3.31)	15.3 (15.43)	9.74 (9.78)
$[ZnL_2]$	75	234	Pale Brown	9.96 (10.02)	54.96 (55.09)	3.28 (3.37)	17.06 (17.14)	
$[CdL_2]$	75	239	Pale Brown	15.98 (16.06)	51.27 (51.39)	3.07 (3.14)	14.46 (14.61)	

6.3.2.2 Magnetic moments

The effective magnetic moment values of the complexes were calculated from the corrected molar susceptibilities obtained after incorporating the diamagnetic corrections. The magnetic moment value of a metal complex usually depends upon the number of unpaired electrons and it helps to determine the oxidation state of the metal ion. The room temperature magnetic moment values of the complexes of Co(II), Ni(II) and Cu(II) are given in Table 6.

The high-spin octahedral Co(II) ion has an observed magnetic moment value greater than the expected spin-only value (3.87 B.M.) due to intrinsic orbital angular momentum contribution. This leads to magnetic moment values between 4.70-5.20 B.M at room temperature. The Co(II) complex reported in this chapter registered a magnetic moment value of 4.87 B.M. This value clearly indicated a high-spin octahedral geometry for $[Co(LH)_2X_2]$ complex.

The high-spin and low-spin octahedral complexes of Ni(II) have two unpaired electrons in them. The room temperature magnetic moment values of Ni(II) complexes fall in the range 2.90-3.30 B.M, which is slightly higher than the spin-only value. In the present case, a magnetic moment value of 3.24 B.M for $[NiL_2(H_2O)_2]$ complex, indicated its octahedral geometry.

A regular octahedral Cu(II) complex has a magnetic moment slightly greater than the spin-only value and it ranges from 1.80-2.10 B.M. The Cu(II) complexes of both octahedral and tetrahedral types almost have the same magnetic moments. For a

regular tetrahedral Cu(II) complex, the observed moments are in the range of 1.95-2.00 B.M. The Cu(II) complexes with distorted octahedral geometry have magnetic moment values slightly greater than the spin-only value of 1.73 B.M. Thus, the observed values are in the range, 1.95-2.00 B.M due to the spin-orbit coupling in the ion. The observed magnetic moment for $[Cu(LH)_2X_2]$ was 1.97 B.M, indicating its distorted octahedral geometry.

Table 6 Magnetic moments of the complexes

Complex	μ_{eff} (B.M)
$[Co(LH)_2X_2]$	4.87
$[NiL_2(H_2O)_2]$	3.24
$[Cu(LH)_2X_2]$	1.97

6.3.2.3 Electronic spectra

The electronic spectra of the complexes give an obvious idea about their electronic structure. They also provide detailed information about the d-d transitions. The position of electronic spectral bands of the complexes and their transition assignments are of great importance, because they are highly dependent on the geometries of the complexes. The electronic spectral bands of Co(II), Ni(II) and Cu(II) complexes of ISISNHY and their assignments are given in Table 7.

The octahedral complex of Co(II) has $^4T_{1g}$ as the ground term with considerable orbital contribution, resulting in a magnetic moment value around 5.20 B.M. Of the three spin-allowed transitions for an octahedral Co(II) complex, the highest energy transition, $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ is the most intense one. The spin allowed transition, $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ occurs usually in the near IR region. The Co(II) complex investigated here, registered bands at 1040, 637 and 428 nm, which may be assigned to $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$, $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ and $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ transitions respectively, corresponding to an octahedral geometry.

The electronic spectral bands corresponding to three spin-allowed transitions in the case of octahedral Ni(II) complexes are due to $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$, $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ and $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$, respectively. The electronic spectral bands corresponding to these transitions are usually found in the region, 1022-1075, 830-740 and 420 nm. In the electronic spectrum of $[NiL_2(H_2O)_2]$, the bands at 1037, 728 and 436 nm may be assigned to these three spin-allowed transitions, respectively. The electronic spectrum together with the magnetic moment value of 3.24 B.M supported an octahedral geometry for $[NiL_2(H_2O)_2]$.

In octahedral Cu(II) complexes only a single broad band due to $^2T_{2g} \rightarrow {}^2E_g$ transition is observed[26,27]. In the present investigation, the spectrum of $[Cu(LH)_2Cl_2]$ showed a broad band at 643 nm, which may be due to $^2T_{2g} \rightarrow {}^2E_g$ transition, expected for an octahedral Cu(II) complex. A magnetic moment value of 1.97 B.M also supported a distorted octahedral structure for it. As expected, the diamagnetic

Zn(II) and Cd(II) complexes did not register any characteristic absorption bands in the visible region.

Table 7 Electronic spectral bands of the complexes of ISISNHY and their assignments

Complex	Spectral Bands λ_{max} (nm)	Assignment
$[Co(LH)_2X_2]$	428	$^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$
	637	$^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$
	1040	$^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$
$[NiL_2(H_2O)_2]$	436	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$
	728	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$
	1037	$^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$
$[Cu(LH)_2X_2]$	643	$^2T_{2g} \rightarrow {}^2E_g$

6.3.2.4 Infrared spectra

The characteristic IR spectral bands of the ligand generally undergo shifts from their positions upon coordination to metal ions in the complexes. These shifts in the bands can provide significant indication regarding the mode of bonding of the ligands. The significant IR spectral bands of the ligand and the complexes along with their probable assignments are given in Table 8.

On analyzing the infrared spectra of the metal complexes of isatin 2-methoxyisonicotinoylhydrazone, it was observed that the absorptions due to $\nu_{(N-H)}$ and $\nu_{(C=O)}$ were not found in the spectra of the

complexes of Ni(II), Zn(II) and Cd(II). The ligand spectrum showed a medium intensity band at 3403 cm^{-1} which can be assigned as $\nu_{(N-H)}$. This band was not found in the spectra of all the complexes, except in those of Co(II) and Cu(II). This indicated that the ligand might have undergone enolisation of –N–NH-C=O to –N–N=C-OH and its subsequent coordination through the deprotonated oxygen to the metal ion. This in turn was recognized by the appearance of new bands in the region 1200 cm^{-1} in the spectra of the complexes of Ni(II), Zn(II) and Cd(II) due to $\nu_{(C-O)}$. However, in the spectra of Co(II) and Cu(II) complexes, the $\nu_{(C=O)}$ has been shifted only slightly to lower wave numbers, which suggested the coordination of –N–NH-C=O in the keto form to these metal centres. The coordination through azomethine nitrogen was demonstrated by the shift of $\nu_{(C=N)}$ from 1612 cm^{-1} in the ligand spectrum towards lower wave numbers in the spectra of all the metal complexes. The band observed at 1018 cm^{-1} in the ligand spectrum due to $\nu_{(N-N)}$ has been found shifted to higher wave numbers, in the range 1030 cm^{-1}, in the spectra of all the complexes. Thus the coordination of the ligand took place through azomethine nitrogen and deprotonated or keto oxygen atom in the complexes. This type of bonding was supported by the appearance of new bands in the low frequency regions, ~553-628 cm^{-1} and ~465 cm^{-1} in the spectra of all the metal complexes which can be assigned to $\nu_{(M-N)}$ and $\nu_{(M-O)}$, respectively.

In the spectrum of Ni(II) complex, a broad band was observed at 3489 cm^{-1}. It can be recognized as $\nu_{(O-H)}$ of the water molecules coordinated to the metal centre. The absorption bands found in the

very low frequency region, ~420 cm^{-1}in the spectra of Co(II) and Cu(II) complexes may be assigned to $\nu_{(M-Cl)}$.

Thus the ligand, isatin 2-methoxyisonicotinoylhydrazone can be considered as a bidentate N, O^-/O- donor in all the complexes.

Table 8 Characteristic IR spectral bands (in cm^{-1}) of the ISISNHY ligand and its complexes

Compound	$\nu_{(N-H)}$	$\nu_{(O-H)}$	$\nu_{(C=N)}$	$\nu_{(N-N)}$	$\nu_{(C=O)}$	$\nu_{(C-O)}$	$\nu_{(M-N)}$	$\nu_{(M-O)}$	$\nu_{(M-Cl)}$
$C_{15}H_{12}N_4O_3$ (LH)	3329		1612	1018	1705				
$[Co(LH)_2X_2]$	3346		1598	1034	1697		627	478	428
$[NiL_2(H_2O)_2]$		3489	1595	1031		1223	576	469	
$[Cu(LH)_2X_2]$	3414		1571	1017	1705		627	454	424
$[ZnL_2]$			1581	1034		1219	569	467	
$[CdL_2]$			1601	1028		1226	634	486	

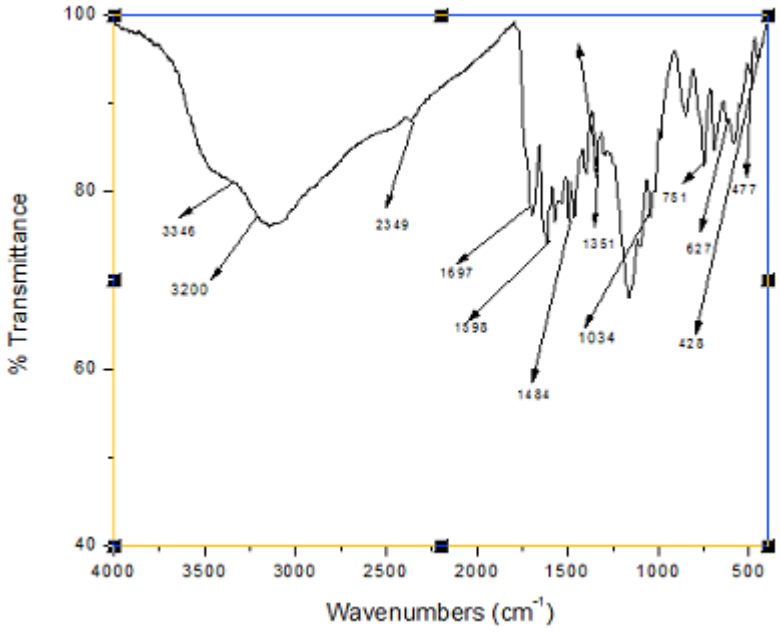


Figure 4

IR spectrum of Co(II) complex of isatin 2-methoxyisonicotinoylhydrazone $[Co(LH)_2X_2]$

