UNIVERSITI PUTRA MALAYSIA

SYNTHESIS, CHARACTERIZATION AND CYTOTOXIC STUDY OF Cu(II) AND Ni(II) CONTAINING AMINO ACID DERIVED SCHIFF BASES

NUR FADHILAH BINTI ABDUL AZIZ

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By

NUR FADHILAH BINTI ABDUL AZIZ

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

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

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November 2016

Chairman : Thahira Begum, PhD
Faculty : Science

Thirty new metal complexes were synthesized from the reaction of Cu(II) acetate and Ni(II) nitrate with Schiff bases derived from the condensation of amino acids (L-phenylalanine, L-valine and glycine) and different heterocyclic aldehydes and ketones. The complexes were characterized by elemental analysis and conductance, magnetic, IR and electronic spectroscopic measurements. The data obtained indicated that the amino acid derived Schiff bases behaved as uninegatively charged bidentate ligands and coordinated with the Cu(II) and Ni(II) ions through their azomethine nitrogen and the deprotonated carboxylate oxygen yielding stable metal complexes as evidenced in their IR spectra. In most of the Cu(II) complexes, only one amino acid derived Schiff base coordinated to the metal centre, while the third and fourth position were occupied by a bidentate -OO- acetate anion. Whereas in two other Cu(II) complexes ($[Cu(ph3t)_2].H_2O$ (Cu(II) complex of 2-[(3-methylthiophene-2-ylmethylene)-amino]-3-phenyl-propionic acid) and $[Cu(glypy)_2].5H_2O$ (Cu(II) complex of [1-(1H-Pyrrol-2-yl)-ethylideneamino]-acetic acid)) and all the Ni(II) complexes, two bidentate -ON- amino acid derived Schiff bases were bonded to the metal centre. Magnetic and spectral evidence supported a four-coordinate geometry for all complexes synthesized. The thermal analysis studies proved the presence of water molecules and methanol outside the inner coordination sphere of some of the complexes synthesized. In turn, the cytotoxic activities of the metal complexes were evaluated against two bladder cancer cell lines which are EJ-28 (invasive human bladder cancer cell line) and RT-112 (minimally-invasive human bladder cancer cell line) and ten of the Ni(II) complexes showed remarkable cytotoxicity against EJ-28 cells while six of the Cu(II) complexes were found to be cytotoxic against RT-112 cells. In conclusion, it appeared that the complexes derived from glycine and 2-acetylpyrrole as their ligands had the best cytotoxic activity in both cancer cell lines.

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

SINTESIS, PENCIRIAN DAN KAJIAN SITOTOKSIK BAGI KOMPLEKS Cu(II) DAN Ni(II) YANG MENGANDUNGI BES SCHIFF TERBITAN ASID AMINO

