UNIVERSITI PUTRA MALAYSIA

SYNTHESIS, CHARACTERISATION AND BIOLOGICAL ACTIVITIES OF NOVEL NITROGEN-SULPHUR MACROCYCLIC LIGANDS AND THEIR TRANSITION METAL COMPLEXES

CHAH CHEE KEONG

FS 2018 11

SYNTHESIS, CHARACTERISATION AND BIOLOGICAL ACTIVITIES OF NOVEL NITROGEN-SULPHUR MACROCYCLIC LIGANDS AND THEIR TRANSITION METAL COMPLEXES

By

CHAH CHEE KEONG

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

October 2017

COPYRIGHT

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

SYNTHESIS, CHARACTERISATION AND BIOLOGICAL ACTIVITIES OF NOVEL NITROGEN-SULPHUR MACROCYCLIC LIGANDS AND THEIR TRANSITION METAL COMPLEXES

By

CHAH CHEE KEONG

October 2017

Chairman : Thahira Begum, PhD
Faculty : Science

Macrocycles have a great importance in macrocyclic and supramolecular chemistry. The term macrocycle is defined as a cyclic macromolecule with more than eight members. Macrocyclic Schiff bases and their transition metal complexes have various interesting biological properties such as cytotoxic, DNA binding, and antibacterial activities. In this project, the first novelty was the synthesis of five series tetradentate nitrogen-sulphur macrocyclic Schiff base derived from terephthaloyl-bis-dithiocarbazate (TDTC) using glyoxal (G), 2,5-hexanedione (H), acetyl acetone (A), 5,5-dimethyl-1,3-cyclohexanedione (D), and malondialdehyde (M). Their complexes were formed *via* reaction with various metal acetate or metal chloride salt [Ni(II), Cu(II), Zn(II), Cd(II), Nb(II), Ru(III), Mo(V), and Pd(II)]. A total of 40 metal complexes were synthesised and these complexes were expected to have a general formula of M_2L or M_3L. These compounds were characterised by various physico-chemical and spectroscopic techniques. Based on the data obtained, the azomethine nitrogen atom and the thiolate sulphur atom from the Schiff base were coordinated to the metal ions. The geometry of Cu(II), Nb(II) and Pd(II) complexes was distorted square planar, but the Ni(II), Zn(II), Cd(II) and Ru(III) complexes was distorted square pyramidal. The Mo(V) complexes showed a distorted pentagonal bipyramidal. The Schiff bases and their metal complexes were evaluated for their cytotoxic activities against the invasive human bladder carcinoma cell line (EJ-28) and the minimum-invasive human bladder carcinoma cell line (RT-112). MTT assay was used in the determination of IC_{50} values. From the data obtained, the macrocyclic Schiff bases were inactive against to both the bladder cancer cell lines but the respective complexes had significantly increased cytotoxic activity. The complexes also showed higher activity against RT-112 than EJ-28. The IC_{50} for PdTGSB and RuTGSB complexes against RT-112 were strongly active with the values of 0.320 and 0.472 μM, respectively. The second novelty in this study was the mechanism of death assays using macrocyclic compounds. The two active complexes were further studied for the

mechanism of death *via* Reactive Oxygen Species (ROS) and Annexin V assays. Migration assay was also carried out on the most inactive compound, which was CuTGSB. The DNA binding interaction of the complexes with calf-thymus DNA (CT-DNA) was investigated *via* electronic absorption spectroscopy, fluorescence spectroscopy, and viscosity measurements. The binding constant, K_b for PdTGSB was 3.79×10^4 M^{-1} and the binding mode was electrostatic binding. Antibacterial studies using three Gram-positive (*B. cereus, S. aureus, Methicillin-resistant S. aureus*) and four Gram-negative (*E. coli, K. pneumonia, S. typhimurium, S. sonnei*) bacteria were carried out. Standard disc diffusion method and determination of minimum inhibitory concentration (MIC) were used in the antibacterial studies. The macrocyclic complexes were generally more active against the Gram-positive bacteria as compared to the Gram-negative bacteria. The MIC values obtained for CdTHSB and CdTASB against *B. cereus* was 2.0 mg/mL. Hence, these complexes were the candidates to be antibacterial agents.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

SINTESIS, PENCIRIAN DAN BIOLOGI AKTIVITI BAGI NITROGEN-SULFUR MAKROKITARAN LIGAN BARU DAN KOMPLEKS LOGAM PERALIHANNYA

Oleh

CHAH CHEE KEONG

Oktober 2017

Pengerusi : Thahira Begum, PhD
Fakulti : Sains

Makrokolekik adalah sangat penting dalam makrokitaran dan kimia supramolekul. Istilah makrokolekik ditakrifkan sebagai makromolekul kitaran dengan lebih daripada lapan ahli. Makrokitaran bes Schiff dan kompleks logam peralihannya mempunyai pelbagai ciri biologi yang menarik seperti aktiviti sitotoksik, pengikatan DNA, dan antibakteria. Dalam projek ini, kebaharuan pertama ialah sintesis lima siri makrokitaran bes Schiff tetradentat nitrogen-sulfur yang diterbitkan daripada terephtaloil-bis-ditiokarbazat (TDTC) dengan menggunakan glioxal (G), 2,5-heksanadion (H), asetil aseton (A), 5,5-dimetil-1,3-sikloheksanadion (D), dan malondialdehid (M). Kompleksnya yang terbentuk adalah melalui tindak balas dengan pelbagai asetat logam atau klorida logam [Ni(II), Cu(II), Zn(II), Cd(II), Nb(II), Ru(III), Mo(V), dan Pd(II)]. Sejumlah 40 kompleks logam telah disintesis dan kompleks ini dijangka mempunyai formula umum iaitu M_2L atau M_3L. Sebatian-sebatian ini telah dicirikan melalui pelbagai teknik fiziko-kimia dan spektroskopi. Berdasarkan data yang diperolehi, atom nitrogen azometin dan atom sulfur thiolat dari bes Schiff dikoordinat kepada ion logam. Geometri untuk kompleks Cu(II), Nb(II) dan Pd(II) adalah segiempat sama terherot, tetapi kompleks Ni(II), Zn(II), Cd(II) dan Ru(III) adalah piramid segiempat terherot. Kompleks Mo(V) menunjukan bipiramid segilima terherot. Bes Schiff dan kompleks logamnya telah dinilai untuk aktiviti sitotoksiknya terhadap sel karsinoma kanser pundi kencing manusia invasif (EJ-28) dan sel karsinoma kanser pundi kencing manusia yang minimum invasif (RT-112). Ujian MTT digunakan dalam penentuan nilai IC_{50}. Daripada data yang diperolehi, makrokitaran bes Schiff tidak aktif terhadap kedua-dua sel kanser pundi kencing tetapi kompleks masing-masing telah meningkatkan aktiviti sitotoksik dengan ketara. Kompleks juga menunjukkan aktiviti yang lebih tinggi terhadap RT-112 daripada EJ-28. IC_{50} untuk kompleks PdTGSB dan RuTGSB terhadap RT-112 adalah sangat aktif dengan nilai masing-masing 0.320 dan 0.472 μM. Kebaharuan kedua dalam kajian ini adalah ujian mekanisme kematian menggunakan sebatian makrokitaran. Dua kompleks yang aktif akan diteruskan bagi kajian lanjutan untuk mekanisme kematian melalui ujian Spesies

Oksigen Reaktif (ROS) dan Annexin V. Ujian penghijrahan juga dilakukan untuk sebatian paling tidak aktif iaitu CuTGSB. Interaksi pengikatan DNA untuk kompleks dengan DNA timus anak lembu (CT-DNA) diselidik melalui spektroskopi penyerapan elektronik, spektroskopi pendarfluor, dan pengukuran kelikatan. Pemalar ikatan, K_b bagi PdTGSB adalah 3.79×10^4 M^{-1} dan mod ikatan adalah ikatan elektrostatik. Kajian antibakteria menggunakan tiga Gram-positif (*B. cereus, S. aureus, Methicillin-resistant S. aureus*) dan empat Gram-negatif (*E. coli, K. pneumonia, S. typhimurium, S. sonnei*) bakteria juga telah dilakukan. Kaedah penyebaran cakera standard dan penentuan kepekatan penghalang minimum (MIC) digunakan dalam kajian antibakteria. Kompleks makrokitaran umumnya lebih aktif terhadap bakteria Gram-positif berbanding dengan bakteria Gram-negatif. Nilai MIC yang diperolehi untuk CdTHSB dan CdTASB terhadap *B. cereus* adalah 2.0 mg/mL. Oleh itu, kompleks tersebut adalah calon untuk menjadi agen antibakteria.

ACKNOWLEDGEMENTS

A very grateful gratitude is dedicated to God who gave me the strength and the motivation that I was finally able to complete this study after so many hurdles and obstacles.

First and foremost, I would like to sincerely thank my supervisor, Dr. Thahira Begum and co-supervisors Assoc. Prof. Abhimanyu Veerakumarasivam, Dr. Mohamed Ibrahim Mohamed Tahir, and Dr. Haslina Ahmad for their invaluable advice, guidance and encouragement toward the completion of this thesis. It means so much and is meaningful to me.

Special thanks to Assoc. Prof. Sharmili Vidyadaran, Assoc. Prof. Rajesh Ramasamy, and Dr. Enoch Kumar Perimal for allowing me to use their lab facilities in the Faculty of Medicine and Health Sciences. Thanks also go to all the laboratory technicians and staff in the Faculty of Science and Faculty of Medicine and Health Sciences for their help and cooperation throughout this study.

Thanks to all lecturers, especially Prof. Emeritus Karen A. Crouse and all staff of the Chemistry Department who have contributed in one way or other. As always, I thank my dear labmates: Shahedeh, Ming Yueh, Georgiana, Ali, Shuhada, Junita, Enis, Lee Chin, Fatihah, Fadilah, and Nabeel for their guidance and contribution inside and outside the lab.

Grateful thanks to my beloved family especially my mother, my father, and my grandmother for their love, support and motivation. Last but not least, I would like to thank to all my friends who gave me moral support to complete this study.

Thank you to those who had contributed their cooperation directly and indirectly during the whole study. Thank you.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Thahira Begum, PhD
Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Chairman)

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

Haslina Ahmad, PhD
Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Member)

Abhimanyu Veerakumarasivam, PhD
Professor
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Member)

ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No.: _____

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013)are adhered to.

