UNIVERSITI PUTRA MALAYSIA

SYNTHESIS, CHARACTERISATION AND BIOLOGICAL STUDIES OF TRANSITION METAL COMPLEXES (Cu, Zn, Ni) DERIVED FROM AMINO ACIDS

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By

LAI LEE CHIN

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

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Chairman : Thahira Begum, PhD
Faculty : Science

The arising of cancer and bacterial infections had become one of the concerns of the public due to high mortality rate. Hence, there is a need of searching for new drugs with higher activity. Metal complexes derived from amino acids have attracted much attention because of biological importance. In view of this, new amino acid-derived Schiff base complexes formed from various amino acids [L-phenylalanine (Phe), L-histidine (His), L-valine (Val), and L-methionine (Met)], dicarbonyl compounds [acetyl acetone (AA), glyoxal (G), 2,5-hexanedione (HD) and 5,5-dimethyl-1,3-cyclohexanedione(MHD)] and metal acetate salts [Cu(II), Ni(II) and Zn(II)] were synthesised. A total of 47 metal complexes were synthesised using template method. These complexes were characterised by various physico-chemical and spectroscopic methods. The FT-IR spectra indicated the presence of the C=N band around the region of 1600 cm^{-1} which proved the successful formation of the Schiff base. The Schiff bases were tetra-coordinated to the metal ion in 1:1 mol ratio *via* the azomethine nitrogen atom and carboxylate oxygen atom in the manner of N_2O_2. Based on the data obtained, the geometry of the complexes were either distorted tetrahedral or distorted square planar for the four-coordinated complexes, square pyramidal for NiGVal and ZnAAVal and distorted octahedral for NiHDPhe, NiMHDHis, NiHDHis, NiGVal, ZnGVal and ZnGMet. The complexes were evaluated against two bladder cancer cell lines (invasive human bladder carcinoma cell line, EJ-28; minimum-invasive human bladder carcinoma cell line, RT-112), and various bacterial strains (*B. cereus*, *S. aureus*, Methicillin-resistant *S. aureus*, *E. coli*, *K. pneumonia*, *S. typhimurium*, and *S. sonnei*) for cytotoxic and antibacterial activity. MTT assay was used to determine the cytotoxic activity of the complexes. From the data obtained, CuAAPhe and CuGPhe were found to be active against RT-112 cells at 13.70 μM and 14.71 μM respectively. The anti-migratory properties of the inactive complexes were studied against EJ-28 cells. CuGPhe, CuMHDVal, ZnGPhe, ZnAAHis, and NiAAVal were found to be potential anti-migration agents for bladder cancer cells. Disc diffusion method and determination of minimum inhibitory concentration (MIC) were used in the antibacterial studies of the

complexes. The data showed that the complexes were more active against Gram-positive bacteria than Gram-negative bacteria. The MIC data obtained for CuAAHis, ZnGPhe, CuMHDHis, ZnGHis, ZnGMet and ZnHDMet against *S. aureus* was 8 mg/mL. The electrochemical behavior of the Cu(II) complexes were determined *via* cyclic voltammetry. Cu(II) complexes derived from L-phenylalanine, L-histidine and L-valine were found to be potential mediators in lactate oxidase (LOx) biosensors.

