UNIVERSITI PUTRA MALAYSIA

STUDIES ON COORDINATION CHEMISTRY AND BIOACTMTY OF Cu(II), Cd(II) AND Zn(II) COMPLEXES CONTAINING SOME NITROGEN-SULPHUR DONOR LIGANDS

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May 2001

Chairman : Associate Professor Md. Tofazzal Hossain Tarafder, Ph.D.

Faculty : Science and Environmental Studies

Several Schiff bases of S-benzyldithiocarbazate (SBDTC) and S-methyldithiocarbazate (SMDTC) have been synthesised. Complexes of Cu(II), Cd(II) and Zn(II) with the pyridine-2-carboxaldehyde Schiff base of SBDTC were prepared. The compounds were characterised by elemental analyses and various physico-chemical techniques. The Schiff bases and the metal complexes were tested for cytotoxicity and antimicrobial and antioxidant activities. Cytotoxic screenings were carried out against T-lymphoblastic leukaemia cells (CEM-SS) and colon cancer cells (HT-29). The antimicrobial screenings were carried out against four bacteria and four fungi. The antioxidative assay was carried out using the ferric thiocyanate (FTC) method. The Cu(II) and Cd(II) complexes were four coordinate while the Zn(II) complex was six coordinate.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

KAJIAN KE ATAS KIMIA KOORDINASI DAN AKTIVITI BIOLOGI KOMPLEKS Cu(II), Cd(II) DAN Zn(II) YANG MENGANDUNGI LIGAN NITROGEN-SULFUR

Oleh

AZAHARI BIN KASBOLLAH

Mei 2001

Pengerusi : Profesor Madya Md. Tofazzal Hossain Tarafder, Ph.D.

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Beberapa bes Schiff dari S-benzilditiokarbazat (SBDTC) dan S-metilditiokarbazat (SMDTC) telah disintesis. Kompleks ion Cu(II), Cd(II) dan Zn(II) bersama bes Schiff piridina-2-karboksaldehid dengan SBDTC telah disediakan. Bahan-bahan yang disintesis telah dianalisis menerusi kaedah analisis unsur dan teknik fizik-kimia yang lain. Kesemua bahan yang disintesis telah diuji terhadap aktiviti sitotoksik, antimikrob dan antioksidan. Ujian sitotoksik dijalankan ke atas sel 'T-lymphoblastic leukemia' (CEM-SS) and sel kanser kolon (HT-29). Ujian antimikrob dijalankan ke atas empat bakteria dan empat kulat. Ujian antioksida dijalankan menggunakan kaedah ferric thiocyanate (FTC). Kompleks Cu(II) dan Cd(II) adalah berkoordinat empat manakala kompleks Zn(II) adalah berkoordinat enam.

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LIST OF ABBREVIATIONS

CEM-SS – T-lymphoblastic leukemic cell type

HT-29 – Colon cancer cell type

DMSO – Dimethylsulfoxide

CHNS Analysis – Carbon, Hydrogen, Nitrogen and Sulphur Analysis

IR Spectroscopy – Infra-red Spectroscopy

NMR Spectroscopy – Nuclear magnetic resonance Spectroscopy

UV/VIS – Ultra violet/Visible Spectroscopy

ROS – Reactive Oxygen Species

FTC – Ferric thiocyanate

SBDTC – S-benzyldithiocarbazate

SMDTC – S-methyldithiocarbazate

BHT – Butylated hydroxytoluene

ORTEP – Oak Ridge Thermal Ellipsoid Plot (from the Program for Crystal Structure Illustration)

NS – Bidentate Nitrogen-Sulphur Donor Ligand

NNS – Tridentate Nitrogen-Nitrogen-Sulphur Donor Ligand

ONS – Tridentate Oxygen-Nitrogen-Sulphur Donor Ligand

SNNS – Quadridentate Sulphur-Nitrogen-Nitrogen-Sulphur Donor Ligand

NNSS – Quadridentate Nitrogen-Nitrogen-Sulphur-Sulphur Donor Ligand

CHAPTER 1

INTRODUCTION

Coordination Chemistry

Coordination chemistry is a branch of chemistry which deals with the study of coordination compounds. The studies include the synthesis or preparation, bonding, structures and reactivities of coordination compounds. A coordination compound may be defined as a compound containing a central atom or ion to which are attached molecules or ions whose number usually exceeds the number corresponding to the oxidation number or valence of the central atom or ion.

