UNIVERSITI PUTRA MALAYSIA

STUDIES ON COORDINATION CHEMISTRY AND BIOACTIVITY OF COMPLEXES OF Ni(II) AND Cu(II) CONTAINING SOME NITROGEN-SULPHUR DONOR LIGANDS

SARAVANAN s/o NAGALINGAM

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By

SARAVANAN s/o NAGALINGAM

Thesis Submitted in Fulfilment of the Requirement for the
Degree of Master of Science in the Faculty of Science and Environmental Studies
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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of requirement for the degree of Master of Science.

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February 2001

Chairman: Associate Professor Dr. Md. Tofazzal Hossain Tarafder

Faculty: Science and Environmental Studies

Several Schiff bases of S-benzyldithiocarbazate (SBDTC) and S-methyldithiocarbazate (SMDTC) have been synthesised. Complexes of Ni(II) and Cu(II) were prepared. These compounds were characterised by elemental analyses and various physico-chemical techniques. The Schiff bases and their metal complexes were tested to evaluate their cytotoxic, antimicrobial and antioxidant activities. Cytotoxic screening was carried out against the T-lymphoblastic leukemic cells (CEM-SS) and colon cancer cells (HT-29). Antimicrobial screening was carried out against four bacteria and four fungi. The nickel(II) complexes are four- and five- coordinated while the copper(II) complex is five-coordinated.

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

KAJIAN KE ATAS KIMIA KOORDINATAN DAN AKTIVITI BIOLOGI KOMPLEKS Ni(II) DAN Cu(II) YANG MENGANDUNGI LIGAN NITROGEN-SULFUR

Oleh

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Februari 2001

Pengerusi: Profesor Madya Dr. Md. Tofazzal Hossain Tarafder

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Beberapa bes Schiff dari S-benzyldithiocarbazate (SBDTC) dan S-methyldithiocarbazate (SMDTC) telah disintesis. Kompleks logam Ni(II) dan Cu(II) disediakan. Sebatian tersebut dicirikan melalui analisis unsur dan beberapa teknik fizik-kimia. Bes Schiff serta kompleks logamnya itu telah diuji untuk menentukan aktiviti sitotoksik, antimikrob and antioksidan. Ujian sitotoksik dijalankan ke atas sel 'T-lymphoblastic leukemia' (CEM-SS) and sel kanser kolon (HT-29). Ujian antimikrob dijalankan ke atas empat jenis bakteria dan empat jenis kulat. Kompleks nikel(II) didapati berkordinat empat dan lima manakala kompleks kuprum(II) berkordinat lima.

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I certify that an Examination Committee met on 1st February 2001 to conduct the final examination of Saravanan s/o Nagalingam on his Master of Science thesis entitled "Studies on Coordination Chemistry and Bioactivity of Complexes of Ni(II) and Cu(II) Containing Some Nitrogen-Sulphur Donor Ligands" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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Date 9/2/2001

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EQUATION

Equation

1 $$\Lambda_m = \frac{(10^3 \times \text{cell constant})(\text{conductance of solution} - \text{conductance of solvent}) \times 10^{-6}}{\text{concentration}}$$

LIST OF ABBREVIATIONS

CEM-SS – T-lymphoblastic leukemic cell type

HT-29 – Colon cancer cell type

DMSO – Dimethylsulfoxide

CHNS Analysis – Carbon, Hydrogen, Nitrogen, Sulphur Analysis

IR Spectroscopy – Infra-red Spectroscopy

NMR Spectroscopy – Nuclear magnetic resonance Spectroscopy

UV/Vis Spectroscopy – 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

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

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

CHAPTER 1

INTRODUCTION

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 [1-36]. 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].

