UNIVERSITI PUTRA MALAYSIA

PREPARATION AND BIOACTIVITIES OF Cu(II) AND Ni(II) COMPLEXES CONTAINING AMINO ACID-DERIVED SCHIFF BASES

NUR FATIHAH BINTI ABAS

FS 2018 18

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By

NUR FATIHAH BINTI ABAS

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

December 2017

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DEDICATION

I would love to dedicate this thesis to my late parents who are always my source of inspiration.

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

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December 2017

Chairman : Thahira Begum, PhD
Faculty : Science

There is an urgent need to discover new drugs with enhanced activity, selectivity, bioavailability and fewer side effects than the current drug regime. In view of this, metal complexes containing amino acid-derived Schiff bases with potentially exciting biological activities and coordination chemistry are attractive candidates for consideration. The research reported in this thesis focused on synthesis and characterisation of new metal complexes containing amino acid-derived Schiff base. Thirty new metal complexes were synthesised from the reaction of Cu(II) chloride and Ni(II) acetate with Schiff bases derived from the condensation of amino acids (L-phenylalanine (P), L-valine (V), L-histidine (H), L-cysteine (C) and L-methionine (M)) and different ketones (2-acetylpyrazine (2APZ), 2-acetylpyridine (2APD) and 2-benzoylpyridine (2BPD)). The synthesised complexes were characterised by various techniques including elemental analysis, molar conductance, magnetic measurements, IR, electronic spectroscopy and thermal analysis. The data obtained indicated that the amino acid-derived Schiff bases behaved as uninegatively charged tridentate NNO ligands and coordinated with the Cu(II) and Ni(II) ions via azomethine nitrogen, pyridine/pyrazine nitrogen and deprotonated carboxylate oxygen yielding stable metal complexes as evidenced in their IR Spectra. In the Cu(II) complexes, only one tridentate amino acid-derived Schiff bases was coordinated to the metal centre, while the fourth position was occupied by chloride ion. In most of the Ni(II) complexes, only one tridentate amino acid-derived Schiff bases was coordinated to the metal centre, while the fourth position was occupied by acetate ion except for $[Ni(H2APZ)_2].H_2O$, $[Ni(M2APZ)_2]$ and $[Ni(M2BPD)_2]$ complexes where they had two tridentate NNO amino acid-derived Schiff bases bonded to the metal centre. Magnetic measurements and spectral evidence supported a four coordinate geometry for Cu(II) complexes and four/six coordinate geometry for the Ni(II) complexes. The thermal analysis proved the presence of water molecules outside the coordination sphere of some of the metal complexes synthesised. The newly synthesised complexes have been screened for activity against two bladder cancer cell lines which are the invasive human bladder carcinoma cell line, EJ-28 and the minimum invasive human bladder carcinoma cell

line, RT-112. It was found that the Cu(II) complexes had better cytotoxic activity against EJ-28 cells compared to RT-112 cells. In general, the Cu(II) and Ni(II) complexes containing amino acid-derived Schiff bases displayed a wide range of activity from non-active to active and could be promising candidates for selective and specific anticancer activity.

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

PENYEDIAAN DAN AKTIVITI BIOLOGI BAGI KOMPLEKS Cu(II) DAN Ni(II) MENGANDUNGI BES SCHIFF YANG DITERBITKAN DARIPADA ASID AMINO

Oleh

NUR FATIHAH BINTI ABAS

Disember 2017

Pengerusi : Thahira Begum, PhD
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Terdapat keperluan segera untuk menemui ubat-ubatan baru dengan aktiviti ditingkatkan, selektiviti, bioavailabiliti dan kesan sampingan yang kurang daripada ubat-ubatan sekarang. Penyelidikan yang dilaporkan dalam tesis ini ditumpukan kepada sintesis dan pencirian kompleks logam baru yang mengandungi bes Schiff yang diterbitkan daripada asid amino. Tiga puluh kompleks logam telah disintesis daripada tindak balas kuprum(II) klorida dan Ni(II) asetat dengan bes Schiff yang diterbitkan daripada tindak balas kondensasi amino asid (L-fenilalanina (P), L-valina (V), L-histidina (H), L-cystina (C) dan L-metionina (M) dan keton yang berbeza (2-asetilpyrazin (2APZ), 2-asetilpyridin (2APD) dan 2-benzoilpyridin (2BPD)). Kompleks yang telah disintesis telah dicirikan dengan pelbagai teknik termasuk analisis unsur, konduktiviti molar, pengukuran kerentanan magnet, inframerah, spektroskopi elektronik dan analisis terma. Hasil pencirian yang diperolehi menunjukkan bahawa bes Schiff yang diterbitkan daripada asid amino berkelakuan sebagai cas uninegatif NNO ligan melalui nitrogen azometin, nitrogen pyridine/pyrazine dan oksigen karboksilat yang dinyahprotonkan yang menghasilkan kompleks logam yang stabil seperti yang dibuktikan dalam spectrum inframerah. Dalam kompleks kuprum(II), hanya satu bes Schiff yang diterbitkan daripada asid amino telah berkoordinat dengan pusat logam, manakala kedudukan keempat dipenuhi oleh ion klorida. Dalam hampir semua kompleks Ni(II), hanya satu bes Schiff yang diterbitkan daripada asid amino telah berkoordinat dengan pusat logam, manakala kedudukan keempat dipenuhi oleh ion asetat kecuali kompleks $[Ni(H2APZ)_2].H_2O$, $[Ni(M2APZ)_2]$ dan $[Ni(M2BPD)_2]$ di mana mereka mempunyai dua tridentat NNO bes Schiff yang diterbitkan daripada asid amino telah berkoordinat dengan pusat logam. Pengukuran kerentanan magnet dan spektra menyokong geometri berkoordinat empat untuk kompleks kuprum(II) dan geometri berkoordinat empat/enam untuk kompleks nikel(II). Analisis terma membuktikan kehadiran molekul air di luar sfera koordinasi dalam beberapa kompleks yang disintesis. Sebatian baru yang disintesis telah disaring untuk tujuan penentuan aktivitinya ke atas dua sel kanser pundi kencing iaitu sel kanser pundi kencing manusia dengan invasif, EJ-28 dan sel kanser pundi kencing manusia dengan invasive rendah,

RT-112. Didapati, kompleks kuprum(II) mempunyai aktiviti sitotoksik yang lebih baik terhadap sel EJ-28 berbanding sel RT-112. Secara umum, kompleks kuprum(II) dan nikel(II) yang mengandungi bes Schiff yang diterbitkan daripada asid amino menunjukkan julat aktiviti daripada tidak aktif kepada aktif dan boleh menjadi calon yang menjanjikan aktiviti antikanser terpilih dan spesifik.

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THANK YOU SO MUCH.

