UNIVERSITI PUTRA MALAYSIA

SYNTHESIS, CHARACTERISATION & BIOLOGICAL ACTIVITIES OF MIXED-LIGAND COPPER(II) COMPLEXES CONTAINING SACCHARIN

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By

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

SYNTHESIS, CHARACTERISATION & BIOLOGICAL ACTIVITIES OF MIXED-LIGAND COPPER(II) COMPLEXES CONTAINING SACCHARIN AS ONE OF THE LIGANDS

By

THAHIRA BEGUM

February 2005

Chairman: Professor Karen A.Crouse, PhD

Faculty: Science

New mixed-ligand copper(II) saccharinate complexes of HNNS Schiff bases of S-methyldithiocarbazate, S-benzyldithiocarbazate, S-2-picolyldithiocarbazate and S-4-picolyldithiocarbazate were synthesized by reacting $[Cu(sac)_2(H_2O)_4]\cdot 2H_2O$ with the appropriate Schiff bases in water-ethanol-methanol mixtures. These complexes were characterized by elemental analysis, conductance, magnetic susceptibility, IR and electronic spectroscopic measurements. Magnetic and spectral results for the complexes support either a four or five-coordinate geometry in which the Schiff bases coordinate as NNS tridentate ligands and the saccharinate anion coordinates as a unidentate N-donor ligand. X-ray crystallographic structural analysis of the copper(II)saccharinate complex of S-methyl-β-N-(6-methylpyrid-2-yl)methylenedithiocarbazate shows that the complex has a distorted square-pyramidal structure in which the Schiff base is coordinated to the copper ion as a tridentate NNS chelating agent *via* the pyridine nitrogen atom, the azomethine nitrogen atom and the thiolate sulphur atom. The fourth and fifth coordination positions of the five-coordinate Cu(II) ion are occupied by the imino

nitrogen of the saccharinate anion and oxygen atom of the aqua ligand. X-ray crystallographic structural analysis of the copper(II)saccharinate complex of S-benzyl-β-N-(2-acetylpyridyl)methylenedithiocarbazate shows that this complex has a distorted square-planar structure in which the Schiff base is also coordinated to the copper ion as a tridentate NNS chelating agent with the fourth coordination position of the four-coordinate Cu(II) ion being occupied by the imino nitrogen of the saccharinate anion. The complexes have been evaluated for their biological activities against seven pathogenic microbials and three cancer cell lines, HL-60 (Human myeloid leukemic cells), MCF-7 (Human breast carcinoma cells with positive estrogen receptor) and Caov-3 (Human ovarian adenocarcinoma cancer cells). Most of the complexes exhibit marked cytotoxicity against the cell lines and display moderate activity against pathogenic bacteria and fungi.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan Ijazah Master Sains

SINTESIS, PENCIRIAN DAN AKTIVITI BIOLOGI BAGI KOMPLEKS KUPRUM(II) BERLIGAN CAMPURAN YANG MENGANDUNGI SAKARIN SEBAGAI SALAH SATU LIGAN

Oleh:

THAHIRA BEGUM

Februari 2005

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Kompleks kuprum(II) sakarinat baru yang mengandungi bes Schiff S-metilditiokarbazat, S-benzilditiokarbazat, S-2-pikolilditiokarbazat serta S-4-pikolilditiokarbazat telah disintesiskan melalui tindak balas diantara $[Cu(sac)_2(H_2O)_4]\cdot 2H_2O$ dengan bes Schiff yang sesuai dalam pelarut campuran air-etanol-metanol. Kompleks ini telah dicirikan melalui analisis unsur dan konduktiviti, kerentanan magnetik, pengukuran spektroskopi elektronik dan IR. Kebanyakan komplex didapati berkoordinatan empat atau lima. Ini dibuktikan melalui nilai kerentanan magnetik dan spektra yang diperolehi. Bes Schiff berkoordinat sebagai ligan tridentat NNS dan ion sakarin berkoordinat sebagai ligan N-penderma unidentat. Analisis struktur hablur sinar X menunjukkan bahawa kompleks kuprum(II) sakarinat bes Schiff S-metil-β-N-(6-metilpirid-2-il) berstruktur piramid segiempat terherot di mana bes Schiff terkoordinat kepada ion kuprum sebagai agen kelat tridentat NNS melalui atom nitrogen piridin, atom nitrogen azometin dan atom sulfur

