CHAPTER 1

THIOSEMICARBAZONES AND THEIR METAL COMPLEXES

1.1. Introduction

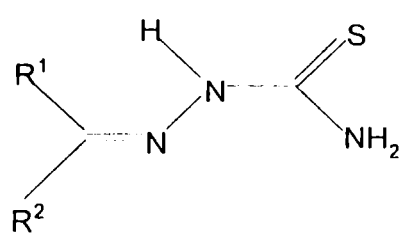
Thiosemicarbazones and their metal complexes exhibit a wide range of biological applications. Owing to the interest they generate in pharmacology, thiosemicarbazones and their metal complexes have been extensively studied. The biological activity of thiosemicarbazones results from their ability to form chelates with metal ions. Thiosemicarbazones have also been used in the analysis of metals [1, 2].

According to the IUPAC recommendations for the nomenclature of organic compounds [3], the derivatives of semicarbazides of the type, RCH=N-NH-CX-NH_2 and R^1R^2C=N-NH-CX-NH_2, which are usually obtained by condensation of semicarbazide or thiosemicarbazide with suitable aldehydes and ketones, may be named by adding the class name 'semicarbazone' (X=O) or 'thiosemicarbazone' (X=S) after the name of the condensed aldehyde RCHO or ketone R^1R^2C=O. It is also usual to include in this class derivatives with substituents [4] on the amide or thioamide nitrogen, R^1R^2C=N-NH-CX-NR^3R^4, on the X atom, R^1R^2C=N-N=C(XR^3)-NH_2 or on the hydrazinic nitrogen, R^1R^2C=N-NR^3-CX-NH_2

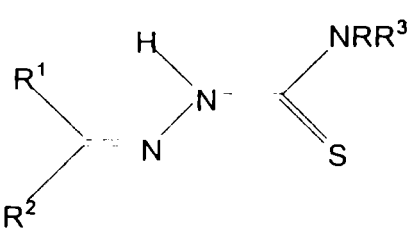
These classes of compounds usually react with metallic cations giving complexes in which the semicarbazones and thiosemicarbazones behave as chelate ligands. Research on the coordination chemistry [5], analytical applications [6], and biological activities [7] of these complexes has been increasing steadily for many years.

1.2. Bonding and stereochemistry

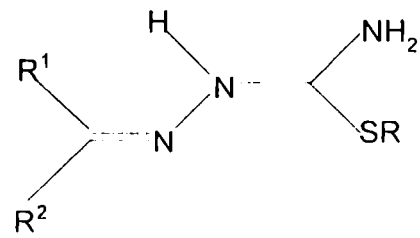
A review of the thiosemicarbazone structures included in the Cambridge structural database (CSD) [8] shows that in the free unsubstituted thiosemicarbazones in the solid state, the C= N- NH- CX- NH_2 backbone is usually almost planar, with the sulfur atom trans to the azomethine nitrogen {configuration *E*; Scheme 1. 1(a)}. Although there are several electronic and steric factors that may contribute to the adoption of this arrangement, the most important is probably that the trans arrangement places the amine and azomethine nitrogen atoms in relative positions suitable for intramolecular hydrogen bonding [9]. In fact thiosemicarbazones in which the amine group is fully substituted crystallizes with the sulfur atom cis to the azomethine nitrogen {*Z* configuration; Scheme 1. 1(b)}. Substitution of the hydrazinic hydrogen seems not to change the usual *E* configuration of the unsubstituted thiosemicarbazone, however, *S*-substituted thiosemicarbazones adopt the *Z* form {(Scheme 1.1(c)}.



Scheme 1.1(a)



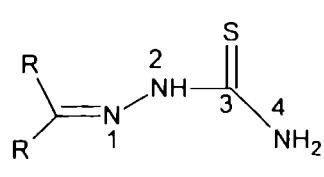
Scheme 1.1(b)



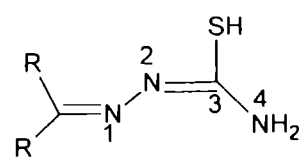
Scheme 1.1(c)

When forming complexes, while in *E* configuration, bonding occurs *via* the sulfur atom as a monodentate ligand. However, in most complexes [10], the thiosemicarbazones coordinate as bidentate ligands *via* the azomethine nitrogen and the thione/thiol sulfur. When an additional coordinating functionality is present in the proximity of the donating centres (eg. 2- heterocyclic thiosemicarbazones), the ligands bond in a tridentate manner. This can be done by either the neutral molecule [11,12] or by the monobasic anion upon the loss of the hydrogen from ^{2}N. There are instances reported, where the heterocyclic atom and the azomethine nitrogen are involved in bidentate coordination [13].