Signature: _____
Name of Chairman of Supervisory Committee: _____

Signature: _____
Name of Member of Supervisory Committee: _____

Signature: _____
Name of Member of Supervisory Committee: _____

Signature: _____
Name of Member of Supervisory Committee: _____

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

SMDTC	S-methyldithiocarbazate
SBDTC	S-benzyldithiocarbazate
HOMO	Highest occupied molecular orbital energies
LUMO	Lowest unoccupied molecular orbital energies
DNA	Deoxyribonucleic acid
mRNA	Messenger ribonucleic acid
PS	Phosphatidylserine
ROS	Reactive oxygen species
ATP	Adenosine triphosphate
NADH	Nicotinamide adenine dinucleotide
RPMI	Roswell Park Memorial Institute medium
FBS	Fetal bovine serum
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
PBS	Phosphate-buffered saline
DCFH-DA	2',7'-dichlorodihydrofluorescin diacetate
DCF	2',7'-dichlorodihydrofluorescin
DPBS	Dulbecco's phosphate buffered saline
PI	Propidium iodide
Tris	Tris(hydroxymethyl)aminomethane
MHA	Mullex Hinton agar
MHB	Mullex Hinton broth
TSA	Tryptic Soy agar
INT	p-iodonitrotetrazolium violet
DMSO	Dimethylsulphoxide

mp	Melting point
TDTC	Terephthaloyl-bis-dithiocarbazate
TGSB	4,11,20,27-tetrathioxo-3,12,19,28-tetrathia-5,6,9,10,21,22,25,26-octaazatricyclo[28.2.2.2^{14,17}]hexatriaconta 1(33),6,8,14(36),15,17(35),22,24,30(34),31-decaene-2,13,18,29-tetraone
THSB	7,10,25,28-Tetramethyl-4,13,22,31-tetrathioxo-3,14,21,32-tetrathia-5,6,11,12,23,24,29,30-octaaza-tricyclo[32.2.2.216,19]tetraconta 1(37),6,10,16(40),17,19(39),24,28,34(38),35-decaene-2,15,20,33-tetraone
TDSB	S-(5,5-dimethyl-1,3-cyclohexanedione)terephthaloyl-bis-dithiocarbazate
TASB	7,9,24,26-Tetramethyl-4,12,21,29-tetrathioxo-3,13,20,30-tetrathia-5,6,10,11,22,23,27,28-octaaza-tricyclo[30.2.2.215,18]octatriaconta-1(35),6,9,15(38),16,18(37),23,26,32(36),33-decaene-2,14,19,31-tetraone
TMSB	4,12,21,29-Tetrathioxo-3,13,20,30-tetrathia-5,6,10,11,22,23,27,28-octaaza-tricyclo[30.2.2.215,18]octatriaconta-1(35),6,9,15(38),16,18(37),23,26,32(36),33-decaene-2,14,19,31-tetraone
L	Ligand
FT-IR	Fourier Transform Infrared
NMR	Nuclear Magnetic Resonance
TMS	Tetramethylsilane
ppm	Parts per million
MS	Mass spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
UV-Vis	Ultraviolet-Visible
EJ-28	Invasive human bladder carcinoma cell line
RT-112	Minimally-invasive human bladder carcinoma cell line
IC_{50}	Inhibition concentration at 50%

ELISA	Enzyme-linked immunosorbent assay
OD	Optical density
BD tube	BD polystyrene round-bottom tube
CT-DNA	Calf thymus- deoxyribonucleic acid
K_b	Binding constant
MRSA	Methicillin resistant *staphylococcus aureus*
B.C.	*Bacillus cereus*
S.A.	*Staphylococcus aureus*
E.C.	*Escherichia coli*
K.P.	*Klebsiella pneumonia*
S.T.	*Salmonella typhimurium*
S.S.	*Shigella sonnei*
cfu	Colony forming units
MIC	Minimum inhibitory concentration
D	Decomposed
M	Metal
Ar.	Aromatic
m/z	Mass/charge ratio
B.M.	Bohr magneton
μ_{eff}	Magnetic moment
LMCT	Ligand to metal charge transfer
MCF-7	Human breast cancer cell lines
DCFH	2', 7'- dichlorodihydrofluorescin
RFU	Relative fluorescence unit
FITC	Fluorescein isothiocyanate

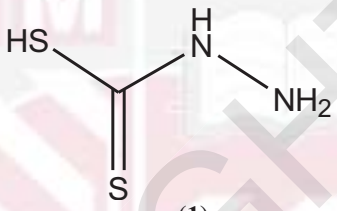
ε_a	Extinction coefficient observed
ε_f	Extinction coefficient of free complex
ε_b	Extinction coefficient of complex that fully bound to CT-DNA
I_o	Fluorescence intensities of complex in the absence of DNA
I	Fluorescence intensities of complex in the presence of DNA
k_{sv}	Stern-Volmer constant
η	Viscosity value
t	Observed flow time of DNA-containing complex solution
t_o	Flow time of CT-DNA alone
LPS	Lipopolysaccharide

CHAPTER 1

INTRODUCTION

1.1 Dithiocarbazate

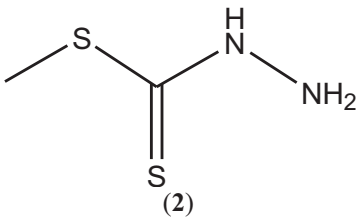
Dithiocarbazate, $NH_2NHCS_2^-$ (**1**) and its substituted derivatives, especially ligands with nitrogen and sulphur as donor atoms have been of great interest to researchers over the past few decades. There have also been studies on Schiff bases prepared through condensation of dithiocarbazate with various aldehydes and ketones that yielded bidentate, tridentate or multidentate chelating agents (Tarafder *et al.*, 2000).



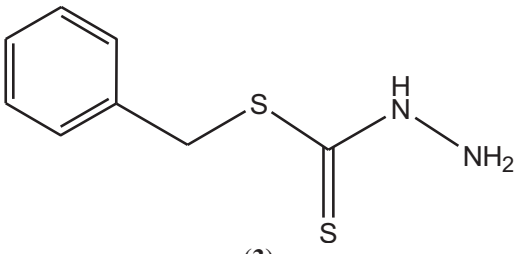
(**1**)

Dithiocarbazates are very promising compounds from the view point of coordination chemistry because of their ability for complexation (Singh *et al.*, 2009). The general examples of dithiocarbazates used are hydrazine or ethyldiamine, NH(H)NHCS(S)CH(H)R (R = methyl/benzyl/2,3 or 4-methylbenzyl groups), and $NH(H)(CH_2)_nNH(H)$ (n = 2 or 3) that act as primary amines.

S-methyldithiocarbazate, SMDTC (**2**) and S-benzyldithiocarbazate, SBDTC (**3**) are examples of substituted derivatives of dithiocarbazate. SBDTC has four potential donor atoms of which two are sterically available for coordination with metal ions (Ali *et al.*, 2006). Thus, in principle, both nitrogen-sulphur and sulphur-sulphur chelated structures are possible.



(**2**)



(**3**)

Dithiocarbazate and its derivatives are known to undergo thione-thiol tautomerism when in solution (Figure 1). They are predominantly in the thione form (NH-C=S) when in the solid state. In solution, dithiocarbazate will convert to the thiol form (N=C-SH) and when in the presence of metal ions, this allows bonding *via* the deprotonation process from the –SH group (Ali *et al.*, 2005).

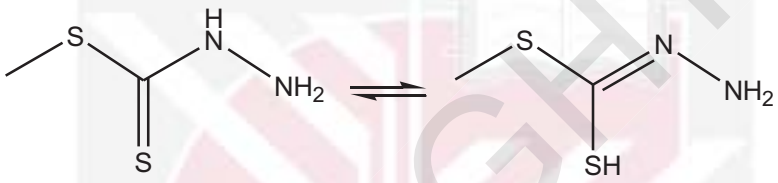


Figure 1: An example of thione-thiol tautomerism

Dithiocarbazate and its derivatives have obtained considerable attention and have been important over the past few decades because of their biological activity (Tarafder *et al.*, 2002). They have unique structural features and potential pharmacological applications. In addition, these compounds have a wide variety of bonding and stereochemistry. Hence, many types of complexes with different geometries and properties can be formed *via* a relatively easy preparative method involving fewer steps and chemicals, hence with lower costs.

1.2 Macrocyclic Schiff Bases

Dithiocarbazates react with carbonyl compounds (either aldehydes or ketones) to synthesise Schiff bases *via* a condensation reaction. Schiff bases are compounds that contain a carbon-nitrogen double bond and are a diverse class of ligands that contain oxygen, nitrogen as well as sulphur as their main donor atoms for chelation on complexation. They are important in the pharmaceutical and medicinal field (Tiwari *et al.*, 2011) and have applications as antibacterial (Azarkish and Sedaghat, 2012), antifungal (Abou-Hussein and Linert, 2012), and antitumor agents (Labisbal *et al.*, 2000), with good reactivity against microbes and cancer cells. Schiff bases have also been reported to find use as flourometric analytical reagents in spectroscopy, in chromatography, as specific metal-ion membrane sensors (Kedy *et al.*, 2015), and in catalysis (Mieczynska *et al.*, 2015).

Many Schiff bases have been designed to mimic the function of natural carriers in transporting specific metal ions and molecules, and in understanding the catalytic activity of metalloenzymes and proteins (Sengottuvelan *et al.*, 2007). The successful application of ligands and their complexes in enzyme mimicking studies, redox catalysts and as potent antibacterial agents, as well as in other uses as radiopharmaceuticals, MRI reagents and fluorescent probes have attracted the attention of many researchers (Sreedaran *et al.*, 2008).

Macrocyclic Schiff bases are synthesised by reacting dithiocarbazates with dicarbonyl compounds. The term macrocycle is defined as a cyclic macromolecule or a cyclic compound with nine or more members (Constable, 1999). In coordination chemistry, the functionally substituted Schiff bases bearing additional donor groups represent the most important class of heteropolydentate ligands capable of forming mono-, bi-, and polynuclear complexes with transition and non-transition metals.

Macrocycles have been of great importance in macrocyclic and supramolecular chemistry (Borisova *et al.*, 2007). Interest in exploring the synthesis of metal ion complexes with macrocyclic ligands has been continuously increasing owing to the recognition of the role played by these structures in metalloproteins. The macrocycle ring enables a molecule to achieve a degree of structural pre-organization, such that key functional groups can interact across extended binding sites in proteins without a major entropic loss on binding (Driggers *et al.*, 2008).

For macrocycles, the hole size represents an additional parameter which may influence greatly the ability to discriminate among the different charged or neutral species to be recognized while for the macroacyclic systems, interesting properties may arise from their higher flexibility (Vigato and Tamburini, 2004). Hence, different donor atoms, the number and size of the chelating rings formed, and the geometries determine the selective binding of the charged metal ions. The progressive enlargement of the coordinating moiety due to the presence of two or more metal ions allowed further understanding of the physico-chemical and biological properties in similar coordinating moieties.

There is increasing interest in the potential applications of macrocylic Schiff bases and their metal complexes. This is because of their mixed soft-hard donor character (Abou-Hussein and Linert, 2012) where, in this work, the nitrogen is the hard donor while sulphur is the soft donor. According to Ali and Livingstone (1974), the permanent dipole moment and coordinating ability toward metal ions normally decreases in the order of $H_2O > ROH > R_2O$. But, this order is reverse for sulphur donors ($H_2S < RSH < R_2S$).