tiolat. Ligan yang keempat ialah ion sakarinat yang terkoordinat melalui nitrogen imino. Manakala kompleks yang terkoordinat lima, ligan yang kelima ialah air. Analisis struktur hablur sinar X bagi kompleks kuprum(II) sakarinat bes Schiff S-benzil-β-N-(2-asetilpirid-2-il)metilinditiokarbazat menunjukkan bahawa kompleks ini mempunyai struktur segiempat planar terherot dimana bes Schiffnya juga terkoordinat kepada ion kuprum sebagai agen kelat tridentat NNS. Pengkoordinatan yang ke empat bagi ion kuprum(II) diduduki oleh nitrogen imino daripada anion sakarinat. Tujuh patogen mikrob terpilih dan tiga jenis sel kanser, [HL-60 (Sel leukemia myeloid), MCF-7 (Sel kanser payudara dengan reseptor estrogen positif) dan Caov-3 (sel kanser ovari adenocarcinoma)] telah digunakan untuk menilai keaktifan biologi. Kebanyakan kompleks tersebut menunjukkan tanda positif sitotoksik terhadap sel kanser dan menunjukkan keaktifan keatas bakteria dan fungi.

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I certify that an Examination Committee met on 25th February 2005 to conduct the final examination of Thahira Begum on her Master of Science thesis entitled " Synthesis, characterization and biological activities of mixed-ligand Copper(II) complexes containing saccharin as one of the 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 any other institution.

_____

THAHIRA BEGUM

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LIST OF ABBREVIATIONS

sac	Saccharinate anion
1D	One-dimensional
FT-IR	Fourier-transform Infrared
B.M	Bohr magneton
LMCT	Ligand to metal charge transfer
MLCT	Metal to ligand charge transfer
DMSO	Dimethylsulphoxide
CD_{50}	Cytotoxic Dose at 50%
DNA	Deoxyribonucleic acid
HL-60	Human myeloid leukemic cells
MCF-7	Human breast carcinoma cells with positive estrogen receptor
Caov-3	Human ovarian adenocarcinoma cancer cells
CHNS	Carbon,Hydrogen, Nitrogen & Sulphur
NNS	Nitrogen-nitrogen-sulphur
UV/Vis	Ultraviolet/ Visible Spectroscopy
L	Ligand
Bpy	Bipyridine
PPh_3	Triphenylphosphine
Ap-SBz	2-acetylpyridine Schiff base of S-benzyldithiocarbazate
HSB-1	S-benzyl-β-N-(pyridine-2-yl)methylenedithiocarbazate.
HSB-2	S-benzyl-β-N-(6-methylpyrid-2-yl)methylenedithiocarbazate.

HSB-3	S-benzyl-β-N-(di-2-pyridylketone)methylenedithiocarbazate.
HSB-4	S-benzyl-β-N-(2-acetylpyridyl)methylenedithiocarbazate.
HSB-5	S-benzyl-β-N-(2-benzoylpyridyl)methylenedithiocarbazate.
HSM-2	S-methyl-β-N-(6-methylpyrid-2-yl)methylenedithiocarbazate.
HSM-3	S-methyl-β-N-(di-2-pyridylketone)methylenedithiocarbazate.
HSM-4	S-methyl-β-N-(2-acetylpyridyl)methylenedithiocarbazate.
HSM-5	S-methyl-β-N-(2-benzoylpyridyl)methylenedithiocarbazate.
HS2P-1	S-2-picolyl-β-N-(pyridine-2-yl)methylenedithiocarbazate.
HS2P-3	S-2-picolyl-β-N-(di-2-pyridylketone)methylenedithiocarbazate.
HS2P-5	S-2-picolyl-β-N-(2-benzoylpyridyl)methylenedithiocarbazate.
HS4P-1	S-4-picolyl-β-N-(pyridine-2-yl)methylenedithiocarbazate.
HS4P-2	S-4-picolyl-β-N-(6-methylpyrid2-yl)methylenedithiocarbazate.
HS4P-3	S-4-picolyl-β-N-(Di-2-pyridylketone)methylenedithiocarbazate.
HS4P-4	S-4-picolyl-β-N-(2-acetylpyridyl)methylenedithiocarbazate.
HS4P-5	S-4-picolyl-β-N-(2-benzoylpyridyl)methylenedithiocarbazate.
[Cu(SB-1)(sac)]	Copper(II) Saccharinate Complex of S-benzyl-β-N-(pyridine-2-yl)methylenedithiocarbazate
[Cu(SB-2)(sac)]	Copper(II) Saccharinate Complex of S-benzyl-β-N-(6-methylpyrid-2-yl)methylenedithiocarbazate
[Cu(SB-3)(sac)]	Copper(II) Saccharinate Complex of S-benzyl-β-N-(di-2-pyridylketone) methylenedithiocarbazate
[Cu(SB-4)(sac)]	Copper(II) Saccharinate Complex of S-benzyl-β-N-(2-acetylpyridyl)methylenedithiocarbazate
[Cu(SB-5)(sac)]	Copper(II) Saccharinate Complex of S-benzyl-β-N-(2-benzoylpyridyl)methylenedithiocarbazate