I certify that a Thesis Examination Committee has met on 6 December 2017 to conduct the final examination of Nur Fatihah binti Abas on her thesis entitled "Preparation and Bioactivities of Cu(II) and Ni(II) Complexes Containing Amino Acid-Derived Schiff Bases" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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

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I hereby confirm that

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This is to confirm that

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Name of Member of
Supervisory Committee: Mohamed Ibrahim Mohamed Tahir

Signature: _____
Name of Member of
Supervisory Committee: Abhimanyu Veearakumarasivam

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LIST OF SCHEMES

LIST OF ABBREVIATIONS

B. M	Bohr Magneton
EJ-28	Invasive human bladder cancer cell line
RT-112	Minimum invasive human bladder cancer cell line
FTIR	Fourier Transform Infrared
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
CT-DNA	Calf thymus-deoxyribonucleic acid
DMSO	Dimethyl sulphoxide
UV-Vis	Ultraviolet-Visible
MCF-7	Human breast carcinoma cells with positive estrogen
MDA-MB-231	Human breast carcinoma cells with negative estrogen
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
HepG-2	Human liver hepatocellular carcinoma cells
NCl-H460	Human large-cell lung carcinoma cells
PC-3	Prostate cancer cells
1D	One dimension
$Cu(S2M2TK)_2$	Cu(II) complex of S-2-methylbenzyl-β-N-(2-thiophenyl)ethylene dithiocarbazate
TG	Thermogravimetric
DTG	Differential thermal gravimetric
calc.	Calculated
NAD(P)H	Reduced form of nicotamide adenine dinucleotide phosphate
NADH	Reduced form of nicotinamide adenine dinucleotide
IC_{50}	Inhibition concentration at 50%

SXRD	Single crystal X-ray diffraction
NNO	Nitrogen-nitrogen-oxygen
Ace	Acetate
P	L-phenylalanine
V	L-valine
H	L-histidine
C	L-cysteine
M	L-methionine
MW	Molecular weight

CHAPTER 1

INTRODUCTION

1.1 Complexes containing Schiff bases

Schiff bases are considered an important class of organic ligands that have wide applications in chemical, biological and pharmacological fields (Nawaz *et al.*, 2009). The condensation of aldehydes or ketones with various amines, diamines or amino acids will lead to bi-, tri- or tetra-dentate Schiff bases with N, O as donor atoms (Mahon *et al.*, 2009). In this research, various amino acids and ketones were used in order to obtain the Schiff bases which were subsequently reacted with metal salts.

The presence of the azomethine group, C=N in the Schiff bases is essential for biological activity. Several azomethine derivatives were reported be versatile pharmacophores for the design and development of various bioactive lead compounds. Schiff bases exhibit useful biological activities such anti-inflammatory, analgesic, antimicrobial, anticonvulsant, antitubercular, anticancer, antioxidant, anthelmintic, and antidepressant activities (Kajal *et al.*, 2013). Amino acid-derived Schiff bases are very effective metal chelators and their metal complexes have been reported as models for a number of important biological systems. They are key intermediates in a variety of metabolic reactions involving amino acids such as decarboxylation, transamination, racemization and C-C bond cleavage, which are catalyzed by enzymes (Karmakar *et al.*, 2005).

In addition, complexes containing amino acid-derived Schiff bases have been known to act as good chelating agents, possess efficient biological activity and behave as good cytotoxic agents (Wang *et al.*, 2002). Moreover, amino acid-derived Schiff base complexes are considered to combine new kinds of potential antibacterial and anticancer agents (Wang *et al.*, 2005). The transition metal complexes containing Schiff bases are also very vital chelates because they are cheap, easy to synthesise, have extensive applications in the fields of medicine and are chemically and thermally stable (Rahman *et al.*, 2014).

1.2 Amino acids

Amino acids are organic molecules containing two main functional groups which are amine, $-NH_2$ and carboxylic acid, -COOH. These functional groups are bonded to the same chiral carbon atom in the molecule. The general formula for an amino acid is $H_2NCHRCOOH$ where R is the side chain that varies for different amino acids. The particularly important amino acids in biochemistry are referred to as α- amino acids.

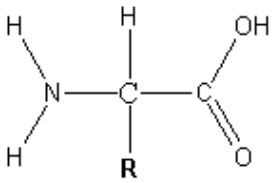


Figure 1:1: General structure of α-amino acids

Amino acids are critical to life and have many functions in metabolism. One particularly important function is to serve as building blocks for proteins (Mho *et al*., 2001). They often act as the essential ingredients of coenzymes and the precursors of heme, which play key roles in biochemistry (Kostel *et al.,* 1997). Amino acids can react with carbonyl compounds to form Schiff bases (Fan *et al*., 2007).

Amino acids also play an important role in many biochemical processes. The metal complexes with amino acids play an important role in understanding biological functions of macromolecules such as proteins in the human body (Chohan *et al*., 2007). As amino acids which have multiple N and O atoms are significant endogenous biological ligands that play an important role in almost all life activities, thus there is great possibility to apply amino acids to explore more effective, lower toxicity and specific metal based drugs (Bartel *et al*., 2012).

1.3 Structure and Background of 2-Acetylpyrazine, 2-Acetylpyridine and 2-Benzoylpyridine

This section focused on structure and background of ketones used as the starting materials which are 2-acetylpyrazine, 2-acetylpyridine and 2-benzoylpyridine. 2-acetylpyrazine (Figure 1.2) is a yellow-brown powder at room temperature and its structure consists of a pyrazine ring and a ketone. It can be found in foods like seeds, nuts and meats. It is used in frozen dairy products such as ice cream and is recognized as safe by the US Food and Drug Administration. The pyrazine ring in the structure of 2-acetylpyrazine is usually fused to form many polycyclic compounds which serve as useful structures in the pharmaceutical, flavouring and perfumery industry (Dubuissona *et al*., 2004).

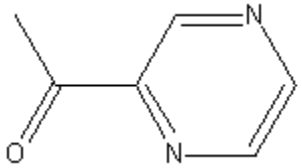


Figure 1.2: Structure of 2-acetylpyrazine

Pyrazine itself has shown numerous physiological effects including as antituberculosis, anthelmenitics, antianginals, anticancer, analgesic, antidepressant, antipsychotic, antidiabetic, antihistamines, hypolipidemic and flavouring agents and these drugs have encouraged medicinal chemists to synthesise a large number of novel chemotherapeutic agents (Meher *et al.*, 2013).

Pyrazine is also a component of the vitamin B_9 compound known as folate or folic acid (Figure 1.3). Folate is essential for numerous bodily functions such as to synthesise and repair deoxyribonucleic acid (DNA). It is very vital in aiding rapid growth and cell division during infancy and pregnancy. Pyrazine derivatives such as phenazine (Figure 1.4) are well known for their antitumor, antibiotic and diuretic activities (Asif, 2015).