Allocation of the charge distribution is complicated in thiosemicarbazones due to the existence of thione (A) and thiol (B) tautomers. Although the thione form predominates in the solid state, solutions of thiosemicarbazone show a mixture of both tautomers (Scheme 1.2). The IUPAC system for numbering the thiosemicarbazones is given in Scheme 1.2.



(A)



(B)

Scheme 1.2

There were reports of metal complexes containing thiosemicarbazones in the uncharged thione form and also complexes in which thiosemicarbazone moiety is closer to that of the thiol form in which the ^{2}N- hydrogen is lost. There were also reports of complexes containing both the neutral and the anionic forms of the ligand bonded to the same metal [14, 15].

1.3. Biological activity of thiosemicarbazones and their metal complexes

Thiosemicarbazones have been extensively studied as they show a variety of biological applications: antitumoral, antiviral, antibacterial, antimalarial and antifungal [16, 17]. Heterocyclic thiosemicarbazones exert their therapeutic properties in mammalian cells by inhibiting ribonucleotide reductase, a key enzyme in the synthesis of DNA precursors [18, 19]. The interaction of thiosemicarbazones with various biochemical systems has been studied to understand the potential antitumor behavior of this agent *in vivo*. For example, the 1:1 Cu(II) complex of 2-formylpyridine thiosemicarbazone has been shown to inhibit the RNA-dependent DNA polymerases [20].

Thiosemicarbazones react as bidentate ligands by bonding through the sulfur and azomethine nitrogen atoms. The additional coordination should take place when another coordinating group is present in the vicinity of the thiosemicarbazone moiety, behaving as a tridentate ligand.

Most thiosemicarbazones and their complexes are highly hydrophobic and their low solubility in water induces experimental limitations in biological studies [21]. The introduction of a hydrophilic group such as $-NH_2$ or -OH in heterocyclic ring systems should permit a soluble acid or sodium salt to be obtained with the goal of increasing the solubility in water [22]. Therefore, since the medicinal activity of thiosemicarbazones, may in part be related to their chelating ability, the

metal complexes are proved to be more biologically active than the thiosemicarbazones. A large number of these complexes involve biologically essential metal ions such as copper, iron and zinc.

It has been revealed that only certain substituted benzaldehyde and heterocyclic thiosemicarbazones possess antitubercular activity [23, 24]. In addition to their antibacterial activities, thiosemicarbazones inhibit growth of both fungi and protozoa. Wiles and Supunchuk [25] reported that heterocyclic derivatives of thiosemicarbazide are active against the growth of *Aspergillus niger* and *Chaetominum globsum* in very low concentrations. Thiosemicarbazones have been tested against a variety of viral infections including herpes virus, adnovirus, polio virus, and RNA tumor virus with mixed results.

An extensive series of thiosemicarbazones obtained from 2-acetylpyridine were tested by Klayman et al [26, 27] for antimalarial activity against *Plasmodium berghei*. The molecular features essential for this activity were found to be a 2-pyridylethylidene moiety, the presence of the thiocarbonyl sulfur and certain cyclic substituents at the terminal ^4N-atom. For example, the 2-acetylpyridine ^4N-dialkylthiosemicarbazones were the most active against *Neisseria gonorrhoeae*.

Cancerostatic properties have been reported for Au(III) complexes with bi- and tridentate thiosemicarbazones as well as with chelating phosphine thiol ligands [28]. A systematic study of the formyl thiosemicarbazones of different heterocyclic ring systems carried out by French and Blanz revealed that the thiosemicarbazone side chain must be adjacent to the heterocyclic nitrogen and a conjugated NNS tridentate ligand system is essential for anticancer activity [29]. As a result, pyridine and isoquinoline ring systems have been most extensively investigated for structure-activity relationships among the antitumor compounds of this series.

1.4. Objective and scope of the present work

Thiosemicarbazones and their metal complexes present a wide range of biological applications [5, 30]. Thiosemicarbazones derived from 2-formyl and 2-acetylpyridine have been extensively investigated by several authors [31-39]. There are also some reports of thiosemicarbazones derived from 2-benzoylpyridine [40-43]. There is another report in the literature on the 4-benzoylpyridine derived analogues [44].

Thiosemicarbazones having a third potential bonding site are found to possess considerable biological activity [45, 46]. In some cases changing the point of attachment of the thiosemicarbazones chain from the 2-position in the pyridine ring to the 3- or 4- position causes a decrease in activity, presumably due to a lower coordination ability but the presence of a bulky group at the terminal nitrogen considerably increases the activity. Earlier works on ^{4}N-substituted thiosemicarbazones have concluded that, the presence of bulky groups at the ^{4}N position of the thiosemicarbazones moiety greatly enhances biological activity [47, 48, 49]. Consequent upon these findings, we have undertaken the work with the following objectives:

- To synthesise two ligands:

  ^{4}N-cyclohexyl-2-benzoylpyridine thiosemicarbazone III.[1] and

  ^{4}N-phenyl-2-benzoylpyridine thiosemicarbazone III.[2]; henceforth referred to as 2-benzoylpyridine N(4)-cyclohexylthiosemicarbazone and 2-benzoylpyridine N(4)- phenyl thiosemicarbazone respectively.

