

**SYNTHESIS, CHARACTERIZATIONS AND BIOLOGICAL
PROPERTIES OF SOME HYDRAZONES AND THEIR METAL
COMPLEXES**

SITI MUNIRAH SAHARIN

**CHEMISTRY DEPARTMENT
FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
KUALA LUMPUR**

2012

ABSTRACT

The aim of the project as presented in this thesis concerns on the preparation, characterization and biological evaluation of Schiff bases derived from 3, 5-dihydroxybenzohydrazide and their Cu(II) and Zn(II) complexes. The Schiff bases were synthesized by the condensation of 3, 5-dihydroxybenzohydrazide and hydroxyacetophenone (BzyHAP-H), 5-bromo hydroxyacetophenone (BzyHAP-Br), 5-chloro hydroxyacetophenone (BzyHAP-Cl), salicylaldehyde (BzySAL-H), 5-bromo salicylaldehyde (BzySAL-Br), and 5-chloro salicylaldehyde (BzySAL-Cl). The BzyHAP series ligands were subsequently reacted with the acetate of Zn(II) whereas the BzySAL series ligands were reacted with Cu(II) and Zn(II) acetate in a 1:2 metal to ligand ratio. The chemical structures of the compounds were characterized by means of elemental analyses, and spectroscopy methods including IR, ^1H NMR, ^{13}C NMR, and UV-Vis. The determination of metal content was carried out using atomic absorption spectroscopy. Single X-ray diffraction data collection is carried out for ligand BzySAL-Br. From the structural analysis, the ligand is found to be planar with an r.m.s. deviation for the non-H atoms of 0.16Å. In the crystal structure, the Schiff base molecules and the water molecules are linked together by intermolecular N-H--O and O-H--O hydrogen bonds, leading to layers parallel to the *bc* plane. An intramolecular O-H--N hydrogen bond involving the imine N atom and a hydroxyl substituent is also observed. The synthesized Schiff base and their metal complexes are screened for the *in vitro* cytotoxicity towards prostate cancer cell line (PC-3), colon cancer cell line (HT-29) lung cancer cell line (A549), and normal cell line (WRL-68). All Zn (II) complex Schiff bases show relatively low cytotoxicity against all tested cell line. Complexes labeled as CuBzySAL-H, CuBzySAL-Br, and CuBzySAL-Cl show encouraging value of EC_{50} against colon cancer cell line (HT-29), lung cancer cell line (A549), and normal cell line (WRL-68). On contrary, the cytotoxicity against prostate

cancer cell line (PC-3) is recorded negative. The evaluation of antioxidant reveals that all compounds are moderate DPPH inhibitor compared to the standard vitamin C.

ABSTRAK

Seperti yang dibentangkan di dalam tesis ini, projek yang dijalankan adalah berkenaan penyediaan, pencirian dan penilaian aktiviti biologi bagi bes Schiff yang diterbitkan daripada 3,5-dihidroksibenzohidrazida dan kompleks Cu(II) dan Zn(II)nya. Bes Schiff berkenaan telah disintesis melalui proses kondensasi sebatian 3,5-dihidroksibenzohidrazida dengan hidroksiasetofenon, 5-bromohidroksiasetofenon, 5-klorohidroksiasetofenon, salisilaldehid, 5-bromosalisilaldehid, dan 5-klorosalisilaldehid. Ligan-ligan ini kemudiannya ditindakbalaskan dengan asetat bagi logam Cu(II) dan Zn(II) dalam nisbah 1:2 metal kepada ligan. Struktur kimia sebatian ini kemudiannya dicirikan dengan analisis unsur dan kaedah spektroskopi termasuklah IR, ^1H NMR, ^{13}C NMR, and UV-Vis. Penentuan kandungan logam ditentukan dengan kaedah spektroskopi penyerapan atom. Analisis kristalografi dijalankan bagi ligan BzySAL-Br.