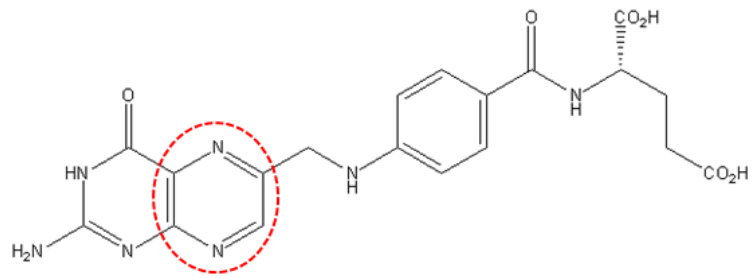


Figure 1.3: Structure of folate

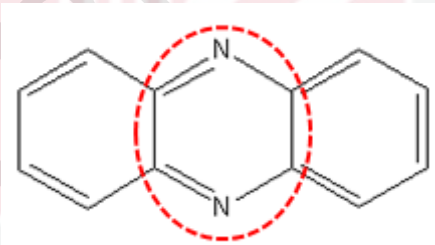
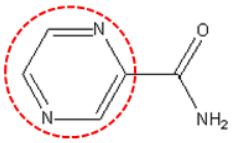
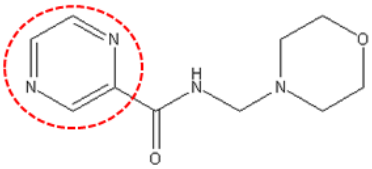


Figure 1.4: Structure of phenazine

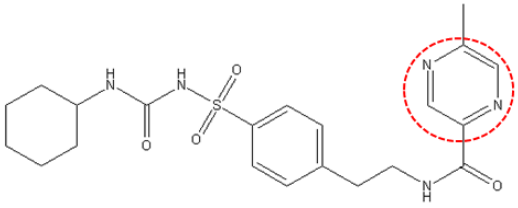
There are several drugs containing the pyrazine moiety such as pyrazinamide (Shi *et al.*, 2012), morinamide (Bonanni *et al.*, 1993), glipizide (Tripathi, 2006), oltipraz (Iida *et al.*, 2007) and telaprevir (Revill *et al.*, 2007). Pyrazinamide (Figure 1.5(a)) and morinamide (Figure 1.5(b)) have been used to treat tuberculosis. Oltipraz (Figure 1.5(d)) acts as a schistosomicide, a drug to treat schistosomiasis which is an acute and chronic disease caused by parasitic worms. Oltipraz also has been shown in rodent models to inhibit the formation of cancers in the bladder, blood, colon, kidney, liver, lung, pancreas, stomach, skin, and mammary tissue. Glipizide (Figure 1.5(c)) is an anti-diabetic drug and telaprevir (Figure 1.5(e)) has been used in the treatment of hepatitis C.



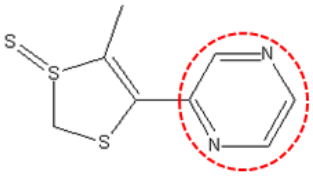
1.5 (a) Structure of pyrazinamide



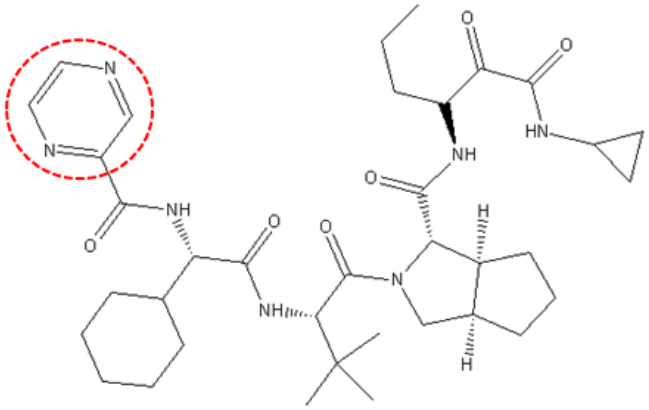
1.5 (b) Structure of morinamide



1.5 (c) Structure of glipizide



1.5 (d) Structure of oltipraz



1.5 (e) Structure of telaprevir

Figure 1.5: Drugs containing the pyrazine moiety

2-acetylpyridine (Figure 1.6) is widely used as a component in processed food products, as flavoring agents and also in aromatherapy. Meanwhile, 2-benzoylpyridine (Figure 1.7) has been used as an intermediate for pharmaceuticals and organic synthesis. These compounds containing the pyridine ring in their structures have been used as precursors to polymers, dyes, antioxidants, agrochemicals and pharmaceuticals (Chaubey *et al.*, 2011).

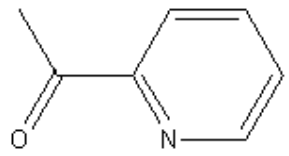


Figure 1.6: Structure of 2-acetylpyridine

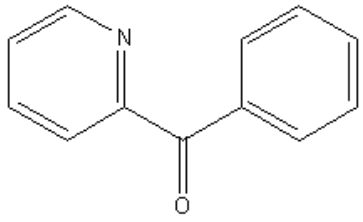


Figure 1.7: Structure of 2-benzoylpyridine

The pyridine structure is found in many important compounds such as niacin (vitamin B3) (Figure 1.8) and pyridoxine (vitamin B6) (Figure 1.9). Niacin or nicotinic acid is required for the biosynthesis of the redox coenzyme nicotine adenine dinucleotide (NAD^+) and pyridoxine is a coenzyme in transaminases (Joule *et al.*, 2010). Moreover, nicotinic acid has been used as a therapeutic agent to increase the relative levels of high-density lipoproteins and thereby reduce the risk of cardiovascular disease (Gille *et al.*, 2008).

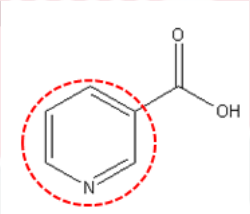


Figure 1.8: Structure of niacin

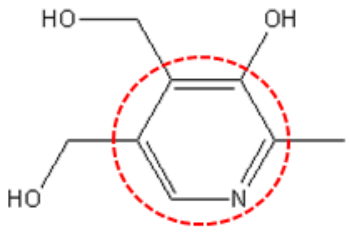
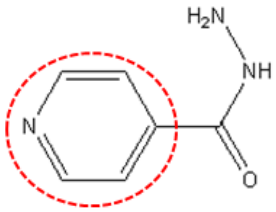
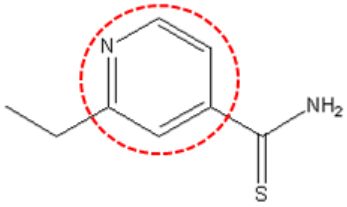