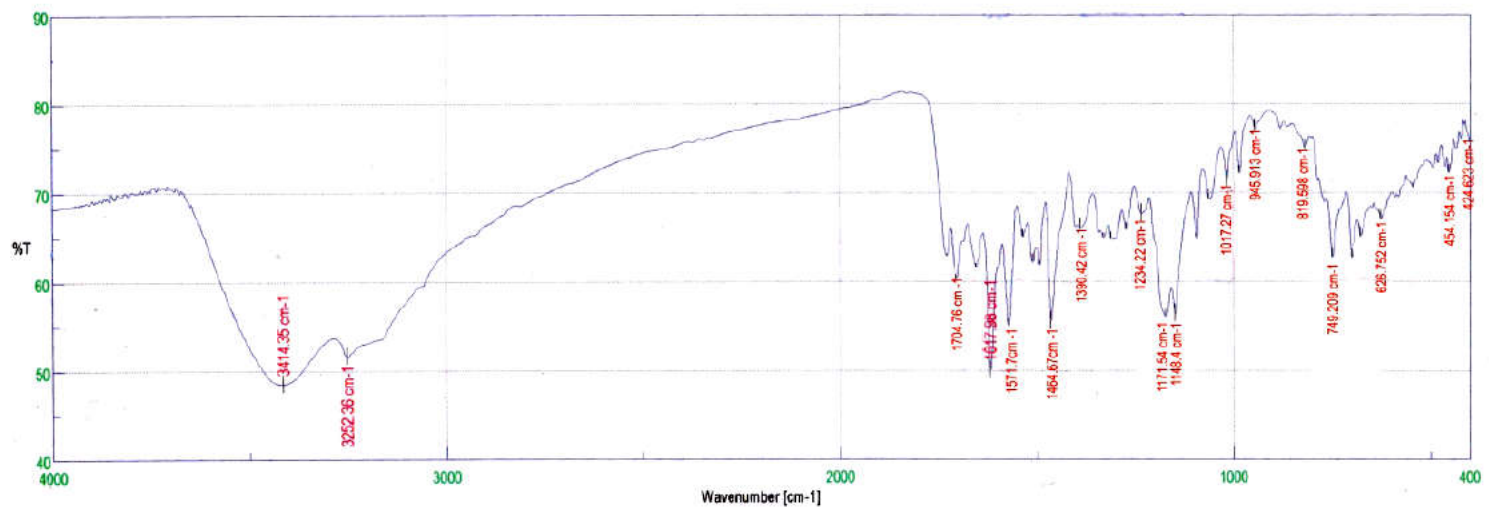


Figure 5

IR spectrum of Cu(II) complex of isatin 2-methoxyisonicotinoylhydrazone

$[Cu(LH)_2Cl_2]$

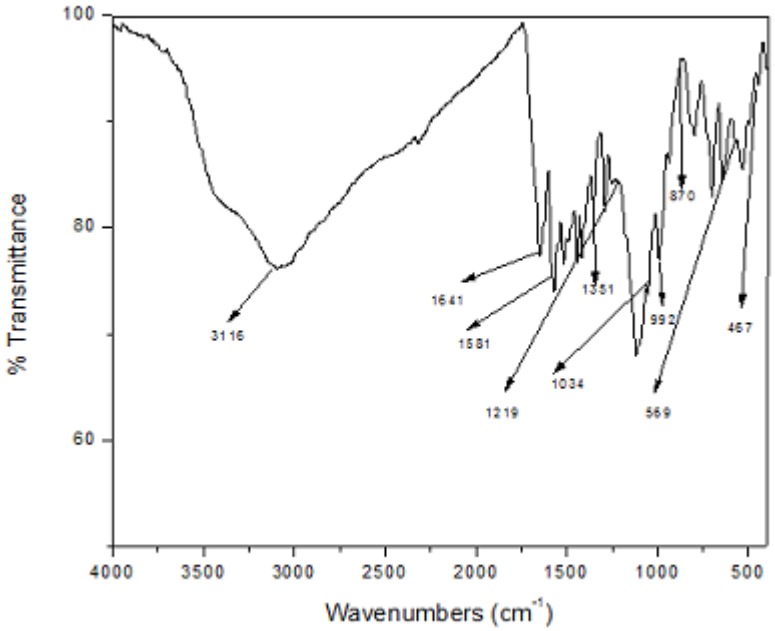


Figure 6

IR spectrum of Zn(II) complex of isatin 2-methoxyisonicotinoylhydrazone

$[ZnL_2]$

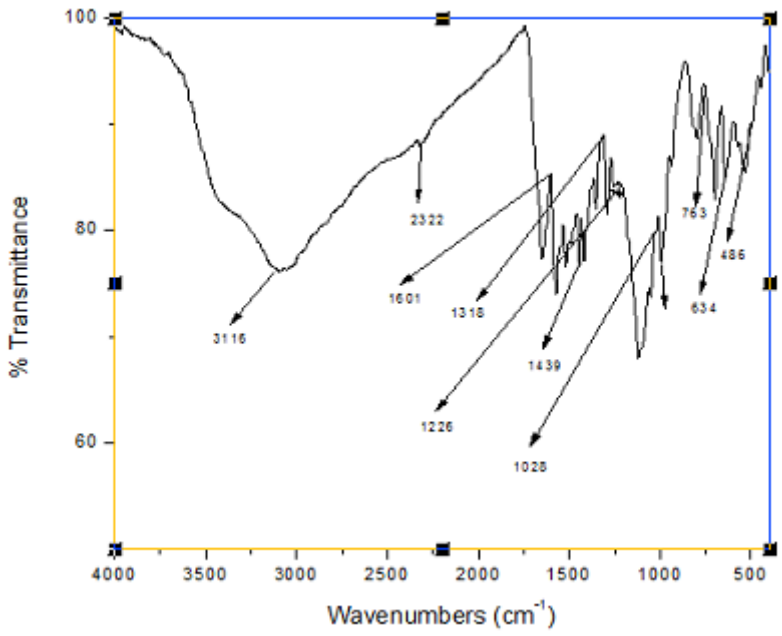


Figure 7

IR spectrum of Cd(II) complex of isatin 2-methoxyisonicotinoylhydrazone

$[CdL_2]$

6.4 Conclusions

In the present investigation, the ligand, isatin 2-methoxyisonicotinoylhydrazone (LH) behaved as an N, O^-/O bidentate ligand. The mode of coordination of the ligand was understood clearly from the spectral studies. The ligand coordinated through azomethine nitrogen and enolate oxygen atom in all the complexes, except those of Co(II) and Cu(II). Magnetic moment values and electronic spectral data suggested that the complexes of Co(II), Cu(II) and Ni(II) have octahedral geometries with general molecular formulae, $[M(LH)_2Cl_2]$ and $[ML_2(H_2O)_2]$ which are shown in Figures 8 and 9. A tetrahedral geometry was assigned for the complexes of Zn(II) and Cd(II) and their structures are shown in Figure 10.

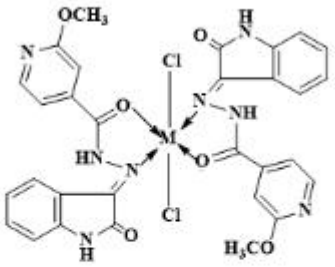


Figure 8

Suggested structure of the complexes of the formula $[M(LH)_2Cl_2]$, where M = Co(II) or Cu(II)

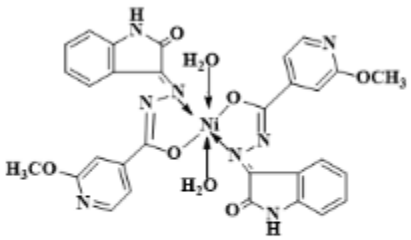


Figure 9

Suggested structure of $[NiL_2(H_2O)_2]$

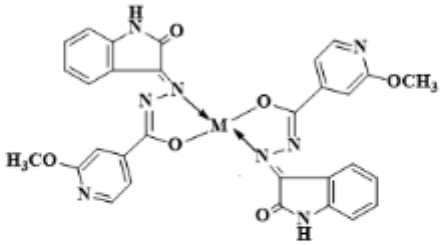


Figure 10

Suggested structure of the complexes of the formula $[ML_2]$,

where M = Zn(II) or Cd(II)

6.5 References

[1] J. R. Anacona, M. Rincones, Spectrochim. Acta A., 141, 169 (2015)

[2] P. Vicini, M. Incerti, I. A. Doytchinova, P. La Colla, B. Busonera, R. Loddo, Eur. J. Med. Chem., 41, 624 (2006)

[3] S. Rollas, S. G. Kucukguzel, Molecules., 12, 1910 (2007)

[4] A. A. Recio Despaigne, J. G. da Silva, A. C. M. do Carmo, F. Sives, O. E. Piro, E. E. Castellano, H. Beraldo, Polyhedron., 28, 3797 (2009)

[5] N. A. Mangalam, S. Sivakumar, M. R. Prathapachandra Kurup, E. Suresh, Spectrochim. Acta A., 75, 686 (2010)

[6] O. Pouralimardan, A. C. Chamayou, C. Janiak, H. Hosseini-Monfared, Inorg. Chim. Acta., 360, 1599 (2007)

[7] C. Basu, S. Chowdhury, R. Banerjee, H. S. Evans, S. Mukherjee, Polyhedron, 26, 3617 (2007)

[8] M. Bakir, O. Green, W. H. Mulder, J. Mol. Struct., 17, 873 (2008)

[9] O. A. El-Gammal, G. A. El-Reash, S. F. Ahmed, J. Mol. Struct., 1, 1007 (2012)

[10] H. Hosseini-Monfared, H. Falakian, R. Bikas, P. Mayer, Inorg. Chim. Acta., 394, 526 (2013)

[11] K. S. Abou-Melha, Spectrochim. Acta A., 70, 162 (2008)

[12] S. Kakimoto, K. Yashamoto, Pharm. Bull., 4, 4 (1956)

[13] V. J. Negi, A. Q. Sharma, J. S. Negi, V. Ram, Int. J. Pharm. Chem., 2, 100 (2012)

[14] M. Asif, Int. J. Adv. Chem., 2, 85 (2014)

[15] F. Martins, S. Santos, C. Ventura, R. Elvas-Leitao, L. Santos, S. Vitorino, M. Reis, V. Miranda, H. F. Correia, J. Aires-de-Sousa, V. Kovalishyn, D. A. R. S. Latino, J. Ramos, M. Viveiros, Eur. J. Med. Chem., 81, 119 (2014)

[16] R. K. Agarwal, D. Sharma, L. Singh, H. Argawal, Bioorg. Chem. Appl., 1, (2006)

[17] F. R. Pavan, P. I. da S. Maia, S. R. A. Leite, V. M. Deflon, A. A. Batista, D. N. Sato, S. G. Franzblau, C. Q. F. Leite, Eur. J. Med. Chem., 45, 1898 (2010)

[18] S. Naskar, D. Mishra, R. J. Butcher, S. K. Chattopadhyay, Polyhedron, 26, 3703 (2007)

[19] S. Naskar, S. Biswas, D. Mishra, B. Adhikary, L. R. Falvello, T. Soler, C. H. Schwalbe, S. K. Chattopadhyay, Inorg. Chim. Acta., 357, 4257 (2004)

[20] D. Mishra, S. Naskar, A.J. Blake, S. K. Chattopadhyay, Inorg. Chim. Acta., 360, 2291 (2007)

[21] Y. X. Ma, Z. Q. Ma, G. Zhao, Y. Ma, M. Yan, Polyhedron, 8, 2105 (1989)

[22] R. K. Agarwal, R. K. Sarin, R. Prasad, Pol. J. Chem., 67, 1947 (1993)

[23] M. Carcelli, C. Pelizzi, G. Pelizzi, P. Mazza, F. Zani, J. Organomet. Chem., 488, 55 (1995)

[24] Z. Y. Yang, R. D. Yang, K. B. Yu, Polyhedron, 15, 3749 (1996)

[25] A. S. Fouda, M. M. Gouda, S. I. Abd El-Rahman, Bull. Korean Chem. Soc., 21, 1085 (2000)

[26] F. Rafat, M.Y. Siddiqi, K.S. Siddiqi, J. Seb. Chem. Soc., 69, 641 (2004)

[27] B. J. Hathaway, J. Chem. Soc. Dalton. Trans., 1196 (1972)

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PART II

BIOTOXIC EVALUATION

SECTION A

ANTIMICROBIAL STUDIES

CHAPTER VII

THIOSEMICARBAZONES AS ANTIMICROBIAL AGENTS

7.1 Introduction

In the current years, the number of life-threatening infectious diseases caused by multi-drug resistant Gram positive and Gram negative pathogen bacteria have increased to an alarming level in many countries around the world[1]. More than 50 million people are infected globally and up to 110,000 of these die every year. Antimicrobial resistance is detected usually in pathogenic bacteria before the contact or it is correlated to an uncontrolled infection after a patient receives that antimicrobial agent. Infections caused by Gram positive bacteria, represent the main health problem obstructing the therapeutic efficacy of antibacterial drugs due to their frequent multidrug resistance[2–6]. The fungal infections categorized as the adaptable fungal ones have also emerged worldwide, which pose a serious and persistent threat to the human life. The World Health Organization (WHO) estimates tuberculosis also as one of the deadliest global diseases associated with the occurrence of multiple drug resistant strains of *Mycobacterium tuberculosis* discovered by Robert Koch in 1882. In 2013, about 9 million people were identified with active tuberculosis and 1.5 million deaths occured due to this disease. In spite of the discovery of Bacillus-Calmette Gu´erin (BCG) vaccine and some main drugs (streptomycin, isoniazid, and rifampicin),

thiacetazone, p-acetamidobenzaldehyde thiosemicarbazone, is one of the oldest and cheapest second line drug for the tuberculosis treatment.

Antibiotics provide the chief source for the therapy of microbial (bacterial and fungal) infections. However, the misuse of antibiotics has become the main factor for the occurrence and spreading of multi-drug resistant strains of several groups of microorganisms[7]. This in turn, enforces an upgrading of the existing antimicrobial drugs and invention of the new ones. Thiosemicarbazones, a large group of thiourea derivatives, have produced significant interest in the areas of chemistry and biology. Traces of interest for these compounds date back to the beginning of 20th century with their first reports on the medical application as drugs against Tuberculosis and leprosy[8,9]. Later their antiviral properties were discovered and a huge amount of research was carried out, eventually leading to the commercialization of methisazone, Marboran to treat small pox[10]. In the recent years, thiosemicarbazones have been the subject of broad investigation for a long while due to their wide range of biological applications[11,12]. Numerous thiosemicarbazone derivatives have been synthesized and evaluated for their antibacterial activities. The recent use of these compounds in the bacterial infections has led to the growth of novel antibacterial drugs[13–16].

7.1.1 Thiosemicarbazones and their transition metal complexes as antimicrobial agents

Thiosemicarbazones and their derivatives are of considerable interest because of their chemistry and potentially useful biological

activities. Thiosemicarbazones are a class of compounds with a wide range of pharmacological applications such as antitumor, antibacterial, antiviral, antimalarial and antiparasitic applications[17-19]. They have therefore, attracted significant pharmaceutical importance[20]. They have been evaluated as antiviral, antibacterial and anticancer therapeutics over the last 50 years, whose biological activities are a function of parent aldehyde or ketone moiety[21]. Thiosemicarbazone derivatives containing a 4-acyl-2-pyrazolin-5-one moiety form an important class of organic compounds because of their structural chemistry and biological activities[22]. It was also demonstrated that upon complexation to copper(II), the antimicrobial properties of thiosemicarbazones were strongly enhanced[23]. The antimicrobial activities of N(4)-phenyl- and N(4)-cyclohexyl substituted thiosemicarbazones derived from 2-benzoylpyridine and a series of its copper(II) complexes were investigated by Joseph *et al*[24]. They observed that almost all the compounds were biologically active ones. Previous reports on N(4)-substituted thiosemicarbazones have established that the presence of bulky groups at the N(4) position of the thiosemicarbazone moiety significantly improves their biological activity[25–27]. Four novel binuclear copper(II) complexes of N(4)-substituted thiosemicarbazones reported by Krishna *et al*[28] have been found to exhibit moderate activity towards Gram-positive bacteria. The DNA binding properties of the complexes with calf thymus (CT) DNA showed greater binding affinity and it may be due to the presence of a phenyl ring at the N(4) position of the thiosemicarbazone that facilitates pi-stacking interaction.