Besides, the strength of bonding to a metal ion is $RO^- > RS^-$ and $R_2O > R_2S$. If Π bonding occurs, the order is reversed to $RO^- < RS^-$ and $R_2O < R_2S$. Furthermore, sulphur donor atoms bind more strongly to (b) class metals (4d transition metals) compared with oxygen donor atoms. The low-spin d^8 ions such as palladium ions and

d^9 ions copper ions have high stability constants when sulphur donor atoms are involved in complexation. Hence, they form strong α-bonds with soft ligands (sulphur as donor atoms) and also d_Π - d_Π bonds by donating a pair of electrons to the ligand. The properties of sulphur ligands also apply to sulphur-nitrogen chelating ligands (Ali and Livingstone, 1974).

1.3 Transition Metal Complexes

A metal complex is a single central atom or ion that is connected to surrounding atoms or molecules through bonding. A ligand is an atom, ion, or molecule that has at least one pair of lone pair electrons to donate to a central metal to form a complex. So, ligands are capable of functioning as the electron pair donor in a coordinate covalent bond (bonding between ligands and central metal) formed with the metal atom or ion. In this study, the macrocyclic Schiff bases act as the ligands.

Normally, complexes require overall neutrality. If the directly-bonded ligands, ie the inner-sphere ligands, do not balance the overall charge on the central atom, it requires ionic interactions with outer-sphere ligands or another set of ions. The inner-sphere ligands arrange themselves in certain geometry to produce geometric structures for the complexes. A polydentate ligand can be attached to the metal atom by bonds from two or more donor atoms (Jolly, 1996). Examples of donor atoms in the ligand are oxygen, nitrogen as well as sulphur. So, when two atoms possessing lone pair electrons are present in a molecule, it may bond *via* both of these atoms, and is termed as a bidentate ligand.

Currently, there are many research reports on the preparation of metal complexes with different geometries and properties. Dithioligands are very promising compounds from the view point of coordination chemistry due to their ability to complex with metal ions easily and also that small changes in the backbone of the structure of these ligands can lead to wide variations in biological activity (Tarafder *et al.*, 2000). The ability to chelate to metal ions, steric effects of the structure, electronic effects and lipophilicity are the four major properties for the effectiveness of the synthesised drug. In addition, the shape, structure or the geometry of a certain molecule are also important factors in drug activity (Gringauz, 1997).

Metal complexes have been found to be more active against several bacterial species than the parent Schiff base especially macrocyclic metal complexes (Mohamed *et al.*, 2009). This was due to the more stable structure of the metal complexes as compared to the unstable parent Schiff base. Normally, metal complexes will have a higher melting point as compared to the Schiff base. Some Schiff bases decompose when heat is applied (Kalia *et al.*, 2011). In this project, three 3d metals were used which were nickel, copper and zinc and five 4d metals were chosen which were niobium, molybdenum, ruthenium, palladium and cadmium.

1.4 Cytotoxicity of Some Sulphur –Nitrogen Ligands and Their Metal Complexes

The biological activity of a chemical species can be explained by experimental and computational methods. There are a lot of theoretical studies about the determination of chemical activity (Sayin and Karakas, 2013). Generally, quantum chemical descriptors are used to determine the ranking of biological activities. The examples of these parameters include the highest occupied molecular orbital energies (HOMO), the lowest unoccupied molecular orbital energies (LUMO), the energy gap between LUMO and HOMO, hardness or softness of the molecules or atoms and the global electronegativity.

The biological activities closely depend on the separation of the LUMO and HOMO in a molecule. The binding ability of an inhibitor to the appropriate molecule will increase with the increase of the HOMO and decrease of the LUMO of complex ions. This is due to the ability of electrons to transfer to the acceptor molecule and the strong electron accepting ability of the molecules (Alexander and Moccari, 1993). The smaller the energy gap between HOMO and LUMO, the more active the molecule is in the term of biological properties (Zhang *et al.*, 2012). This is because the electrons are easily excited from the lower energy orbital to higher energy orbital. Besides that, soft complexes (complexes in which sulphur atoms act as donor atoms) have a small energy gap between the molecular orbital and can interact easily with biological molecules. Hence, the biological activity is increased with the increase of softness of the complexes.

Cytotoxicity of some sulphur –nitrogen ligands and their metal complexes is based on four main criteria. Firstly, the complex should be reasonably labile. Zinc and cadmium complexes are the most labile with d^{10} configurations (Shriver and Atkins, 1999). Secondly, the metal chelate should have reasonably high thermodynamic stability. The metals used on complexation should be (b) class metals (4d metals), in particular palladium and platinum due to its similarity to cisplatin, a common anticancer drug used in cancer treatment. Complexes or ligands with sulphur acting as donor atoms are the most likely to be effective drugs. This is because they allow for lipid solubility of the stable metal complexes (Ali and Livingstone, 1974).

1.5 Mechanism of Death Studies on Synthetic Complexes

Obstruction of the clinical bladder cancer management is due to the high rates of recurrence and various series of muscle invasive. The bladder cancer is range from low risk non-invasive to muscle-invasive tumors. More than 70% of the bladder cancer's patients will recurrence within five years after the treatments. Currently, various clinically reliable drugs for the treatment of bladder cancer are discover to overcome the resistance mechanisms involved in the treatments such as chemotherapy.

The chemotherapy drugs used to treat bladder cancer are cisplatin, gemcitabine, methotrexate, vinblastine, doxorubicin, and mitomycin C. Survival rate can be increased with combination chemotherapy. Currectly, the combination of gemcitabine and cisplatin with lower toxicity has been used in the treatment of bladder cancer. Mitomycin C also been used in the treatment. Treatment using mitomycin C resulted in decreased recurrence rates (Maase *et al.*, 2000; Tolley *et al.*, 1996).

Apoptosis is usually the expected mechanism of death of the synthesised anti-cancer compounds or drugs. Apoptosis is a mechanism or process of programmed cell death without damaging normal cells that occurs in multicellular organisms (Green *et al.*, 2011). When apoptosis occurs, it will cause a change in cell morphology that leads to the blebbing process, cell shrinkage, nuclear fragmentation, chromosomal DNA fragmentation, and chromatin condensation (Cohen *et al.*, 1992; Martin and Green, 1995). The apoptosis process in cancer cells is triggered by body signals. The cell begins to shrink and the proteins in the body are activated to break down cellular components. Subsequently, enzymes will start to fragment down the nucleus and the cell starts blebbing which causes the cell to break into several smaller pieces. The macrophages recognise the cell parts and then will remove them from the body. Many different signals and pathways lead to apoptosis, but there is only one mechanism that actually causes the death of a cell. It undergoes organised degradation of cellular organelles by activated proteolytic caspases after a cell receives stimulus. In addition to the destruction of cellular organelles, mRNA is rapidly and globally degraded by a mechanism that is not yet fully characterised (Thomas *et al.*, 2015). mRNA decay is triggered very early in apoptosis. There are various biochemical techniques for the analysis of cell death caused by apoptosis such as phosphatidylserine exposure, reactive oxygen species generation, caspase activation, and DNA fragmentation.

Annexin V binding assay is one of the techniques used to study apoptosis in terms of phosphatidylserine (PS) exposure. PS exposure during apoptosis reviews biodirectional trafficking between plasma membrane and cytoplasm in organisms (Fadok *et al.*, 1992). In addition, PS exposure on the external leaflet of the plasma membrane is widely observed during the apoptosis process and forms the basis for the Annexin V binding assay to detect apoptotic cell death (Lee *et al.*, 2013). The activation of a calcium-mediated and phospholipid scramblase trafficking of lysosomes to the cell surface are currently two main potential mechanisms to explain PS exposure. A two-step model has been proposed, in which the first step is the internalisation of the plasma membrane to form cytoplasmic vesicles occurs as cells shrink during apoptosis. This is followed by Ca^{2+} dependent trafficking of some of these vesicles back to the cell surface, leading to PS externalisation (Mirnikjoo *et al.*, 2009).

In the reactive oxygen species (ROS) generation, the significance of ROS as aggravating or primary factors in numerous pathologies is widely recognized. The mitochondrion is considered the major intracellular source of ROS (Orrenius *et al.*, 2007). Generally, there are several harmful effects of reactive oxygen species on the cell such as deletions and mutations (Murphy, 2009), mitochondrial dysfunction, damage of DNA, and lipid peroxidation. Hydrogen peroxide, H_2O_2 being a chief messenger molecule is involved in physiological signaling cascades regulating various

cellular and organ functions (Stone and Yang, 2006). The origin of ROS species produced in mitochondria is thought to be superoxide (Andreyev *et al.*, 2005). Superoxide, $O_2^{\bullet-}$ does not easily permeate cell and mitochondrial membranes because of the presence of the negative charge. The rate of ROS production is measured by identifying major sites of ROS production and comparing the rates of ROS production in mitochondria isolated from normal and diseased tissue (cancerous tissue). The most recent and reliable method to measure low levels of H_2O_2 *in vitro* is horseradish peroxidase which can trap emittion of H_2O_2 with high selectivity and affinity. Amplex Red Ultra (a derivative of 10-acetyl-3,7- dihydroxyphenoxazine) acts as a sensitive fluorescent probe for H_2O_2 (Zhou *et al.*, 1997). The rate of $O_2^{\bullet-}$ production is lower and the production sites are uncertain when mitochondria actively generates adenosine triphosphate, ATP. This suggests that *in vivo* conditions leading to RET or an accumulation of NADH will favour $O_2^{\bullet-}$ production.

This chapter presents an overview of basic explanations for the key terms in this thesis. Five different macrocyclic Schiff bases and 40 different macrocyclic metal complexes were synthesised and their biological activities including the anticancer, DNA binding interaction, and antibacterial properties were studied. Macrocyclic complexes that are biologically relevant and may have potential to be used as anticancer or antibacterial agents due to the various properties observed were fully synthesised and characterised. To further study the behavior of a compound against the cancerous cells, mechanism of death studies were conducted to study the apoptosis process. The overall structure, coordination, geometry, lipophilicity and planarity were the important aspects resulting in their selection for a more detailed study of the biological activities.

1.6 Problem Statements

Synthetic drugs such as macrocyclic Schiff bases and their metal complexes are important due to their interesting biological properties such as anticancer and antibacterial. Hence, macrocyclic Schiff bases derived from dithiocarbazate were synthesised *via* a [2+2] type condensation reaction. There are many types of cancers based on the cell in which the cancer originates. From the literature, insufficient study was found on macrocyclic compounds tested against various cancer cell lines especially bladder cancer. Bladder cancer is considered to be the fourth most common type of cancer in men. Cisplatin-based drugs are still used as the frontline chemotherapy drug in the treatment of bladder cancer. Hovewer, the side effects of cisplatin can be very severe. Therefore, the cytotoxic activity of macrocyclic compounds was determined against bladder cancer. Besides, no mechanism of death studies were performed to investige the apoptosis process for the macrocyclic compounds especially nitrogen-sulphur macrocyclic complexes. Hence, these studies were conducted to predict the macrocyclic complexes as potential pharmaceutical drugs.