Oleh

NUR FADHILAH BINTI ABDUL AZIZ

November 2016

Pengerusi : Thahira Begum, PhD
Fakulti : Sains

Tiga puluh kompleks logam baru telah disintesis daripada tindak balas kuprum(II) asetat dan nikel(II) nitrat dengan bes Schiff yang diterbitkan daripada tindak balas kondensasi asid amino (L-fenilalanina, L-valina dan glisina) dan pelbagai aldehid dan keton heterosiklik. Kompleks tersebut telah dicirikan melalui analisis unsur dan konduktiviti, kerentanan magnet,inframerah dan pengukuran spektroskopi eletronik. Hasil pencirian yang diperolehi menunjukkan bahawa bes Schiff ligan yang diterbitkan daripada asid amino berkelakuan sebagai cas uninegatif ligan bidentat dan berkoordinat dengan kuprum(II) dan nikel(II) ion melalui nitrogen azometin dan oksigen karboksilat yang dinyahprotonkan yang menghasilkan kompleks logam stabil seperti yang dibuktikan dalam spektrum inframerah mereka. Dalam hampir semua kompleks kuprum(II), hanya satu ligan bes Schiff yang diterbitkan daripada asid amino berkoordinat dengan pusat logam, manakala kedudukan ketiga dan keempat dipenuhi oleh anion bidentat -OO- asetat. Sedangkan dalam dua kompleks kuprum(II) yang lain ($[Cu(ph3t)_2].H_2O$ (kompleks kuprum(II) asid 2-[(3-metiltiofena-2-ilmetilena)-amino]-3-fenil-propionik) dan $[Cu(glypy)_2].5H_2O$ (kompleks kuprum(II) asid [1-(1H-Pirrol-2-il)-etilidenaamino]-asetik)) dan dalam semua kompleks nikel(II), dua bidentat -ON- bes Schiff ligan yang diterbitkan daripada asid amino telah berkoordinat dengan pusat logam. Nilai kerentanan magnet dan spektra menyokong geometri semua kompleks yang disintesis berkoordinat empat. Kajian analisis terma membuktikan kehadiran molekul air dan metanol di luar sfera koordinasi dalam beberapa kompleks yang disintesis. Kemudiannya, aktiviti sitotoksik kompleks logam telah dikaji terhadap dua jenis sel kanser pundi kencing iaitu EJ-28 (sel kanser pundi kencing manusia dengan invasif tinggi) dan RT-112 (sel kanser pundi kencing manusia dengan invasif rendah) dan sepuluh kompleks nikel(II) menunjukkan aktiviti sitotoksik yang luar biasa terhadap sel EJ-28 manakala enam kompleks kuprum(II) didapati sitotoksik terhadap sel RT-112. Konklusinya, kelihatan bahawa kompleks yang diterbitkan daripada glisina dan 2-asetilpirrol sebagai ligan mereka mempunyai aktiviti sitotoksik yang terbaik terhadap kedua-dua sel kanser.

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I certify that a Thesis Examination Committee has met on 8 November 2016 to conduct the final examination of Nur Fadhilah binti Abdul Aziz on her thesis entitled "Synthesis, Characterization and Cytotoxic Study of Cu(II) and Ni(II) 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 is to confirm that;

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- Supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

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Signature: _____
Name of Member of Supervisory Committee: Dr. Mohamed Ibrahim B. Mohamed Tahir

Signature: _____
Name of Member of Supervisory Committee: Dr. Abhimanyu Veerakumarasivam

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LIST OF ABBREVIATIONS

B.M	Bohr Magneton
CHNS	Carbon, Hydrogen, Nitrogen & Sulphur
NMR	Nuclear Magnetic Resonance
DMSO	Dimethylsulphoxide
FT-IR	Fourier-Transform Infrared
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectroscopy
IC_{50}	Inhibitory concentration at 50%
LMCT	Ligand to metal charge transfer
MCF-7	Human breast carcinoma cells with positive estrogen receptor
MDA-MB-231	Human breast carcinoma cells with negative estrogen receptor
HCT117	Human Colon Carcinoma Cell Line
EJ-28	Invasive Human Bladder Cancer Cell Line
RT-112	Minimally-Invasive Human Bladder Cancer Cell Line
ON	Oxygen-Nitrogen
OO	Oxygen-Oxygen
ONO	Oxygen-Nitrogen-Oxygen
N,N	Nitrogen-Nitrogen
SXRD	Single Crystal X-ray Diffraction
UV/Vis	Ultraviolet/Visible Spectroscopy
XRD	X-Ray Diffraction
L	Ligand
LOR	Lornoxicam
Phen	Phenanthtroline
bipy	2,2'-bipyridine
$[Cu(L^1)(Phen)].9H_2O$	Cu(II) complexes of 2-[(2-Hydroxy-3-methoxy-benzylidene)-amino]-4-methylsulfanyl-butyric acid
$[Cu(L^2)(Phen)].3H_2O$	Cu(II) complex of 2-[(2-Hydroxy-naphthalen-1-ylmethylene)-amino]-3-methyl-butyric acid
CuOVHIS	Cu(II) complex of 2-[(2-Hydroxy-3-methoxy-benzylidene)-amino]-3-(1H-imidazol-4-yl)-propionic acid
dpktch	Thiophene-2-carboxylic acid (di-pyridin-2-yl-methylene)-hydrazide
$(Et_3P)_2Ni(NO_3)_2$	Ni(II) complex of triphenylphosphine
CuS2M2TK	N'-(1-Thiophen-2-yl-ethylidene)-hydrazinecarbodithioic acid 2-methyl-benzyl ester
Glypy	[1-(1H-Pyrrol-2-yl)-ethylideneamino]-acetic acid
Ac	Acetate
ph	L-phenylalanine
va	L-valine
gly	glycine
3t	3-methylthiophene-2-carbaldehyde
5t	5-methylthiophene-2-carbaldehyde