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

SINTESIS, PENCIRIAN DAN PENGAJIAN BIOLOGI BAGI KOMPLEKS LOGAM (Cu, Zn, Ni) DARI ASID AMINO

Oleh

LAI LEE CHIN

November 2018

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Kemunculan kanser dan jangkitan bakteria telah menjadi salah satu kebimbangan dalam kalangan masyarakat disebabkan kadar kematian yang tinggi. Oleh itu, terdapat keperluan untuk mencari ubat baharu dengan aktiviti yang lebih tinggi. Kompleks logam terbitan asid amino telah menarik banyak perhatian kerana kepentingan dalam biologi. Memandangkan situasi ini, kompleks bes Schiff baharu dari asid amino disintesiskan daripada pelbagai asid amino (L-phenylalanine, L-histidine, L-valine, dan L-methionine), sebatian dikarbonil (asetil aseton, glioxal, 2,5-heksanadion, dan 5,5-dimetil-1,3-sikloheksanadion) dan asetat logam [Cu(II), Ni(II) dan Zn(II)]. Sejumlah 47 kompleks logam telah disintesis dengan menggunakan kaedah template. Kompleks tersebut telah dicirikan melalui pelbagai kaedah fiziko-kimia dan spektroskopi. FT-IR spektra menunjukkan kehadiran regangan C=N dalam linkungan 1600 cm^{-1} yang mana berjaya membuktikan pembentukan bes Schiff. Ligan bes Schiff adalah tetra-koordinat kepada ion logam dalam nisbah mol 1: 1 melalui atom nitrogen azometin dan atom oksigen karbosilat dengan cara N_2O_2. Berdasarkan data yang diperolehi, geometri untuk kompleks adalah tetrahedron terherot atau segiempat satah terherot bagi kompleks empat koordinasi, piramid segiempat terherot bagi NiGVal dan ZnAAVal dan oktahedron terherot bagi NiHDPhe, NiMHDHis, NiHDHis, NiGVal, ZnGVal dan ZnGMet. Kompleks telah diuji terhadap dua jenis sel kanser pundi kencing (sel karsinoma kanser pundi kencing manusia invasif, EJ-28; sel karsinoma kanser pundi kencing manusia yang minimum invasif, RT-112), dan pelbagai jenis bakteria (*B. cereus, S. aureus,* Methicillin-resistant *S. Aureus, E. coli, K. pneumonia, S. typhimurium, S. Sonnei*) untuk menilai aktiviti sitotoksik dan antibakteria. Ujian MTT digunakan dalam menentukan aktiviti sitotoksik bagi kompleks. Daripada data yang diperolehi, CuAAPhe dan CuGPhe didapati aktif terhadap sel RT-112 pada 13.70 μM dan 14.71 μM. Sifat anti-migrasi untuk kompleks yang tidak aktif dikajikan ke atas sel EJ-28. CuGPhe, CuMHDVal, ZnGPhe, ZnAAHis, dan NiAAVal didapati berpotensi dijadikan sebagai agen anti-migrasi bagi sel kanser pundi kencing. Kaedah cakera penyebaran dan penentuan kepekatan penghalang minimum (MIC) digunakan dalam kajian antibakteria untuk kompleks. Data yang diperoleh menunjukkan bahawa kompleks lebih aktif terhadap

bakteria Gram-positif berbanding dengan bakteria Gram-negatif. Nilai MIC yang diperolehi untuk CuAAHis, ZnGPhe, CuMHDHis, ZnGHis, ZnGMet dan ZnHDMet terhadap *S. aureus* adalah 8 mg / mL. Sifat elektrokimia kompleks Cu(II) ditentukan melalui voltametri kitaran. Kompleks Cu(II) disintesis dari L-phenylalanine, L-histidine dan L-valine didapati berpotensi dijadikan sebagai mediator dalam laktat oksida biosensor (LOx).

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Last but not least, special thanks to my beloved family especially my parents for their love, support and motivation. Thank you to those who had contributed their cooperation directly and indirectly during my study. Thank you.

I certify that a Thesis Examination Committee has met on 22 November 2018 to conduct the final examination of Lai Lee Chin on her thesis entitled "Synthesis, Characterisation and Biological Studies of Transition Metal Complexes (Cu, Zn, Ni) Derived from Amino Acids" 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 Doctor of Philosophy.

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Phe	L-phenylalanine
His	L-histidine
Val	L-valine
Met	L-methionine
AA	Acetyl acetone
G	Glyoxal
HD	2,5-hexanedione
MHD	5,5-dimethyl-1,3-cyclohexanedione
LOx	Lactate oxidase
FT-IR	Fourier transform infrared
ICP-OES	Inductively coupled plasma optical emission spectrometry
ppm	Parts per million
UV-Vis	Ultraviolet-visible
DMSO	Dimethylsulphoxide
TGA	Thermogravimetric analysis
EJ-28	Invasive human bladder carcinoma cell line
RT-112	Minimally-invasive human bladder carcinoma cell line
RPMI	Roswell park memorial institute medium
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
PBS	Phosphate-buffered saline
FBS	Fetal bovine serum
ELISA	Enzyme-linked immunosorbent assay
IC_{50}	Inhibition concentration at 50%
BC	*Bacillus cereus*