Figure 1.9: Structure of pyridoxine

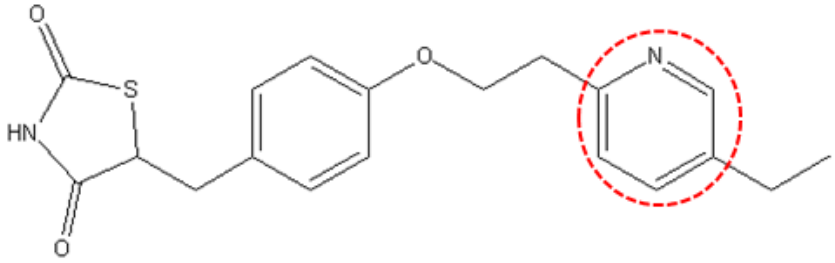
In the pharmaceutical industry, pyridine forms the nucleus of over 7000 existing drugs (Henry *et al.*, 2004). The pyridine moiety is found in anti-tuberculosis drugs like isoniazid (Figure 1.10(a)) (Timmins *et al.*, 2004) and ethionamide (Figure 1.10(b)) (Vanneli *et al.*, 2002). The other important species containing pyridine moiety are pioglitazone (Figure 1.10(c)) and rosiglitazone (Figure 1.10(d)), which have been used as anti diabetic drugs in the thiazolidinedione class of drugs. To be more specific, pioglitazone (Lincoff *et al.*, 2007) and rosiglitazone (Richter *et al.*, 2007) are currently being used for treating patients with type 2 diabetes. These pharmaceutical agents act as binders to the peroxisome proliferator-activated receptors that migrate upon activation to the DNA to regulate the transcription of specific genes which control the metabolism of carbohydrates and fatty acids (Baumann *et al.*, 2013)



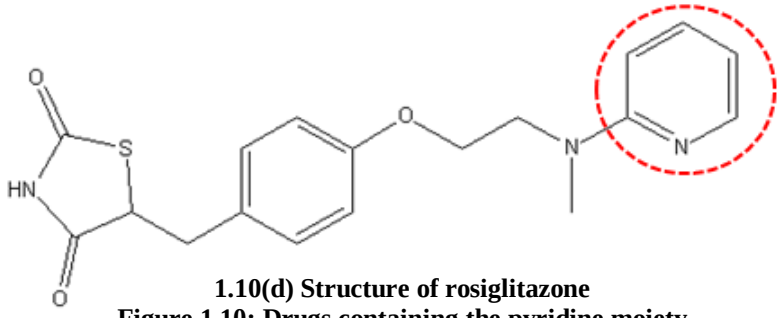
1.10(a) Structure of isoniazid



1.10(b) Structure of ethionamide



1.10(c) Structure of pioglitazone



1.10(d) Structure of rosiglitazone

Figure 1.10: Drugs containing the pyridine moiety

1.4 Copper and Nickel

Copper is the third most abundant trace metal in the human body after iron and zinc and the total amount of copper in the body is only 75-100 milligrams (Willis *et al.,* 2005). Copper is also an important dietary nutrient although only small amounts of the metal are needed for well being (Araya *et al.,* 2006). Copper plays an important role in our metabolism, largely because it allows many critical enzymes to function properly. Moreover, copper plays a role in the production of hemoglobin, myelin, melanin and it also keeps thyroid gland functioning normally (Harris, 2001). In addition, copper can act as both an antioxidant and a pro-oxidant. Free radicals occur naturally in the body and can damage cell walls, interact with genetic material, and contribute to the development of a number of health problems and diseases. As an antioxidant, Cu scavenges or neutralize free radicals and may reduce or help prevent some of the damage they cause (Davis, 2003).

Nickel is one of the micronutrients or trace minerals in the human body since it is present in very small amounts, however it plays an important role in bodily processes. Nickel is present in the ribonucleic acid (RNA) and DNA of the human body where it functions in association with nucleic acids. It probably has a role in stabilizing the RNA structure (Petzold *et al.,* 2011). It may activate certain enzymes related to the breakdown or utilization of glucose. In addition, nickel may aid in prolactin production which is involved in human breast milk production. Nickel also aids in iron absorption and plays a role in the production of red blood cells. Since it is a trace element, deficiency is rare. However, it has been found that low amounts of nickel in the bodies of some individuals can lead to certain liver and kidney diseases (Wilfred, 2012).

In this research, carbonyl compounds and amino acids formed in-situ Schiff bases that acted as ligands for the ligands for the complexation of metal ions. A compilation of literature reports focusing on previously synthesised amino acid-derived Schiff base complexes and their significant bioactivities are discussed in Chapter 2.

1.5 Problem statement

Bladder cancer is a disease in which the cells lining the urinary bladder lose the ability to regulate their growth and start to multiply abnormally. This uncontrollable growth results in tumor growth which then becomes cancer. It is the most common malignancy of the urinary tract and is seen more often in men. Based on national statistics, it is the fourth most common malignancy in males after lung, colorectal and nasopharynx cancers (Jayendran *et al*., 2007). The current drugs used in treatment of bladder cancer are Bacillus Calmette-Guerin (BCG) and mitomycin C (Figure 1.11). However, treatment with these drugs can lead to various side effects including kidney damage and burning sensation in the bladder. The side effects of bladder cancer treatment vary according to the treatment which is further discussed in the next paragraph.

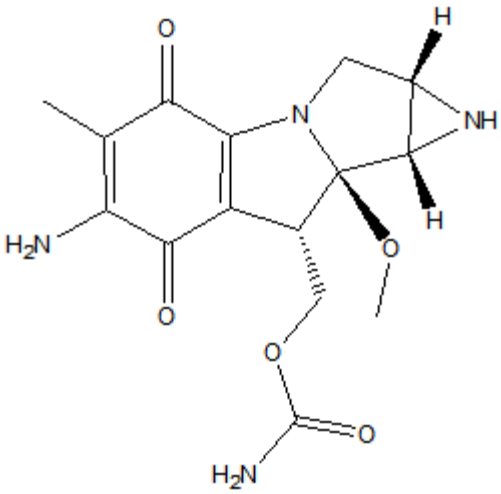


Figure 1.11: Structure of mitomycin C

Bacillus Calmette-Guerin (BCG) is the main intravesical immunotherapy for treating early-stage bladder cancer. Immunotherapy causes the body's own immune system to attack the cancer cells. BCG is a germ that is related to the one that causes tuberculosis but it doesn't usually cause serious disease. However, treatment with BCG can cause symptoms that feel like having the flu, such as fever, chills, and fatigue. It also can cause a burning feeling in the bladder. Rarely, BCG can spread through the body, leading to a serious infection called systemic BCG reaction. A systemic BCG reaction can cause pneumonitis, hepatitis, prostatitis and respiratory distress.

Mitomycin C or mutamycin(trade name) is the drug used most often for intravesical chemotherapy. Chemotherapy is the use of drugs to destroy cancer cells, usually by stopping the cancer cells' ability to grow and divide. Side effects of chemotherapy depend on the individual and the dose used, but they can include fatigue, risk of infection, nausea and vomiting, hair loss, loss of appetite, and diarrhea. Prolonged use may result in permanent bone-marrow damage. It also can cause lung fibrosis and renal damage. These facts have led researchers to continuously synthesise new drugs to treat bladder cancer. Researchers are looking for drugs which can help lower the risk of the cancer coming back and is better or safer than currently used drugs.