pyr	2-acetylpyrrole
Ace	3-acetylthiophene
Az	2-acetylthiazole
$[Cu(ph3t)_2].H_2O$	Cu(II) complex of 2-[(3-methylthiophene-2-ylmethylene)-amino]-3-phenyl-propionic acid
$[Cuph5tAc].3H_2O$	Cu(II) complex of 2-[(5-methylthiophene-2-ylmethylene)-amino]-3-phenyl-propionic acid
[CuphpyrAc]	Cu(II) complex of 3-Phenyl-2-[1-(1H-pyrrol-2-yl)-ethylideneamino]-propionic acid
$[CuphAceAc].H_2O$	Cu(II) complex of 3-Phenyl-2-(1-thiophene-3-yl-ethylideneamino)-propionic acid
[CuphAzAc]	Cu(II) complex of 3-Phenyl-2-(1-thiazol-2-yl-ethylideneamino)-propionic acid
$[Cuva3tAc].\ 2H_2O$	Cu(II) complex of 3-Methyl-2-[(3-methylthiophene-2-ylmethylene)-amino]-butyric acid
$[Cuva5tAc].\ H_2O$	Cu(II) complex of 3-Methyl-2-[(5-methylthiophene-2-ylmethylene)-amino]-butyric acid
$[CuvapyAc].2H_2O$	Cu(II) complex of 3-Methyl-2-[1-(1H-pyrrol-2-yl)-ethylideneamino]-butyric acid
[CuvaAcAc]	Cu(II) complex of 3-Methyl-2-(1-thiophene-3-yl-ethylideneamino)-butyric acid
[CuvaAzAc]	Cu(II) complex of 3-Methyl-2-(1-thiazol-2-yl-ethylideneamino)-butyric acid
$[Cugly3tAc].H_2O$	Cu(II) complex of [(3-methylthiophene-2-ylmethylene)-amino]-acetic acid
$[Cugly5tAc].H_2O$	Cu(II) complex of [(5-methylthiophene-2-ylmethylene)-amino]-acetic acid
$[Cu(glypyr)_2].2H_2O$	Cu(II) complex of [1-(1H-Pyrrol-2-yl)-ethylideneamino]-acetic acid
$[CuglyAceAc].2H_2O$	Cu(II) complex of (1-thiophene-3-yl-ethylideneamino)-acetic acid
$[CuglyAzAc].2H_2O$	Cu(II) complex of (1-thiazol-2-yl-ethylideneamino)-acetic acid
$[Ni(ph3t)_2].5H_2O$	Ni(II) complex of 2-[(3-methylthiophene-2-ylmethylene)-amino]-3-phenyl-propionic acid
$[Ni(ph5t)_2].5H_2O$	Ni(II) complex of 2-[(5-methylthiophene-2-ylmethylene)-amino]-3-phenyl-propionic acid
$[Ni(phpyr)_2].6H_2O.2CH_3OH$	Ni(II) complex of 3-phenyl-2-[1-(1H-pyrrol-2-yl)-ethylideneamino]-propionic acid
$[Ni(phAce)_2].5H_2O$	Ni(II) complex of 3-phenyl-2-(1-thiophene-3-yl-ethylideneamino)-propionic acid
$[Ni(phAz)_2].4H_2O$	Ni(II) complex of 3-phenyl-2-(1-thiazol-2-yl-ethylideneamino)-propionic acid
$[Ni(va3t)_2].5H_2O$	Ni(II) complex of 3-methyl-2-[(3-methylthiophene-2-ylmethylene)-amino]-butyric acid
$[Ni(va5t)_2].5H_2O$	Ni(II) complex of 3-methyl-2-[(5-methylthiophene-2-ylmethylene)-amino]-butyric acid
$[Ni(vapyr)_2].3H_2O.2CH_3OH$	Ni(II) complex of 3-methyl-2-[1-(1H-pyrrol-2-yl)-ethylideneamino]-butyric acid
$[Ni(vaAce)_2].5H_2O.3CH_3OH$	Ni(II) complex of 3-methyl-2-(1-thiophene-3-yl-