Analisis struktural, mendapati bahawa ligan adalah planar dengan nilai sisihan r.m.s. bagi atom bukan hidrogen adalah 0.16Å. Dalam struktur ligan berkenaan, didapati bahawa ianya terhubung dengan molekul air melalui ikatan hidrogen N-H--O dan O-H--O menghasilkan lapisan yang selari dengan satah *bc*. Turut terlibat ialah ikatan hidrogen intramolekul antara atom nitrogen kumpulan imina dan kumpulan penukarganti hidroksi. Kemudiannya, bes Schiff dan kompleks logamnya disaring untuk sitotoksiti secara *in vitro* terhadap sel kanser prostat (PC-3), sel kanser kolon (HT-29), sel kanser paru-paru (A549), dan sel normal (WRL-68). Kesemua kompleks Zn(II) menunjukkan keisotoksian yang rendah secara relatif terhadap semua sel yang diuji. Kompleks Cu(II) berlabel CuBzySAL-H, CuBzySAL-Br, and CuBzySAL-Cl pula menunjukkan nilai EC_{50} yang menggalakkan terhadap sel kanser kolon (HT-29), sel kanser paru-paru (A549), dan sel normal (WRL-68). Sebaliknya, sitotoksiti terhadap sel kanser prostat (PC-3) adalah

negatif. Penyaringan terhadap sifat antioksidasi menunjukkan semua sebatian merupakan perencat DPPH yang sederhana berbanding vitamin C sebagai standard.

ACKNOWLEDGEMENTS

First and foremost, praise only to Allah, the most gracious and merciful, for His blessing that I am able to making this thesis a reality.

I would like to express my sincere appreciation to my supervisor, Prof. Dr. Hapipah Mohd Ali for her guidance, advice and constant support throughout the study. One simply could not wish for a better or friendlier supervisor.

I want to thank the staff members of Chemistry Department, University of Malaya for their co-operation and assistance in carrying out my research work. My sincere thanks is also extended to my lab mates; Dr. Hamid, Azimah, and Suleiman for their encouragement and constant help. Not forgetting all my friends for their understanding and support. I am also very thankful to the financial assistance from University of Malaya (PPP009/2008A) and Tutorship Scheme.

Last but not least, the greatest appreciation is dedicated to my family to whom I am indebted the most. The tender love, care and support are the reasons I become what I am today.

CONTENTS

ABSTRACT	ii
ABSTRAK	iv
ACKNOWLEDGEMENTS	vi
CONTENTS	vii
LIST OF FIGURES	x
LIST OF TABLES	xiii
ABBREVIATIONS	xiv
CHAPTER 1: INTRODUCTION	1
1.1 Introduction of Schiff base.	1
1.2 The Chemistry of Schiff base.....	1
1.3 Cancer.....	3
1.3.1 Anticancer drugs.....	4
1.4 Antioxidant	5
CHAPTER 2: LITERATURE REVIEW	7
2.1 The Schiff bases.	7
2.2 The Importance of benzohydrazone-related compounds.....	9
2.3 Salicylaldehyde	9
2.4 Acetophenone	10
2.5 Biological Importance of salicylaldehyde and acetophenone.....	11
2.6 The biological importance of Polyhydroxy Benzohydrazone.....	12
2.7 The biological importance of Salicylaldehyde Benzoylhydrazone and hydroxylacetophenone Benzoylhydrazone.	13
2.8 The objective of Study.....	16
CHAPTER 3: RESEARCH METHODOLOGY	18
3.1 Reagents and Solvents.....	18
3.2 Experimental Instrumentation.....	18
3.3 General preparation of ligands and their metal complexes.....	19
3.4 Preparation of starting material.....	20
3.5 Series 1: Hydroxyacetophenone Benzohydrazone	21

3.5.1	(BzyHAP-H)	21
3.5.2	(BzyHAP-Br)	22
3.5.3	(BzyHAP-Cl).....	23
3.5.4	Zn(BzyHAP-H)	24
3.5.5	Zn(BzyHAP-Br)	24
3.5.6	Zn(BzyHAP-Cl)	25
3.6	Series 2: Salicylaldehyde Benzohydrazones.....	26
3.6.1	(BzySAL-H).....	26
3.6.2	(BzySAL-Br).....	27
3.6.3	(BzySAL-Cl).....	28
3.6.4	Cu(BzySAL-H)	29
3.6.5	Zn(BzySAL-H).....	29
3.6.6	Cu(BzySAL-Br)	30
3.6.7	Zn(BzySAL-Br).....	30
3.6.8	Cu(BzySAL-Cl).....	31
3.6.9	Zn(BzySAL-Cl).....	31
3.7	Experimental Procedures for Cytotoxicity Test.....	32
3.7.1	Cell culture.....	32
3.7.2	MTT assay.....	32
3.8	Experimental Procedures for Anti-oxidant Activity.....	33
3.8.1	DPPH assay.....	33
3.8.2	FRAP Assay.....	33
CHAPTER 4: RESULTS AND DISCUSSIONS		35
4.1	Ligands and complexes of BzyHAP series.....	36
4.1.1	IR Spectral Data	37
4.1.2	¹ HNMR Spectral Data.....	43
4.1.3	¹³ C NMR Spectral Data.....	47
4.1.4	UV-Vis Spectral Data	50