Thiosemicarbazones have emerged mainly as an important class of sulfur donor ligands for transition metal ions because of their mixed hard–soft donor character and versatile coordination behaviour[29]. They usually chelate with transition metal ions by bonding through the sulfur and the azomethine nitrogen atoms resulting in the formation of a five-membered chelate ring. Therefore, they provide a diverse range of compounds with varying modes of bonding and different activities[30-33]. The exploration of pharmacological properties of these compounds has initiated many investigations related to the catalysis and biological importance[34-36].

In many circumstances, upon coordination to metal ions, the pharmacological activities of thiosemicarbazones improved. The mechanism of action of the metal ions may involve their binding to proteins and interaction with the microbial membrane which cause changes in its structure and permeability. These interactions with the nucleic acids prevent the microbial replication[37]. Thus, the complexes of nitrogen and sulfur donor ligands may be considered as potent biological agents. The antibacterial screening of the complexes of 3-acetylpyridine ^{4}N-(2-pyridyl)thiosemicarbazone done by El-Ayaan revealed that all the complexes have maximum activities against Gram positive and Gram negative bacterial strains[38]. Prathima *et al*[39] established the biological activities of Mn(II) and Co(II) complexes of benzyloxybenzaldehyde-4-phenyl-3-thiosemicarbazone and found that the metal complexes were more active than the ligand. Chandra *et al*[40] conducted the antibacterial studies of Ni(II) and Cu(II) complexes of 2-carboxybenzaldehyde thiosemicarbazone. They established that the copper complex inhibited the growth of bacteria to a greater extent

than the nickel complex. *In vitro* antimicrobial activity and DNA cleavage studies of a series of complexes of Cr(III), Mn(II), Ni(II), Zn(II) and Hg(II) of pyridyl thiosemicarbazone were carried out by Yousef *et al*[41]. The results showed that the metal complexes were more effective in antibacterial activity than the parent Shciff base ligand. Studies on a series of cobalt(II) complexes of various thiosemicarbazones of 4-aminoantipyrine by Prasad *et al*[42] revealed that the complexes show enhanced inhibitory activity compared to their parent ligands. Costa *et al*[43] conducted a study on the antifungal activity of 2-benzoylpyridine thiosemicarbazone as well as its N(4)-methyl and N(4)-phenyl analogues and their Fe(II), Ni(II) and Zn(II) complexes against *Candida albicans.*

7.2 Scope of the present investigation

Recently, it has been the most important global problem to treat the microbial diseases caused by bacteria and fungi owing to the sudden growth of their resistance towards the antibacterial and antifungal drugs. This is due to the over use of available pharmacological drugs and their unwanted side effects[44]. Thus, in the light of the rapid spread of resistant clinical isolates, the requirement to discover new antimicrobial agents is of utmost importance. There has been a major attention focused onto the thiosemicarbazones due to an extensive range of their biological activities[45,46]. Thiosemicarbazones and their complexes have been widely studied because they have a broad range of potential medical applications[47–49] including antiparasital[50], antibacterial[51], antitumor[52], antiviral[53], fungicidal[54] and

antineoplastic activities[55]. It has been recognized that some drugs exhibit better activity when administered as metal complexes[56,57]. Numerous metal chelates have been shown to inhibit the tumor growth[58].

Thiosemicarbazones derived from cinnamaldehyde and cuminaldehyde together with their complexes were able to inhibit the cell proliferation[59]. Isatin derived thiosemicarbazones have also been found to possess a variety of physiological properties including antibacterial, antifungal, antineoplastic, antiulcer, antiviral and enzymatic inhibition[60, 61]. As a result of the major pharmacological effects of isatin thiosemicarbazones, there is a growing interest in the biotesting of these compounds[62–66]. Thus, in our present investigation, the antibacterial- and antifungal activities of thiosemicarbazones and their complexes were carried out and the results of the studies were analysed. The procedure and the explanations are presented in the following chapter.

CHAPTER VIII

ANTIMICROBIAL STUDIES OF N(4)-SUBSTITUTED THIOSEMICARBAZONES AND THEIR COMPLEXES

Thiosemicarbazones and their complexes have been the subject of several structural and medicinal investigations, due to their interesting and potentially useful biological activities. It was also well known from the studies that the presence of bulky groups at the terminal nitrogen, N(4)-position of the thiosemicarbazone moiety considerably improved their activity. In view of these facts, it has been decided to evaluate the antibacterial- and antifungal activities of some of the N(4)-substituted thiosemicarbazones and their metal complexes that have been synthesized during our study. Those studies are presented as two sub-sections in this chapter.

SUB-SECTION I

ANTIBACTERIAL ACTIVITIES OF CROTONALDEHYDE N(4)-METHYL(PHENYL)THIOSEMICARBAZONE (CRTSC), ISATIN N(4)-METHYL(PHENYL)THIOSEMICARBAZONE (ISTSC) AND THEIR COMPLEXES

Evaluation of the antibacterial activities of crotonaldehyde N(4)-methyl(phenyl)thiosemicarbazone (CRTSC), isatin N(4)-methyl(phenyl)thiosemicarbazone (ISTSC) and their Ni(II) and Cu(II) complexes were done by Disc Diffusion or Kirby Bauer method. The structures of the ligands, CRTSC and ISTSC are shown in Figures 1 and 2.

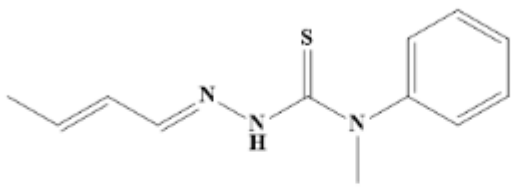

Figure 1

Crotonaldehyde N(4)-methyl(phenyl)thiosemicarbazone (CRTSC) (LH)*

*IUPAC name

(1E)-1-((E)-but-2-enylidene)-4-methyl-4-phenylthiosemicarbazide

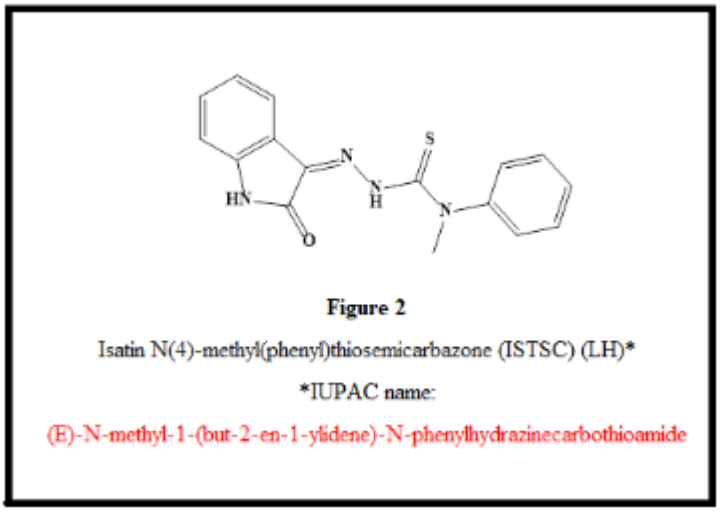

Figure 2

Isatin N(4)-methyl(phenyl)thiosemicarbazone (ISTSC) (LH)*

*IUPAC name:

(E)-N-methyl-1-(but-2-en-1-ylidene)-N-phenylhydrazinecarbothioamide

8.1 Materials and methods

The bacterial cultures used in this study were three gram positive bacteria; *Staphylococcus*, *Bacillus*, *Streptococcus* and three gram negative bacteria; *Escherichia coli*, *Pseudomonas*, *Klebsiella*. The pure cultures were obtained from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

8.1.1 Antibacterial activity by Disc Diffusion or Kirby Bauer Method

Antibacterial tests were done by disc diffusion method (Collins & Lyner., 1987)[69] with some modifications. The bacterial cultures were maintained in nutrient broth and it was prepared from the yeast extract (1.5 g), sodium chloride (5 g) and peptic digest of animal tissue

(5 g) in 1000mL distilled water (pH 7.3). Nutrient agar used for keeping these culture media was prepared from the yeast extract (1.5 g), sodium chloride (5 g), peptic digest of animal tissue (5 g) and agar (15 g) in 1000mL distilled water (pH 7.3). The uniform distribution of cultures was done on the nutrient agar plates using sterile swabs. Sterile filter paper discs of 3.2 mm diameter were placed on the surface of nutrient agar plates at a distance of 2 cm using sterile forceps. The drug dissolved in 2% DMSO was found to have no unfavorable effect on the bacterial cultures. Suitable concentration of the drug was prepared from the stock solution in DMSO. A drug of concentration 200 μg/ml was added on each disc using a micropipette. Disc with DMSO, but without the drug was used as the control. Then the plates were incubated at 37^0C for 24 hrs. After incubation, zone diameter was measured and found to be greater for the compounds than for the control and this indicated their antibacterial activity.

8.2 Results

Antibacterial activities of the compounds were tested against a set of six bacterial strains. The experimental results showed that the complexes were more active towards the various organisms than the ligand. The complexes of Ni(II) and Cu(II) of CRTSC exhibited moderate activity for the Gram positive bacteria and the ligand showed low activity towards all the bacterial strains studied. The results obtained from the disc diffusion method for CRTSC and its complexes are presented in Table 1.

Table 1 Antibacterial activity of crotonaldehyde N(4)-methyl(phenyl)thiosemicarbazone and its complexes					
Sl. No	**Organism**	**Ligand CRTSC**	**$[NiL_2(H_2O)_2]$**	**$[CuL(H_2O)Cl]$**	**DMSO**
1	Staphylococcus	4	10	15	-
2	Bacillus	-	5	13	-
3	Streptococcus	4	5	11	-
4	Escherichia coli	-	6	9	-
5	Pseudomonas	-	-	-	-
6	Klebsiella	5	-	8	-

The experimental results of isatin N(4)-methyl(phenyl)thiosemicarbazone and its complexes are given in Table 2 and Figures 3 and 4. A comparison of the antibacterial activities of the ligand and its complexes done against the six bacterial cultures showed that the complexes of ISTSC exhibited higher antibacterial effects than the ligand. The studies revealed that almost all the compounds showed considerable activity against all the tested organisms.

Table 2 Antibacterial activity of isatin N(4)-methyl(phenyl)thiosemicarbazone and its complexes					
Sl. No	**Organism**	**Ligand ISTSC**	**$[NiL_2(H_2O)_2]$**	**$[CuL(H_2O)Cl]$**	**DMSO**
1	Staphylococcus	9	20	25	-
2	Bacillus	6	16	24	-
3	Streptococcus	8	14	21	-
4	Escherichia coli	6	12	17	-
5	Pseudomonas	8	15	16	-
6	Klebsiella	7	11	14	-

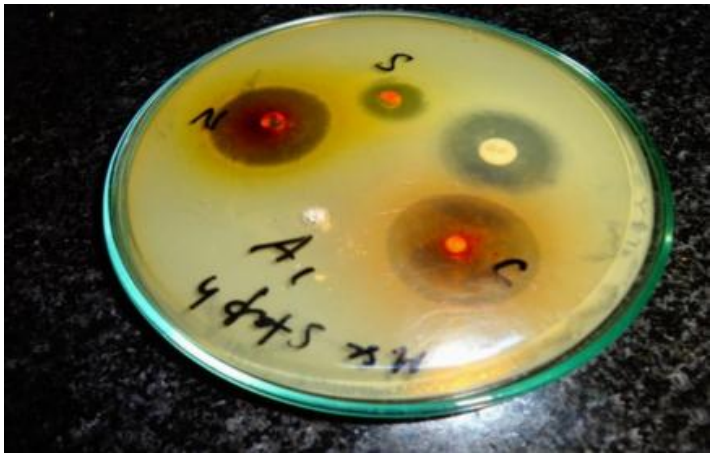

Figure 3

Antibacterial activity of the Ni(II) and Cu(II) complexes of ISTSC on Staphylococcus species

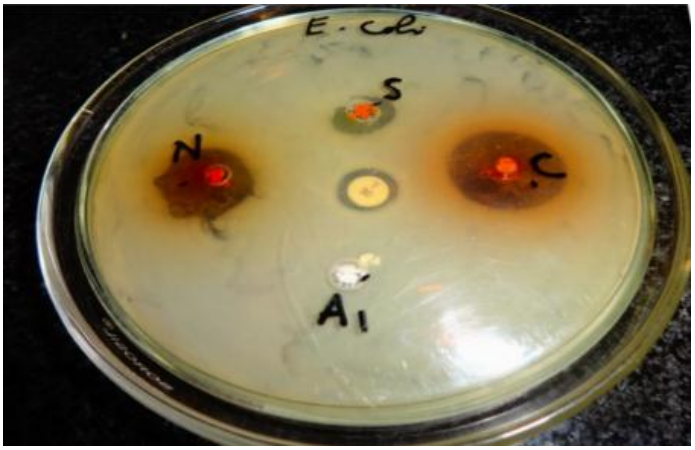

Figure 4

Antibacterial activity of the Ni(II) and Cu(II) complexes of ISTSC on Escherichia coli species

8.3 Discussion

It was found that the Ni(II) and Cu(II) complexes of crotonaldehyde N(4)-methyl(phenyl)thiosemicarbazone exhibited higher activity when compared to the parent ligand, CRTSC. The results revealed that the Cu(II) complex showed higher activity towards most of the bacterial strains than the Ni(II) complex. Another observation was that the Cu(II) complex exhibited greater activity towards Staphylococcus species but no activity towards Pseudomonas.