	ethylideneamino)-butyric acid
$[Ni(vaAz)_2].7H_2O$	Ni(II) complex of 3-methyl-2-(1-thiazol-2-yl-ethylideneamino)-butyric acid
$[Ni(gly3t)_2].7H_2O$	Ni(II) complex of [(3-methylthiophene-2-ylmethylene)-amino]-acetic acid
$[Ni(gly5t)_2].3H_2O.2CH_3OH$	Ni(II) complex of [(5-methylthiophene-2-ylmethylene)-amino]-acetic acid
$[Ni(glypyr)_2].5H_2O$	Ni(II) complex of [1-(1H-Pyrrol-2-yl)-ethylideneamino]-acetic acid
$[Ni(glyAce)_2].5H_2O$	Ni(II) complex of (1-thiophene-3-yl-ethylideneamino)-acetic acid
$[Ni(glyAz)_2].H_2O$	Ni(II) complex of (1-thiazol-2-yl-ethylideneamino)-acetic acid

CHAPTER I

INTRODUCTION

1.1 Structure and Properties of Amino Acids

Amino acids are the building blocks of proteins. Amino acids are compounds containing amine, carboxylic acid functional groups and a side chain (R group) that differs in each amino acid. Amino acids having both amine and carboxylic acid groups attached to the first carbon atom are known as α-amino acids (Figure 1) with a generic formula of $H_2NCHRCOOH$ where R is an organic substituent.

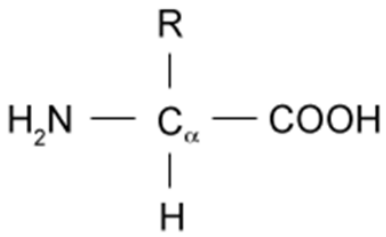

Figure 1: General structural formula for α-amino acids

Amino acids (except glycine) occur in two isomeric forms which are L and D forms, which analogous to a left-handed and right-handed configuration. However in this study, L-amino acids were used instead of D-amino acids since only L-amino acids are constituents of proteins (Berg *et al.*, 2002). The enzymes in body only recognize L-amino acids and thus L-amino acids become abundant and selectively advantageous compared to D-amino acids. Glycine is the simplest amino acid which has just a hydrogen atom in its side chain. Glycine, with a chemical formula of H_2NCH_2COOH is the only achiral amino acid since it has two hydrogen atoms bonded to the α-carbon atom. The structure of glycine is shown in Figure 2.

