UNIVERSITI PUTRA MALAYSIA

SYNTHESIS, CHARACTERISATION AND BIOLOGICAL ACTIVITIES OFMIXED-LIGAND COPPER@) COMPLEXES CONTAINING SACCHARIN

THAHIRA BEGUM

FPSK(M) 2005 21

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

By

THAHIRA BEGUM

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

February 2005

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

Pengerusi: Profesor Karen A.Crouse, PhD

Fakulti: Sains

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.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my deepest gratitude and appreciation to my supervisor, Prof. Karen A.Crouse and co-supervisor Dr.Mohamed Ibrahim Mohamed Tahir for their constant help and for all the advice, suggestions and morale booster talks during the course of my work. Many thanks also for all those after-lab activities that we enjoyed. To Dr Akbar Ali, Universiti Brunei Darussalam, mere thanks is not enough. I am indeed lucky to have you as one of my mentors. I am also indebted to the staff of the Chemistry Department of UPM for helping me with the various analyses of my samples.

To the Organometallica group (Teng Jin, Kar Beng, Eddy, Mat,Liza & Fiona) you guys are the best!! I'll never forget all the fun we had, both in the lab and outside. The trips to Penang, Kelantan and Port Dickson will be forever imprinted in my mind as part of the sweet memories in Malaysia. Special thanks to Teng Jin for all those car rides during the semester breaks and all the gruff advice that meant so much to me although I pretended that it just flew over my head☺. Thanks also goes to Mat and Eddy for running the cytotoxic tests on my complexes, and Fiona and Liza for running the antimicrobial tests on my complexes.

To my family-without your support and encouragement, I would have never made it. To my father and mother, thank you for always wishing me the best and encouraging me, even when I have trouble believing in myself at times. To my sisters, Abidha – your advice and practical way of seeing things often pulled my feet back on the ground and made me realize my goals and ambitions. You're a great source of inspiration to me. To Husna, your wackiness often kept me from going mad over the little pitfalls I had during the course of my work. To Sharifa, for being so sweet and for making me smile. To Dhekra from Yemen, thank you so much for being the best friend I ever had in Malaysia. We went through a lot together. I will never forget you. Last, but certainly not least, I would like to thank Pradeep, my best friend, for being there for me, both physically and emotionally and for encouraging me to be the best I can be, and for wishing good things for me always. It means a lot to me. Thank you.

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, Characterisation and Biological Activities of Mixed-Ligand Copper (II) Complexes Containing Saccharin" 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:

Mohd Basyaruddin Abdul Rahman, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Sidik Silong, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Mohd Zaizi Desa, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Hapipah Mohd Ali, PhD
Associate Professor
Faculty of Science
Universiti Malaya
(External Examiner)

GULAM RUSUL RAHMAT ALI, PhD
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 19 MAY 2005

The thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

Karen A.Crouse, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Chairperson)

Mohamed Ibrahim Mohamed Tahir, D.Phil.
Lecturer
Faculty of Science
Universiti Putra Malaysia
(Member)

AINI IDERIS, PhD
Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 09 JUN 2005

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

THAHIRA BEGUM

Date: 18 05 2005

TABLE OF CONTENTS

Page

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

LIST OF TABLES

LIST OF FIGURES

APPENDIX A

APPENDIX B

EQUATIONS

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

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.

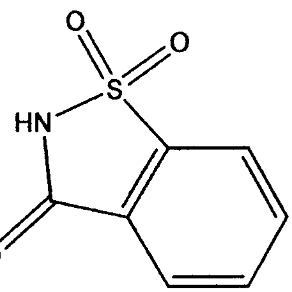

Figure 1: Structure of Saccharin

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.