The results of the antibacterial activity of isatin N(4)-methyl(phenyl)thiosemicarbazone and its complexes revealed that they possess significant activity. It was observed that the complexes of Ni(II) and Cu(II) exhibited higher inhibition effect than the parent ligand towards all the six bacterial strains. On the other hand, the Cu(II) complex showed the highest activity towards all the species. It possessed greater inhibition effect towards the Gram positive bacteria than the Ni(II) complex and the parent ligand. Since both the complexes were active towards most of the bacterial strains, a comprehensive study in this regard will be interesting.

SUB-SECTION II

ANTIFUNGAL ACTIVITY OF CROTONALDEHYDE N(4)-METHYL(PHENYL)THIOSEMICARBAZONE (CRTSC) AND ITS COMPLEXES

Evaluation of the antifungal activities of crotonaldehyde N(4)-methyl(phenyl)thiosemicarbazone (CRTSC) and its Ni(II), Cu(II) and Zn(II) complexes was done by Disc Diffusion or Kirby Bauer method.

8.4 Materials and methods

The fungal cultures used in this study were *Penicillium*, *Fusarium* and *Aspergillus species*. The pure cultures were taken from the stock culture maintained in the Department of Microbiology, St. Mary's College, Thrissur, Kerala, India.

8.4.1 Antifungal activity by Disc Diffusion or Kirby Bauer Method

Antifungal activity was studied by Disc diffusion method (Collins & Lyner 1987)[69]. The fungal cultures were maintained in Sabouraud's dextrose broth. It was prepared from mycological peptone (10 g) and dextrose (40 g) in 1000 mL distilled water (pH 5.7). Sabouraud's dextrose agar used for keeping these culture media was prepared from mycological peptone (10 g), dextrose (40 g) and agar (15 g) in 1000 mL distilled water (pH 5.7).

The fungal cultures were maintained in Sabouraud's dextrose broth. Each culture of about 0.1 mL was distributed homogeneously on Sabouraud's dextrose agar (SDA) plates. Sterile filter paper discs of 2 mm diameter were placed on the surface of SDA plates. Different concentrations of the drug were added to each disc using a micropipette. The control used was 2% DMSO. The plates were kept at room temperature for 2 days. After incubation, the antifungal activities of the ligand and its complexes were compared by measuring the diameters of zones of inhibition.

8.5 Results

The antifungal activities of crotonaldehyde N(4)-methyl(phenyl) thiosemicarbazone (CRTSC) and its Ni(II), Cu(II) and Zn(II) complexes stuided by disc diffusion method were evaluated against three fungal species, *Penicillium*, *Fusarium* and *Aspergillus*. The diameters of the zones of inhibition of these compounds were measured after incubation. The experimental results of the samples are presented in Tables 1, 2, 3 and 4. The complexes showed higher antifungal activity than the parent ligand, crotonaldehyde N(4)-methyl(phenyl)thiosemicarbazone (CRTSC).

Table 1 Antifungal activity of the ligand, CRTSC			
Drug	**Diameter of zone of inhibition (mm)**		
(µg/ml)	***Pencillium***	***Fusarium***	***Aspergillus***
DMSO	-	-	-
100	-	-	-
250	-	-	5
500	6	-	7

Table 2 Antifungal activity of $[NiL_2(H_2O)_2]$			
Drug	**Diameter of zone of inhibition (mm)**		
(µg/ml)	***Pencillium***	***Fusarium***	***Aspergillus***
DMSO	-	-	-
100	-	-	7
250	6	-	8
500	9	-	12

Table 3 Antifungal activity of $[CuL(H_2O)Cl]$			
Drug	**Diameter of zone of inhibition (mm)**		
(µg/ml)	***Pencillium***	***Fusarium***	***Aspergillus***
DMSO	-	-	-
100	5	5	17
250	7	8	24
500	12	10	29

Table 4 Antifungal activity of $[ZnL_2]$			
Drug	**Diameter of zone of inhibition (mm)**		
(µg/ml)	***Pencillium***	***Fusarium***	***Aspergillus***
DMSO	-	-	-
100	-	8	-
250	-	10	-
500	7	13	-

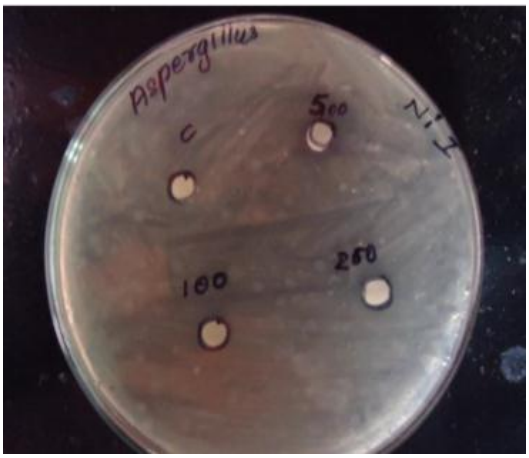

Figure 1

Antifungal activity of Ni(II) complex on the Aspergillus species

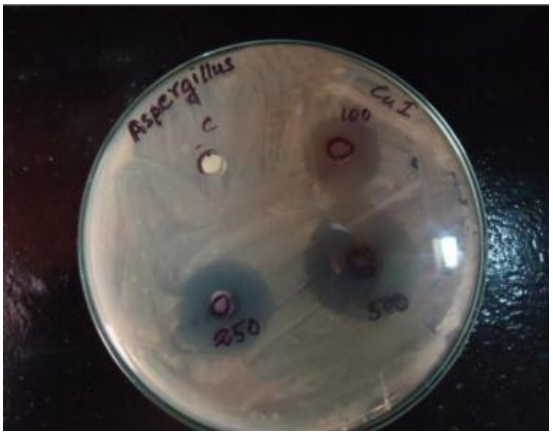

Figure 2

Antifungal activity of Cu(II) complex on the Aspergillus species

8.6 Discussion

The antifungal activities of all the compounds were compared on the basis of the consolidated Table 5.

Table 5 Antifungal activity of the ligand, CRTSC and its complexes (500µg/ml)

Sl.No.		Diameter of zone of inhibition (mm)		
	Compound	*Pencillium*	*Fusarium*	*Aspergillus*
1	Ligand, CRTSC	6	-	7
2	$[NiL_2(H_2O)_2]$	9	-	12
3	$[CuL(H_2O)Cl]$	12	10	29
4	$[ZnL_2]$	7	13	-

The antifungal studies revealed that the complexes were more active than the parent ligand. The studies showed that all the

compounds were active towards the three fungal strains in different manners. From the studies, it was observed that the Cu(II) complex exhibited higher activity than the Ni(II) and Zn(II) complexes. While the ligand showed a lesser activity, the Ni(II) and Zn(II) complexes exhibited a moderate inhibition effect.

8.7 References

[1] I. Berber, C. Cokmus, E. Atalan, Mikrobiologia, 72, 54 (2003)

[2] M. A. M. S. El-Sharief, S. Y. Abbas, K. A. M. El-Bayouki, E. W. El-Gammal, Eur. J. Med. Chem., 67, 263 (2013)

[3] A. P. MacGowan, J. Antimicrob. Chemother., 62, 105 (2008)

[4] M. Martinez, P. Silley, Antimicrobial Drug Resistance, Springer, Heidelberg, Germany (2010)

[5] D. Armenise, M. Muraglia, M. A. Florio, Archiv der Pharmazie., 345, 407 (2012)

[6] N. Woodford, D. M. Livermore, J. Infect., 59, 14 (2009)

[7] H. Harbottle, S. Thakur, S.D.G. Zhao, Anim. Biotechol., 17, 111 (2006)

[8] E. M. Bavin, R. J. W. Rees, J. M. Robson, M. Seiler, D. E. Seymour, D. Suddaby, J. Pharm. Pharmacol., 2, 764 (1950)

[9] O. Koch, G. Stuttgen, Naunyn Schmiedebergs Arch Exp Pathol Pharmakol., 210, 409 (1950)

[10] G. A. Kune, Br. Med. J., 2, 621 (1964)

[11] R. P. Gupta, N. L. Narayana, Pharmaceutica Acta Helvetiae., 72, 43 (1997)

[12] S. A. Khan, A. M. Asiri, A. A. Khan, K. A. Khan, M. A. M. Zayed, Asian J. Chem., 25, 8643 (2013)

[13] N. A. Mohamed, R. R. Mohamed, and R. S. Seoudi, Int. J. Biol. Macromol., 63, 163 (2014)

[14] M. M. Aly, Y. A. Mohamed, K. A. M. El-Bayouki, W. M. Basyouni, S. Y. Abbas, Eur. J. Med. Chem., 45, 3365 (2010)

[15] R. K. Agarwal, L. Singh, D. K. Sharma, Bioinorg. Chem. Applications, Article ID 59509, (2006)

[16] N. Parul, N. Subhangkar, M. Arun, Int. Res. J. Pharm., 3, 351 (2012)

[17] J. F. M. da Silva, S. J. Garden, A. C. Pinto, J. Braz. Chem. Soc., 12, 273 (2001)

[18] E. M. Jouad, G. Larcher. M. Allain. A. Riou, G. M. Bouet, M. A. Khan, X. Do Thanh, J. Inorg. Biochem., 86, 565 (2001)

[19] H. Beraldo, D. Gambino, Mini Rev. Med. Chem., 4, 31 (2004)

[20] W. X. Hu, W. Zhou, C. N. Xia, X. Wen, Bioorg. Med. Chem. Lett., 16, 2213 (2006)

[21] X. Du, C. Guo, E. Hansall, P. S. Doyle, C. R. Caffrey, T. P. Holler, J. H. McKerrow, F. E. Cohen, J. Med. Chem., 45, 2695 (2002)

[22] A. K. Rana, N. R. Parekh, Eur. J. Chem., 6, 747 (2009)

[23] I. C. Mendes, J. P. Moreira, A. S. Mangrich, S. P. Balena, B. L. Rodrigues, H. Beraldo, Polyhedron, 26, 3263 (2007)

[24] M. Joseph, M. Kuriakose, M. R. P. Kurup, E. Suresh, A. Kishore, S. G. Bhat, Polyhedron, 25, 61 (2006)

[25] S. K. Jain, B. S. Garg, Y. K. Bhoon, Spectrochim. Acta A, 42, 959 (1986)

[26] M. E. Hossain, M. N. Alam, J. Begum, M. A. Ali, M. Nazimudhin, F. E. Smith, R. C. Hynes, Inorg. Chim. Acta., 249, 207 (1996)

[27] H. Beraldo, D. Gambino, Mini Rev. Med. Chem., 4, 159 (2004)

[28] P. M. Krishna, B. S. Shankara, N. S. Reddy, Int. J. Inorg. Chem., 1 (2013)

[29] F. Basuli, S.M. Peng, S. Bhattacharya, Inorg. Chem., 39, 120 (2000)

[30] A. E. Liberta, D. X. West, Biometals, 5, 121 (1992)

[31] S. Padhye, R. C. Chikate, P. B. Sonawane, Coord. Chem. Rev., 123, 49 (1993)

[32] J. G. Cory, A. H. Cory, G. Rappa, Biochem. Pharmacol., 48, 335 (1994)

[33] D. S. Raja, N. S. P. Bhuvanesh, K. Natarajan, Eur. J. Med. Chem., 46, 4584 (2011)

[34] M. A. Hussein, T. S. Guan, R. A. Haque, M. B. K. Ahamed, A. M. S. A. Majid, J. Coord. Chem., 67, 714 (2014)

[35] M. Khandani, T. Sedaghat, N. Erfani, M. R. Haghshenas, H. R. Khavasi, J. Mol. Struct., 1037, 136 (2013)

[36] B. Demoro, R. F. M. de Almeida, F. Marques, C. P. Matos, L. Otero, J. Costa Pessoa, I. Santos, A. Rodriguez, V. Moreno, J. Lorenzo, D. Gambino, A. I. Tomaz, Dalton Trans., 42, 7131 (2013)

[37] J. Sheng, P.T.M. Nguyen, R.E. Marquis. Arch. Oral Biol., 50, 747 (2005)

[38] U. El-Ayaan J. Coord. Chem., 65, 629 (2012)

[39] B. Prathima, Y. Subba Rao, G.N. Ramesh, M. Jagadeesh, Y.P. Reddy, P.V. Chalapathi, A. Varada Reddy, Spectrochim. Acta Part A, 79, 39 (2011)