In terms of metal-based drugs, the platinum drug cisplatin was introduced clinically in 1971 and was approved by the US Food and Administration Authority (FDA) in late 1978. Cisplatin (Figure 1.12) has been the most effective metal-based anticancer drug in the market. For these studies, copper and nickel were used as metal centre as an alternative to platinum. These might open up new breakthroughs in the development of clinically useful drugs. Furthermore, there is an urgency to discover and characterise new drugs with enhanced activity, selectivity, bioavailability and fewer side effects than the current drug regime.

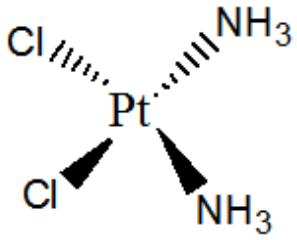


Figure 1.12: Structure of cisplatin

Synthesised complexes derived from amino acid Schiff bases have been reported to show good biological activities as antibacterial, antifungal and antitumor agents (Galal *et al.*, 2009). Transition metal ions play an important role in a vast number of different biological processes (Priya *et al.*, 2009). Some drugs show increased activity when administered as metal chelates and could inhibit the growth of tumors (You *et al.*, 2004).

In this work, several amino acids were chosen to condense with various ketones to form Schiff bases and then reacted with transition metal ions, copper, Cu(II) and nickel, Ni(II). The cytotoxic activities of the synthesised compounds were then evaluated against the invasive human bladder carcinoma cell lines, EJ-28 and the minimum invasive human bladder carcinoma cell lines, RT-112.

1.6 Objectives

The objectives of this research work were:

1. To synthesise metal complexes derived from various amino acids and ketones (2-acetylpyrazine, 2-acetylpyridine and 2-benzoylpyridine) using a one pot method.
2. To characterise the new complexes by physico-chemical techniques, including elemental analysis, magnetic susceptibility and molar conductivity measurements, and spectroscopic methods.
3. To determine the cytotoxic activities of the synthesised complexes against the EJ-28 and RT-112 human bladder carcinoma cell lines.

BIBLIOGRAPHY

Abdel-Rahman, L. H., El-Khatib, R. M., Nassr, L. A. E., Abu-Dief, A. M., Ismael, M., and Seleem, A. A. (2014). Metal based pharmacologically active agents: synthesis, structural characterization, molecular modeling, CT-DNA binding studies and in vitro antimicrobial screening of iron(II) bromosalicylidene amino acid chelates. *Spectrochimica Acta-Part A: Molecular and Biomolecular Spectroscopy*, **117**: 366–378.

Abdel-Rahman, L. H., El-Khatib, R. M., Nassr, L.A.E., and Abu-dief, A. M. (2013). DNA binding ability mode , spectroscopic studies , hydrophobicity , and *in vitro* antibacterial evaluation of some new Fe(II) complexes bearing ONO donors amino acid Schiff bases. *Arabian Journal of Chemistry*, **10(2)**: 1878–5352.

Ali, M. A., Haroon, C. M., Nazimuddin, M., Majumder, S.M.M., Tarafder, M.T.H. and Khair, M.A. (1992*), Transition Metal Chem*istry, **17**: 133-134.

Ali, M. A., and Tarafder, M. (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(10)**, 1785-1791.

Ali, M. A., Mirza, A. H., M.H.S., Aminath, N., and Bernhardt, P. V. (2012). Synthesis, characterization and X-ray crystal structures of thiolate sulphur-bridged dimeric copper(II) complexes of the 2-aminoacetophenone Schiff base of S-methyldithiocarbazate. *Polyhedron*, **49(1)**, 277-283.

Alsalme, A., Laeeq, S., Dwivedi, S., Khan, M. S., Al Farhan, K., Musarrat, J., and Khan, R. A. (2016). Synthesis, characterization of α-amino acid Schiff base derived Ru/Pt complexes: Induces cytotoxicity in HepG2 cell via protein binding and ROS generation. *Spectrochimica Acta-Part A: Molecular and Biomolecular Spectroscopy*, **163**: 1–7.

Araya, M., Pizarro, F., Olivares, M., Arredondo, M., González, M., and Méndez, M.(2006). Understanding copper homeostasis in humans and copper effects on health. *Biological Research*, **39(5)**: 183–187.

Asif, M. (2015). Piperazine and Pyrazine containing molecules and their diverse pharmacological activities. *International Journal of Advances in Scientific Research*, **01(01)**: 5–11.

Bakhtiar, R., and Ochiai, E. –I. (1999). Pharmacological applications of inorganic complexes. *General Pharmacology: The Vascular System*, **32(5)**, 525-540.

Bartel, C., Bytzek, A. K., Scaffidi-Domianello, Y. Y., Grabmann, G., Jakupec, M. A., Hartinger, C. G., and Keppler, B. K. (2012). Cellular accumulation and DNA interaction studies of cytotoxic trans -platinum anticancer compounds. *Journal of Biology Inorganic Chemistry*, **17**: 465–474.

Barton, J. K., and Rapheal, A. L. (1984). Photoactivated stereospecific cleavage of double-helical DNA by cobalt(III) complexes. *Journal of the American Chemical Society*, **106**: 2466-2468.

Baumann, M., and Baxendale, I. R. (2013). An overview of the synthetic routes to the best selling drugs containing 6-membered heterocycles. *Beilstein Journal of Organic Chemistry*, **9**: 2265–2319.

Boerner, L. J. K., and Zaleski, J. M. (2005). Metal complex- DNA interactions:from transcription inhibition to photoactivated cleavage. *Current opinion in chemical biology*, **9(2)**:135-144.

Bonanni, G., Ciccariello, M., Mancini, P., Pace, V., and Sagliaschi, G. (1993). Concomitant ceco-appendicular and urinary tuberculosis. Description of two rare cases: Physiopathological and diagnostic remarks. *European Review for Medical and Pharmacological Sciences,* **15(3-4)**: 171–174.

Chaubey, A., and Pandeya, S. N. (2011). Asian Journal of Pharmaceutical and Clinical Research. *Asian Journal of Pharmaceutical and Clinical Research*, **4(4)**: 5–8.

Chew, S.T., Lo, K.M., Lee, S. K., Heng, M.P., Teoh, W.Y, Sim, K.S., and Tan, K.W. (2014). Copper complexes with phosphonium containing hydrazone ligand: topoisomerase inhibition and cytotoxicity study. *European Journal of Medicinal Chemistry*, **76**: 397-407.

Chohan, Z.H., Arif, M., and Sarfraz, M.(2007). Metal-based antibacterial and antifungal amino acid derived Schiff bases: their synthesis, characterization and in vitro biological activity. *Applied Organometallic Chemistry*, **21(4)**: 294–302.

Cozzi, P. G. (2004). Metal-Salen Schiff base complexes in catalysis: practical aspects. *Chemical Society Reviews*, **33(7)**: 410-421

Davis, C. D. (2003). Low Dietary Copper Increases Fecal Free Radical Production, Fecal Water Alkaline Phosphatase Activity and Cytotoxicity in Healthy Men. *Nutrition*, (October 2002), 522–527.