4.1.5	Metal analysis by Atomic Absorption Spectroscopy (AAS).....	53
4.1.6	Proposed Structures of BzyHAP Series and their Zn(II) complexes	54
4.2	Ligands and complexes of BzySAL Series.....	56
4.2.1	IR Spectral Data	57
4.2.2	¹ H NMR Spectral Data.....	63
4.2.3	¹³ C NMR Spectral Data.....	67
4.2.4	UV-Vis Spectral Data	70
4.2.5	Metal analysis by Atomic Absorption Spectroscopy (AAS).....	75
4.2.6	X-ray Crystallographic Analyses of Schiff Base.....	76
4.2.7	Proposed Structures of BzyHAP Series and their Zn(II) complexes	79
4.3	Cytotoxicity	83
4.3.1	Cytotoxicity of BzyHAP and BzySAL series Compounds.....	83
4.4	Antioxidant Activity.....	84
4.4.1	DPPH Scavenging Activity of Ligands and complexes of BzyHAP series	85
4.4.2	FRAP Value of Ligands and complexes of BzyHAP series.....	86
4.4.3	DPPH Scavenging Activity of Ligands and complexes of BzySAL series.....	87
4.4.4	FRAP Value of Ligands and complexes of BzyHAP series.....	88
	CONCLUSIONS	89
	REFERENCES.....	90
	APPENDIX.....	97

LIST OF FIGURES

		Page
4.1	BzyHAP series ligand	36
4.2	The keto and enol structure of the ligand	38
4.3	IR Spectra of BzyHAP-Brv	41
4.4	IR Spectra of Zn(BzyHAP-Br)	42
4.5	Proton numbering scheme of BzyHAP Series ligands	44
4.6	¹ H NMR Spectra of (BzyHAP-Cl)	45
4.7	¹ H NMR Spectra of Zn(BzyHAP-Cl)	46
4.8	Carbon numbering scheme of BzyHAP Series ligands	47
4.9	¹³ C NMR Spectral of BzyHAP-H	49
4.10	UV Spectra of BzyHAPCl	52
4.11	UV Spectra of ZnBzyHAPCl	52
4.12	Proposed structure of 3,5-dihydroxy-N' - [(2-hydroxyphenyl) methylidene] benzohydrazide	54
4.13	Proposed structure of 3,5-dihydroxy-N' - [(5-bromo-2-hydroxyphenyl)methylidene] benzohydrazide	54
4.14	Proposed structure of 3,5-dihydroxy-N' - [(5-chloro-2-hydroxyphenyl)methylidene] benzohydrazide	54
4.15	Proposed structure of Zn[3,5-dihydroxy-N' - [(2-hydroxyphenyl) methylidene] benzohydrazide]	55
4.16	Proposed structure of Zn[3,5-dihydroxy-N' - [(5-bromo -2-hydroxyphenyl) methylidene] benzohydrazide]	55
4.17	Proposed structure of Zn[3,5-dihydroxy-N' - [(5-chloro -2-hydroxyphenyl) methylidene] benzohydrazide]	55
4.18	BzySAL series ligand	56
4.19	IR spectrum of BzySAL-H	60
4.20	IR spectrum of Cu(BzySAL-H)	61