[40] S. Chandra, Vandana, Spectrochim Acta A Mol. Biomol. Spectrosc., 129, 333 (2014)

[41] T. A. Yousef, G. M. Abu El-Reash, O. A. El-Gammal, R. A. Bedier, J. Mol. Struct., 1035, 307 (2013)

[42] S. Prasad, R. K. Agarwal, Transition Met. Chem., 32, 143 (2007)

[43] R. F. F. Costa, A. P. Rebolledo, T. Matencio, H. D. R. Calado, J. D. Ardisson, M.E. Cortes, B. L. Rodrigues, H. Beraldo, J. Coord. Chem., 58, 1307 (2005)

[44] S. Berger, Horm. Metab. Res., 17, 111 (1985)

[45] B. Lakshmi, P. G. Avaji, K. N. Shivananda, P. Nagella, S. H. Manohar, K. N. Mahendra, Polyhedron, 30, 1507 (2011)

[46] J. Chan, Y. Huang, G. Liu, Z. Afrasiabi, E. Sinn, S. Padhye, Y. Ma, Toxicol. Appl. Pharmacol., 40, 197 (2004)

[47] J. R. Dilworth, R. Hueting, Inorg. Chim. Acta., 3, 389 (2012)

[48] A. Karakucuk- Iyidogan, D. Tasdemir, E. E. Oruc-Emre, J. Balzarini, Eur. J. Med. Chem., 46, 5616 (2011)

[49] R. J. Glisoni, M. L. Cuestas, V. L. Mathet, J. R. Oubina, A. G. Moglioni, A. Sosnik, Eur. J. Pharm. Sci., 47, 596 (2012)

[50] X. Du, C. Guo, E. Hansel, P. S. Doyle, C. R. Caffrey, T. P. Holler, J. H. McKerrow, F. E. Cohen, J. Med. Chem., 45, 2695 (2002)

[51] D. Kovala-Demertzi, M. A. Demertzis, E. Filiou, A. A. Pantazaki, P. N. Yadav, J. R. Miller, Y. Zheng, D. A. Kyriakidis, Biometals, 16, 411 (2003)

[52] J. P. Scovill, D. L. Klayman, D. G. Franchino, J. Med. Chem., 25, 1261 (1982)

[53] L. Klayman, J. P. Scovill, J. F. Bartosevich, J. Bruce, J. Med. Chem., 26, 35 (1983)

[54] D. K. Demertzi, M. A. Demertzis, J. R. Miller, C. Papadopoulou, C. Dodorou, G. Filousis, J. Inorg. Biochem., 86, 555 (2001)

[55] P. K. Singh, D. N. Kumar, Spectrochim. Acta. Part A, 64, 853 (2006)

[56] D. R. Williams, Chem. Rev., 72, 203 (1972)

[57] A. Furst, R. T. Haro, Prog. Exp. Tumor Res., 12, 102 (1969)

[58] F. B. Dwyer, E. Mayhew, E. M. F. Roe, A. Shulman, Br. J. Cancer., 19, 195 (1965)

[59] F. Bisceglie, S. Pinelli, R. Alinovi, M. Goldoni, A. Mutti, A. Camerini, L. Piola, P. Tarasconi,

[60] G. Pelosi, J. Inorg. Biochem., 140, 111 (2014)

[61] J. F. M. da Silva, S. J. Garden, A. C. Pinto, J. Braz. Chem. Soc., 12, 273 (2001)

[62] S. N. Pandeya, S. Smitha, M. Jyoti, S. K. Sridhar, Acta Pharm., 55, 27 (2005)

[63] N. Karali, Eur. J. Med. Chem., 37, 909 (2002)

[64] I. Chiyanzu, E. Hansell, J. Gut, P. J. Rosenthal, J. H. McKerrow, K. Chibale, Bioorg. Med. Chem. Lett., 13, 3527 (2003)

[65] T. R. Bal, B. Anand, P. Yogeeswari, D. Sriram, Bioorg. Med. Chem. Lett., 15, 4451(2005)

[66] I. Chiyanzu, C. Clarkson, P. J. Smith, J. Lehman, J. Gut, P. J. Rosenthal, K. Chibale, Bioorg. Med. Chem., 13, 3249 (2005)

[67] A. Rai, S. K. Sengupta, O. P. Pandey, Spectrochim Acta., 61 A, 2761(2005)

[68] Collins and Lyne's Microbiological Methods 8th Edn (1987)

SECTION B

ANTITUMOUR STUDIES

CHAPTER IX

COMPLEXES OF THIOSEMICARBAZONES AS ANTITUMOUR AGENTS

9.1 Introduction

Cancer has been the main killer disease throughout the human history. Cancer is the second leading cause of death worldwide and is a multi-step disease characterized by physical, environmental, metabolic, chemical and genetic factors. They play a direct and/or indirect role in the initiation and worsening of cancers. According to World Health Organization (WHO), death due to cancer over the globe is 8.2 million and expected to rise over to 13.1 million in 2030 (about 70% increase)[1]. Cancer is caused by the changes in a cell's DNA, either genetically or by outside exposures, which are often referred to as environmental factors. The substances that are capable of producing tumours may cause the cells to divide at a faster rate, thereby, augmenting the chances of DNA alteration. In general, cancer cells have more genetic changes, such as mutations in DNA, than the normal cells.

There are about 200 types of cancers which include carcinoma, sarcoma, leukemia, central nervous system cancer, lymphoma and myeloma. Cancer can start almost anywhere in the human body, which has trillions of cells. According to the body requirements, human cells normally grow and divide to form new cells. Once cancer develops, this orderly process breaks down and the uncontrolled growth of

abnormal cells takes place leading to cancer cells, or malignant cells, or tumor cells. Since the newly formed cancer cells are less specialized, they continue to divide forming mostly solid tumors, except for leukemia. Cancerous tumors are malignant, with a potential to invade or spread into the nearby tissues. This process of cancer cells leaving an area and growing in another body area is termed metastatic spread or metastasis. Unlike malignant tumors, benign tumors do not penetrate into the normal body tissues.

9.1.1 Causes of cancer and its treatment

Cancer is a disease caused by the genetic changes that alter the cell function, thereby leading to uncontrolled cell growth and tumor formation. Some of these genetic changes occur naturally by the DNA replication during the process of cell division. However, most of the cancers are related to environmental, lifestyle, or behavioral exposures that cause damage to DNA[2]. Cancer is caused by certain viruses and bacteria known as Oncoviruses and Helicobacterpylom (cancer bacteria). The word "environmental", as used by cancer researchers, refers to everything outside that interacts with the human body[3]. The environment is not limited to the biophysical environment (for example, exposure to factors such as air pollution or sunlight), but also includes lifestyle and behavioral factors[4]. Over one third of world wide cancer death (and about 75-80% in the United States) is possibly preventable by reducing the exposure to these known factors[5,6]. Common environmental factors that add to the cancer death include exposure to different chemical- and physical agents (tobacco use

accounts for 25–30% of cancer death), environmental pollutants, diet and obesity (30–35%), infections (15–20%), and radiation (both ionizing and non-ionizing, up to 10%)[7]. These factors will act, atleast moderately, by changing the function of genes within the cells[8].

Although it is often impossible to determine the instigating actions that cause cancer to develop in a specific person, the major reason is from the exposures to these substances, such as the chemicals, or radiation. Chemical or carcinogenic compounds include benzene, asbestos, aflatoxin, those in tobacco smoke, etc., which may induce DNA alterations. Cancer is also caused by the ionizing radiations such as ultraviolet rays from the sunlight, radiations from alpha, beta, gamma, and X-ray-emitting sources. Certain pathogens are being identified as possible agents such as Human papillomavirus (HPV), hepatitis viruses B and C, Merkel cell polyomavirus and Helicobacter pylori. Diet comprising of charred fats and low quality fruits and vegetables also play a crucial role in causing different types of cancer.

A number of cancers related to human genes, known as carcinoma type include breast-, ovarian-, skin cancers and melanoma. Leukemia (lymphoblastic and T-cell) that starts in the blood-forming tissue such as bone marrow, causes a large number of abnormal blood cells to be produced and spread in the blood. Cancer that begins in the bone or cartilage are known as "bone, soft tissue cancers" or osteosarcoma.

In 2011, the World Health Organization categorized the low energy radiations from cell phones as “possible carcinogenic”.

However, it is at a very low risk level and can be compared with the same risk levels from caffeine and pickled vegetables.

Now a days there is an immense need to fight against the most dangerous cancer disease, which is the most complex one due to its multiple causes. Therefore, cancer must be fought to the end with the help of all possible therapies. In the case of solid tumours, surgery is a procedure in which the cancer is removed from the body. Radiation therapy is particularly effective for the head and neck cancer that uses high doses of radiation to kill the cancer cells and thus shrink the tumors. Chemotherapy is also a type of treatment for the breast cancer and pancreatic cancer, whereby drugs are used to kill the cancer cells. Immunotherapy is another type of treatment that helps the immune system to fight against the cancer. Targeted therapy is a type of another cancer treatment that targets the changes in the cancer cells which help them to grow, divide and spread. Hormone therapy is a treatment that helps to slow down or to stop the growth of breast and prostate cancers that use the hormones to grow. Stem cell transplants are the procedures that restore the blood-forming stem cells in the cancer patients that had been destroyed by the very high doses of chemotherapy or radiation therapy.

9.1.2 Thiosemicarbazones and their metal complexes as potent anticancer agents

The identification of new anticancer therapeutic agents is one of the important goals in the field of medicinal chemistry. But cytotoxicity and genotoxicity of anticancer drugs to the normal cells

are the major problems in cancer therapy and they produce the risk of inducing secondary malignancy[9]. In the recent years, there has been an apprehensive search for the discovery and development of novel anticancer agents, devoid of the numerous unpleasant side effects of conventional ones. The resistance to anticancer drugs is the main clinical challenge for an effective treatment of cancer, since some of the tumour cells develop a particular phenotype, called multi drug resistance [MDR], which makes these cells resistant to anticancer agents which were used previously to treat cancers[10]. However, there has been a remarkable development in the chemotherapy of cancer and researches are still developing novel and more effective drugs to fight this disease.

Heterocyclic molecules are well recognized for their critical role in pharmaceutical drug design. Now a number of heterocyclic compounds are commercially available as anticancer drugs and huge efforts have been made for the identification and discovery of novel anticancer drugs. In the last few years there has been a growing attention towards thiosemicarbazones due to their extensive ranges of biological properties, specifically as antifungal, antiviral, antibacterial and anticancer agents[11-14]. Recently, several thiosemicarbazone derivatives have been synthesized and their antitumor activities have also been evaluated[15]. Biological properties of thiosemicarbazones have been studied since 1956 when Brockman *et al*[16] reported the antitumour properties of thiosemicarbazones derived from 2-formylpyridine. The biological activities of the thiosemicarbazones have been attributed to their ability to form complexes with trace metal ions and the compounds thus formed have been found to possess

improved therapeutic properties[17]. For example, the iron complexes of 2-hydroxy-1, 4-naphthoquinone-1-thiosemicarbazone and its C-3 methyl derivative have been shown to be active against P388 leukemia as well as MCF-7 breast cancer cells[18,19]. Similarly, metal complexes of 3-benzoyl-α-methylbenzene acetic acid thiosemicarbazone have been shown to be effective against human MCF-7 breast cancer cell lines[20].

Thiosemicarbazones in their neutral or deprotonated form behave as N, N, S chelate towards metal ions and displays antiproliferative activity on different tumors cell lines, which has been a common feature of all the compounds with carcinogenic potency. Moore *et al*[21] found that the antitumor activity of thiosemicarbazones is due to their ability to inhibit ribonucleotide reductase (RR), a necessary enzyme for DNA synthesis. Therefore, it has been suggested that an inhibitor to RR would be worth for the treatment of cancer[22].

It has been observed that various substituents at N(4)-position have a significant effect on the biological efficiency of thiosemicarbazones and it has also been proposed that the conjugated N-N-S tridentate system of the thiosemicarbazone moiety enhances the antitumor activity[23,24]. Thus, a number of thiosemicarbazone derivatives were reported as potential anticarcinogenic, mainly by varying the substituents at the thioamide nitrogen atom. All such compounds screened against human neuroblastoma (SK-N-MC), human feta lung fibroblast (MRC-5), liver carcinoma (HepG2) and cervical cancer (HeLa) cell lines showed significant activity[25-26]. For example, the heterocyclic-N(4)-thiosemicarbazones have displayed promising activity against breast cancer. The presence of aromatic

group at the N(4)-position of isatin-β thiosemicarbazones derivatives has shown improved cytotoxicity against the parental KB-3-1 cell lines and the P-glycoprotein-expressing cell line KB-V1[27,28].

Thiosemicarbazones and their metal complexes possess a wide range of applications in analytical chemistry, pharmacology and nuclear medicine[29–32]. The existence of imine and thione groups makes them potential polydentate ligands and thus it is not surprising that various complexes of thiosemicarbazone have been prepared and characterized[33,34]. Thiosemicarbazones and their complexes are emerging as anticancer chemotherapeutic agents due to their ability to form complexes with metal ions in enzymes. In the recent years, there has been a great attention on the complexes of substituted thiosemicarbazones, due to their wide range of biological activities and also as a suitable model for various bioinorganic processes[35,36]. Literature survey has also revealed that the pharmaceutical importances of thiosemicarbazones are due to their ability to interfere with the biosynthesis of DNA[37]. It has been reported that the copper complexes of aromatic thiosemicarbazones can act as potential antitumor agents owing to their ability to interfere in the complicated mechanism of leukemic transformations, by inhibiting their replication and triggering apoptotic processes[38].