Dubuissona, M. L. N., Reesa, J.-F., and Marchand-Brynaert, J. (2004). Discovery and validation of a new family of antioxidants: the aminopyrazine derivatives. *Mini Reviews in Medicinal Chemistry*, **4(4)**: 421–35.

Dziegielewska, B., Kolwalski, D., and Beerman, T. (2004). SV40 DNA replication inhibition by the monofunctionl DNA alkylato Et743. *Biochemistry*, **43(44)**: 14228-14237

El-Sonbati, A. Z., Diab, M. A., El-Bindary, A.A., and Morgan, S. M. (2014). Supramolecular spectroscopic and thermal studies of azodye complexes. *Spectrochimica Acta-Part A: Molecular and Biomolecular Spectroscopy*, **127**: 310-328.

El-Sonbati, A. Z., Diab, M. A., El-Bindary, A. A., Eldesoky, A. M., and Morgan, S. M (2014). Correlation between ionic radii of metals and thermal decomposition of supramolecular structure of azodye complexes. *Spectrochimica Acta- Part A: Molecular and Biomolecular Spectroscopy*, **135**: 774-791

Fan, Y. H., Zou, Y. N., Bi, C. F., Wang, A. D., and Guo, F. (2007). Synthesis, crystal structure, and kinetics of thermal decomposition of the Zn(II) complex with vanillin. *Russian Journal of Coordination Chemistry*, **33(8)**: 570–575.

Foye,W. O. (1995). *Cancer Chemotherapeutic Agents* (Vol. 34, No. 27-28). American Chemical Society.

Galal, S. A., Hegab, K. H., Kassab, A. S., Rodriguez, M. L., Kerwin, S. M., El-khamry, A., and Diwani, H. I. El. (2009). New transition metal ion complexes with benzimidazole-5-carboxylic acid hydrazides with antitumor activity. *European Journal of Medicinal Chemistry*, **44(4)**: 1500–1508.

Genc, Z. K., Selcuk, S., Sandal, S., and Colak, N. (2014). Spectroscopic, antiproliferative and antiradical properties of Cu(II), Ni(II), and Zn(II) complexes with amino acid based Schiff bases. *Medicinal Chemistry Research*, **23**: 2476–2485.

Gille, A., Bodor, E. T., Ahmed, K., and Offermanns, S. (2008). Nicotinic Acid: Pharmacological Effects and Mechanisms of Action. *Annual Review of Pharmacology and Toxicology*, **48**: 79–106.

Hadjoudis, E., and Mavridis, I. M. (2004). Photochromism and thermochromism of Schiff base in the solid state: structural aspects. *Chemical Society Reviews*, **33(9)**: 579-588.

Hambley, T. W. (2007). Developing new metal-based therapeutics: challenges and opportunities. *Dalton Transactions*, **43**: 4929-4937.

Harris, E. (2001). Copper Homeostasis : The Role of Cellular Transporters. *Nutrition Reviews*, **59(9)**: 281–285.

Hegg,E.L., and Burstyn, J. N. (1998). Toward the development of metal-based synthetic nucleases and peptidases: a rationale and progress report in applying the principles of coordination chemistry. *Coordination Chemistry Reviews*, **173(1)**: 133-165.

Henry, G. D. (2004). De novo synthesis of substituted pyridines. *Tetrahedron*, **60(683)**: 6043–6061.

Hosny, N. M., and El-Dossoki, F. I. (2008). Schiff Base Complexes Derived from 2-Acetylpyridine, Leucine, and Some Metal Chlorides: Their Preparation, Characterization, and Physical Properties. *Journal of Chemical and Engineering Data*, **53(11)**: 2567–2572.

Hosny, N. M., Hussien, M. A., Radwan, F. M., and Nawar, N. (2014). Spectrochimica Acta Part A : Molecular and Biomolecular Spectroscopy Synthesis , spectral

characterization and DNA binding of Schiff-base metal complexes derived from 2-amino-3-hydroxyprobanoic acid and acetylacetone. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **132**: 121–129.

Ito, T., Thyagarajan,S., Karlin,K.D., and Rokita,S. E. (2005). Recognition of guanines at a double helix–coil junction in DNA by a trinuclear copper complex. *Chemical Communications*, **38**: 4812-4814.

Jayendran, D and Manoj, S. (2007) retrieved from Malaysian Oncological Website, www.malaysiaoncology.org/article.php?aid=9

Jiang, Q., Xiao,N., Shi,P., Zhu,Y., and Guo, Z. (2007). Design of artificial metallonucleases with oxidative mechanism. *Coordination Chemistry Reviews*, **251(15)**: 1951-1972.

Johnstone, T. C., Park, G. Y., and Lippard, S.J. (2014). Understanding and Improving Platinum Anticancer Drugs-Phenathriplatin. *Anticancer Research*, **34(1)**: 471-476.

Joule, J. A., and Mills, K. (2010). *Heterocyclic Chemistry , Fifth Edition.*

Kajal, A., Bala, S., Kamboj, S., Sharma,N., and Saini, V.(2013).Schiff Bases:A Versatile Pharmacophore. *Journal of Catalysts*, *2013* (Article ID 893512)

Karmakar, R., Choudhury, C. R., Mitra, S., and Dahlenburg, L. (2005). One Novel Cu(II)--Amino Acid Schiff Base Complex Derived from Salicylaldehyde and L-Serine: Identification of Unusual Monodentate 4,4'-Bipyridine. *Structural Chemistry*, **16(6)**: 611–616.

Kostel, K. L., and Lunte, S. M. (1997). Evaluation of capillary electrophoresis with post-column derivatization and laser-induced fluorescence detection for the determination of substance P and its metabolites. *Journal of Chromatography B: Biomedical Applications*, **695**: 27–38.

Kruszynska, A. T. (2012). Copper complex of glycine Schiff base: In situ ligand synthesis, structure, spectral, and thermal properties. *Journal of Molecular Structure*, **1017**: 72–78.

Li, A., Liu, Y. H., Yuan, L. Z., Ma, Z. Y., Zhao, C. L., Xie, C. Z., Xu, J. Y. (2015). Association of structural modifications with bioactivity in three new copper(II) complexes of Schiff base ligands derived from 5-chlorosalicylaldehyde and amino acids. *Journal of Inorganic Biochemistry*, **146**: 52–60.

Lippard, S. J. (2006). The inorganic side of chemical biology. *Nature chemical biology*, **2(10):** 504-507.

Lincoff, A. M., Wolski, K., Nicholls, S. J., and Nissen, S. E. (2007). Pioglitazone and Risk of Cardiovascular Events in Patients With Type 2 Diabetes Mellitus. *American Medical Association*, **298(10)**: 1180–1188.