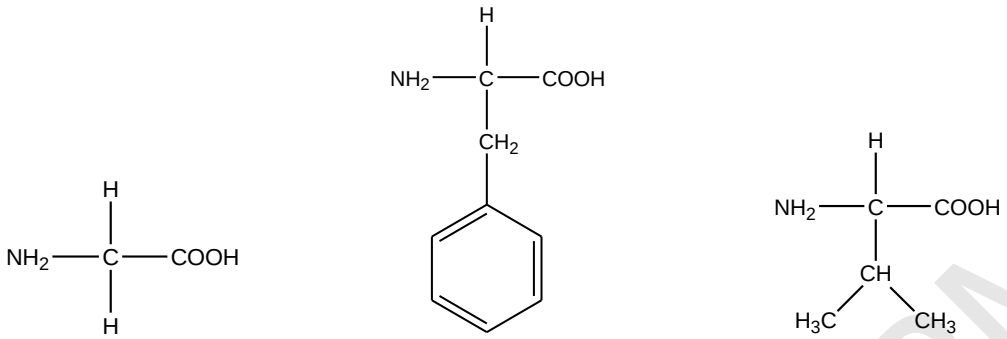

Figure 2: Structures of glycine, phenylalanine and valine

Larger hydrocarbon side chains are found in phenylalanine and valine. Phenylalanine with a chemical formula of $H_2NCHCH_2C_6H_5COOH$ contains a phenyl ring as its side chain. On the other hand, valine with a chemical formula of $H_2NCHCH(CH_3)_2COOH$ has an isopropyl group as its side chain. The larger aliphatic side chains are hydrophobic where they tend to cluster together rather than contact water. The three-dimensional structures of water-soluble proteins are stabilized by this tendency of hydrophobic groups to come together. The structures of phenylalanine and valine are shown in Figures 2 above.

1.2 Amino Acid-derived Schiff Bases

Schiff bases are compounds containing azomethine nitrogen or a carbon-nitrogen double bond (RHC=N-R') as a functional group where R and R' can be an alkyl, aryl or a heterocyclic group. The first Schiff bases synthesized were reported by Hugo Schiff in 1864. The Schiff bases are formed by condensation of an aldehyde or ketone with a primary amine (Cozzi *et al.,* 2004). Amino acids containing uncharged amino groups may undergo Schiff base formation which presents another potential mechanism for metal complexes (Mahmud *et al.,* 2010). An example of an amino acid-derived Schiff base formed from the reaction between an amino acid and a carbonyl compound is shown in the figure below:

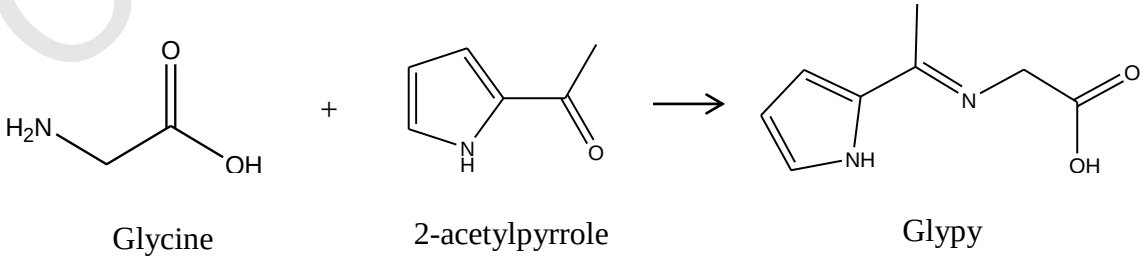

Figure 3: Preparation of amino acid-derived Schiff base (Glypy) from the reaction between an amino acid (glycine) and a carbonyl compound (2-acetylpyrrole)

One of the most important amino acids derivatives are Schiff base complexes which have been known as anti-cancer, anti-bacterial, anti-tumor, anti-fungal and anti-inflammatory agents (Wang *et al.,* 2005). The Schiff bases are active against a wide range of organisms since they play an important role in living organisms (Sari *et al.,* 2014). The Schiff bases and their metal complexes also have wide biological applications and they have been reported to inhibit superoxide anion radicals (Yi *et al.,* 2001).