SA	*Staphylococcus aureus*
MRSA	Methicillin resistant *staphylococcus aureus*
EC	*Escherichia coli*
KP	*Klebsiella pneumonia*
SS	*Shigella sonnei*
ST	*Salmonella typhimurium*
TSA	Tryptic Soy agar
NA	Nutrient agar
MHA	Mullex Hinton agar
MHB	Mullex Hinton broth
OD	Optical density
INT	P-iodonitrotetrazolium violet
MIC	Minimum inhibitory concentration
ITO	Indium tin oxide
d	Decomposed
B.M.	Bohr magneton
μ_{EFF}	Magnetic moment
M_{SO}	Spin-only
HOMO	Highest occupied molecular orbital energies
LUMO	Lowest unoccupied molecular orbital energies
LMCT	Ligand to metal charge transfer
ROS	Reactive oxygen species

CHAPTER 1

INTRODUCTION

There is a rise in the different types of cancers around the world due to the adoption of different lifestyles behaviour such as smoking, poor diet, physical inactivity, reproductive changes and some other environmental risk factors including occupation, infections, and radiation as shown in Figure 1.1 (Parkin *et al*., 2011).

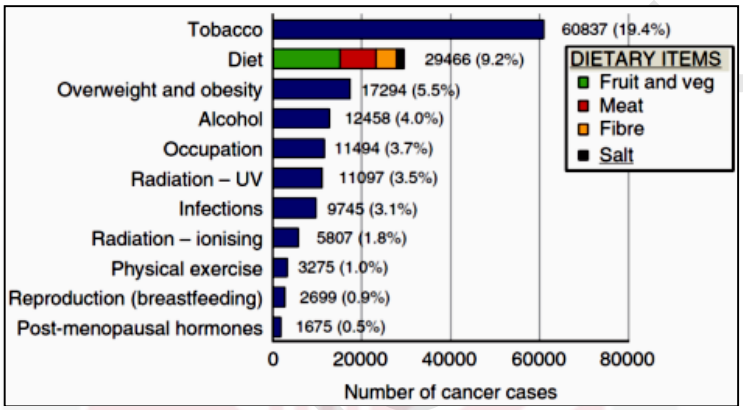

Figure 1.1: Number and percentage of cancer cases in the UK attributable to different exposures (Parkin *et al*., 2011)**.**

Among the cancers, bladder cancer is the ninth most common cancer worldwide based on the data from International Agency for Research on Cancer and the World Health Organisation (Antoni *et al*., 2017). Besides, the high risk of recurrence and the absence of reliable biomarkers for the prediction of cancer progression have resulted in a high cost of treatment (van Rhijn *et al*., 2009). The current treatments for non-muscle-invasive bladder cancer are complete resection of the tumour followed by Bacillus Calmette-Guérin (BCG) vaccine induction or intravesical chemotherapy (Kamat *et al,*. 2016) while muscle-invasive bladder cancer include multimodal treatments which include radical cystectomy with neoadjuvant chemotherapy (Plimack *et al.,* 2014), bladder-sparing trimodality treatment consisting of transurethral resection with chemoradiation (James *et al*., 2012; Gogna *et al*., 2006; Mitin *et al*., 2013) and systemic cisplatin-based chemotherapy or immunotherapy (Sternberg *et al*., 2001; De Santis *et al*., 2012; Bellmunt *et al*., 2009). However, failure in BCG and ineligible in receive cisplatin were reported in some patients (Kamat *et al*., 2016; Dash *et al*., 2006). Thus, carboplatin-based combinations were used in treatment (De Santis *et al*., 2009). However, inferior results were obtained when compared with cisplatin-based regimens (Dogliotti *et al*., 2007). Since the number of new drugs being evaluated for bladder cancer treatment are relatively low in comparison with other cancer types, and therefore more attempts were required in searching for new therapeutic agents.

Other than cancers, bacterial infections that are on the rise had also received the attention of researchers in finding more efficient antibacterial drugs. This was due the rapid increase of bacteria resistant to currently available antibacterial drugs. As reported by Chambers and DeLeo (2009), four waves of antibiotic resistance were observed for *Staphylococcus aureus* (Figure 1.2) whereby the penicillin resistance was encountered in the mid of 1940s, only a few years after penicillin introduction into clinical practice. Later, it was followed by the methicillin resistance which began almost immediately upon the introduction of methicillin into clinical practice. In 1970s, the new MRSA strains so-called MRSA-II and III. Community-associated MRSA (CA-MRSA) strains were identified in the mid-to-late 1990s.