Transition metal complexes of these ligands are also widely studied because of their potential for therapeutic use [1-3, 8, 9, 15, 21-23, 31-32, 36]. For example, the Schiff base of 2-benzoylpyridine with S-methyldithiocarbazate (HBP-SMe) inhibits the growth of bacteria *E.coli* and *S.aureus* to some extent while that with S-benzyldithiocarbazate (HBP-SBz) shows no effect at all on the two mentioned bacteria [21]. To date no pattern has emerged to enable the activity to be predicted on the basis of structure or substituents. The bioactivities of the ligands and the metal complexes

such as cytotoxicity and antimicrobial activities have not yet been widely studied. The mode of interaction of these compounds with the cancer cells and microbes are yet to be investigated. 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:

I. 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 opposite in character].

II. The polarizabilities 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 which implies that thiolo ligands can coordinate to metal ions in a negatively charged manner.

III. 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, $H_2S < RSH < R_2S$.

IV. The strength of bonding to a metal (considering both electrostatic models 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 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 required of metal chelates in order to be an effective antiviral agent. 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. Since cancer growth depends very much on the reproduction of malignant cells having a kinetic advantage over the body's defence mechanism, 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. For this reason iridium complexes are unlikely to be effective.
ii. The metal chelate should have reasonably high thermodynamic stability.
iii. The metal should be a (b) class metal.
iv. 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 class (b) 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. An extended-spectrum antibiotic is one that, as a result of chemical modification, affects additional types of bacteria, usually gram-negative bacteria.

Whether an antimicrobial agent affects a microorganism depends on several factors. Medicinal chemists are getting better at seeking new bacterial targets for attack. 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 [37]. 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 or enzyme. 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 will destroy the bacteria [38]. An observation carried by Ali *et al.* [3] reveals that the greater activity of the metal chelates compared to that of the free ligand may be attributed to the enhanced conjugation of the deprotonated ligand.

Antifungal Activity

Fungi appear in two morphological forms:

I. A single cell that is round or oval (yeast)

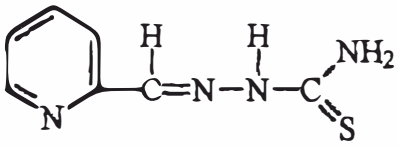
II. A filamentous form (mold)

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 material occupies the cytoplasm densely [39]. Unlike bacteria, fungi have a nucleus surrounded by a membrane, an endoplasmic reticulum and mitochondria. These differences between the bacteria and fungi are reflected in the use of different chemotherapeutic agents. Both antibiotics and chemical agents are used in the chemotherapy of fungal disease [38].

Results of previous antifungal screening experiments by Ali *et al.* [3] indicate that metal complexes show higher antifungal activity against *A. alternata*, *F. moniliforme* and *D. orezae* than the free ligands. Copper(II) complexes display better antifungal properties than nickel(II) complexes. *A. alternata* showed the highest susceptibility compared to the other two. The mixed ligand complexes were found to have antifungal activities comparable with those of mono ligand metal complexes.

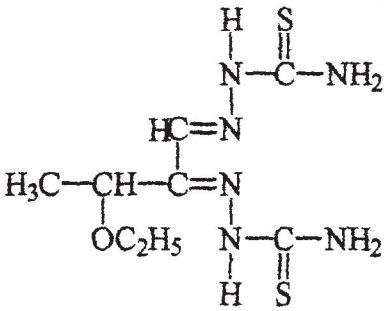
Carcinostatic Activities of Some Sulphur-Nitrogen Ligands and Their Complexes

In 1956, it was reported that pyridine-2-carboxaldehyde thiosemicarbazone (**I**) displays carcinostatic (anti-cancer) activity in the lymphoid leukaemia-1210 test [11].



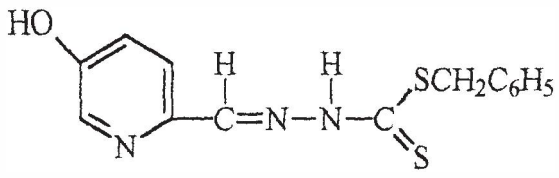
(**I**)

Kethoxal bis(thiosemicarbazone) (KTS) (**II**) was reported to show carcinostatic action against leukemic cells [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.