[Cu(SM-2)(sac)]	Copper(II) Saccharinate Complex of S-methyl-β-N-(6-methylpyrid-2-yl)methylenedithiocarbazate
[Cu(SM-3)(sac)]	Copper(II) Saccharinate Complex of S-methyl-β-N-(di-2-pyridylketone)methylenedithiocarbazate
[Cu(SM-4)(sac)]	Copper(II) Saccharinate Complex of S-methyl-β-N-(2-acetylpyridyl)methylenedithiocarbazate
[Cu(SM-5)(sac)]	Copper(II) Saccharinate Complex of S-methyl-β-N-(2-benzoylpyridyl)methylenedithiocarbazate
[Cu(S2P-1)(sac)]	Copper(II) Saccharinate Complex of S-2-picolyl-β-N-(pyridine-2-yl)methylenedithiocarbazate
[Cu(S2P-2)(sac)]	Copper(II) Saccharinate Complex of S-2-picolyl-β-N-(6-methylpyrid-2-yl)methylenedithiocarbazate
[Cu(S2P-3)(sac)]	Copper(II) Saccharinate Complex of S-2-picolyl-β-N-(di-2-pyridylketone)methylenedithiocarbazate
[Cu(S2P-4)(sac)]	Copper(II) Saccharinate Complex of S-2-picolyl-β-N-(2-acetylpyridyl)methylenedithiocarbazate
[Cu(S2P-5)(sac)]	Copper(II) Saccharinate Complex of S-2-picolyl-β-N-(2-benzoylpyridyl)methylenedithiocarbazate
[Cu(S4P-1)(sac)]	Copper(II) Saccharinate Complex of S-4-picolyl-β-N-(pyridine-2-yl)methylenedithiocarbazate
[Cu(S4P-2)(sac)]	Copper(II) Saccharinate Complex of S-4-picolyl-β-N-(6-methylpyrid-2-yl)methylenedithiocarbazate
[Cu(S4P-3)(sac)]	Copper(II) Saccharinate Complex of S-4-picolyl-β-N-(di-2-pyridylketone)methylenedithiocarbazate
[Cu(S4P-4)(sac)]	Copper(II) Saccharinate Complex of S-4-picolyl-β-N-(2-acetylpyridyl)methylenedithiocarbazate
[Cu(S4P-5)(sac)]	Copper(II) Saccharinate Complex of S-4-picolyl-β-N-(2-benzoylpyridyl)methylenedithiocarbazate
$[Cu(sac)_2(OH_2)_4].2H_2O$	Copper(II) Saccharinate

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B28	FT-IR Spectrum of [Cu(S4P-1)(sac)]	157
B29	FT-IR Spectrum of HS4P-2	158
B30	FT-IR Spectrum of [Cu(S4P-2)(sac)]	158
B31	FT-IR Spectrum of HS4P-3	159
B32	FT-IR Spectrum of [Cu(S4P-3)(sac)]	159
B33	FT-IR Spectrum of HS4P-4	160
B34	FT-IR Spectrum of [Cu(S4P-4)(sac)]	160
B35	FT-IR Spectrum of HS4P-5	161
B36	FT-IR Spectrum of [Cu(S4P-5)(sac)]	161