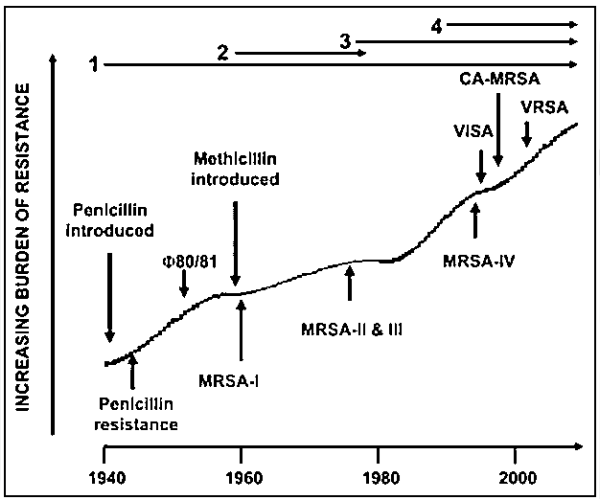

Figure 1.2: A timeline of the four waves of antibiotic resistance in *Staphylococcus aureus* (Chambers and DeLeo, 2009)**.**

Besides that, some other types of bacteria were also reported to have resistance against the antibiotics such as *Escherichia coli* against antibiotics as cephalosporin and fluoroquinolones, *Klebsiella pneumoniae* against cephalosporin and carbapenems, and *Shigella* species against fluoroquinolones (World Health Organisation, 2014). According to World Health Organisation (2016), people with MRSA (methicillin-resistant *Staphylococcus aureus*) were estimated to be 64% more likely to die than people with a non-resistant form of the infection. Undeniably, the increasing resistance of bacteria against the antibacterial drugs has become a serious issue to the public health. Hence, the efforts in identifying new antibiotics has become top research and development priority among pharmaceutical companies especially the microbes have found their ways to circumvent different structural classes of drugs and were less susceptible to most.

Herein, synthetic drugs play a crucial role in the pharmaceutical companies because it does not face the difficulties in access and supply as compared to natural products. Besides, small changes could be made on synthetic compounds by introducing with different organic substituents and hence results in different drug activity (Iftikhar *et al*.,

2018; Cvijetić *et al*., 2018). Therefore, in this work, different amino acid Schiff bases were synthesised from various amino acids and dicarbonyl compounds in order to explore the biological activity of the ligands upon complexation with various metal ions. This is also important as inorganic chemistry usually offers a wide spectrum of design for metal-based drugs (Rajarajeswari *et al*., 2013).

Besides the pharmaceutical application, metal complexes were also widely applied in sensors. As reported by Gou *et al.* (2015), 1,10-phenanthroline copper(II) (PhenCu) complex was non-covalently functionalised on reduced graphene oxide (RGO) as an electrochemical chiral sensor for tryptophan (Trp) enantiomers. Besides, a low temperature NO_2 gas sensor was also reported on the $(CuTu)Cl{\cdot}0.5H_2O$ nanowires based on metal-thiourea complex (Han *et al*., 2018). Thereby, in this work, the copper(II) complexes synthesised were employed for the application of lactate oxidase (LOx) biosensor. L-lactate is a biochemical compound that produced from pyruvate in liver, muscles and kidney due to insufficient supply of oxygen (Zhao *et al*., 2015). Besides, its concentration plays a crucial role in clinical diagnostics (Loaiza *et al*., 2015), sport medicine (Faude *et al*., 2009) and the food processing industry (Zanini *et al*., 2011).

All in all, Schiff base amino acid complexes synthesised from amino acid are believed to be safer due to their natural presence that could safely inhibit the bladder cancer cells but also bacteria. Not only that, the copper(II) complexes derived from amino acid were hoped to be applied as lactate oxidase (LOx) biosensor in L-lactate detection.

1.1 Amino Acids

Amino acids (Figure 1.3) are organic compounds that contain at least one amine group, NH_2 and carboxyl group, COOH.