9.1.3 Copper complexes of thiosemicarbazones as antitumor agents

Copper is a vital micronutrient for the fundamental life processes and are found in all the life forms. The ability of copper to catalyze the oxidation-reduction (redox) reactions can involuntarily

lead to the production of reactive oxygen species (ROS), which in turn, demands the limited homeostatic regulation of copper within the body[39]. Numerous cancer types show increased intratumoral copper or completely altered copper distribution. The recognition that copper serves as a limiting factor for the multiple aspects of tumor progression, including growth, angiogenesis and metastasis, has encouraged the development of copper-specific chelators as therapies, to inhibit these processes. Alternative therapeutic approach employs the specific ionophores, which transport copper to the cells to increase the intracellular copper levels. Due to this, the therapeutic gap between the normal and cancerous cells has led to the development of copper-ionophores with anticancer properties. Currently, the replacement of platinum by copper in coordination complexes for the mainstream chemotherapies is also under investigation.

Even though platinum-based complexes had been the major emphasis of research on chemotherapy agents[40–42], the interests in this field have been moved to non-platinum-based agents[43–46], in order to discover the metal complexes with lesser side effects and with similar or better cytotoxicity. Thus, an extensive range of metal complexes, based on titanium, gallium, germanium, palladium, gold, cobalt, ruthenium and tin are being studied tremendously as platinum replacements[47–50]. Moreover, copper(II)-based complexes appear to be very promising candidates for anticancer therapy; an idea reinforced by a huge number of research articles describing the synthesis and cytotoxic activities of various copper(II) complexes[51–54].Thus, in contrast to platinum-based drugs, these promising copper coordination complexes may be more effective anticancer agents, with reduced

toxicity toward normal cells and they may possibly avoid the chemo resistance associated with the recurring platinum treatment.

The coordinated ligands also seem to be important as the choice of the metal, because being the essential part of biologically active complexes, these organic molecules (ligands) can exert a biological activity of their own[55]. Numerous indole derivatives, commonly present endogenously in both human and other mammalian tissues, are the main structural motif found in several pharmacologically active compounds. Isatin, an indole derivative has potentially important biological activity by virtue of its typical size and privileged electronic properties[56-58]. Vine and co-workers reported a variety of substituted isatins and they were screened against a panel of five human cancer cell lines. Generally, these compounds showed better selectivity toward leukemia and lymphoma cells over breast, prostate and colorectal carcinoma cell lines[59]. *In vitro* studies of the complexes of Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) of isatin β-thiosemicarbazone on human leukaemic ells have shown that the free ligand and the Cu(II) complex were more active in the antiproliferation of cells and no compound was able to induce apoptosis[60].

9.2 Scope of the present investigation

Cancer is definitely one of the main health concerns facing our society and is found to be the second leading cause of death. All the predictions made by the scientists about cancer that it will be curable in the 21stcentury, turned out to be wrong. Even though the long-term goal is to make this dangerous disease a curable one, there remains a

short-term goal to extend the survival time of the cancer patients with a maximum of life quality. Therefore, in the recent years, tremendous research and discoveries have been going on for the development of novel anticancer drugs in the area of medicinal chemistry, to produce newer drugs devoid of the unpleasant side effects of the conventional ones.

Thiosemicarbazone is an important structural motif that exhibit potentially useful biological applications. Due to the N-N-S-ligating system of thiosemicarbazones, which is a common feature of all the compounds with carcinogenic potency, they display antiproliferative activity on different tumour cell lines. This conjugated system has the ability to form chelates with metal ions which in turn enhances their antitumor activity. Many of the thiosemicarbazone complexes are potential anticancer- and chemotherapeutic agents due to their ability to inhibit the DNA biosynthesis and cell division[61]. They block the biosynthesis by binding to the nitrogen base pairs of DNA and make lesions in the DNA double helix by an oxidative rupture[62,63]. Thus, a strong correlation was found between tumor growth rate and the enzyme Ribonucleotide Reductase[64]. Literature review revealed that a variety of isatin based thiosemicarbazones and their Cu(II) complexes showed excellent *in vitro* antioxidant effect. This persuaded us to conduct the cytotoxic and antitumour studies of isatin N(4)-methyl(phenyl)thiosemicarbazone (ISTSC) and its complexes, especially copper(II) complex. The copper(II) complex was also subjected to the *in vivo* antitumour studies.

CHAPTER X

MATERIALS AND METHODS

The experimental and procedural details of the antitumour studies are described in this chapter.

10.1 Materials

10.1.1 Chemicals

All the chemicals and solvents of analytical grade reagents were purchased from Merck were used without further purification. The hydrated metal salts were used for the preparation of the complexes. Solvents like methanol, ethanol, petroleum ether and dimethyl sulphoxide were used for the preparation, extraction and recrystallization of the ligand and its complexes. The solvents used for the spectral studies were of spectroscopic grade.

10.1.2 Synthesis of isatin N(4)-methyl(phenyl)thiosemicarbazone

Equimolar amounts of isatin and N(4)-methyl(phenyl) thiosemicarbazide were refluxed on a water bath for less than 2 h to obtain the ligand, isatin N(4)-methyl(phenyl)thiosemicarbazone (ISTSC). The detailed procedure for the synthesis of the ligand is described in Chapter V.

10.1.3 Synthesis of the thiosemicarbazone complexes

The complexes of Mn(II), Fe(III), Co(II), Ni(II) and Cu(II) of ISTSC were prepared by refluxing equimolar amounts of the respective metal salt with the ligand for 4 h. However, the complexes of Zn(II) and Cd(II) were prepared by taking the ethanolic solution of the ligand and methanolic solution of the metal acetates in 2:1 molar ratio.

10.1.4 Characterization of the ligand and its complexes

Physical characterization of the ligand and the complexes were done (details given in Chapter V) and their structures were confirmed from infrared and electronic spectral studies and magnetic susceptibility measurements. The structures of the ligand and its Cu(II) complex are shown in Figures 1 and 2.

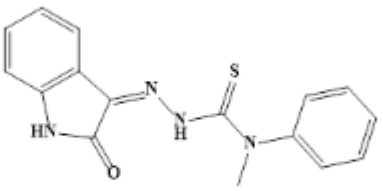

Figure 1

Isatin N(4)-methyl(phenyl)thiosemicarbazone (ISTSC) (LH)

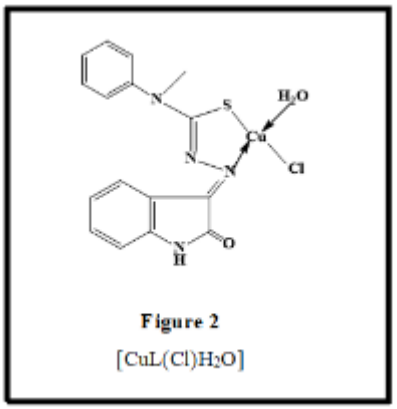

Figure 2

[CuL(Cl)H_2O]

10.1.5 Requirements of Cytotoxicity studies

The preparation of the drug was done by dissolving 50 mg of the copper complex in 1 mL DMSO. This solution was then diluted with distilled water to get the desired concentrations for *in vitro* and *in vivo* cytotoxicity studies. The cell lines of Dalton's Lymphona Ascites (DLA) and Ehrlich's Ascites Carcinoma (EAC) were embedded into the intraperitoneal cavity of the mouse. The cell lines for the above cytotoxic studies were obtained from Adayar Cancer Institute, Chennai.

Female mice (24-29 g) of Swiss albino type required for the study were obtained from the Small Animal Breeding Station (SABS), Mannuthy, Thrissur, Kerala. These animals were kept under the standard conditions in the animal house of Amala Cancer Research Centre. They were nurtured with standard mouse chow (Sai Durga Feeds and Foods, Bangalore, India) and water. These female mice were kept in ventilated cages (six each) in air-controlled rooms (25^0C). The animals were approved from the Institutional Animal Ethics

Committee (IAEC), and the experiments were done strictly according to the strategy of Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) constituted by the Animal Welfare Division, Government of India.

The tumour cells used for the study were Dalton's Lymphona Ascites (DLA) and Ehrlich's Ascites Carcinoma (EAC) cell lines and they were aspirated from the intraperitoneal cavity of tumour bearing mice.

The phosphate buffered saline (PBS) prepared from NaCl (8.00 g), KCl (0.20 g), $Na_2HPO_4.2H_2O$ (1.44 g) and KH_2PO_4 (0.20 g) was dissolved in distilled water and further diluted upto 1 litre, which has a pH of 7.2.

The normal saline was prepared by dissolving AR NaCl (0.85 g) in 100 mL distilled water.

10.2 Methods

10.2.1 Trypan blue exclusion method for short term *in vitro* cytotoxic studies

The short-term *in vitro* cytotoxic studies of isatin N(4)- methyl(phenyl)thiosemicarbazone and its complexes were done using Dalton's lymphoma ascites (DLA) cells. The tumour cells were extracted from the peritoneal cavity of tumour bearing mice and then washed three times with PBS or normal saline. Cell viability was tested by Trypan blue exclusion method. Viable cell suspensions ($1x10^6$ cells in 0.1 mL) added to different concentrations of the drug were made

upto 1 mL with PBS and incubated for 3 h at 37^0C. The cell suspension, mixed with 0.1 mL of 1% Trypan blue, was loaded after 2-3 minutes on a haemocytometer. The blue colour will be taken up by the dead cells while the live cells will not. The percentage of dead cells was determined with a haemocytometer, by counting separately the number of stained and unstained cells.

10.2.2 Cytotoxic studies of the complexes of ISTSC

Twenty four Swiss albino female mice were collected as four groups (6 animals per group). According to the dosage of the drug treatment, they were grouped as follows,

Group 1: 5 mg/kg,

Group 2: 10 mg/kg,

Group 3: 20 mg/kg

Group 4: 25 mg/kg.

The drug was administered once in a day (i.p) and continued upto 6 weeks, in order to note their mortality rate.

10.2.3 *In vivo* studies of copper(II) complex of isatin N(4)-methyl(phenyl)thiosemicarbazone on animals bearing ascites tumour

This study was carried on 6-8 weeks old female mice weighing 24-29 g, in five groups containing six in each group. Viable EAC, one million cells in 0.1 mL PBS were extracted from the peritoneal cavity and injected intraperitoneally to the animal for the development of

ascites tumour. There were five groups of six animals each, with the control and standard drug (cyclophosphamide) in Group 1 and 2, respectively. Groups 3, 4 and 5 were, respectively, treated with drug concentrations of 5 mg, 10 mg and 20 mg per kg body weight of the animal. Various concentrations of these test compounds and the standard drug (cyclophosphamide) were given intraperitoneally. The death patterns of the animals were noted and the percentage of life span increase was calculated using the equation given below:

% ILS = [T-C/C x 100]

where, T and C are survival rate of treated and control mice, respectively.

10.2.4 *In vivo* studies of copper(II) complex of isatin N(4)-methyl(phenyl)thiosemicarbazone on the expansion of solid tumour

This study was carried out on five groups of Swiss albino female mice (6-7 weeks old), with six animals in each group. Tumour induction was done by injecting 0.1 mL of one million DLA cells into the right hind leg of each mouse. The control and cyclophosphamide treated groups were 1 and 2, whereas, groups 3, 4 and 5 were treated with the different concentrations of Cu(II) complex of ISTSC. The tumour growth in animals of each group was determined, right from the 7^{th} day of tumour induction. This was done by measuring the tumour growth diameter in two vertical planes by a digital vernier caliper, and it continued up to 34^{th} day. The size of the tumour growth

was calculated using the equation, $V=4/3\pi r_1^2 r_2$, where, r_1 and r_2 are the minor- and major diameters obtained from the vernier caliper[65].

CHAPTER XI

CYTOTOXIC STUDIES OF ISATIN N(4)-METHYL(PHENYL)THIOSEMICARBAZONE AND ITS COMPLEXES

In 2030 around 12 million deaths due to cancer are predictable in the world. Breast cancer, one of the highest cases, affects more than one million women every year[66]. The second most common form of cancer in pediatric patients is the Malignant gliomas, which is the most common and lethal cancers originating in the central nervous system[67]. The therapies can produce only a short- or moderate survival time. Furthermore, the low tolerance of the central nervous system to the conventional chemotherapeutic agents (*cis*-platin) impairs the effectiveness of the treatment[68]. Thus, it is an essential thing to overcome these challenges by exploring novel drug candidates, with improved pharmacological properties and a broader range of antitumor activities.