1.7 Objectives

This study was conducted in the synthesis and characterisation of novel dithiocarbazate, macrocyclic Schiff bases and macrocyclic complexes. Besides, this study hypothesised that the macrocyclic compounds were able to have various biological activities. Therefore, this study was focused at the determination of cytotoxic and antibacterial studies. The specific objectives of this study include:

- To synthesise novel macrocyclic Schiff bases derived from dithiocarbazate and their transition metal complexes [M = Ni(II), Cu(II), Zn(II), Cd(II), Nb(II), Mo(V), Ru(III) and Pd(II)].
- To characterise the macrocyclic Schiff bases and transition metal complexes *via* various physico-chemical techniques (elemental analysis, magnetic susceptibility, and molar conductivity) and spectroscopic techniques.
- To determine the cytotoxic, antibacterial, and DNA binding properties of the Schiff bases and their complexes.
- To investigate the mechanism of death of the active compounds *via* Annexin V binding and ROS assays.

REFERENCES

Abou-Hussein, A.A.A. and W. Linert (2012). Synthesis, spectroscopic and biological activities studies of acyclic and macrocyclic mono and binuclear metal complexes containing a hard-soft Schiff base, *Spectrochimica Acta Part A*, **95**: 596–609

Agarwal, R. and S.D. Chase (2002). Rapid, fluorimetric–liquid chromatographic determination of malondialdehyde in biological samples, *Journal of Chromatography B*, **775**: 121–126

Alexander, D.B. and A.A. Moccari (1993). Evaluation of corrosion inhibitors for component cooling water systems, *Corrosion*, **49**: 921-928

Ali, M.A. and S.E Livingstone (1974). Metal complexes of sulphur-nitrogen chelating agents, *Coordination Chemistry Reviews*, **13**: 101-132

Ali, M.A. and M.T. Tarafder (1977). Metal complexes of sulphur and nitrogen-containing ligands: complexes of S-benzyldithiocarbazate and a Schiff base formed by its condensation with pyridine-2-carboxaldehyde, *Journal of Inorganic and Nuclear Chemistry*, **39**: 1785-1791

Ali, M.A., A.H Mirza, M.H.S.A. Hamid and P.V. Bernhardt (2005). Diphenyltin(IV) complexes of the 2-quinolinecarboxaldehyde Schiff bases of S-methyl- and S-benzyldithiocarbazate (Hqaldsme and Hqaldsbz): X-ray crystal structures of Hqaldsme and two conformers of its diphenyltin(IV) complex, *Polyhedron*, **24**: 383-390

Ali, M.A., M.H. Mirza, F. HjBujang, M.H.S.A. Hamid and P.V. Bernhardt (2006). Synthesis, characterization and X-ray crystallographic structural study of copper(II) and nickel(II) complexes of the 2-quinolinecarboxaldehyde Schiff base of *S*-methyldithiocarbazate (Hqaldsme), *Polyhedron*, **25**: 3245-3252

Anbu, S., M. Kandaswamy, P. Suthakaran, V. Murugan and B. Varghese (2009). Structural, magnetic, electrochemical, catalytic, DNA binding and cleavage studies of new macrocyclic binuclear copper(II) complexes, *Journal of Inorganic Biochemistry*, **103**: 401–410

Andreyev, A.Y., Y.E. Kushnareva and A.A. Starkov (2005). Mitochondrial metabolism of reactive oxygen species, *Biochemistry (Moscow)*, **70**: 200-214

Azarkish, M. and T. Sedaghat (2012). Synthesis, spectral studies, thermal behavior, and antibacterial activity of Ni(II), Cu(II), and Zn(II) complexes with an ONO tridentate Schiff base, *Chinese Chemical Letters*, **23**: 1063-1066

Baguley, B.C. and M.L. Bret (1984). Quenching of DNA-ethidium fluorescence by amsacrine and other antitumor agents: a possible electron-transfer effect, *Biochemistry*, **23**: 937-943

Balhausen, C. J. (1962). An introduction to ligand field, McGraw Hill, New York

Banerjee, S., A.K. Mukherjee, I. Banerjee, R.L. De, M.A. Neumann and D. Louer (2005). Synthesis, spectroscopic studies and *ab-initio* structure determination from X-ray powder diffraction of bis-(N-3-acetophenylsalicylaldiminato)copper(II), *Crystal Research and Technology*, **40**: 815-821

Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck (1966). Antibiotic susceptibility testing by a standardized single disc method, *American Journal of Clinical Pathology*, **45**: 493-496

Bayer, E. and G. Schenk (1960). Rearrangement of organic complex formers and specificity of complex formation, *Chemische Berichte*, **93**: 1184-1193

Bertrand, J.A. and R.I. Kaplan (1966). A study of bis(hexafluoroacetylacetonato)copper(II), *Inorganic Chemistry*, **5**: 489-491

Bohn, H., N. Inaba, N. Sato, M. Ijichi, I. Fukazawa, A. Nito, H. Takamizawa and G. Luben (1984). The immunocytochemical location of two membraneassociated placental tissue proteins in human and cynomolgus monkey placentae, *Tumour Biology*, **5**: 75–85

Borisova, N.E., M.D. Reshetova and Y.A. Ustynyuk (2007). Metal-free methods in the synthesis of macrocyclic Schiff bases, *Chemical Reviews*, **107**: 46-79

Boulikas, T. and M. Vougiouka (2003). Cisplatin and platinum drugs at the molecular level, *Oncology Reports*, **10**: 1663–1682

Brauchi, S., C. Cea, J.G. Farias, J. Bacigalupo and J.G. Reyes (2006). Apoptosis induced by prolonged exposure to odorants in cultured cells from rat olfactory epithelium, *Brain Research*, **1103**: 114–122

Brauers, A. and G. Jakse (2000). Epidemiology and biology of human urinary bladder cancer, *Journal of Cancer Research and Clinical Oncology*, **126**: 575-583

Buttke, T.M. and P.A. Sandstrom (1994). Oxidative stress as a mediator of apoptosis, *Immunology*, **15**: 7-10

Cakir, S., I. Bulut, P. Naumov, E. Bicer and O. Cakir (2001). Synthesis and spectroscopic studies of novel Cu(II), Co(II), Ni(II) and Zn(II) mixed ligand complexes with saccharin and nicotinamide, *Journal of Molecular Structure*, **560**: 1-7

Cao, D.X., B. Qiao, Z.Q. Ge and Y.J. Yuan (2005). Amplification loop cascade for increasing caspase activity induced by docetaxel, *Journal of Cellular Biochemistry*, **96**: 810–820

Cater, M. A., H.B. Pearson, K.Wolyniec, P.Klaver, M. Bilandzic, B.M. Paterson, A.I. Bush, P.O. Humbert, S.L. Fontaine, P.S. Donnelly and Y. Haupt (2013). Increasing intracellular bioavailable copper selectively targets prostate cancer cells, *American Chemical Society Chemical Biology*, **8**: 1621–1631

Chand, D.K., H.J. Schneider, J.A. Aguilar, F. Escartí, E. García-España and S.V. Luis (2001). Copper complexes of polyaza[*n*]cyclophanes and their interaction with DNA and RNA, *Inorganica Chimica Acta*, **316**: 71-78

Chandra, S. and L.K. Gupta (2005). Spectroscopic studies on Co(II), Ni(II) and Cu(II) complexes with a new macrocyclic ligand: 2,9-dipropyl-3,10-dimethyl-1,4,8,11-tetraaza-5,7:12,14-dibenzocyclotetradeca-1,3,8,10-tetraene, *Spectrochimica Acta Part A*, **61**: 1181-1188

Chandra, S., P. Pipil and S. Agarwal (2015). Synthesis of macrocyclic ligands and their cobalt(II) complexes spectral characterization and antimicrobial studies, *International Journal of Pharmaceutical Sciences and Research*, **6**: 1258-1265

Cheng, C.H., D.F. Chen, H.B. Song and L.F. Tang (2013). Synthesis and catalytic activity of N-heterocyclic carbene silver complexes derived from 1-[2-(pyrazol-1-yl)phenyl]imidazole, *Journal of Organometallic Chemistry*, **726**: 1-8

Chen, Q., T. Yano, H. Matsumi, Y. Osuga, N. Yano, J. Xu, O. Wada, K. Koga, T. Fujiwara, K. Kugu and Y. Taketani (2005). Cross-talk between Fas/Fas ligand system and nitric oxide in the pathway subserving granulosa cell apoptosis: a possible regulatory mechanism for ovarian follicle atresia, *Endocrinology*, **146**: 808–815

Chohan, Z.H., C.T. Supuran and A. Scozzafava (2005). Metal binding and antibacterial activity of ciprofloxacin complexes, *Journal of Enzyme Inhibition and Medicinal Chemistry*, **20**: 303–307

Chohan, Z.H., M.U. Hassan, K.M. Khan and C.T. Supuran (2005). *In-vitro* antibacterial, antifungal and cytotoxic properties of sulfonamide—derived Schiff's bases and their metal complexes, *Journal of Enzyme Inhibition and Medicinal Chemistry*, **20**, 181-188

Cohen, G.N. (2011). The outer membrane of Gram-negative bacteria and the cytoplasmic membrane, Springer, 13-21

Cohen, J.J., R.C. Duke, V.A. Fadok and K.S. Sellins (1992). Apoptosis and programmed cell death in immunity, *Annual Review of Immunology*, **10**: 267-293

Constable, E.C. (1999). Coordination chemistry of macrocyclic compounds, Oxford University Press Inc, New York

Cotton, F.A. and G. Wilkinson (1988). Advanced inorganic chemistry, fifth edition, Wiley, New York

Crouse, K.A., K.B. Chew and M.T.H. Tarafder (2004). Synthesis, characterization and bio-activity of *S*-2-picolyldithiocarbazate (S2PDTC), some of its Schiff bases and their Ni(II) complexes and X-ray structure of *S*-2-picolyl-β-*N*-(2-acetylpyrrole)dithiocarbazate, *Polyhedron*, **23**: 161-168

Díaz, T.G., A. Guiberteau, M.D.L. Soto and J.M. Ortiz (2006). Determination of copper with 5,5-dimethylcyclohexane-1,2,3-trione 1,2-dioxime 3-thiosemicarbazone in olive oils by adsorptive stripping square wave voltammetry, *Food Chemistry*, **96**: 156–162

Ding, C.X., J. Ni, Y.H. Yang, S.W. Ng, B.W. Wang and Y.S. Xie (2012). Mono-, tetra- and octanuclear transition metal complexes of in situ generated Schiff base ligands containing up to 12 coordinating atoms: syntheses, structures and magnetism, *Crystal Engineering Communications*, **14**: 7312–7319

Domijan, A.M., J. Ralić, S.R. Brkanac, L. Rumora and T. Žanić-Grubišić (2014). Quantification of malondialdehyde by HPLC-FL – application to various biological samples, *Biomedical Chromatography*, **29**: 41–46

Donnelly, P.S., J.R. Liddell, S. Lim, B.M. Paterson, M.A. Cater, M.S. Savva, A.I. Mot, J.L. James, I.A. Trounce, A.R. White and P.J. Crouch (2012). An impaired mitochondrial electron transport chain increases retention of the hypoxia imaging agent diacetylbis(4-methylthiosemicarbazonato)copper^{II} , *Proceedings of the National Academy of Sciences*, **109**: 47–52