Coordination compounds are compounds containing coordinate bonding while ligands are the compounds that have excess electrons and can form coordinate linkages upon interaction with metal centres. The ligands may be neutral molecules or they may be ions. Ligands are attached to the central atom by means of what are called coordinate bonds or coordinate covalent bonds. In an ordinary covalent bond each of the bonded atoms contributes one electron to the electron pair that forms the bond. In the coordinate bond, on the other hand, the coordinating atom or ligand, called the donor, donates a pair of electrons to the central atom, called the acceptor. The bond is often depicted by an arrow proceeding from the donor atom to the acceptor atom. Interaction between

metal ions and ligands results in the formation of complexes. The entire aggregate of central atom and ligands is sometimes called a complex.

Ligands may be unidentate, that is, they may possess only one coordinating atom. The ammonia molecule and the chloride ion are examples of unidentate ligands. Ligands may also be bidentate or chelate, that is, they may possess two coordinating atoms. Polydentate or multidentate ligands containing more than two coordinating atoms are also possible. Donor atoms are usually nonmetals, the most common being nitrogen, oxygen and sulfur.

Nitrogen-Sulphur Donor Ligands

Coordination compounds have always been a challenge to inorganic chemists. In the early days of chemistry they seemed unusual (hence the name "complex" ions) and seemed to defy the usual rules of valence. Today, scientists deal with a large body of inorganic research and one of the very active fields is the study of complexes containing nitrogen-sulphur donor ligands.

The study of nitrogen-sulphur donor ligands continues to be of great interest to researchers. Dithiocarbazate, $NH_2NHCS_2^-$, and its substituted derivatives have been synthesised and investigated over the past few decades [1-37]. Dithiocarbazic acid and the Schiff bases derived from its S-alkyl and S-benzyl esters form an interesting series of ligands and metal complexes. Researchers in this area have been continuing the

syntheses of new nitrogen-sulphur donor ligands through Schiff base condensation with aldehydes and ketones. The properties of these ligands can be greatly modified through the introduction of organic substituents. The number of ligands synthesised continues to increase because of the intriguing observation that different ligands show different biological properties, although they may differ only slightly in their molecular structures [1-6, 8, 9, 24-29]. However, no pattern has emerged to enable the activity to be predicted on the basis of structure or substituents.

Transition metal complexes of these ligands are also widely studied because of their potential for therapeutic use [1-3, 8, 9, 21-23, 29, 31, 32, 36]. For instance, antimicrobial tests of the Schiff base of salicylaldehyde with S-benzyldithiocarbazate and the five metal complexes of Cu^{II}, Ni^{II}, U^{VI}, Zn^{II} and Sb^{III} show that they are strongly active against bacteria. Ni^{II} and Sb^{III} complexes were the most effective against *pseudomonas aeruginosa* (gram negative), while the Cu^{II} complex proved to be best against *bacillus cereus* (gram positive bacteria) [36]. The bioactivity of the ligands and their metal complexes, such as cytotoxicity, antimicrobial and antioxidant activities, has not been widely studied. The mode of the interaction of these compounds with cancer cells and microbes are yet to be studied. In addition, there has been no previous report on the bioactivity of the starting ligands, S-benzyldithiocarbazate (SBDTC) and S-methyldithiocarbazate (SMDTC) although these compounds were first synthesised decades ago.

Properties Associated with Sulphur and Nitrogen as Donor Ligands

Ligands with sulphur as donor atoms have the following characteristics [11]:

- Those with sulphur bind more strongly to (b) class metals than do oxygen donors [Class (a) metals ions are small, not very easily polarised and have a greater affinity for F^- than for I^-. Class (b) metal ions are essentially the opposite in character].
- The polarizability of sulphur donors and the number of lone pairs decrease in the order $S^{2-} > RS^- > R_2S$. Consequently, thiolo ligands are more polarizable but not as effective d_π electron acceptors as thioethers.
- Normally, the permanent dipole moment and the coordinating ability decrease in the order: $H_2O > ROH > R_2O$. However, the reverse order holds for sulphur, $H_2S < RSH < R_2S$.
- The strength of bonding to a metal (considering both electrostatic and covalent models) is in the following order: $RO^- > RS^-$ and $R_2O > R_2S$. However, sulphur has vacant d orbitals that can be used for d_π - d_π bonding such as can occur with the later transition metals and with early transition metals in unusually low oxidation states.