Thiosemicarbazones form an interesting class of compounds with an extensive range of pharmacological applications[69]. Thiosemicarbazones act as chelating agents for various metal ions by bonding through the sulfur- and the imine nitrogen atoms. The well documented biological activities of numerous heterocyclic thiosemicarbazones have been recognized as often due to their ability to form chelates with the transition metal ions[70,71]. The previous studies showed that the antitumor activity of copper complexes of α(N)-heterocyclic thiosemicarbazones has been attributed to their ability to

block the enzyme, ribonucleotide reductase (RR), that catalyzes the conversion of ribonucleotides into deoxyribonucleotides[72–74].

The common feature of compounds with the carcinostatic potency is the presence of NNS or ONO donor atoms. Thus, the anticancer studies conducted on isatin N(4)-methyl (phenyl)thiosemicarbazone and its transition metal complexes with the same nature have established their cytotoxic potentiality. The copper complex of isatin N(4)-methyl(phenyl)thiosemicarbazone has been selected for the antitumour studies.

11.1 Results

The results obtained from the short term *in vitro* cytotoxic studies, *in vivo* cytotxic studies on the ascites tumour and solid tumour development are presented here.

11.1.1 Short term *in vitro* cytotoxic studies

The short term *in vitro* cytotoxic studies of the ligand and its complexes on DLA cells showed a remarkable activity (Table 1). The copper complex exhibited an exceptionally high cytotoxicity with an IC_{50} value of 38 μg/mL (Figure 1), while the free ligand showed 70% cytotoxic activity. The cytotoxic action of the isatin N(4)-methyl(phenyl)thiosemicarbazone in comparison with its complexes is shown in Figure 2 and the comparison of the cytotoxic activity of Cu(II) complex with its parent ligand is shown in Figure 3.

Table 1 *In vitro* cytotoxicity of the ligand and its complexes						
Drug Concentration (µg/ml)	**Percentage cytotoxicity of ISTSC and its complexes**					
	Fe(III)	**Co(II)**	**Ni(II)**	**Cu(II)**	**Zn(II)**	**ISTSC ligand**
10	4	7	4	35	6	4
20	8	14	11	42	12	18
50	16	26	28	60	30	32
100	32	32	41	83	58	56
200	48	40	60	95	72	70

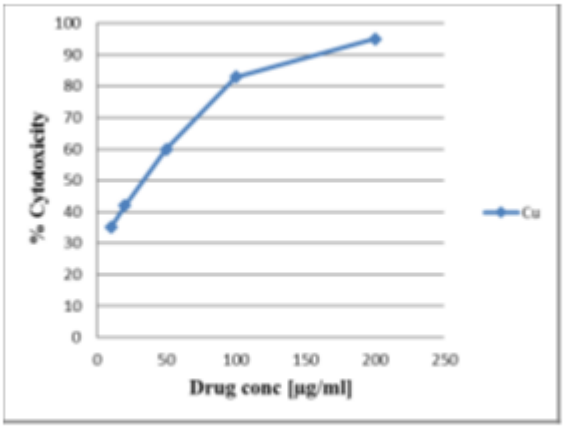

Figure 1

Cytotoxic action of Cu(II) complex of isatin N(4)-methyl(phenyl)thiosemicarbazone

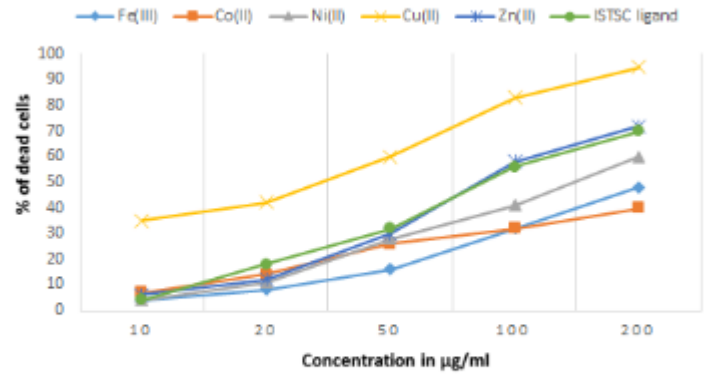

Figure 2

Cytotoxic action of isatin N(4)-methyl(phenyl)thiosemicarbazone and its complexes

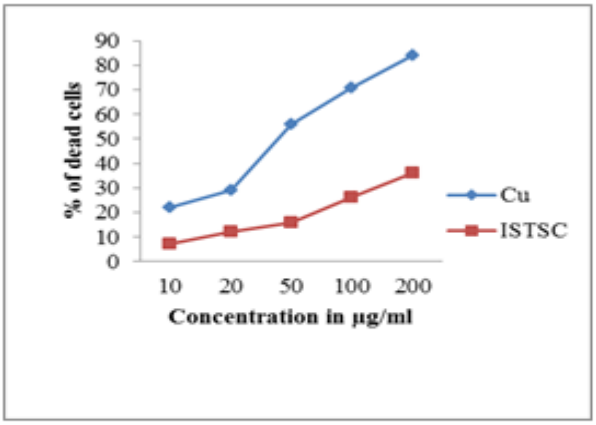

Figure 3

Cytotoxicity of isatin N(4)-methyl(phenyl)thiosemicarbazone and its Cu(II) complex

11.1.2 Toxicity studies

The toxicity studies of the copper(II) complex performed on four groups of twenty four Swiss albino mice, at four different concentrations of 5, 10, 20 and 25 mg/kg, revealed that the last concentration of 25 mg/kg was toxic to the animals. Hence, the *in vivo* studies were done using 5, 10 and 20 mg/kg concentrations, avoiding the last concentration of 25 mg/kg.

11.1.2.1 *In vivo* studies of copper(II) complex of isatin N(4)-methyl(phenyl)thiosemicarbazone on the development of ascites tumour

The ascites tumour bearing animals of the control group survived for 15 days. While the animals treated with cyclophosphamide drug survived up to 24.7 days (Table 2). The dosage of the test compound at 5, 10 and 20 mg/kg increased the continued survival of animals by 19.6, 21.2 and 18.3 days, respectively (Figure 4). Hence, the copper(II) complex of ISTSC was found to be capable of increasing the duration of life of the animals by 30.7, 41.3, and 22%, respectively, at 5, 10 and 20 mg/kg concentrations (Table 3).

Table 2 Effect of copper complex of ISTSC on the survival rate of ascites tumour bearing mice	
Drug treatment	**Survival rate**
Control	15
5 mg/kg b.wt	19.6
10 mg/kg b.wt	21.2
20 mg/kg b.wt	18.3
Standard (Cyclophosphamide)	24.7

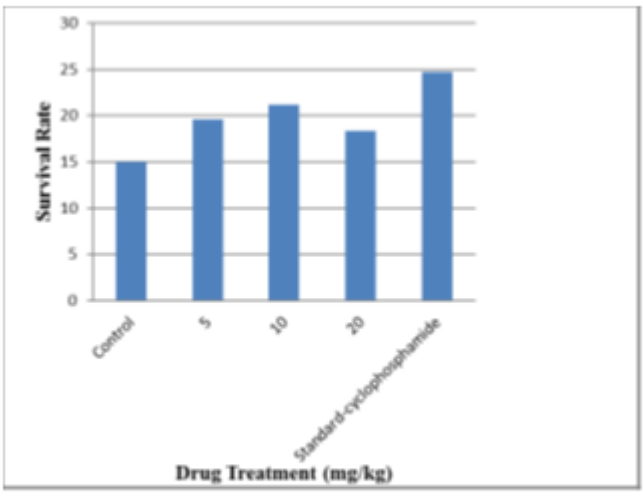

Figure 4

Effect of copper(II) complex on the survival rate of ascites tumour bearing mice

Table 3 Effect of copper complex of ISTSC on the percentage of life span of mice bearing ascites tumour	
Drug treatment(mg/kg b.wt)	**% of life span**
Control	-
5	30.7
10	41.3
20	22
Standard (Cyclophosphamide)	24.7

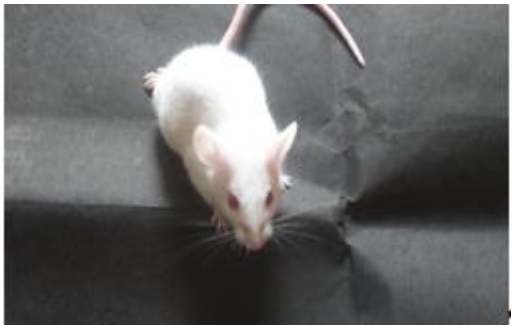

Figure 5

Normal mice

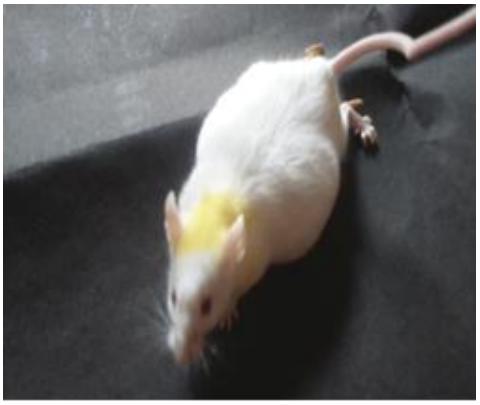

Figure 6

Ascites tumour bearing mice

11.1.2.2 *In vivo* studies of copper(II) complex of isatin N(4)-methyl(phenyl)thiosemicarbazone on the development of solid tumour

The copper(II) complex treated animals showed a significant reduction in the tumour volume. But the tumour volume got enlarged to 2.873 cm^3 on the 34^{th} day in the control group animals, whereas, the tumour volume reduced to 0.486 cm^3 in the cyclophosphamide (standard drug) treated group (Figure 7). The tumour volumes at concentrations of 5 and 20 mg/kg were, respectively, 1.942 and 1.373 cm^3, while the tumour volume was 1.073 cm^3 at 10 mg/kg (Table 4).

Table 4 Effect of copper complex of ISTSC on the decrease of tumour volume (cm^3)								
Drug Treatment (mg/kg)	**Number of days of observation (tumour volume in cm^3)**							
	Initial	**10**	**14**	**18**	**22**	**26**	**30**	**34**
Control	0.082	0.584	0.725	1.972	2.124	2.423	2.724	2.873
5	0.07	0.703	1.024	1.446	1.685	1.539	1.631	1.942
10	0.069	0.432	0.563	0.872	0.925	1.134	1.237	1.073
20	0.072	0.512	0.641	1.312	1.523	1.775	1.146	1.373
Standard	0.074	0.225	0.251	0.384	0.421	0.485	1.063	0.486

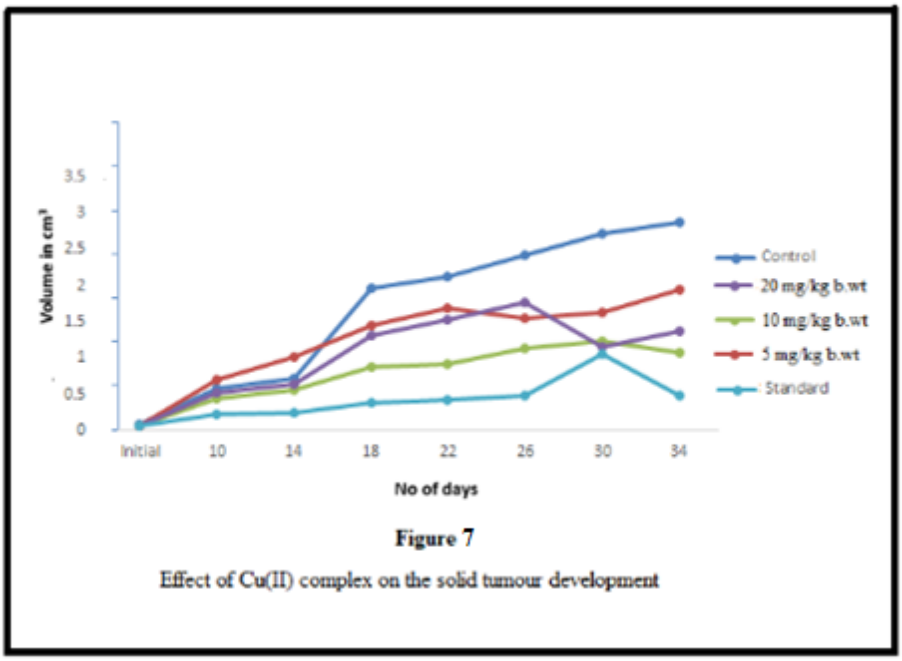

Figure 7

Effect of Cu(II) complex on the solid tumour development

Figure 8

Control

Figure 9

Copper complex treated (10 mg/kg b.wt)

11.2 Discussion

The common feature of the compounds with carcinostatic potency is the presence of NNS or ONO donor atoms. Thus, the

anticancer studies on isatin N(4)-methyl(phenyl)thiosemicarbazone and its transition metal complexes have established their cytotoxic potentiality against EAC and DLA cell lines. Ehrlich ascetic tumour grows in a rapid and aggressive manner in almost all types of mice[75]. The implantation of EAC induces a local inflammation which results in an intense edema formation and a progressive ascetic fluid formation, which is an essential and direct nutrient source for the tumour cells[76,77]. The copper(II) complex was found to be capable of acting against DLA and EAC induced tumours and it showed the maximum cytotoxicity with an IC_{50} value of 38 μg/ml. In both the cell lines, a concentration of 10 mg/kg b.wt of the drug showed a noticeable effect than the two other concentrations, of 20 and 5 mg/kg b.wt. Thus the current study has shown the copper(II) complex of isatin N(4)-methyl(phenyl)thiosemicarbazone as a potential anticancer agent at a moderate concentration.