Driggers, E.M., S.P. Hale, J. Lee and N.K. Terrett (2008). The exploration of macrocycles for drug discovery - an underexploited structural class, *Nature Reviews Drug Discovery*, **7**: 608-624

Du, D., Z. Jiang, C. Liu and A.M. Sakho (2011). Macrocyclic organotin(IV) carboxylates based on benzenedicarboxylic acid derivatives: syntheses, crystal structures and antitumor activities, *Journal of Organometallic Chemistry*, **696**: 2549-2558

El-Asmy, A.A., A.Z. Al-Abdeen, W.M.A. El-Maaty and M.M. Mostafa (2010). Synthesis and spectroscopic studies of 2,5-hexanedione *bis*(isonicotinylhydrazone) and its first raw transition metal complexes, *Spectrochimica Acta Part A*, **75**: 1516-1522

El-Boraey, H.A. and El-Gammal O.A. (2015). New 15-membered tetraaza (N4) macrocyclic ligand and its transition metal complexes: spectral, magnetic, thermal and anticancer activity, *Spectrochimica Acta Part A*, **138**: 553–562

El-Gammal, O.A., G.M.A. El-Reash, S.E. Ghazy and A.H. Radwan (2012). Synthesis, characterization, molecular modeling and antioxidant activity of (1E,5E)-1,5-bis(1-(pyridin-2-yl)ethylidene)carbonohydrazide (H_2APC) and its zinc(II), cadmium(II) and mercury(II) complexes, *Journal of Molecular Structure*, **1020**: 6–15

El-Gammal, O.A., M.M. Bekheit and S.A. El-Brashy (2015). Synthesis, characterization and in vitro antimicrobial studies of Co(II), Ni(II) and Cu(II) complexes derived from macrocyclic compartmental ligand, *Spectrochimica Acta Part A*, **137**: 207–219

Esterbauer, H., R.J. Schaur and H. Zollner (1991). Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes, *Free Radical Biology and Medicine*, **11**: 81–128

Fadok, V.A., D.R. Voelker, P.A. Campbell, J.J. Cohen, D.L. Bratton and P.M. Henson (1992). Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages, *The Journal of Immunology*, **148**: 2207-2216

Fogelman, A.M., I. Shechter, J. Seager, M. Hokom, J.S. Child and P.A. Edward (1980). Malondialdehyde alteration of low density lipoproteins leads to cholesteryl ester accumulation in human monocyte- macrophages, *Proceedings of the National Academy of Sciences*, **77**: 2214-2218

Frisch, M.J., G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A. Petersson and et al. (2009). Gaussian 09, Revision C.01, Gaussian Inc., Wallingford

Gasinski, J.P., J.R. Bianchani, J.C. Cueva, F.A. El-Saied, A.A. El-Asmy and D.X. West (2003). Spectral and structural studies of the copper(II) complexes of 3, 4-hexanedione bis(3-azacyclothiosemicarbazones), *Journal of Inorganic and General Chemistry*, **629**: 202-206

Geary, W.J. (1971). The use of conductivity measurements in organic solvents for the characterisation of coordination compounds, *Coordination Chemistry Reviews*, **7**: 81–122

Gottlieb, H.E., V. Kotlyar and A. Nudelman (1997). NMR chemical shifts of common laboratory solvents as trace impurities, *The Journal of Organic Chemistry*, **62**: 7512–7515

Green, D. (2011). Means to an End: Apoptosis and other Cell Death Mechanisms, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press

Gringauz, A (1997). Drugs and the cardiovascular diseases, *Introduction to Medicinal Chemistry: How Drugs Act and Why*, 450-461

Grotto, D., L.D.S. Maria, S. Boeira, J. Valentini, M.F. Charao, A.M. Moro, P.C. Nascimento, V.J. Pomblum and S.C. Garcia (2007). Rapid quantification of malondialdehyde in plasma by high performance liquid chromatography–visible detection, *Journal of Pharmaceutical and Biomedical Analysis*, **43**: 619–624

Hadizadeh, S., N. Najafzadeh, M. Mazani, M. Amani, H. Mansouri-Torshizi and A. Niapour (2014). Cytotoxic effects of newly synthesized palladium(II) complexes of diethyldithiocarbamate on gastrointestinal cancer cell lines, *Biochemistry Research International*, **2014**: 1-9

Halliwell, B and S. Chirico (1993). Lipid peroxidation: its mechanism, measurement, and significance, *American Journal of Clinical Nutrition*, **57**: 715-725

Haudenschild, C.C. and S.M. Schwartz. Endothelial regeneration. II. restitution of endothelial continuity, *Laboratory Investigation*, **41**: 407–418

Hazra, S., S. Majumder, M. Fleck, R. Koner and S. Mohanta (2009). Syntheses, structures, absorption and emission properties of a tetraiminodiphenol macrocyclic ligand and its dinuclear Zn(II) and Pb(II) complexes, *Polyhedron*, **28**: 2871-2878

Helsel, M.E. and K.J. Franz (2015). Pharmacological activity of metal binding agents that alter copper bioavailability, *Dalton Transactions*, **44**: 8760-8770

He, X.F., H. Chen, L. Xu and L.N. Ji (1998). Synthesis, characterization and DNA binding study of Co(III) polypyridyl mixed-ligand complexes, *Polyhedron*, **17**: 3161-3166

Hollstein, U (1974). Actinomycin. Chemistry and mechanism of action, *Chemical Reviews*, **74**: 625–652

Hossain, M.E., M.N. Alam, J. Begum, M.A. Ali, M. Nazimuddin, F.E. Smith and R.C. Hynes (1996). The preparation, characterization, crystal structure and biological activities of some copper(II) complexes of the 2-benzoyalpyridine Schiff bases of S-methyl-N-S-benzyl dithiocarbazate, *Inorganica Chimica Acta*, **249**: 207–210

Hossain, M.E., M.N. Alam, M.A. Ali and M. Nazimuddin (1996). The synthesis, characterization and bioactivities of some copper(II) complexes of the 2-acetylpyridine schiff bases of s-methyl- and s-benzyldithiocarbazate, and the x-ray crystal structure of the nitrato(s-benzyl-β-n-(2-acetylpyridyl) methylenedithiocarbazato)copper(II) complex, *Polyhedron*, **15**: 973-980

Huang R., A. Wallqvist and D.G. Covell (2005). Anticancer metal compounds in NCI's tumor-screening database: putative mode of action, *Biochemical Pharmacology*, **69**: 1009-1039

Ilhan, S. (2008). Preparation and characterization of binuclear Cu(II) complexes derived from diamines and dialdehydes, *Journal of Coordination Chemistry*, **61**: 2884-2895

Jadamus, H., Q. Fernando and H. Freiser (1964). Metal ion induced rearrangements of bisbenzthiazolines to Schiff-base chelates, *Journal of the American Chemical Society*, **86**: 3056-3059

Jaffe H.H., S.J. Yeh and R.W. Gardner (1958). The electronic spectra of azobenzene derivatives and their conjugate acids, *Journal of Molecular Spectroscopy*, **2**: 120-136

Jeffrey, H. (2008). Organic Structure Determination using 2-D NMR spectroscopy: a problem-based approach, Elsevier, 384-394

Jeragh, B. and A.A. El-Asmy (2014). Spectroscopic and structural study of some 2,5-hexanedione bis(salicyloylhydrazone) complexes: Crystal structures of its Ni(II) and Cu(II) complexes and N-(2,5-dimethyl-1H-pyrrol-1-yl)-2-hydroxybenzamide, *Spectrochimica Acta Part A*, **129**: 307–313

Jeragh, B., M.S. Ali and A.A. El-Asmy (2015). Synthesis, crystal structure, characterization and biological activity of 2,5-hexanedione bis(isonicotinylhydrazone) and N-(2,5-dimethyl-1H-pyrrol-1-yl)isonicotinamide complexes, *Spectrochimica Acta Part A*, **150**: 504–513

Jolly, W.L. (1996). Review of Submarine Escape Action Levels for Selected Chemicals, Modern Inorganic Chemistry, Berkeley: McGraw-Hill Book Company: 341-351

Kalia, S.B., K. Lumba and P. Sankhyan (2011). Magnetic and spectral studies on nickel(II) and copper(II) dithiocarbazates derived from isoniazid, *Journal of Coordination Chemistry*, **64**: 1216–1228

Kamath, A., K. Naik, S.P. Netalkar, D.G. Kokare and V.K. Revankar (2013). Pyrazole-bridged late first row transition metal complexes derived from hexadentate compartmental ligand: synthesis, characterization, antibacterial activity, and DNA binding/cleavage studies, *Medicinal Chemistry Research*, **22**: 1948–1956

Karmakar, I., S. Mandal and A. Mitra (2015). Evaluation of antimicrobial and insect repellent properties of two novel zinc (II), and nickel(II) complexes containing a tetradentate Schiff Base, *Inorganic Letters*, **2**: 6-13

Kedy, S., N. Almhna and F. Kandil (2015). Synthesis and characterization of new macrocyclic Schiff bases by the reaction of: 1,7-bis (6-methoxy-2-formylphenyl)-1,7-dioxaheptane and their use in solvent extraction of metals, *Arabian Journal of Chemistry*, **8**: 93-99

Kelly, J.M., A.B. Tossi, D.J. McConnell and C. OhUigin (1985). A study of the interactions of some polypyridylruthenium(II) complexes with DNA using fluorescence spectroscopy, topoisomerisation and thermal denaturation, *Nucleic Acids Research*, **13**: 6017-6034

Kemp, S., N.J. Wheate, D.P. Buck, M. Nikac, J.G. Collins and J.R. Aldrich-Wright (2007). The effect of ancillary ligand chirality and phenanthroline functional group substitution on the cytotoxicity of platinum(II)-based metallointercalators, *Journal of Inorganic Biochemistry*, **101**: 1049–1058

Keypour H., H. Khanmohammadi, K.P. Wainwright and M.R. Taylor (2004). Synthesis, crystal structure, NMR and ab initio molecular-orbital studies of some magnesium(II) macrocyclic Schiff-base complexes, with two 2-aminoethyl pendant arms, *Inorganica Chimica Acta*, **357**: 1283–1291

Keypour, H., H. Goudarziafshar, A.K. Brisdon and R.G. Pritchard (2007). New macrocyclic Schiff base complexes incorporating a phenanthroline unit: part 1; template synthesis of three cadmium(II) complexes and crystal structure, NMR and ab initio studies, *Inorganica Chimica Acta*, **360**: 2298-2306

Keypour, H., M. Rezaeivala, M. Mirzaei-Monsef, K. Sayin, N. Dilek and H. Unver (2015). Synthesis and characterization of Co(II), Ni(II), Cu(II) and Zn(II) complexes with a new homopiperazine macrocyclic Schiff base ligand, *Inorganica Chimica Acta*, **432**: 243–249