4.21	IR spectrum of Zn(BzySAL-H)	62
4.22	Phenoxy bridging of the metal complexes	64
4.23	Proton numbering scheme of BzySAL Series ligand	65
4.24	¹ H NMR Spectra of BzySAL-H	66
4.25	¹ H NMR Spectra of Zn(BzySAL-H)	67
4.26	Carbon numbering scheme of BzySAL Series Ligands	69
4.27	¹³ C NMR Spectral of BzySAL	73
4.28	UV Spectra of BzySALCl	73
4.29	UV Spectra of Cu(BzySALCl)	74
4.30	UV Spectra of Zn(BzySALCl)	78
4.31	Thermal ellipsoids plot of 3,5-dihydroxy- <i>N'</i> -[(5-bromo-2-hydroxyphenyl) ethylidene] benzohydrazide.	78
4.32	Packing view looking down the crystallographic b unit cell edge.	79
4.33	Proposed structure of 3,5-dihydroxy- <i>N'</i> -[(2-hydroxyphenyl) ethylidene] benzohydrazide.	79
4.34	Proposed structure of 3,5-dihydroxy- <i>N'</i> -[(5-bromo-2-hydroxyphenyl) ethylidene] benzohydrazide.	79
4.35	Proposed structure of 3,5-dihydroxy- <i>N'</i> -[(5-chloro-2-hydroxyphenyl) ethylidene] benzohydrazide.	80
4.36	Proposed structure of Cu[3,5-dihydroxy- <i>N'</i> -[(2-hydroxyphenyl) ethylidene] benzohydrazide].	80
4.37	Proposed structure of Zn[3,5-dihydroxy- <i>N'</i> -[(2-hydroxyphenyl) ethylidene] benzohydrazide].	81
4.38	Proposed structure of Cu[3,5-dihydroxy- <i>N'</i> -[(5-bromo-2-hydroxyphenyl) ethylidene] benzohydrazide].	81
4.39	Proposed structure of Zn[3,5-dihydroxy- <i>N'</i> -[(5-bromo-2-hydroxyphenyl) ethylidene] benzohydrazide].	82
4.40	Proposed structure of Cu[3,5-dihydroxy- <i>N'</i> -[(5-chloro-2-hydroxyphenyl) ethylidene] benzohydrazide].	82

4.41 Proposed structure of Zn[3,5-dihydroxy-*N*'-(5-chloro-2-hydroxyphenyl) ethylidene] benzohydrazide]. 85

LIST OF TABLE

	Page
4.1 Analytical data and physical properties of ligands and complexes of BzyHAP Series	36
4.2 Selected IR spectral data of BzyHAP series	40
4.3 ¹ H NMR spectral data of BzyHAP series and their Zn(II) complexes	44
4.4 ¹³ C NMR spectral data of BzyHAP series	48
4.5 UV-Vis spectral data of BzyHAP series and their Zn(II) complexes	51
4.6 Experimental and theoretical metal composition of the Zn(II) complexes	53
4.7 Analytical data and physical properties of ligands and complexes of BzySAL Series	56
4.8 Selected IR spectral data of BzySAL series and their Cu(II) and Zn(II) complexes	59
4.9 ¹ H NMR spectral data of BzySAL series and their Cu(II) and Zn(II) complexes	64
4.10 ¹³ C NMR spectral data of BzySAL series	68
4.11 UV-Vis spectral data of BzySAL series and their Cu(II) and Zn(II) complexes	72
4.12 Experimental and theoretical metal composition of the Cu(II) and Zn(II) complexes	75
4.13 Crystal data and structure refinement of BzySAL-Br	77
4.14 Hydrogen-bond geometry (Å, °)	77

ABBREVIATIONS

%	Percent
π	Pi
λ	Wavelength
δ	Chemical shift
ν	Stretching vibration
\AA	Angstrom
^{13}C NMR	Carbon nuclear magnetic resonance
^1H NMR	Proton nuclear magnetic resonance
AAS	Atomic absorption spectroscopy
Abs	Absorbance
BHT	Butylated hydroxytoluene
Cu	Copper
DMSO	Dimethyl sulphoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
EC ₅₀	Half maximal effective concentration
EtOH	Ethanol
FRAP	Ferric reducing ability of plasma
IR	Infra-red
M	Mole per litre
m	Multiplet
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
s	singlet
U _{eq}	Equivalent isotropic displacement parameter
UV-Vis	Ultraviolet-visible
Zn	Zinc