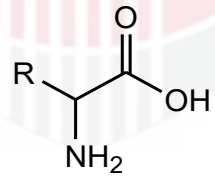

Figure 1.3: The general structure of amino acid.

Amino acids are the basic unit of the protein and are found in structural tissues of the body. Amino acids play an important role in the life activity such as performing some critical biological roles including neurotransmitters and transport in the body (Li *et al.*, 2014).

Moreover, amino acids are one of the essential and indispensable nutrients in vivo. Herein, various functions are supported by amino acids in the body depending on the proper amino acid metabolism. For instance, methionine metabolism is subject to many

impairments, resulting in a variety of disorders, including neural tube defects, cardiovascular diseases, neuropsychiatric disorders, and osteoporosis. Furthermore, amino acids are widely used as therapeutic agents. For example, phenylalanine could be used as an anti-depression medication (Beckmann *et al.*, 1977) whereas valine for muscle building (Carunchio *et al.*, 2010; Tokunaga *et al.*, 2004).

In addition, amino acids are an excellent chelating agent since they have multiple N and O atoms that allowed them to act as donors to the metal ion. Herein, a stable complex of five-membered chelate rings could be formed *via* the amino and carboxylate groups (Laurie, 1995). Therefore, amino acids were used to form Schiff bases and further reacted with metal ions as to produce effective and lower toxicity metal based drugs.

1.2 Schiff bases

Schiff bases are named after Hugo Schiff, which contain a functional group of C=N (Schiff, 1864). Structurally, it is also known as imine or azomethine nitrogen. It is generally formed by reacting amino and carbonyl compound under acid or base catalysis or with heat. This process is known as a condensation reaction whereby water molecule will be eliminated. The general reaction scheme can be represented as in Figure 1.4:

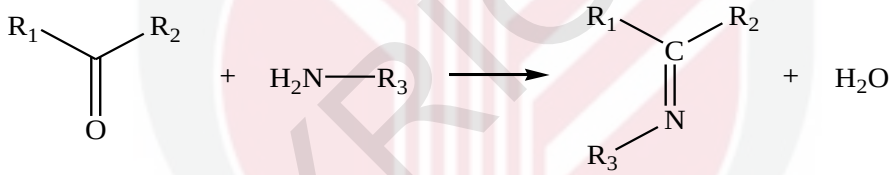

Figure 1.4: Reaction scheme for formation of Schiff base.

There are three different routes for the preparation of Schiff base complexes which are (Yamada, 1966):

I. Metal salt + aldehyde/ketone + amine
II. Metal salt + Schiff base
III. Metal-aldehyde/ketone + amine

Method I is known as 'one-pot' or template reaction whereby the Schiff base is not isolated out from the solution. The reaction is carried out by an *in situ* addition of metal salt in forming a Schiff base complex. This method is widely applied for amino acid Schiff bases as ligands. Method II is the most preferable method if the Schiff base was able to be isolated. This is because the structure of the pure Schiff base can also be determined. The last method is mostly applicable to the synthesis of macrocyclic compounds.

Schiff bases have been widely studied by researchers due to the ease of preparation and broad applications in pharmaceutical, agriculture, catalysis, and industry chemistry. The biological properties of Schiff bases were one of the main focuses since they have been found to possess remarkable antibacterial, antifungal, anticancer and diuretic activities. In addition, the imine or azomethine groups are found in various natural, natural-derived, and non-natural compounds such as Ancistrocladidine (antimalarial activity) (Bringmann *et al.*, 2004), Chitosan-derived Schiff base (antifungal activity) (Guo *et al.*, 2007) and N-(Salicylidene)-2-hydroxyaniline (antibacterial activity) (de Souza *et al*., 2007) respectively.

From the chemical and biological point of view for Schiff bases, the azomethine nitrogen was sp^2 hybridised with the presence of a lone pair of electrons (Patai, 1970). Hydrogen bond can be formed between the active centers of cellular entities and thus resulting in interference in normal cell processes (Venugopala and Jayashree, 2003; Vashi and Naik, 2004).