Khan, T.A, S. Naseem, S.N. Khan, A.U. Khan and M. Shakir (2009). Synthesis and spectral characterization of 14- and 16-membered tetraazamacrocyclic Schiff base ligands and their transition metal complexes and a comparative study of interaction of calf thymus DNA with copper(II) complexes, *Spectrochimica Acta Part A*, **73**:622-629

Khojasteh, R.R. and S.J. Matin (2015). Synthesis, characterization, and antibacterial activities of some metal complexes of heptadentate Schiff base ligand derived from acetylacetone, *Russian Journal of Applied Chemistry*, **88**: 921−925

Kim, Y.K., M.A. Arai, T. Arai, J.O. Lamenzo, E.F. Dean, N. Patterson, P.A. Clemons and S.L. Schreiber (2004). Relationship of stereochemical and skeletal diversity of small molecules to cellular measurement space, *Journal of the American Chemical Society*, **126**: 14740–14745

Kirkpatrick, G.J., J.A. Plumb, O.B. Sutcliffe, D.J. Flint and N.J. Wheate (2011). Evaluation of anionic half generation 3.5–6.5 poly(amidoamine) dendrimers as delivery vehicles for the active component of the anticancer drug cisplatin, *Journal of Inorganic Biochemistry*, **105**: 1115-1122

Klajner, M., C. Licona, L. Fetzer, P. Hebraud, G. Mellitzer, M. Pfeffer, S. Harlepp and C. Gaiddon (2014). Subcellular localization and transport kinetics of ruthenium organometallic anticancer compounds in living cells: a dose-dependent role for amino acid and iron transporters, *Inorganic Chemistry*, **53**: 5150-5158

Koch, A.L. (2003). Bacterial wall as target for attack past, present, and future research, *Clinical Microbiology Reviews*, 16: 673-687

Koh, L.L., O.L. Kon, K.W. Loh, Y.C. Long, J.D. Ranford, A.L.C. Tan and Y.Y. Tjan (1998). Complexes of salicylaldehyde acylhydrazones: cytotoxicity, QSAR and crystal structure of the sterically hindered t-butyl dimer, *Journal of Inorganic Biochemistry*, **72**: 155-162

Konstantakou, E.G., G.E. Voutsinas, P.K. Karkoulis, G. Aravantinos, L.H. Margaritis and D.J. Stravopodis (2009). Human bladder cancer cells undergo cisplatin-induced apoptosis that is associated with p53-dependent and p53-independent responses, *International Journal of Oncology*, **35**: 401–416

Koopman, G., C.P. Reutelingsperger, G.A. Kuijten, R.M. Keehnen, S.T. Pals and M.H. van Oers (1994). Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis, *Blood*, **84**: 1415– 1420

Kumar, G., S. Devi, R. Johari, and D. Kumar (2012). Synthesis, spectral characterization and antimicrobial evaluation of Schiff base Cr (III), Mn (III) and Fe (III) macrocyclic complexes, *European Journal of Medicinal Chemistry*, **52**: 269-274

Labisbal, E., A. Sousa, A. Castiñeiras, J.A. García-Vázquez, J. Romero and D.X. West (2000). Spectral and structural studies of metal complexes of isatin 3-hexamethyleneiminylthiosemicarbazone prepared electrochemically, *Polyhedron*, **19**: 1255-1262

Lakowicz, J.R. and G. Webber (1973). Quenching of protein fluorescence by oxygen. Detection of structural fluctuations in proteins on the nanosecond time scale, *Biochemistry*, **12**: 4171-4179

LaPensee, E.W., T.R. Tuttle, S.R. Fox and N.B. Jonathan (2009). Bisphenol A at low nanomolar doses confers chemoresistance in estrogen receptor-α–positive and –negative breast cancer cells, *Environmental Health Perspect*ives, **117**: 175–180

Lawrence, P.G., P.L. Harold and O.G. Francis (1980). Cefotaxime concentration in ascites, *Antibiotics and Chemotherapy*

Lee, S.H., X.W. Meng, K.S. Flatten, D.A. Loegering and S.H. Kaufmann (2013). Phosphatidylserine exposure during apoptosis reflects bidirectional trafficking between plasma membrane and cytoplasm, *Cell Death and Differentiation*, **20**: 64–76

LePecq, J.B. and C. Paoletti (1967). A fluorescent complex between ethidium bromide and nucleic acids: physical-chemical characterization, *Journal of Molecular Biology*, 27: 87-106

Lever, A.B.P. (1968). Inorganic electronic spectroscopy, Amsterdam: Elsevier

Li, H.Y., C.S Lai, J. Wu, P.C. Ho, D. Vos, E.R.T. Tiekink (2007). Cytotoxicity, qualitative structure–activity relationship (QSAR), and anti-tumor activity of bismuth dithiocarbamate complexes, *Journal of Inorganic Biochemistry*, **101**: 809-816

Lipinski, C.A., F. Lombardo, B.W. Dominy and P.J. Feeney (2001). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, *Advanced Drug Delivery Reviews*, **46**: 3–26

Li, P., Q.L. Zhao, L.H. Wu, P. Jawaid, Y.F. Jiao, M. Kadowaki and T. Kondo (2014). Isofraxidin, a potent reactive oxygen species (ROS) scavenger, protects human leukemia cells from radiation-induced apoptosis *via* ROS/mitochondria pathway in p53-independent manner, *Apoptosis*, **19**: 1043-1053

Liu, Jie, T.X. Zhang, T.B. Lu, L.H. Qu, H. Zhou, Q.L. Zhang and L.N. Ji (2002). DNA-binding and cleavage studies of macrocyclic copper(II) complexes, *Journal of Inorganic Biochemistry*, **91**: 269–276

Liu, Z.H., K.Z. Yue, S.F. Ma, X.S. Sun and J.H. Tan (2003). Effects of pregnant mare serum gonadotropin (eCG) on follicle development and granulosa-cell apoptosis in the pig, *Theriogenology*, **59**: 775–785

Macías, B., I. García, M.V. Villa, J. Borrás, M. González-Álvarez and A. Castiñeiras (2003). Oxidative DNA damage of mixed copper(II) complexes with sulfonamides and 1,10-phenanthroline, *Journal of Inorganic Biochemistry*, **96**: 367–374

Mallinson, J. and I. Collins (2012). Macrocycles in new drug discovery, *Future Medicinal Chemistry*, **4**: 1409–1438

Manogaran, S. and D.N. Sathyanarayana (1983). ^{1}H and ^{13}C NMR and Infrared spectroscopic study of the conformational equilibria in S-methyldithiocarbazate, *Journal of Molecular Structure*, **99**: 261-273

Mao, J.W., H. Zhou, Y.F. Chen, G.Z. Cheng and Z.Q. Pan (2012). Synthesis, structure, DNA interaction, and hydrolytic function toward bis(*p*-nitrophenyl) phosphate of a heterobinuclear macrocyclic complex, *Transition Metal Chemistry*, 37: 385–391

Marsault, E. and M.L. Peterson (2011). Macrocycles are great cycles: applications, opportunities, and challenges of synthetic macrocycles in drug discovery, *Journal of Medicinal Chemistry*, **54**: 1961–2004

Martin, S.J., C.P.M. Reutelingsperger, A.J. McGahon, J.A. Rader, R.C.A.A. van Schie, D.M. LaFace and D.R. Green (1995). Earlier distribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: inhibition by overexpression of Bcl-2 and Abl, *Journal of Experimental Medicine*, **182**: 1545-1556

Mieczyn´ska, E., J. Lisowski and A.M. Trzeciak (2015). A macrocyclic Pd(II)–Ni(II) complex in Heck and Suzuki reactions, *Inorganica Chimica Acta*, 431: 145-149

Mirnikjoo, B., K. Balasubramanian and A.J. Schroit (2009). Suicidal membrane repair regulates phosphatidylserine externalization during apoptosis, *The Journal of Biological Chemistry*, **284**: 22512–22516

Mohamed, G.G., M.M. Omar and A.M.M. Hindy (2005). Synthesis, characterization and biological activity of some transition metals with Schiff base derived from 2-thiophene carboxaldehyde and aminobenzoic acid, *Spectrochimica Acta Part A*, **62**: 1140–1150

Mohamed, G.G., M.M. Omar and A.A. Ibrahim (2009). Biological activity studies on metal complexes of novel tridentate Schiff base ligand. Spectroscopic and thermal characterization, *European Journal of Medicinal Chemistry*, **44**: 4801-4812

Mohanan, K. and S.N. Devi (2006). Synthesis, characterization, thermal stability, reactivity, and antimicrobial properties of some novel lanthanide(III) complexes of 2-(N-salicylideneamino)-3-carboxyethyl-4,5,6,7-tetrahydrobenzo[b]thiophene, *Russian Journal of Coordination Chemistry*, **32**: 600-609

Mohan, M., P. Sharma and N.K. Jha (1985). Metal(II) chelates of 4-methyl-5-amino-1-formylisoquinoline thiosemicarbazone: Their preparation, characterization and antitumour activity, *Inorganic Chimica Acta*, **106**: 197-201

Mohapatra, R.K., M. Dash, U.K. Mishra, A. Mahapatra and D.C. Dash (2014). Synthesis, spectral characterization, and fungicidal activity of transition metal complexes with benzimidazolyl-2-hydrazones of glyoxal, diacetyl, and benzil, *Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry*, **44**: 642–648

Mokhtary, M. and S.A.M. Langroudi (2014). Polyvinylpolypyrrolidone-supported boron trifluoride: a mild and efficient catalyst for the synthesis of 1,8-dioxooctahydroxanthenes and 1,8-dioxodecahydroacridines, *Chemical Monthly*, **145**: 1489–1494

Morishima, N., K. Nakanishi, H. Takenouchi, T. Shibata and Y. Yasuhiko (2002). An endoplasmic reticulum stress-specific caspase cascade in apoptosis. Cytochrome c-independent activation of caspase-9 by caspase-12, *The Journal of Biological Chemistry*, **277**: 34287–34294

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, *Journal of Immunological Methods*, **65**: 55-63

Murphy, M.P. (2009). How mitochondria produce reactive oxygen species, *Biochemical Journal*, **417**: 1-13

Murugan, P., P. Shanmugasundaram, V.T. Ramakrishnan, B. Venkatachalapathy, N. Srividya, P. Ramamurthy, K. Gunasekaran and D. Velmurugan (1998). Synthesis and laser properties of 9-alkyl-3,3,6,6-tetramethyl-1,2,3,4,5,6,7,8,9,10-decahydroacridine-1,8-dione derivatives, *Journal of the Chemical Society, Perkin Transactions* **2**: 999-1004

Nair, M.S. and R.S. Joseyphus (2007). Synthesis and characterization of Co(II), Ni(II), Cu(II) and Zn(II) complexes of tridentate Schiff base derived from vanillin and DL-α-aminobutyric acid, *Spectrochimica Acta Part A*, **70**: 749-753

Nair, U., H. Bartsch and J. Nair (2007). Lipid peroxidation-induced DNA damage in cancer-prone inflammatory diseases: a review of published adducts types and levels in humans, *Free Radical Biology and Medicine*, **43**: 1109–1120