The Schiff bases have been used extensively as ligands because of their intramolecular hydrogen bonds between the oxygen and azomethine nitrogen atoms which play an important role in the formation of metal complexes. In addition, Schiff bases have appeared to be an important intermediate in many enzymatic reactions involving an interaction of the enzyme with amino or carbonyl group of the substrate (Elerman *et al.,* 2002).

1.3 Metal Complexes of Amino Acid-derived Schiff Bases

Amino acid-derived Schiff bases are used as ligands for the preparation of metal complexes having a series of different structures. The complexes containing amino acid-derived Schiff bases have gained importance from the inorganic point of view because of their physiological and pharmacological activities (Wang *et al.,* 1990). Previous studies showed the amino acid-derived Schiff base behaves as a flexidentate ligand and commonly coordinates through the deprotonated carboxylate oxygen and the nitrogen atom of azomethine. Schiff bases which could act as polydentate ligands and assisted by metal ions, provide highly organized supramolecular metal complexes and possess binding sites and cavities for various cations, anions and organic molecules (Dixit *et al.,* 2009). It has been reported that binding of a drug to a metallo-element enhances its activity which can lead it to possessing even more healing properties than the parent drug (Lippard *et al.,* 1999).

There has been considerable interest in metal complexes with Schiff bases since they have a variety of applications including biology, medical imaging, magnetism and catalysis (Zhang *et al.,* 2012). The Schiff bases play an important role in the development of coordination chemistry as they form stable complexes with most of the transition metals. In bioinorganic chemistry, they provide synthetic models for the metal-containing sites in metalloproteins or enzymes and contribute widely to the development of medicinal chemistry, cancer diagnosis and treatment of tumor (Quian *et al.,* 2004). The active species of drugs of metal complexes can spontaneously undergo biological reactions such as ligand substitutions and redox reactions (Dilip *et al.,* 2013). The bonding modes and the geometry of the metal complexes in ligand chelation environments serve as models to enzyme containing metal ions (Sujarani *et al.,* 2014).

Complexes containing N and O donor atoms are effective as stereospecific catalysts for oxidation and reduction, biocidal activity, hydrolysis and transformation of organic and inorganic chemistry (Sengupta *et al.*, 2001). The examples of metal complexes containing amino acid-derived Schiff bases that have been proven to have remarkable cytotoxicity against human breast (MDA-MB231 and MCF-7) and prostate (PC-3) cancer cells are shown in Figures 4 and 5 below. The complexes were synthesized from the ligands derived from amino acids of L-methionine (C1) and L-valine (C2), and 1,10-phenanthtroline as their second ligand.

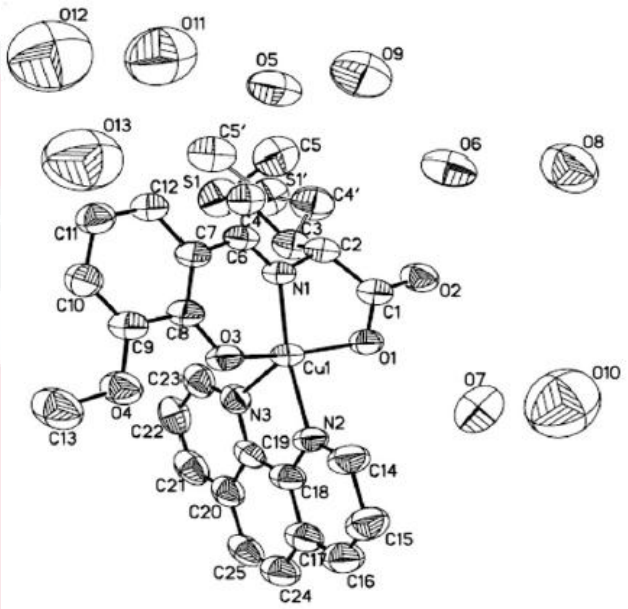

Figure 4: ORTEP diagram of C1, [Cu(L^1)(Phen)].9H_2O (Zuo *et al.*, 2013)