Lu, Y., Yeung, N., Sieracki, N., and Marshall, N. M., (2009). Design of functional metalloproteins. *Nature*, **460(7257)**: 855-862

Mahmoud, W. H., Mohamed, G. G., and El-Dessouky, M. M. I. (2015). Synthesis, structural characterization, in vitro antimicrobial and anticancer activity studies of ternary metal complexes containing glycine amino acid and the anti-inflammatory drug lornoxicam. *Journal of Molecular Structure*, **1082**: 12–22.

Mahmoud, W. H., Mahmoud, N. F., Mohamed, G. G., El-Sonbati, A. Z., and El-Bindary, A. A. (2015). Ternary metal complexes of guaifenesin drug: Synthesis, spectroscopic characterization and in vitro anticancer activity of the metal complexes. *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy*, **150**: 451–60.

Mahon, M. F., Mcginley, J., Rooney, A. D., and Walsh, J. M. D. (2009). Unusual copper (II) coordination mode from a potential Schiff-base reaction. *Inorganica Chimica Acta*, **362(7)**: 2353–2360.

Mancin, F., Scrimin, P., Tecilla, P., and Tonellato, U. (2005). Artificial metallonucleases. *Chemical Communications*, **20**: 2540-2548.

Medici, S., Peana, M., Nurchi, V.M., Lachowicz, J.I., Crisponi, G., and Zoroddu, M.A. (2015). Noble Metals in medicine; Latest Advances. *Coordination Chemistry Reviews*, **284**: 329-350.

Meher, C., Rao, A., and Omar, M. (2013). Piperazine-pyrazine and their multiple biological activities. *Asian Journal of Pharmaceutical Science*, **3(1)**: 43–60.

Mho, S., and Johnson, D. C. (2001). Electrocatalytic response of amino acids at Cu Mn alloy electrodes. *Journal of Electroanalytical Chemistry*, **495**: 152–159.

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

Nawaz, H., Akhter, Z., Yameen, S., Siddiqi, H. M., Mirza, B., and Rifat, A. (2009). Synthesis and biological evaluations of some Schiff-base esters of ferrocenyl aniline and simple aniline. *Journal of Organometallic Chemistry*, **694(14)**: 2198–2203.

Patel, M. N., Patel, C, R., and Joshi, H. N. (2012). Interaction of drug based copper(II) complexes with Herring Sperm DNA and their biological activities. *Spectrochimica Acta-Part A: Molecular and Biomolecular Spectroscopy*, **97**: 66-73.

Petzold, K., and Al-Hashimi, H. M. (2011). RNA structure: Adding a second dimension. *Nature Chemistry*, **3(12)**: 913–915.

Pozo-Guisado, E.,Alvarez-Barrientos, A., Mulero-Navarro., S., Santago-Josefat, B., and Fernandez-Salguero, P.M. (2002). The antiproliferative activity of resveratrol results in apoptosis in MCF-7 and MDA-MB-231 human breast

cancer cells: cell-specific alteration of the cell cycle. *Biochemical Pharmacology*, **64**: 1375 – 1386.

Pogozelski, W.K., and Tullius, T.D. (1998). Oxidative strand scission of nucleic acids: routes initiated by hydrogen abstraction from the sugar moiety. *Chemical Reviews*, **98(3)**: 1089-1108.

Prakash, A., Kabir, S., Agrawal, M., Sharma, R., and Gupta, A. K. (2014). Coordination of Cobalt(II), Nickel(II) and Copper(II) with Schiff bases derived from Pyridine-2-Carboxaldehyde and Amino acids. *International Journal of ChemTech Research*, **6(2)**: 1276–1280.

Priya, N. P., Arunachalam, S., Manimaran, A., Muthupriya, D., and Jayabalakrishnan, C. (2009). Molecular and Biomolecular Spectroscopy Mononuclear Ru(III) Schiff base complexes : Synthesis , spectral , redox , catalytic and biological activity studies. *Spectrochimica Acta- Part A: Molecular and Biomolecular Spectroscopy*, **72**: 670–676.

Raja, G., Butcher, R.J., and Jayabalakrishnan, C. (2012). Studies on synthesis, characterization, DNA interaction and cytotoxicity of ruthenium(II) Schiff base complexes. *Spectrochimica Acta- Part A: Molecular and Biomolecular Spectroscopy*, **94:** 205-210.

Raman, N., Sakthivel, A., and Pravin, N. (2014). Exploring DNA binding and nucleolytic activity of few 4-aminoantipyrine based amino acid Schiff base complexes: A comparative approach. *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy*, **125:** 404–413.

Ramli, S., (2016). Synthesis, Electrochemical, Characterisation and Cytotoxic Studies of Bidentate Dithiocarbazate Schiff Base and their Metal Complexes. Master Thesis, Universiti Putra Malaysia

Reddy, P.R., and Shilpa, A. (2011). Oxidative and hydrolytic DNA cleavage by Cu(II) complexes of salicylidene tyrosine schiff base and 1, 10-phenanthroline/bipyridine. *Polyhedron.* **30(4)**: 565–572.

Reddy, P. R., Shilpa, A., Raju, N., and Raghavaiah, P. (2011). Synthesis, structure, DNA binding and cleavage properties of ternary amino acid Schiff base-phen/bipy Cu(II) complexes. *Journal of Inorganic Biochemistry*, **105(12)**: 1603–1612.

Revill, P., Serradell, N., Bolos, J., and Rosa, E. (2007). Telaprevir: HCV NS3 protease inhibitor treatment of hepatitis C. *Drugs of the Future*, **32(9)**: 788–798.

Richter, B., Bergerhoff, K., Clar, C., Richter, B., Bandeira-echtler, Ebrahim, S. H. (2009). Rosiglitazone for type 2 diabetes mellitus (Review). *Cochrane Database of Systematic Reviews*, **(3)**: 2007–2009.

Rimbu, C., Danac, R., and Pui, A. (2014). Antibacterial Activity of Pd(II) Complexes with Salicylaldehyde-Amino Acids Schiff Bases Ligands. *Chemical and Pharmaceutical Bulletin*, **62(1)**: 12–15.

Sakiyan, I., Elif, L., and Seza, A. (2004). Antimicrobial activities of N-(2-hydroxy-1-naphthalidene)-amino acid (glycine , alanine , phenylalanine , histidine , tryptophane) Schiff bases and their manganese(III) complexes. *BioMetals*, **17**: 115–120.

Sakiyan, I., and Hamza, Y. (2003). Manganese(III) Complexes of Some Amino Acid Derived from 2-Hydroxy-1-naphthaldehyde. *Synthesis and Reactivity in Inorganic and Metal-Organic Chemistry*, **33(6)**: 971–983.

Sakiyan, I., Ozdemir, R.,and Ogutcu, H. (2014). Synthesis and Reactivity in Inorganic , Metal-Organic , and Nano-Metal Chemistry Synthesis , Characterization , and Antimicrobial Activities of New N- (2-hydroxy-1-naphthalidene) -amino Acid (L-Tyrosine , L-Arginine , and L-Lysine) Schiff Bases and their Manganese(III) Complexes. *Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry*, **44(3)**: 417–423.