The previous reports consider the copper complexes of thiosemicarbazones as the most active antitumour agents. Hence, copper chelators have been developed as anticancer agents. The cytotoxic potential of the thiosemicarbazone chelated copper complex in comparison with the ligand, ISTSC may be due to the lipophilicity of the drug, which arises due to the metal coordination. This may lead these molecules to cross the cell wall barriers and form hydrogen bonds with the nitrogen base pairs of DNA and thereby reducing the normal cell metabolism by blocking the base replication and inhibit the tumour growth[78]. Though the real pathway by which the copper(II) complex exhibits antitumour activity is not known, but it is assumed

that this will be taking place on a structure activity relationship, like in the case of *cis*-platin[79].

11.3 Conclusion

The *in vitro* cytotoxic and antitumour studies of the copper complex of isatin N(4)-methyl(phenyl)thiosemicarbazone (ISTSC) suggest its potential use as anticancer agent.

11.4 References

[1] World Health Organisation. www.worldhealthorganisation.in.

[2] B. W. Stewart, C. P. Wild, eds. Cancer etiology World Cancer Report, World Health Organization 16 (2014)

[3] Cancer and the Environment: What you Need to Know, What You Can Do. NIH Publication National Institutes of Health 3, 2039 (2003)

[4] J. Kravchenko, I. Akushevich, K. G. Manton, Cancer mortality and morbidity patterns from the U. S. population: an interdisciplinary approach. Berlin, Springer (2009)

[5] R. Doll, R. Peto, J. Natl. Cancer Inst., 66, 1191 (1981)

[6] D. C. Whiteman, L. F. Wilson, Cancer Epidemiol., 44, 203 (2016)

[7] P. Anand, A. B. Kunnumakkara, C. Sundaram, K. B. Harikumar, S. Tharakan, O. S. TLai, B. Sung, B. B. Aggarwal, Pharmaceutical Research, 25, 2097 (2008)

[8] World Cancer Report, World Health Organization. Chapter 1.1 (2014)

[9] N. Aydemir, R. Bilaloglu, Mutat. Res., 537, 43 (2003)

[10] S. Arora, S. Agarwal, S. Singhal, Int. J. Pharm. Pharm. Sci., 6,34 (2014)

[11] I. C. Mendes, F. B. Costa, G. M. de Lima, J. D. Ardisson, I. G.-Santos, A. Castineiras, Polyhedron, 28,1179 (2009)

[12] K.-Demertzi, A. Papageorgiou, L. Papathanasis, A. Alexandratos, P. Dalezis, J. R. Miller, Eur. J. Med. Chem., 44, 1296 (2009)

[13] M. B.-Ferrari, F. Bisceglie, A. Buschini, S. Franzoni, G. Pelosi, S. Pinelli, J. Inorg. Biochem., 104, 199 (2010)

[14] M. Li, J. Zhou, H. Zhao, C. Chen, J. Wang, J. Coord. Chem., 62, 1423 (2009)

[15] K. Hu, Z.-J. Yang, S.-S. Pan, H.-J. Xu, J. Ren, Eur. J. Med. Chem., 45, 3453 (2010)

[16] J. G.-Tojal, J. G.-Joca, R. Cortos, T. Rojo, M. K. Urtiago, I. Arriortua, Inorg. Chim. Acta., 249, 25 (1996)

[17] U. Abram, K. Ortner, K. Sommer, J. Chem. Soc. Dalton Trans., 735 (1999)

[18] S. Padhye, S. Chikate, A. Kumbhar, J. M. Shallom, M. P.Chitnis, Biometals, 5, 67 (1991)

[19] D. K. Saha, S. Padhye, S. B. Padhye, Metal-Based Drugs, 8, 73 (2001)

[20] D. K. Saha, S. B. Padhye, C. J. Newton, E. Sinn, Indian J. Chem,, 41A, 279 (2002)

[21] E. C. Moore, M. S. Zedeck, K. C. Agrawal, A. C. Sartorelli, Biochem., 9, 4492 (1970)

[22] H. L. Elford, M. Freese, E. Passamani, H. P. Morris, J. Biol. Chem., 245, 5228 (1970)

[23] P. V. Bernhardt, P. C. Sharpe, M. Islam, D. B. Lovejoy, D. S. Kalinowski, D. R. Richardson, J. Med. Chem., 52, 407 (2008)

[24] I. C. Mendes, M. A. Soares, R. G. dos Santos, C. Pinheiro, H. Beraldo, Eur. J. Med. Chem., 44, 1870 (2009)

[25] A. Y. Lukmantara, D. S. Kalinowski, N. Kumar, D. R. Richardson, Bioorg. Med. Chem. Lett., 23, 967 (2013)

[26] R. Pingaew, S. Prachayasittikul, S. Ruchirawat, V. Prachayasittikul, Med. Chem. Res., 22, 267 (2013)

[27] B. M. Zeglis, V. Divilov, J. S. Lewis, J. Med. Chem., 54, 2391 (2011)

[28] M. D. Hall, K. R. Brimacombe, M. S. Varonka, K. M. Pluchino, J. K. Monda, J. Li, M. J. Walsh, M. B. Boxer, T. H. Warren, H. M. Fales, M. M. Gottesman, J. Med. Chem., 54, 5878 (2011)

[29] D. X. West, J. K. Swearingen, J. V.-Martinez, S. H.-Ortega, A. K. El-Sawaf, F. van Meurs, Polyhedron, 18, 2919 (1999)

[30] P. Tarasconi, S. Capacchi, G. Pelosi, M. Cornia, R. Albertini, A. Bonati, Bioorg. Med. Chem., 8,157 (2000)

[31] S. E. Ghazy, M. A. Kabil, A. A. El-Asmy, Y. A. Sherief, Anal. Lett., 29,1215 (1996)

[32] J. R. Dilworth, A. H. Cowley, P. S. Donnelly, A. D. Gee, J. M. Heslop, J. Chem. Soc. Dalton Trans., 2404 (2004)

[33] A. A. Abou-Hussen, N. M. El-Metwally, E. M. Saad, A. A. El-Asmy, J. Coord. Chem., 58, 1735 (2005)

[34] T. S. Lobana, R. Sharma, G. Bawa, S. Khanna, Coord. Chem. Rev., 253, 977 (2009)

[35] N. K. Singh, S. B. Singh, A. Shrivastava, S. M. Singh, In. Proceedings of the Indian Academy of Sciences, Chem. Sci., 113, 257 (2001)

[36] Z. Afrasiabi, E. Sinn, J. Chen, Y. Ma, A. L. Rheingold, L. N. Zakharov, Inorg. Chim. Acta., 357, 271 (2004)

[37] M. Baldini, M. Belicchi, F. Bisceglle, P. P. D. Agllo, G. Pelos, S. Pinelli ,J. Inorg. Chem., 43, 7170 (2004)

[38] R. H. U. Borges, E. Paniago, H. Beraldo, J. Inorg. Biochem., 65, 2267 (1997)

[39] D. Denoyer, S. Masaldan, S. La Fontaine, M. A. Cater, Metallomics, 7, 1459 (2015)

[40] B. Lippert, Cisplatin: Chemistry and Bioc-hemistry of a Leading Anticancer Drug, Wiley Inter-science (1999)

[41] A. S. Abu-Surrah, M. Kettunen, Curr. Med. Chem., 13, 1337 (2006)

[42] C. S. Allardyce, P. J. Dyson, Platinum Met. Rev., 45, 62 (2001)

[43] I. Ott, R. Gust, Arch. Pharm. Chem. Life, 340, 117 (2007)

[44] M. A. Jakupec, M. Galanski, V. B. Arion, C. G. Hartinger, B. K. Keppler, Dalton Trans., 183 (2008)

[45] P. Yang, M. Guo, Coord. Chem. Rev., 185, 189 (1999)

[46] S. K. Hadjikakou, N. Hadjiliadis, Coord. Chem. Rev., 253,235 (2009)

[47] K. Strohfeldt, M. Tacke, Chem. Soc. Rev., 37, 1174 (2008)

[48] P. M. Abeysinghe, M. M. Harding, Dalton Trans., 3474 (2007)

[49] R. Gust, D. Posselt, K. Sommer, J. Med. Chem., 47,5837 (2004)

[50] C. G. Hartinger, P. J. Dyson, Chem. Soc. Rev., 38,391 (2009)

[51] M. B. Ferrari, F. Bisceglіea, G. G. Favaa, G. Pelosia, P. Tarasconi, R. Albertini, S. Pinelli, J. Inorg. Biochem., 89,36 (2002)

[52] M. B. Ferrari, F. Bisceglіea, G. Pelosi, P. Tarasconia, R. Albertini, A. Bonati, P. Lunghi, S. Pinelli, J. Inorg. Biochem., 83,169 (2001)

[53] M. R. Arguelles, M. B. Ferrari , F. Bisce-gli, C. Pelizzi, G. Pelosi, S. Pinelli, M. Sassi, J. Inorg. Biochem., 98,313 (2004)

[54] M. B. Ferraria, F. Bisceglie, A. Buschini, S. Franzoni, G. Pelosi, S. Pinelli, P. Tarasconi, M. Tavone, J. Inorg. Biochem., 104,199 (2010)

[55] V. Jevtovic, G. Pelosi, S. Ianelli, R. Kovacevic, S. Kaisarevic, Acta. Chim. Slov., 57, 363 (2010)

[56] L. Zhou, Y. Liu, W. Zhang, P. Wei, C. Huang, J. Pei, Y. Yuan, L. Lai, J. Med. Chem., 49, 3440 (2006)

[57] R. Ghahremanzadeh, M. Sayyafi, S. Ahadi, A. Bazgir, J. Comb. Chem., 11, 393 (2009)

[58] S. K. Sridhar, S. N. Pandeya, J. P. Stables, A. Ramesh, Eur. J. Pharm. Sci., 16, 129 (2002)

[59] K. L. Vine, J. M. Locke, M. Ranson, S. G. Pyneb, J. B. Bremner Bioorg. Med. Chem., 15, 931 (2007)

[60] M. C. Rodriguez-Argiielles, A. Sanchez, M. B. Fermi, G. G. Fava, C. Pelizzi, G. Pelosi, R. Albertini, P. Lunghi, S. Pinelli, J. Inorg. Biochem., 73, 7 (1999)

[61] D. P. Saha, S. Padhye, E. Sinn, C. Newton, Indian J. Chem., 41A, 279 (2002)

[62] S. Yolles, R. M. Roat, M. F. Sartori, C. L. Washburre, ACS symp.Ser., 186, 233 (1982)

[63] K. C. Agarwal, A. C. Sartorelli. Prog. Med. Chem., 15, 321 (1978)

[64] H. L. Elford, M. Freese, E. Passamani, H. P. Morris, J. Biol. Chem., 245, 5228 (1970)

[65] Y. Ma, T. Mizuno, H. Ito, Agric. Biol. Chem., 55, 2701 (1991)

[66] S. S. Coughlin, D. U. Ekwueme, Cancer Epidemiol., 33, 315 (2009)

[67] D. N. Louis, H. Ohgaki, O. D. Wiestler, W. K. Cavenee, P. C. Burger, A. Jouvet, B. W. Scheithauer, P. Kleihues, Acta Neuropathol., 114, 97 (2007)

[68] B. O. Colli, Carlotti, C. G., Jr J. Bras. Neurocir. 12, 36 (2001)

[69] H. Beraldo, D. Gambino, Mini-Rev. Med. Chem., 4, 31 (2004)

[70] A. E. Liberta, D.X. West, Biometals, 5, 121 (1992)

[71] U. Abram, K. Ortner, K. Sommer, J. Chem. Soc. Dalton Trans., 735 (1999)

[72] R. A. Finch, M. Liu, S. P. Grill, W. C. Rose, R. Loomis, K. M. Vasquez, Y. Cheng, A. C. Sartorelli, Biochem. Pharmacol., 59, 983 (2000)

[73] L. Gojo, M. L. Tidwell, J. Greer, N. Takebe, K. Seiter, M. F. Pochron, B. Johnson, M. Sznol, J. E. Karp, Leuk. Res., 31, 1165 (2007)

[74] J. Shao, B. Zhou, B. Chu, Y. Yen, Cancer Drug Targets, 6, 409 (2006)

[75] J. A. Segura, I. G. Barbro, J. Marquez, Immunol. lett., 74, 111 (2000)

[76] D. Fechhio, P. Siriosis, M. Russo, S. Janear, Immunol. lett., 14, 125 (1990)

[77] M. Shimizu, C. Azuma, T. Tainguchi, T. Muruyama, J. Pharm. Sci., 96, 234 (2004)

[78] L. A. Saryan, K. Mailer, C. Krishnamurti, W. Antholine, D. H. Petering, Biochem. Pharmacol., 30, 1595 (1981)

[79] J. Kuncheria, S. Jayasree, K. K. Aravindakshan, G. Kuttan, Indian J. Pharm. Sci., 56, 37 (1994)