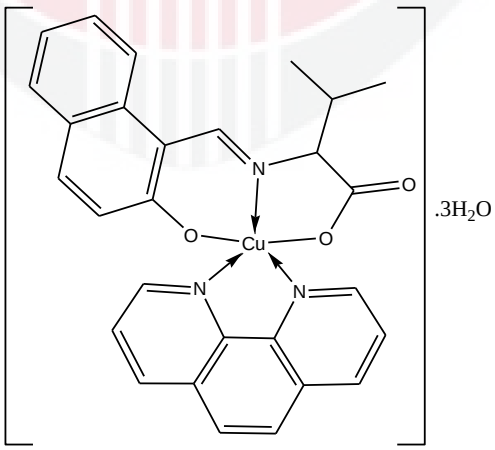

Figure 5: Proposed structure of C2, [Cu(L^2)(Phen)].3H_2O (Zuo *et al.*, 2013)

1.4 Problem Statement

Malignant tumors or commonly known as cancer, is a generic term for a large group of diseases that can affect any part of the body. Cancers figure among the leading causes of morbidity and mortality worldwide. Statistics have shown that approximately 14 million new cancer cases were reported and resulted in 8.2 million deaths in 2012 (World Cancer Report 2014). Terrifyingly, the number of new cancer cases is expected to rise by about 70% over the next two decades. Bladder cancer is one of the most common causes of cancer deaths and is increasing in developing countries. In fact, bladder cancer is the ninth most common cancer in the world with 430, 000 new cases diagnosed in 2012 (World Cancer Research Fund International, 2015). Bladder cancer is the most expensive cancer to treat because of its high rate of recurrence and progression. Thus there is a need to find new therapeutic avenues to manage bladder cancer. The Table 1 below gives the estimated number of new cases and deaths for each common cancer type.

Previous studies have shown that different chemical properties can affect the biological activities of metal complexes as drugs. Therefore, these findings will open up the opportunity for the discovery of metal-based drugs with minimal side effects and toxicity. The potential biological activities of metal complexes containing amino acid-derived Schiff bases have rarely been studied. Thus in this study, metal complexes containing heterocyclic amino acid-derived Schiff bases were synthesized and their cytotoxic activity against two bladder cancer cell lines, EJ-28 (invasive human bladder cancer cell line) and RT-112 (minimally-invasive human bladder cancer cell line) was investigated. Three different amino acids were used which are glycine, L-phenylalanine and L-valine. These amino acids were chosen since they have been proved in many previous studies (Reddy *et la.,* 2011), (Zuo *et al.,* 2013) and (Alsalme *et al.,* 2016) to have remarkable cytotoxic activities against various cancer cell lines.

Table 1: Estimated Number of New Cancer Cases and Deaths for the 10 Most Common Cancers (American Cancer Society: Cancer Facts and Figures 2016. Atlanta, GA: American Cancer Society, 2016).

Rank	Cancer Type	Estimated New Cases	Estimated Deaths
1	Breast (Female – Male)	246,660 – 2,600	40,450 – 440
2	Lung (Including Bronchus)	224,390	158,080
3	Prostate	180,890	26,120
4	Colon and Rectal (Combined)	134,490	49,190
5	**Bladder**	**76,960**	**16,390**
6	Melanoma	76,380	10,130
7	Non-Hodgkin Lymphoma	72,580	20,150
8	Thyroid	64,300	1,980
9	Kidney (Renal Cell and Renal Pelvis) Cancer	62,700	14,240
10	Leukemia (All Types)	60,140	24,400

1.5 Objectives

1. To synthesize Cu(II) and Ni(II) complexes containing amino acid Schiff bases derived from L-phenylalanine, glycine and L-valine with different heterocyclic aldehydes and ketones.

2. To characterize the newly synthesized compounds by using various physico-chemical techniques and spectroscopic techniques.

3. To determine the *in vitro* cytotoxic activity of metal complexes containing amino acid-derived Schiff bases against bladder cancer.

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