EQUATIONS

No.		Page
1	$[ML \cdot Cl] + DMSO \longrightarrow [ML(DMSO)]^{+} + Cl^{-}$ [M= Ni(II), Cu(II), Zn(II) and Sn(II), L= NNS]	48
2	$[M'(NNS)Cl_2 \cdot H_2O] + 3DMSO \longrightarrow [M'(NNS)(DMSO)_3]^{2+} + 2\ Cl^{-} + H_2O$ [M' = Cr(III), Sb(III)]	48

CHAPTER I

INTRODUCTION

Saccharin – Structure and Historical Background

The structure of saccharin (sac), 1,2-benzisothiazoline-3-(2H)one 1,1-dioxide or *o*-sulphobenzoimide is shown in Figure 1.

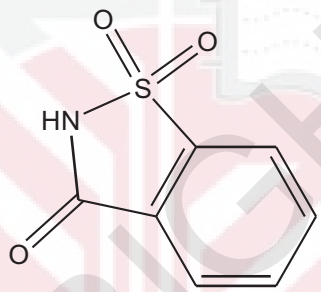

Figure 1: Structure of Saccharin

Saccharin is the world's oldest low-calorie sweetener and is 500 times as sweet as sugar. It was discovered in 1879 by researchers lead by Prof. Ira Remsen at John Hopkins University. Initially, consumption of saccharin was primarily confined to diabetics who could then enjoy sweetened foods without the extra calories, or glucose reaction associated with many sweeteners (Watkins(a), 2004). Subsequently, because of sugar rationing during the World Wars, a strong need for a sugar substitute both in the U.S and Europe developed and this need was met by saccharin. Even after World War II, saccharin continued to be used as a popular alternative to sugar, as people's interest in weight control developed. Its usefulness remained significant until the 1970s. Due to the

synergistic and functional properties of saccharin, and its low cost, it remains a valuable low-calorie sweetener today. Saccharin continues to be important for a wide range of low-calorie and sugar-free food and beverage applications (Watkins(a), 2004).

Saccharin has been the subject of extensive scientific research. It is one of the most studied food ingredients. Although evidence indicates that saccharin is safe for human consumption, there has been controversy over its safety. The basis for the controversy was the indication of the development of bladder tumors in male rats fed high doses of sodium saccharinate. Consequently, a ban was imposed on saccharin and its usage, although the male rats used in the study were fed the human equivalent of the sodium saccharinate in hundreds of cans of diet soft drink a day for a lifetime (Watkins(b),2004). However, extensive research on human populations has established no association between saccharin and cancer even at consumption levels above that of the average user of less than one ounce of the sweetener each year (Watkins(c), 2004).

The scientific data supporting safety of saccharin include the following:

1. Extensive research on human populations has established no association between saccharin and cancer. More than 30 human studies have been completed and indicate saccharin's safety at human levels of consumption.
2. In fourteen single-general animal studies involving several species of animals, saccharin was not shown to induce cancer in any organ, even at exceptionally high doses.

3. Saccharin is not metabolized and does not react with DNA, meaning that saccharin lacks two of the major characteristics of a classical carcinogen (Stolberg, 1997).

4. Other research indicates that the bladder tumors developed by male rats fed high doses of sodium saccharinate are related to very high doses of the sodium salt and not saccharin *per se*. Sodium ascorbate (vitamin C) and sodium citrate, found in many foods and beverages, demonstrate similar effects (Stolberg, 1997).

5. Research showed that the sodium form of saccharin combines with rat urine to create crystal-like stones in the bladder of the animal. Those stones, in turn, lead to cellular changes that cause cancer. However this seems to be a rat-specific phenomenon (Stolberg, 1997) as human urine is vastly different from rat urine and does not react with saccharin in the same way (Stolberg, 1997).

In 1991, the American Food and Drug Administration (FDA) withdrew its 1977 proposal to ban the use of saccharin (Watkins(b), 2004). In July 1997, the National Institute of Health announced that its National Toxicology Program (NTP) was reviewing data that could delist saccharin from FDA's list of carcinogens. In this announcement, NTP noted that saccharin was never listed as a known carcinogen and human studies had showed no link between saccharin and bladder cancer. The lack of effect of sodium saccharinate in mice and in monkeys further supported these findings.