Moreover, Schiff bases are also versatile metal complexing agents. They are able to coordinate with all kind of metals with different oxidation states in forming stable metal complexes with either five or six membered chelate rings (Hameed *et al.*, 2016). Furthermore, Schiff bases have been proven to have improved biological properties upon complexation (Chohan *et al*., 1997).

1.3 Transition metal complexes

From the view point of chemistry, transition metal complexes are known to have huge structural diversity. Unlike organic compounds, a carbon atom with four different substituents can only exist as two different stereoisomers as it can only be in planar and tetrahedral geometries. In contrast with carbon atoms, metal ions can formed a variety of geometries as they have partially filled and energetically accessible d-orbital which allows the formation of more than four bonds. Besides, the types of geometries formed upon complexation were greatly influenced by the types of ligands and metal ions. Herein, the ligand types can be divided into monodentate, bidentate, tridentate, polydentate ligands and those with at least two donor atoms are called chelating ligands. For a tetradentate ligand, the formation of either a tetrahedral or square planar complex could result (Neelakantan *et al*., 2008; Cisterna *et al*.; 2017). The structural diversity, tunable properties and the ability to tailor-made metal-organic-ligand interaction of transition metal complexes (Tian *et al*., 1998) contributed to the approach in finding new metal based drug.

1.3.1 Copper

Copper is one of the few metals that occur in nature metallic form but commonly found in the earth's crust as copper-iron-sulphide and copper sulphide minerals such as

chalcopyrite ($CuFeS_2$), bornite (Cu_5FeS_4) and chalcocite (Cu_2S) (Biswas and Davenport, 2013).

Besides, copper is essential for all living organisms as a trace dietary mineral. To this, copper is the third most abundant trace metal in the body with the amount of 70 to 100 mg (Willis *et al*., 2005). It is required for a number of enzymes which are necessary for normal metabolic function such as cytochrome c oxidase, lysyl oxidase, feroxidase, monoamine oxygenase, superoxide dismutase (Angelova *et al*., 2011). It contributes to a number of key physiological processes such as iron export from the cells, the production of neuroendocrine peptides and neurotransmitters, pigmentation, blood clotting and others (Gupta and Lutsenko, 2009). Deficiency of copper will decrease the enzymatic activity and thus affecting the corresponding physiological processes that may result in an extensive range of symptoms (Emsley, 2011). For instance, irregular heart beat (Zhou *et al*., 2009), low body temperature (Bonham *et al*., 2002), iron deficiency anemia (Groff *et al*., 1995) and poor thyroid function (Araya *et al*., 2006).

Copper ion is known to have three common oxidation states including +1, +2 and +3. Both +1 and +3 oxidation states are mostly unstable in biological systems, but for the +2 state, copper usually forms stable complexes with coordination numbers of 4, 5 or 6 (Szymański *et al*., 2012). Besides, the interest in the anticancer field has rapidly grown in recent years, as illustrated in Figure 1.5 with an increasing number of publications started from 2000 to 2012 (Santini *et al*., 2014).

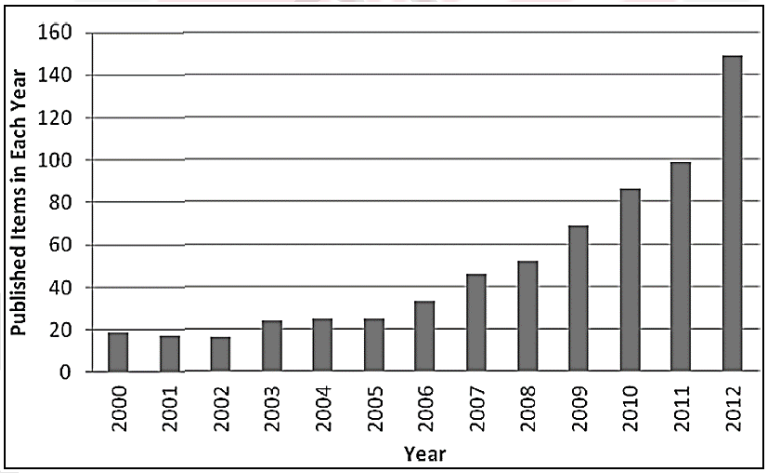

Figure 1.5: Number of articles in Web of Science on the topic "copper and anticancer" from 2000 to 2012 (Santini *et al*., 2014)**.**

1.3.2 Nickel

Nickel is the earth's 22nd most abundant element and the 7th most abundant transition metal. It commonly occurs in two types of ore deposits which are nickel sulphides and nickeliferrous laterites (Alexander, 2009).