The properties of complexes of sulphur donor ligands apply also to the complexes of nitrogen-sulphur chelating agents. However, there are additional characteristics in the case of the latter due to the presence of nitrogen in these complexes. In general, the presence of nitrogen tends to lower the solubility of complexes in non-polar solvents. This causes the complexes of nitrogen-sulphur ligands to be either sparingly soluble or

completely insoluble in non-polar solvents. Nitrogen-sulphur ligands seem to cause a smaller reduction in the interelectronic repulsion energy than do sulphur-sulphur ligands. It is assumed that this is due to the lower position of nitrogen in the nephelauxetic series compared to sulphur [11].

Biological Activity

Cytotoxicity

It is believed that some cancers are actually caused by viruses [11]. This means that an anticancer drug may actually be an antiviral agent. The protein and nucleic acid portions of viruses are effective chelating agents. Therefore, the aim of metallotherapeutic designers is to alter the virus by metal chelation so that the viral activity will be lessened. Several characteristics are also required of metal chelates in order to be effective antiviral agents.

These metal chelates are to be moderately stable, since the metal ion must not be so weakly bound as to be free enough to be complexed by non-viral chelating agents such as amino acids and enzymes present in the body. The chelating agent should also be able to be displaced by the virus. The metal ion has to be selective in regard to benign and malignant viruses. Cancer growth depends very much on the reproduction of malignant cells having a kinetic advantage over the body's defence mechanism. Due to this, it is evident that kinetic consideration is of greater importance compared to the

thermodynamic stability of the metal chelates. Therefore, the metal complex has to be labile enough to outpace cancer growth [11].

The following criteria are important in determining whether a metal complex will have carcinostatic activity:

i. The complex should be reasonably labile.
ii. The metal chelate should have reasonably high thermodynamic stability.
iii. The metal should be a (b) class metal.
iv. The complex should be soluble in biological media.

Ligands with sulphur donors are likely to be the most effective, since they usually confer lipid solubility on the metal complex and they form stable complexes with (b) class and borderline metals [11].

Antibacterial Activity

Antibacterial agents are categorised as narrow-, broad-, or extended-spectrum agents. Narrow-spectrum agents (e.g. penicillin G) affect primarily gram-positive bacteria. Broad-spectrum antibiotics, such as tetracyclines and chloramphenicol, affect both gram-positive and some gram-negative bacteria. Extended-spectrum agents usually affect gram-negative bacteria with a chemical modification.

Whether an antimicrobial agent affects a microorganism depends on several factors. The ability of medicinal chemists to find new bacterial targets for attack is

improving. Rather than block the functions that bacteria perform in a petri dish, chemists are learning to hit at what bacteria need to do when fighting to survive and thrive in a human host. Antibacterials in use today attack microorganisms by interfering with biosynthesis of proteins, DNA or cell wall material [38]. The drug must be delivered to a sensitive site in the cell, such as an enzyme that is involved in the synthesis of a protein. Bacteriostatic drugs inhibit the growth and multiplication of bacteria but do not kill them. They act by interfering with enzyme systems essential to normal metabolic and growth patterns of bacteria. Bactericidal drugs destroy the bacteria.

It has been observed by Ali *et al.* [3] that the greater activity of metal chelates compared to free ligands may be attributed to the enhanced conjugation of the deprotonated ligand.

Antifungal Activity

Fungi appear in two morphological forms:

- A single cell that is round or oval (yeast)
- A filamentous form (mold)

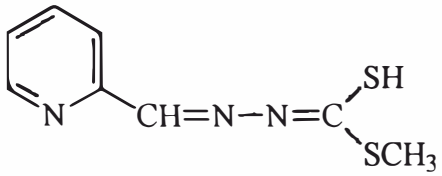
Fungi differ from bacteria in several ways, including the chemical composition of the cell wall and cell membrane. Bacteria have no apparent nucleus membrane. The nucleus occupies the cytoplasm densely [39]. Unlike bacteria, fungi have a nucleus surrounded by a membrane, an endoplasmic reticulum and mitochondria. These

differences between bacteria and fungi are reflected in the use of different chemotherapeutic agents [40].

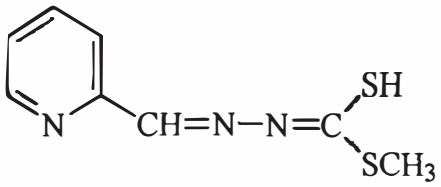
Results of previous antifungal screening experiments by Ali *et al.* [3] indicated that metal complexes were more active against *A. alternata*, *F. moniliforme* and *D. orezae* than their free ligands. Copper(II) complexes displayed better antifungal properties than nickel(II) complexes.