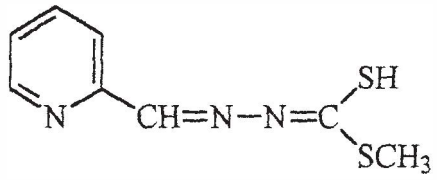
(**II**)

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



(III)

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, S-methyl-N-(2-pyridyl)methylenedithiocarbazate (**IV**), and the complexes $Pd(Me_2C{=}NN{=}CSSMe)_2$ and $CuCl(o\text{-}C_5H_4N\text{-}CH{=}NNMeCSSMe)$ have shown confirmed cytotoxic activity in the 9KB test system [11].



(IV)

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].

Antioxidant Activity

Antioxidants are substances that when present in foods or in the body at low concentrations compared to 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 first-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 effect lipid cell membranes to produce lipid peroxides and reactive oxygen species (ROS) which are linked to a variety of diseases (Figure 1.1) [40].

Some ROS are generated by "accidents of chemistry". For example, superoxide radical ($O_2^{\cdot-}$) 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 ($OH^{\bullet}$) [40].

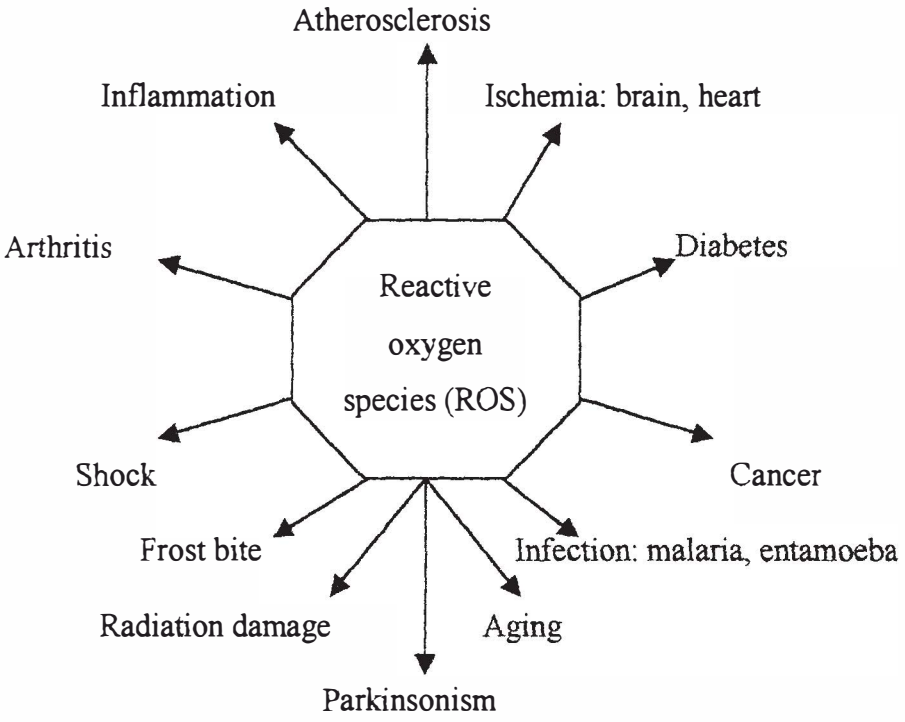


Figure 1.1: Diseases promoted by ROS [40]

Antioxidants can be divided into two classes: chain breaking (primary) antioxidants which can add or donate H or e^{-} to chain carrying radicals and preventive (secondary) antioxidants which decompose ROOH, bind metals, scavenge oxygen or capture UV-radiation. Some common primary antioxidants used are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), tertiary butylhydroquinine (TBHQ) and d1-Alpha-tocopherol (Vitamin E). Some secondary antioxidants are ethylenediaminetetraacetic acid (EDTA), ascorbic acid and isopropyl citrate (IPC) [41].