In May 2000, the NTP released the 9th edition of its report on carcinogens and announced that saccharin had been delisted. The NTP report was submitted to the American Congress, and in December 2000, U.S President, Bill Clinton signed a bill that removed

the warning label that had been required on saccharin-sweetened products since 1977 (Watkins(b), 2004).

Despite the controversy, saccharin continued to be a major part of the non-nutritive sweetener market worldwide. In 2001, a report from a market research firm, Frost and Sullivan showed that the US food additive market was worth US $3000 million in 1999 and was predicted to rise to over US $5000 million by 2006. It was estimated that the non-nutritive sweetener segment of this market is worth about US $498 million of which saccharin accounts for about 45%.

Metal-Saccharinate Complexes - Their Biological Relevance

Studies revolving around saccharin and its metal complexes have been carried out to investigate the effect of saccharin consumption on humans and also living systems in general. Reports on the ability of saccharin to act as an inhibitor for certain enzyme reactions have been published (Supuran and Banciu, 1991). Carbonic anhydrase is a zinc-containing enzyme that converts carbon dioxide to soluble hydrogen carbonate in living systems. Complex-type inhibitors of this enzyme containing zinc were reported [Supuran *et al*. 1993(a)] and their mechanism of action investigated (Luca *et al*., 1991). These complexes have been found to contain aromatic or heterocyclic sulphonamides and a divalent or trivalent cation, a structure similar to that found for the metal complexes of saccharin. Such complexes are stronger inhibitors than the unsubstituted sulfonamides

due to their mechanism of action and interference with both steps of carbonic anhydrase catalytic turnover (Silverman and Lindskog, 1988).

Saccharin complexes have also been reported to have superoxide dismutase (SOD)-like behaviour. Copper-zinc superoxide dismutase, a metalloprotein found in living systems, was first found to contain copper by Mann and Keilin (1938). The presence of zinc in this protein was established later. Since the discovery of the copper-zinc enzyme (McCord and Fridovich, 1973), systems containing manganese and iron as the active metal centre have been isolated (Keele *et al*.,1970, DaSilva and William, 1991). The superoxide anion, produced in cells as a by-product of aerobic metabolism, is toxic to living systems. These enzymes protect the living systems by catalyzing the dismutation of the superoxide anion to generate oxygen.

The superoxide dismutase activity of the saccharinate complexes of manganese, iron, cobalt, nickel, copper and zinc has been investigated (Apella *et al*.,1993). The results were compared with those for the native superoxide dismutase. The copper saccharinate complex was found to have the highest SOD-like activity, with a value almost double that of the nickel, cobalt and zinc complexes, and very much higher than the values for the iron and the manganese complexes (Apella *et al*.,1993).

Properties Associated with Sulphur and Nitrogen as Donor Ligands

Ali and Livingstone (1974) have summarized the characteristics of ligands with sulphur as donor atoms as follows:

1. The permanent dipole moment and coordinating ability normally decreases in the order: H_2O › ROH › R_2O, but the reverse order holds for sulphur, i.e. H_2S ‹ RSH ‹ R_2S.

2. 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 $d_\pi - d_\pi$ bonding with the later transition metals and the early transition metals in unusually low oxidation states.

3. The polarisabilities of sulphur donors and the number of lone pairs decrease in the order S^{2-} › RS^- › R_2S. Consequently, thiolo ligands are more polarisable but not as effective d_π electron acceptors as thioethers, which is why most dithiocarbazate Schiff bases coordinate in their thiolate forms.

4. Sulphur donors bind more strongly to (b) class metals than do oxygen donors. [Class (a) metals ions are small, not very easily polarized and have a greater affinity for F^- than for I^-. Class (b) metal ions are essentially opposite in character].

5. Sulphur ligands occupy a late position in the nephalauxetic series (a measure of the degree of covalent bonding between metal and ligand). The series of donor atoms is: F‹ O‹ N‹ Cl‹ Br‹ S≈I ‹ Se.

6. Sulphur atoms in heterocyclic rings have very poor coordinating ability due to the pseudo-aromatic nature of the ring, which has the two-fold effect of causing the lone pairs on the sulphur atom to be less available for donation and the π -orbitals to be less capable of accepting electrons from the metal (Ali and Livingstone, 1974).