Sathyaraj, G., Weyhermuller, T., and Nair, B. U. (2010). Synthesis, characterization and DNA binding studies of new ruthenium(II) bisterpyridine complexes. *European Journal of Medicinal Chemistry*, **45(1)**:284-291.

Satyanarayana, S., and Nagasundara, K. R. (2004). Synthesis and Spectral Properties of the Complexes of Cobalt(II), Nickel(II), Copper(II), Zinc(II), and Cadmium(II) with 2-(Thiomethyl-2′-benzimidazolyl)-benzimidazole. *Synthesis and Reactivity in Inorganic and Metal-Organic Chemistry*, **34**: 883-895.

Shaikh, J. A. (2014). Synthesis, Spectral Characterization and X-Ray Diffraction Studies of Some Pd(II) Complexes with Schiff Bases. *International Letters of Chemistry, Physics and Astronomy*, **17(3)**: 272-280.

Shi, W., Zhang, X., Jiang, X., Ruan, H., Barry, C. E., Wang, H., Ying, Z. (2012). Pyrazinamide inhibits trans-translation in Mycobacterium tuberculosis: a potential mechanism for shortening the duration of tuberculosis chemotherapy. *Science*, **333(6049)**: 1630–1632.

Tarafder, M., Saravanan, N., Crouse, K., Yamin, B., Raj, S., Razak, I., and Fun, H. –K. (2002). Coordination chemistry and bioactivity of some metal complexes containing two isomeric bidentate NS Schiff bases derived from S-benzyldithiocarbazate and the X-ray crystal structures of S-benzyl-β-N-(5-methyl-2-furylmethylene) dithiocarbazate and bis[S-benzyl-β-N-(2-furylmethylketone) dithiocarbazato] cadmium(II). *Polyhedron*, **21(27)**: 2691-2698.

Thompson, K.H., and Orvig, C. (2006). Metal complexes in medicinal chemistry: new vistas and challenges in drug design. *Dalton Transactions*, **6**: 761-764.

Timmins, G. S., Master, S., Rusnak, F., and Deretic, V. (2004). Nitric Oxide Generated from Isoniazid Activation by KatG:Source of Nitric Oxide and Activity against Mycobacterium tuberculosis. *Antimicrobial Agents and Chemotherapy*, **48(8)**: 3006–3009.

Tripathi, K. D. (2008). Essentials of Medical Pharmacology Sixth Edition.

Tyagi, P., Chandra, S., Saraswat, B. S., and Yadav , D. (2015). Design, spectral characterization, thermal, DFT studies and anticancer cell line activities of Co(II), Ni(II) and Cu(II) complexes of Schiff bases derived from 4-amino-5-(pyridine-4-yl)-4H-1,2,4-triazole-3-thiol. *Spectrochimica Acta- Part A: Molecular and Biomolecular Spectroscopy*, **145**:155-164

Vanco, J.,Marek, J.,Travnicek, Z., Racanska, E.,Muselík, J., and Svajlenova, O. (2008). Synthesis, structural characterization, antiradical and antidiabetic activities of copper(II) and zinc(II) Schiff base complexes derived from salicylaldehyde and β-alanine. *Journal of Inorganic Biochemistry*, **102(4)**: 595–605.

Vannelli, T. A., Dykman, A., Montellano, P. R. O. De, Proc, I. I. I., Acad, N., and Chem, J. B. (2002). The Antituberculosis Drug Ethionamide Is Activated by a Flavoprotein Monooxygenase. *The Journal of Biological Chemistry by The American Society for Biochemistry and Molecular Biology*, **277(15)**: 12824–12829.

Vo, N. H., Xia, Z., Hanko, J., Yun, T., Bloom, S.,Shen, J., Koya, K., Sun, L., and Chen, S. (2014). Synthesis, crystallographic characterization and electrochemical property of a copper(II) complex of the anticancer agent elesclomol. *Journal of Inorganic Biochemistry*, **130**: 69-73.

Wang, M., Meng, Z., Liu, B., and Cai, G. (2005). Novel tumor chemotherapeutic agents andtumor radio-imaging agents : Potential tumor pharmaceuticals of ternary copper(II) complexes. *Inorganic Chemistry Communications*, **8**: 368–371.

Wang, Z., Lin, H., Zhu, S., Liu, T., and Chen, Y. (2002). Spectroscopy , cytotoxicity and DNA-binding of the lanthanum(III) complex of an L-valine derivative of 1,10-phenanthroline. *Journal of Inorganic Biochemistry*, **89**: 97–106.

Wilfred, R.C. (2012). Nickel : The trace mineral that aids in iron absorption, as well as adrenaline and glucose metabolism. www.blissreturned. wordpress.com/2012/02/29/nickel

Willis, M.S.,Monaghan,S.A., Miller, M. L., Mckenna, R. W., Perkins, W.D., Levinson, B. S., Kroft, S. H. (2005). Zinc-Induced Copper Deficiency A Report of Three Cases Initially Recognized on Bone Marrow Examination. *American Society for Clinical Pathology*, **123**: 125–131.

Yang, C.T., Moubaraki, B., Murray, K.S. and Vittal, J.J. (2003). Synthesis, characterization and properties of ternary copper(II) complexes containing reduced Schiff base N-(2-hydroxybenzyl)-α-amino acids and 1, 10-phenanthroline. *Dalton Transactions*, **5**: 880-889.

You, Z., Zhu, H., and Liu, W. (2004). Solvolthermal Syntheses and Crystal Structures of Three Linear Trinuclear Schiff Base Complexes of Zinc(II) and Cadmium(II). *Zeitschrift Fur Anorganische Allgemeine Chemie*, **630**: 1617–1622.

Zahran, M. M., Aboul-Enein, A. M., and Abol-Ella,F.M. (2005). Molecular Changes on Cancer Cells as Affected by Willow Extracts. *Research Journal of Agricultre and Biological Sciences,* **1(3)**: 284-287.

.

Zhang, N., Fan, Y., Bi, C., and Zuo, J. (2013). Synthesis , crystal structure , and DNA interaction of magnesium(II) complexes with Schiff bases. *Journal of Coordination Chemistry*, **66(11)**: 1933–1944.

Zhang, N., Fan, Y., Zhang, Z., Zuo, J., Zhang, P., Wang, Q., Bi, C. (2012). Syntheses, crystal structures and anticancer activities of three novel transition metal complexes with Schiff base derived from 2-acetylpyridine and l-tryptophan. *Inorganic Chemistry Communications*, **22**: 68–72.

Zuo, J., Bi, C., Fan, Y., Buac, D., Nardon, C., Daniel, K. G., and Dou, Q. P. (2013). Cellular and computational studies of proteasome inhibition and apoptosis induction in human cancer cells by amino acid Schiff base-copper complexes. *Journal of Inorganic Biochemistry*, **118**: 83–93