Nielsen, F., B.B. Mikkelsen, J.B. Nielsen, H.R. Andersen and P. Grandjean (1997). Plasma malondialdehyde as biomarker for oxidative stress: reference interval and effects of life-style factors, *Clinical Chemistry*, **43**: 1209–1214

Nikolaev, A.V., V.A. Lagvienko and I. Myachina (1967). Thermal analysis, Academic Press, New York

Nomiya K., K. Sekino, M. Ishikawa, A. Honda, M. Yokoyama, N.C Kasuga, H. Yokoyama, S. Nakano and K. Onodera (2004). Syntheses, crystal structures and antimicrobial activities of monomeric 8-coordinate, and dimeric and monomeric 7-coordinate bismuth(III) complexes with tridentate and pentadentate thiosemicarbazones and pentadentate semicarbazone ligands, *Journal of Inorganic Biochemistry*, **98**: 601–615

Nordén, B. and F. Tjerneld (1982). Structure of methylene blue–DNA complexes studied by linear and circular dichroism spectroscopy, *Biopolymers*, **21**: 1713-1734

Nunes, P., N.V. Nagy, E.C.B.A. Alegria, A.J.L. Pombeiro and I. Correia (2014). The solvation and electrochemical behavior of copper acetylacetonate complexes in ionic liquids, *Journal of Molecular Structure*, **1060**: 142–149

O' Brien, I.E.W., C.P.M. Reutelingsperger and K.M. Holdaway (1997). The use of Annexin V and TUNEL to monitor the progression of apoptosis in plants, *Cytometry*, **29**: 28–33

Ogawa, Y, H. Shimizu and S.U. Kim (1996). 2,5-hexanedione induced apoptosis in cultured mouse DRG neurons, *International Archives of Occupational and Environmental Health*, **68**: 495–497

Op den Kamp, J.A.F (1979). Lipid asymmetry in membranes, *Annual Review of Biochemistry*, **48**: 47-71

Orrenius S., V. Gogvadze and B. Zhivotovsky (2007). Mitochondrial oxidative stress: implications for cell death, *Annual Review of Pharmacology and Toxicology*, **47**: 143–183

Özer, C.K., H. Arslan, D. VanDerveer and N. Külcü (2009). Synthesis and characterization of N-(arylcarbamothioyl)-cyclohexanecarboxamide derivatives: the crystal structure of N-(naphthalen-1-ylcarbamothioyl)cyclohexanecarboxamide, *Molecules*, **14**: 655–666

Pavia, D.L., G.M. Lampman and G.S. Kriz (2001). Introduction to spectroscopy, Thomson Leaning Inc., California

Philip, V., V. Suni and M.R.P. Kurup (2006). Copper(II) complexes derived from di-2-pyridyl ketone *N*(4),*N*(4)-(butane-1,4-diyl)thiosemicarbazone: crystal structure and spectral studies, *Polyhedron*, **25**: 1931-1938

Prasad, R.N., M. Mathur and A. Upadhyay (2007). Synthesis and spectroscopic studies of Cr(III), Fe(III) and Co(II) complexes of hexaazamacrocycles, *Journal of the Indian Chemical Society*, **84**: 1202-1204

Parsaeimehr, A., E. Sargsyan and K. Javidnia (2012). A comparative study of the antibacterial, antifungal and antioxidant activity and total content of phenolic compounds of cell cultures and wild plants of three endemic species of *Ephedra*, *Molecules*, **15**: 1668-1678

Patton, S. and G.W. Kurtz (1951). 2-thiobarbituric acid as a reagent for detecting milk fat oxidation, *Journal of Dairy Science*, **34**: 669-674

Pawar, V., S. Joshi and V. Uma (2013). Antibacterial and antioxidant properties of macrocyclic Schiff bases with vanadium(V) complexes, *Asian Journal of Chemistry*, **25**: 1497-1500

Pierce, G.B., R.E. Parchment and A.L. Lewellyn (1991). Hydrogen peroxide as a mediator of programmed cell death in the blastocyst, *Differentiation*, **46**: 181–186

Pinchas, S., B.L. Silver and I. Laulicht (1967). Infrared absorption spectra of the ^{18}O-labeled acetylacetonates of Cr(III) and Mn(III), *The Journal of Chemical Physics*, **46**, 1506-1510

Puckett, C.A. and J.K. Barton (2007). Methods to explore cellular uptake of ruthenium complexes, *Journal of the American Chemical Society*, **129**: 46-47

Pyle, A.M., J.P. Rehmann, R. Meshoyrer, C.V. Kumar, N.J. Turro and J.K. Barton (1989). Mixed-ligand complexes of ruthenium(II): factors governing binding to DNA, *Journal of the American Chemical Society*, **111**: 3051-3058

Qing, J., W. Gu, H. Liu, F.X. Gao, L. Feng, S.P. Yan, D.Z. Liao and P. Cheng (2007). The first dinuclear copper(II) and zinc(II) complexes containing novel Bis-TACN: syntheses, structures, and DNA cleavage activities, *Dalton Transactions*, **10**: 1060–1066

Qiu, X.Y., S.Z. Li, A.R. Shi, Q.Q. Li and B. Zhai (2012). Synthesis, crystal structure and cytotoxic activity of a zinc (II) complex of the Schiff base derived from S-benzyldithiocarbazate, *Chinese Journal of Structural Chemistry*, **31**: 555–561

Rabie U.M, A.S.A. Assran and M.H.M. Abou-El-Wafa (2008). Unsymmetrical Schiff bases functionalize as bibasic tetradentate (ONNO) and monobasic tridentate (NNO) ligands on complexation with some transition metal ions, *Journal of Molecular Struct*ure, **872**: 113-122

Ramadan, A.E.M.M. (2012). Macrocyclic nickel(II) complexes: synthesis, characterization, superoxide scavenging activity and DNA-binding, *Journal of Molecular Structure*, **1015**: 56–66

Ramakrishnan, S., E. Suresh, A. Riyasdeen, M.A. Akbarsha and M. Palaniandavar (2011). Interaction of *rac*-$[M(diimine)_3]^{2+}$ (M=Co, Ni) complexes with CT DNA: role of 5,6-dmp ligand on DNA binding and cleavage and cytotoxicity, *Dalton Transactions*, **40**: 3245-3256

Raman, N. and J. Joseph (2009). Synthesis, spectral characterization and antimicrobial activity of macrocyclic Schiff-base copper (II) complexes containing polycrystalline nanosized grains, *Journal of Coordination Chemistry*, **62**: 1162-1171

Raman, N., S.J. Raja and A. Sakthivel (2009). Transition metal complexes with Schiff-base ligands: 4-aminoantipyrine based derivatives–a review, *Journal of Coordination Chemistry*, **62**: 691-709

Raman, N., S. Sobha and L. Mitu (2013). Design, synthesis, DNA binding ability, chemical nuclease activity and antimicrobial evaluation of Cu(II), Co(II), Ni(II) and Zn(II) metal complexes containing tridentate Schiff base, *Journal of Saudi Chemical Soc*iety, **17**: 151-159

Rana, V.B., D.P. Singh, P. Singh and M.P. Teotia (1981). Divalent nickel, cobalt and copper complexes of a tetradentate N_6 macrocyclic ligand, *Transition Metal Chemistry*, **6**: 36–39

Rao, C.N.R. (1963). Chemical applications of infrared spectroscopy, Academic Press, New York

Rao, D.P. and H.S. Yadav (2013). In-situ synthesis and antibacterial activity of novel macrocyclic complexes of cisdioxomolybdenum (VI), *Biointerface Research in Applied Chemistry*, **3**: 533-540

Rathi, P., K. Sharma and D.P. Singh (2014). Molecular modelling, spectroscopic characterization and biological studies of tetraazamacrocyclic metal complexes, *Spectrochimica Acta Part A*, **130**: 72–75

Reutelingsperger, C.P.M., G. Hornstra and H.C. Hemker (1985). Isolation and partial purification of a novel anticoagulant from arteries of human umbilical cord, *European Journal of Biochemistry*, **151**: 625-629

Rezai, T., B. Yu, G.L. Millhauser, M.P. Jacobson and R.S. Lokey (2006). Testing the conformational hypothesis of passive membrane permeability using synthetic cyclic peptide diastereomers, *Journal of the American Chemical Society*, **128**: 2510–2511

Ricci, J.E., R.A. Gottlieb and D.R. Green (2003). Caspase-mediated loss of mitochondrial function and generation of reactive oxygen species during apoptosis, *Journal of Cell Biology*, **160**: 65-75

Saghatforoush, L., K. Adil, E. Sahin, S. Babaei and J. Musevi (2011). Synthesis, spectroscopy, thermal behavior, and X-ray crystal structure of two lead(II) complexes with 4′-(4-tolyl)-2,2′;6′,2″-terpyridine (ttpy), *Journal of Coordination Chemistry*, **64**: 4421-4433

Salinas, F., J.C.J. Sánchez and T.G. Díaz (1986). Spectrophotometric determination of iron in wines, foods, and minerals with 5,5-dimethyl-1,2,3-cyclohexanetrione 1,2-dioxime 3- thiosemicarbazone, *Analytical Chemistry*, **58**: 824–827

Sallam, S.A. (2006). Binuclear copper(II), nickel(II) and cobalt(II) complexes with N_2O_2 chromophores of glycylglycine Schiff-bases of acetylacetone, benzoylacetone and thenoyltrifluoroacetone, *Transition Metal Chemistry*, **31**: 46-55

Sayin, K. and D. Karakas (2013). Quantum chemical studies on the some inorganic corrosion inhibitors, *Corrosion Science*, **77**: 37-45

Sengottuvelan, N., D. Saravanakumar and M. Kandaswamy (2007). Electrochemical, magnetic, catalytic and DNA cleavage studies of binuclear copper(II) complexes derived from pendant substituted tetraaza macrobicyclic compartmental ligands, *Polyhedron*, **26**: 3825-3832

Shakir, M., N. Shahid, N. Sami, M. Azam and A.U. Khan (2011). Synthesis, spectroscopic characterization and comparative DNA binding studies of Schiff base complexes derived from l-leucine and glyoxal, *Spectrochimica Acta Part A*, **82**: 31– 36

Shanker, K., R. Rohini, V. Ravinder, P.M. Reddy and Y.P. Ho (2009). Ru(II) complexes of N_4 and N_2O_2 macrocyclic Schiff base ligands: their antibacterial and antifungal studies, *Spectrochimica Acta Part A*, **73**: 205–211

Shiota, M., N. Sugai, M. Tamura, R. Yamaguchi, N. Fukushima, T. Miyano and H. Miyazaki (2003). Correlation of mitogen-activated protein kinase activities with cell survival and apoptosis in porcine granulose cells, *Zoological Science*, **20**: 193–201

Shriver, D.F. and P.W. Atkins (1999). Inorganic Chemistry, Third Edition, Oxford: Oxford University Press