The properties of sulphur ligands also apply to sulphur-nitrogen chelating ligands. In general, sulphur-nitrogen ligands appear to give rise to a smaller reduction in the interelectronic repulsion energy than sulphur-sulphur ligands. 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 (Ali and Livingstone, 1974).

Cytotoxicity of Some Sulphur-Nitrogen Ligands and Their Metal Complexes

The following criteria can be used to evaluate whether a metal complex is carcinostatically active:

1. The complex should be reasonably labile
2. The metal chelate should have reasonably high thermodynamic stability
3. The metal should be a class (b) metal.
4. Ligands with sulphur donors are most likely to be effective, since they allow for lipid solubility of the metal complex and form stable complexes with class (b) and borderline metals (Ali and Livingstone, 1974).

In 1956, it was reported that pyridine-2-carboxaldehyde thiosemicarbazone (Fig 2) exhibited carcinostatic activity in the lymphoid leukemia-1210 test.

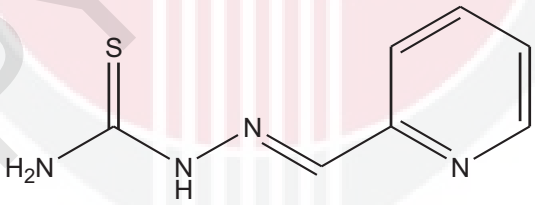

Figure 2: Structure of Pyridine-2-carboxaldehyde Thiosemicarbazone

Kethoxal bis(thiosemicarbazone) (Fig 3) was also reported to exhibit carcinostatic action, and its cytotoxicity was enhanced by the presence of copper and zinc ions (Ali and Livingstone, 1974).

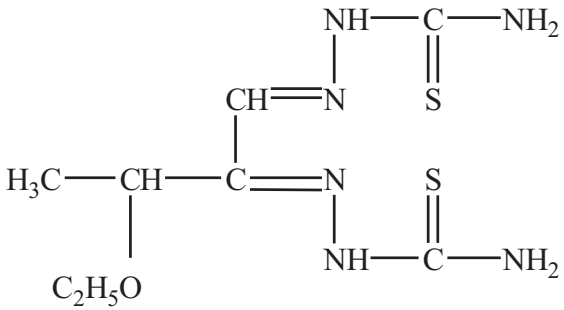

Figure 3: Structure of Kethoxal bis(thiosemicarbazone)

Cytotoxic and antimicrobial tests were carried out on metal complexes of ligands derived from dithiocarbazic acid and most have been found to be biologically active. For example, Hossain *et.al* (1996) carried out biological tests on the copper(II) complexes of the 2-acetylpyridine Schiff base of S-benzyldithiocarbazate on different types of fungi and bacteria and observed that in general, chelation of the Schiff base to the copper(II) complex enhanced its antifungal activities and the activities approached that of commercially available antibiotics. Biological tests carried out on dithiocarbazate Schiff bases and their metal complexes are discussed in more detail in Chapter II.

Due to the controversy surrounding saccharin and the various biological studies carried out on it throughout the years, and the strong antibacterial and antifungal activities shown by nitrogen-sulphur complexes, this work strives to produce complexes that are biologically relevant and may have potential use as antifungal/ antibacterial/ anticancer agents. Copper(II) was chosen as the transition metal due to its importance in biological systems, and its presence in many enzymes, which are essential to life.

Hence, as part of the ongoing study of metal complexes of dithiocarbazate derivatives, the synthesis, characterization and bioactivity of some mixed-ligand ternary Cu(II)

complexes of some NNS donor ligands formed by condensation of several dithiocarbazate Schiff bases with the saccharinate anion and water as co-ligands (in some cases) are reported. Since very little work has been reported on metal-saccharinate complexes, the present work is expected to shed some light on the coordination biological activities of this industrially important sweetener.

OBJECTIVES

The objectives of this project were:

- ✓ To synthesize copper (II) complexes of saccharin of the type [Cu(NNS)(sac)], where NNS^- is a uninegatively charged tridentate sulphur-nitrogen chelating agent and sac^- is the saccharinate anion.
- ✓ To characterize the new complexes by various physico-chemical techniques, including partial elemental analysis, magnetic susceptibility, molar conductance and spectroscopic techniques and X-ray diffraction analysis where possible
- ✓ To study the antimicrobial and cytotoxic activities of the metal complexes.

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