Carcinostatic Activities of Some Sulphur-Nitrogen Ligands and Their Complexes

Metal complexes of ligands derived from dithiocarbazic acid have been reported to show carcinostatic activity [11]. The complexes $Pd(H_2NN{=}CSSMe)_2$, $Cr(C_5H_4NCH{=}NNMeCSSMe)Cl_3$ and $Cu(C_5H_4NCH{=}NN{=}CSSMe)Cl$ show antitumour activity. The ligand (**I**) and the complexes $Pd(Me_2C{=}NN{=}CSSMe)_2$ and $CuCl(o\text{-}C_5H_4N\text{-}CH{=}NNMeCSSMe)$ have shown confirmed cytostatic activity in the 9KB test system – which is an *in vitro* test system giving better indication of the carcinostatic activity of a compound over a wider range of cancers than other test systems [11].



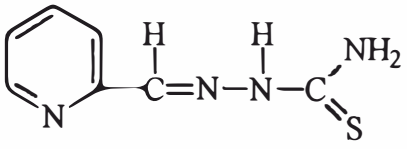
(**I**)



(**I**)

The nickel(II) complex of the 2-acetylpyridine Schiff base of S-methyldithiocarbazate, [Ni(NNS)Cl] has been shown to exhibit marked activity in the P388 lymphocytic leukaemia test system. The analogous 2-acetylpyridine Schiff bases of N-substituted thiosemicarbazides and their nickel(II) and copper(II) complexes have been extensively investigated because of their bioactivies [9].

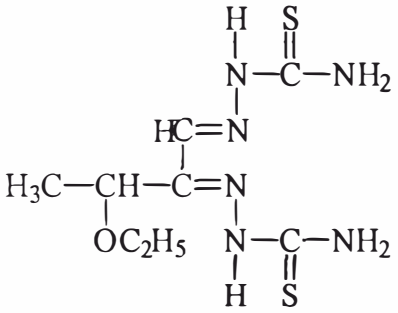
It was also reported that pyridine-2-carboxaldehyde thiosemicarbazone (**II**) displays carcinostatic activity in the lymphoid leukaemia-1210 test [11].



(**II**)

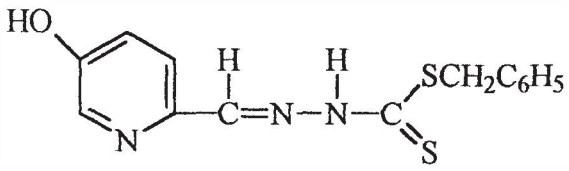
Kethoxal bis(thiosemicarbazone) (KTS) (**III**) was reported to show carcinostatic action [11]. The cytotoxicity of KTS is enhanced by the presence of copper and zinc

ions and it has been shown that the copper(II) chelate of KTS is involved in the cytotoxic action of KTS.



(**III**)

The most active anti-leukaemia reagent among all the thiosemicarbazide derivatives tested to date is 5-hydroxypyridine-2-carboxaldehyde thiosemicarbazone (**IV**) [11].



(**IV**)

Antioxidants

Antioxidants are substances that when present in foods or in the body at low concentrations compared with that of an oxidizable substrate markedly delay or prevent the oxidation of that substrate. Antioxidants have been of interest to biochemists and health professionals because they may help the body protect itself against damage caused by reactive oxygen species and degenerative diseases. Antioxidants may act by decreasing oxygen concentration, intercepting singlet oxygen, preventing chain initiation by scavenging initial radicals such as hydroxyl radicals, binding metal ion catalysts, decomposing primary products to non-radical compounds, and chain-breaking to prevent continued hydrogen abstraction from substrates. In the human body, excess production of oxygen radical species, particularly hydroxyl radicals, can affect lipid cell membranes to produce lipid peroxides and reactive oxygen species (ROS) which are linked to a variety of diseases (Figure 1.1) [41].

Some reactive oxygen species (ROS) are generated by "accidents of chemistry". For example, superoxide radical ($O_2^{\bullet}$) and hydrogen peroxide (H_2O_2) can arise *via* the direct oxidation of several biomolecules by O_2. In addition, humans are exposed to radiation from the environment, both natural (e.g., radon gas, cosmic radiation) and from man-made sources. Low-wavelength electromagnetic radiation (e.g. gamma rays) can split water in the body to generate the viciously reactive hydroxyl radicals ($OH^{\bullet}$) [41].