Nickel is one of the micronutrients in our body. It also helps to increase the iron uptake in the body and used in treating weak bones (Anke *et al*., 1984). Besides, it is an important cofactor to various enzymes. For instance, it takes part in the reaction catalysed by hydrolyses and oxidoreductases such as urease (Hennig *et al*., 1978). Urease is reported to be an important virulence determinant in the pathogenesis of many clinical conditions such as urolithiasis, pyelonephritis and hepatic ncephalopathy, hepatic coma, and urinary catheter encrustation (Konieczna *et al*., 2012; Follmer, 2010; Mobley *et al*., 1995).

1.3.3 Zinc

Zinc is one of the abundant elements on earth where about two billion tons of zinc ore are available for mining. Among the most common ores is zinc sulphide, which is also known as sphalerite or zinc blende (Burdge and Overby, 2012).

Zinc is the second most abundant trace metal in the body with around 2 to 4 grams distributed in the body (Wapnir, 1990). Other than that, it is also the only metal which appears in all enzyme classes (Broadley *et al*., 2007) and is required for the catalytic activity of more than 200 enzymes (Sandstead, 1994). Examples of the enzymes are carbonic anhydrase that regulates the processes of carbon dioxide (Lindskog, 1997) and carboxypeptidase that digests the proteins by cleaving the peptide linkage (Zumdahl, 1998).

In addition, it also plays a significant role in immune function, wound healing (McCarthy *et al*., 1992), protein synthesis and cell division (Prasad, 1995). Hence, the deficiency of zinc could cause taste abnormalities (Heyneman, 1996), weight loss, delayed healing of wounds (Maret and Sandstead, 2006 and Prasad, 2004), and in some serious cases it could lead to hair loss, diarrhea, delayed sexual maturation, and depressed immune function (Shankar and Prasad, 1998).

In summary, the selection of the three biologically relevant 3d metals (copper, nickel and zinc) in this study was due to their importance in biological systems, and their presence in many enzymes, which are essential to life. Thus, a less toxic and improved pharmacological properties of metal complexes could be formed.

1.4 Problem Statements

Cancer is one of the leading cause of death globally as there are many types of cancers based on the cell in which the cancer originates. Bladder cancer is considered to be the the ninth most common cancer worldwide. Cisplatin is a platinium containing metal based drug that has been proven to be effective in treating various types of cancers. However, the usage of cisplatin was found to cause severe side effect such as nephrotoxicity, neurotoxicity and ototoxicity. Thus, metal complexes containing amino acid derived Schiff bases were synthesised and tested for their cytotoxic activity and antimigration properties against bladder cancer. Besides, the complexes were also tested for their antibacterial activity by screening against various types of Gram-positive and Gram-negatve bacterias. In this study, the incorporation of amino acids and use of biologically relevant metal ions in forming new kind of amino acid Schiff base complexes were expeted to be less toxic and efficient biological active agents.

1.5 Objectives

The objectives in this project were:

- to synthesise metal complexes [Cu(II), Ni(II), Zn(II)] containing Schiff bases derived from amino acid (L-phenylalanine, L-histidine, L-valine, L-methionine).
- to characterise the amino acid Schiff base complexes by various physico-chemical (elemental analysis, magnetic susceptibility, molar conductivity, and thermo gravimetric analysis) and spectroscopic techniques [fourier transform-infrared (FT-IR), UV-Vis spectroscopy (UV-Vis), and inductively coupled plasma – optical emission spectrometry (ICP-OES)].
- to investigate the cytotoxic activity of the synthesised complexes against EJ-28 and RT-112 bladder cancer cell lines and antibacterial activity against *Bacillus cereus* (BC), *Staphylococcus aureus* (SA), Methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli* (EC), *Shigella sonnei (SS), Klebsiella pneumonia* (KP), and *Salmonella typhimurium* (ST).
- to determine the anti-migratory properties of the inactive complexes against EJ-28 *via* scratch assay.
- to evaluate the electrochemical properties of copper(II) complexes *via* cyclic voltammetry.

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