Singh, D.P., R. Kumar, V. Malik and P. Tyagi (2007). Template synthesis, spectroscopic studies and biological activities of macrocyclic complexes derived from thiocarbohydrazide and glyoxal, *Journal of Enzyme Inhibition and Medicinal Chemistry*, **22**: 177–182

Singh, M., V. Aggarwal, U.P. Singh and N.K. Singh (2009). Synthesis, characterization and spectroscopic studies of a new ligand [N′-(2-methoxybenzoyl)hydrazinecarbodithioate] ethyl ester and its Mn(II) and Cd(II) complexes: X-ray structural study of Mn(II) complex, *Polyhedron*, **28**: 107-112

Singh, R.V., P. Chaudhary, S. Chauhan and M. Swami (2009). Microwave-assisted synthesis, characterization and biological activities of organotin (IV) complexes with some thio Schiff bases, *Spectrochimica Acta Part A*, **72**: 260-268

Sinha, K., J. Das, P.B. Pal and P.C. Sil (2013). Oxidative stress: the mitochondria-dependent and mitochondria-independent pathways of apoptosis, *Archives of Toxicology*, **87**: 1157-1180

Snyder, H.R., R.G. Handrick and L.A. Brooks (1942). Imidazole, *Organic Syntheses*, **3**: 471-477

Spruck, C.H., P.F. Ohneseit, M. Gonzalez-Zulueta, D. Esrig, N. Miyao, Y.C. Tsai, S.P. Lerner, C. Schmutte, A.S. Yang, R. Cote and et al. (1994). Two molecule pathways to transitional cell carcinoma of the bladder, *Cancer Research*, **54**: 784-788

Sreedaran, S., K. ShanmugaBharathi, A. KalilurRahiman, L. Jagadish, V. Kaviyarasan and V. Narayanan (2008). Novel unsymmetrical macrocyclic dicompartmental binuclear copper(II) complexes bearing 4- and 6-coordination sites: Electrochemical, magnetic, catalytic and antimicrobial studies, *Polyhedron*, **27**: 2931-2938

Stang, P.J., D.H. Cao, S. Saito and A.M. Arif (1995). Self-assembly of cationic, tetranuclear, Pt(II) and Pd(II) macrocyclic squares. X-ray crystal structure of $[Pt^{2+}(dppp)(4,4'\text{-bipyridyl})^{2-}OSO_2CF_3]_4$, *Journal of the American Chemical Society*, **117**: 6273-6283

Stone, J.R. and S.P. Yang (2006). Hydrogen peroxide: a signaling messenger, *Antioxidants and Redox Signaling*, **8**: 243-270

Sun, Y., Y. Lin, H. Li, J. Liu, X.H. Sheng and W.C. Zhang (2012). 2,5-hexanedione induces human ovarian granulosa cell apoptosis through BCL-2, BAX, and CASPASE-3 signaling pathways, *Archives of Toxicology*, **86**: 205–215

Szilagyi, G., L. Simon, P. Koska, G. Telek and Z. Nagy (2006). Visualization of mitochondrial membrane potential and reactive oxygen species *via* double staining, *Neuroscience Letters*, **399**: 206-209

Tait, J.F., D. Gibson and K. Fujikawa (1989). Phospholipid binding properties of human placental anticoagulant protein- 1, a member of the lipocortin family, *Journal of Biological Chemistry*, **264**: 7944-7949

Tarafder, M.T.H., A.M. Ali, M.S. Elias and K.A. Crouse (2000). Coordination chemistry and biological activity of bidentate and quadridentate nitrogen-sulphur donor ligands and their metal complexes, *Transition Metal Chemistry*, **25**: 706-710

Tarafder, M.T.H., N. Saravanan, K.A. Crouse and A.M. Ali (2001). Coordination chemistry and biological activities of complexes of Ni(II) and Cu(II) ions with some nitrogen-sulphur ligands derived from S-benzyldithiocarbazate (SBDTC), *Transition Metal Chemistry*, **26**: 613-618

Tarafder, M.T.H., T.J. Khoo, K.A. Crouse, A.M. Ali, B.M. Yamin and H.K. Fun (2002). Coordination chemistry and bioactivity of Ni^{2+}, Cu^{2+}, Cd^{2+}, and Zn^{2+} complexes containing bidentate Schiff bases derived from S-benzyldithiocarbazate and the X-ray crystal structure of bis[S-benzyl-β-N-(5-methyl-2-furylmethylene)dithiocarbazato]cadmium(II), *Polyhedron*, **21**: 2547-2554

Thahira, B.S.A.R., K.A. Crouse, M.I.M. Tahir, A.R. Cowley and M.A. Ali (2007). Synthesis, characterization and bioactivity of mixed-ligand Cu(II) complexes containing Schiff bases derived from S-benzyldithiocarbazate and saccharinate ligand and the X-ray crystal structure of the copper-saccharinate complex containing S-benzyl-β-N-(acetylpyrid-2-yl) methylenedithiocarbazate, *Polyhedron,* **26**: 1159–1165

Thomas, G. (2000). Medicinal chemistry: an introduction in anticancer agents, John Wiley and Sons, New York, 279-282

Thomas, M.P., X. Liu, J. Whangbo, G. McCrossan, K.B. Sanborn, E. Basar, M. Walch and J. Lieberman (2015). Apoptosis triggers specific, rapid, and globalmrna decay with 3' uridylated intermediates degraded by DIS3L2, *Cell Reports*, **11**: 1079-1089

Tiwari, A.D., A.K. Mishra, S.B. Mishra, B.B. Mamba, B. Maji and S. Bhattacharya (2011). Synthesis and DNA binding studies of Ni(II), Co(II), Cu(II) and Zn(II) metal complexes of N1,N5-bis[pyridine-2-methylene]-thiocarbohydrazone Schiff-base ligand, *Spectrochimica Acta Part A*, **79**: 1050-1056

Todaro, G.J., G.K. Lazar and H. Green (1965). The initiation of cell division in a contact-inhibited mammalian cell line, *Journal of Cellular Physiology*, **66**: 325-333

Torigoe, T., H. Izumi, H. Ishiguchi, Y. Yoshida, M. Tanabe, T. Yoshida, T. Igarashi, I. Niina, T. Wakasugi, T. Imaizumi, Y. Momii, M. Kuwano and K. Kohno (2005). Cisplatin resistance and transcription factors, *Current Medicinal Chemistry - Anti-Cancer Agents*, **5**: 15–27

Ulukaya, E., F.M. Frame, B. Cevatemre, D. Pellacani, H. Walker, V.M. Mann, M.S. Simms, M.J. Stower, V.T. Yilmaz and N.J. Maitland (2013). Differential cytotoxic activity of a novel palladium-based compound on prostate cell lines, primary prostate epithelial cells and prostate stem cells, *PLOS ONE*, **8**: 1-13

Umano, K., K.J. Dennis and T. Shibamoto (1988). Analysis of free malondialdehyde in photoirradiated corn oil and beef fat *via* a pyrazole derivative, *Lipids*, **23**: 811-814

Vigato, P.A. and S. Tamburini (2004). The challenge of cyclic and acyclic schiff bases and related derivatives, *Coordination Chemistry Reviews*, **248**: 1717-2128

Vigato, P.A., S. Tamburini and L. Bertolo (2007). The development of compartmental macrocyclic Schiff bases and related polyamine derivatives, *Coordination Chemistry Reviews*, **251**: 1311–1492

Vrekoussis, M., F. Wittrock, A. Richter and J.P. Burrows (2009). Temporal and spatial variability of glyoxal as observed from space, *Atmospheric Chemistry and Physics*, **9**: 4485–4504

Wang, X.L., H. Chao, X.L. Hong, Y.J. Liu and L.N. Ji (2005). Bis(2,2′-bipyridine)cobalt(III) complexes containing asymmetric ligands: synthesis, DNA-binding and photocleavage studies, *Transition Metal Chemistry*, **30**: 305-311

West, D.X., A.K. El-Sawaf and G.A. Bain (1997). Metal complexes of N(4)-substituted analogues of the antiviral drug methisazone {1-methylisatin thiosemicarbazone}, *Transition Metal Chemistry*, **23**: 1-6

Wheate, N.J., C.R. Brodie, J.G. Collins, S. Kemp and J.R. Aldrich-Wright (2007). DNA intercalators in cancer therapy: organic and inorganic drugs and their spectroscopic tools of analysis, *Mini Reviews in Medicinal Chemistry*, **7**: 627-648

Wolfe, A., G.H. Shimer and T. Meehan (1987). Polycyclic aromatic hydrocarbons physically intercalate into duplex regions of denatured DNA, *Biochemistry*, **26**: 6392-6396

Wolkenberg, S.E and D.L. Boger (2002). Mechanisms for in-situ activation for DNA-targeting antitumor agents, *Chemical Reviews*, **102**: 2477 - 2497

Wu, X.R. (2005). Urothelial tumorigenesis: a tale of divergent pathways, *Nature Reviews Cancer*, **5**: 713-725

Yang, P., M.L. Guo and B.S. Yang (1994). Interaction of titanocene dichloride on DNA, *Chinese Science Bulletin*, **39**: 997-1002

Yousefi S., D.R. Green, K. Blaser and H.U. Simon (1994). Protein-tyrosine phosphorylation regulates apoptosis in human eosinophils and neutrophils. *Proceedings of the National Academy of Science*, **91**: 10868–10872

Zhang, F., Y.M. Tang, Z.Y. Cao, W.H. Jing, Wu Z.L. and Y.Z. Chen (2012). Performance and theoretical study on corrosion inhibition of 2-(4-pyridyl)-benzimidazole for mild steel in hydrochloric acid, *Corrosion Science*, **61**: 1-9

Zhang, G.H., V. Gurtu, S.R. Kain and G.C. Yan (1997). Early detection of apoptosis using a fluorescent conjugate of Annexin V, *BioTechniques*, **23**: 525-531

Zhang H., C.S Liu, X.H Bu and M. Yang (2005). Synthesis, crystal structure, cytotoxic activity and DNA-binding properties of the copper(II) and zinc(II) complexes with 1-[3-(2-pyridyl)pyrazol-1ylmethyl]naphthalene, *Journal of Inorganic Biochemistry*, **99**: 1119-1125

Zhivotovsky, B (2003). Caspases: the enzymes of death, *Essays in Biochemistry*, **39**: 25–40

Zhou, M.J., Z.J. Diwu, N. Panchuk-Voloshina and R.P. Haugland (1997). A stable nonfluorescent derivative of resorufin for the fluorometric determination of trace hydrogen peroxide: applications in detecting the activity of phagocyte NADPH oxidase and other oxidases, *Analytical Biochemistry*, **253**: 162–168

Zhu, S.S., D.R. Cefalo, D.S. La, J.Y. Jamieson, W.M. Davis, A.H. Hoveyda and R.R. Schrock (1999). Chiral Mo-binol complexes: activity, synthesis, and structure. Efficient enantioselective six-membered ring synthesis through catalytic metathesis, *Journal of the American Chemical Society*, **121**: 8251-8259