- To characterize the ligands using elemental analysis, IR, electronic, ^{1}H NMR, ^{13}C NMR, COSY and HMQC spectral studies.
- To synthesise Cu(II), Fe(III), Mn(II), Ni(II), Zn(II), Cd(II) and Hg(II) complexes and characterize these complexes using magnetic susceptibility

measurements, molar conductivity measurements, electronic, infrared, EPR and ^{1}H NMR spectral studies.

- To carry out single crystal X-ray diffraction studies of the ligand HL^1 and some copper(II) complexes.
- To study the antimicrobial activities of the two ligands and metal complexes.
- To find out the structure activity relationship based on the EPR parameters of the complexes.

1.5. Analytical methods

1.5.1. Estimation of carbon, hydrogen and nitrogen

The analyses of carbon, hydrogen and nitrogen were done on a Heracus elemental analyzer at Central Drug Research Institute, Lucknow.

1.5.2. Magnetic susceptibility measurements

The magnetic susceptibility measurements were carried out at Indian Institute of Technology, Roorkee, in the polycrystalline state at room temperature on a Par model 155 Vibrating Sample Magnetometer at 5 K Oersted field strength. Diamagnetic corrections were made using Pascal's constants.

1.5.3. Conductance measurements

The molar conductances of the complexes in dimethylformamide (10^{-3} M) at room temperature were measured on a Centuary CC-601 digital conductivity meter, at the Centre for research in Chemistry, Nirmala College, Muvattupuzha.

Kerala. A dip type conductivity cell with platinised platinum electrodes (cell constant 0.999 cm^{-1}) was used.

1.5.4. Electronic spectra

The diffuse reflectance spectra at room temperature in magnesium oxide diluents were recorded with Ocean Optics DRS Spectrophotometer. The Electronic spectra in solutions were recorded on a Shimadzu model UV-visible 160 A Spectrophotometer in 200-800 nm range.

1.5.5. Infrared spectra

The IR spectra were recorded on a Shimadzu DR 8001 series FT-IR instrument using KBr pellets, in 4000-400 cm^{-1} range at CDRI, Lucknow. The far IR spectra were recorded on a NICOLET MAGNA 550 FT-IR spectrometer using polyethylene pellets in the range 500- 50 cm^{-1} at RSIC, IIT, Bombay.

1.5.6. Nuclear magnetic resonance (NMR) spectra

The ^{1}H and ^{13}C NMR spectra were recorded in a Brucker DRX 500 instrument using $CDCl_3$ as the solvent and TMS as the internal reference at Sophisticated Instruments Facility, Indian Institute of Science, Bangalore. COSY homonuclear and HMQC heteronuclear spectra were also recorded with AMX 400 at the same centre.

1.5.7. Electron paramagnetic resonance (EPR) spectra

The EPR spectra were recorded on Varian E-112 X-band spectrometer operating with 100 KHz modulation frequency using tetracyanoethylene (TCNE) as a standard at RSIC, IIT, Bombay. The EPR spectra of the polycrystalline sample at 298 K and those of the solutions at 298 and 77 K were recorded in the X band; the g factors were quoted relative to the standard marker (g = 2.00277). The EPR spectra of the iron(III) complexes were recorded in a Bruker ESP300, X-band CW spectrometer operating at 9.52 GHZ equipped with a liquid nitrogen cryostat at the Physical chemistry Lab., ETH-Honggerderg, Zurich, Switzerland. The spectra were measured with modulation amplitude of 0.05 (0.01) mT and 100 kHz modulation frequency, and the field was calibrated by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) with a g value of 2.0036.

1.5.8. X-ray diffraction studies

Single crystal X- ray crystallographic analysis of the compounds were carried out using a Siemens SMART CCD area-detector diffractometer at School of Physics, Universiti Sains Malaysia, and at the Department of Inorganic and Physical Chemistry, Indian Institute of Science, Bangalore. The intensity data were collected by ω-scan mode for hkl. Empirical absorptions were employed by using ψ- scan technique. The structures were solved by direct methods and refined by least-square on F_0^2 using the SHELXTL software package [50]. The collected data were reduced using SAINT program [51] and the empirical absorption was carried out using the SADABS program. Graphics quality plots were made by using the packages ORTEP and PLATON [52].

1.5.9. Biological studies

Antimicrobial studies of the ligands and the complexes were done at the Department of Biotechnology, Cochin University of Science and Technology. Disc diffusion method was used for studying the antimicrobial property and determining the MIC (minimum inhibitory concentration) of the compounds. The ligands and the complexes were screened against two Gram positive and three Gram negative bacteria.

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