SYNTHESIS, STRUCTURE ELUCIDATION AND SELECTED BIOLOGICAL ACTIVITIES OF Ag(I) AND Au(I) COMPLEXES CONSTRUCTED FROM THIOSEMICARBAZONE AND PHOSPHINE LIGANDS

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SYNTHESIS, STRUCTURE ELUCIDATION AND SELECTED BIOLOGICAL ACTIVITIES OF Ag(I) AND Au(I) COMPLEXES CONSTRUCTED FROM THIOSEMICARBAZONES AND PHOSPHINE LIGANDS

ABSTRACT

New Ag(I) and Au(I) mixed ligand complexes were synthesised using thiosemicarbazone and phosphine in search of a new biologically active compounds. CHN elemental analysis, powder X-ray diffraction (PXRD), single crystal X-ray diffraction as well as several spectroscopic techniques such as FT-IR, Energy-dispersive X-ray (EDX), ^{1}H, ^{13}C, and $^{31}P\{^{1}H\}$ NMR were performed to elucidate the structure of these complexes. Elemental analysis suggested that the stoichiometry of the complexes formed by the reaction of Ag nitrate with thiosemicarbazone in the presence of phosphine was indeed 1:2:1 molar ratio, while for the Au to be in 1:1:1 molar ratio. The Ag and Au were discovered to be coordinated to the sulphur of thiosemicarbazone and phosphorus of phosphine, having a tetrahedral and linear geometry respectively. The *in-vitro* antiproliferative activity of these Ag(I) complexes was investigated towards the MDA-MB-231 and MCF-7 breast cancer cell lines, as well as the HT-29 colon cancer cell line, which yielded IC_{50} values in low micromolar range. The antiplasmodial activity of these Ag complexes was also examined against chloroquine-resistant *P. falciparum* parasite which demonstrated good activity and further tested for their selectivity index. As for the Au(I) complexes and its free ligands, their antibacterial activity were tested against *E. coli* and *S. aureus* and the result shown that the ligands exhibited higher activity. Preliminary results of transforming the gold complexes to metal clusters by chemical reduction are also discussed in this thesis.

Keywords: thiosemicarbazone; phosphine; Ag; Au.

SINTESIS, ELUSIDASI STRUKTUR DAN AKTIVITI BIOLOGI TERPILIH BAGI KOMPLEKS Ag(I) DAN Au(I) TERBINA DARI LIGAN TIOSEMIKARBAZON DAN FOSFINA

ABSTRAK

Kompleks baru Ag(I) dan Au(I) telah disintesis menggunakan ligan campuran tiosemikarbazon dan fosfina dalam pencarian sebatian baru yang aktif biologinya. Analisis unsur CHN, belauan sinar-x serbuk (PXRD), belauan sinar-x hablur tunggal serta beberapa teknik spektroskopi seperti FT-IR, tenaga-penyebaran sinar-x (EDX), ^{1}H, ^{13}C, and $^{31}P\{^{1}H\}$ NMR telah dilakukan untuk elusidasi struktur kompleks tersebut. Analisis unsur mencadangkan bahawa stoikiometri kompleks yang terhasil daripada tindak balas antara Ag nitrat dan tiosemikarbazon dengan kehadiran fosfina adalah dalam nisbah molar 1:2:1, manakala untuk Au, nisbah molarnya adalah 1:1:1. Ag dan Au didapati terkoordinasi kepada sulfur dari tiosemikarbazon dan fosforus dari fosfina, dengan masing-masing mempunyai geometri tetrahedron dan linear. Aktiviti antiproliferatif *in vitro* bagi kompleks Ag(I) telah disiasat menggunakan sel kanser payudara MDA-MB-231 dan MCF-7, termasuk juga sel kanser kolon HT-29 yang mana menghasilkan nilai IC_{50} dalam julat micromolar yang rendah. Aktiviti antiplasmodial untuk kompleks Ag ini juga telah diperiksa terhadap tentangan klorokuina parasit P. *falciparum* yang mana menunjukkan aktiviti yang bagus dan seterusnya penentuan indeks selektif dilakukan. Manakala, bagi kompleks Au(I) dan ligan bebasnya, aktiviti antibakterial telah diuji terhadap *E. coli* dan *S. aureus* yang menunjukkan ligan mempamerkan aktiviti lebih tinggi berbanding kompleks. Keputusan awal dari transformasi kompleks Au kepada gugusan logam melalui penurunan kimia juga dibincangkan didalam tesis ini.

Kata kunci: thiosemicarbazone; fosfina; Ag; Au.

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LIST OF SYMBOLS AND ABBREVIATIONS

Å	:	Armstrong
^{13}C	:	Carbon 13
$\{H\}^{31}P$	:	Decouple Phosphorus 31
°	:	Degree
°C	:	Degree Celsius
δ	:	Delta
=	:	Double bond
µM	:	Micro molar
%	:	Percentage
^{1}H	:	Proton
—	:	Single bond
ν	:	Wavenumber
⋯	:	Weak bond
AMR	:	Antimicrobial resistance
ATR	:	Attenuated Total Reflection
AuNP	:	Gold nanoparticle
Brcatsc	:	2-bromo-3-phenylpropenalthiosemicarbazone
DMEM	:	Dulbecco's modified Eagle's medium
EC_{50}	:	Half maximal effective concentration
EDX	:	Energy-dispersive X-ray
FBS	:	Fetal bovine serum
FTIR	:	Fourier Transform Infra-Red
HEPES	:	N-2-hydroxyethylpiperazine-N-ethanesulfonic acid
HRP	:	Horseradish peroxidase

HRPII	:	Histidine-rich protein II (unique protein produced exclusively by *P. falciparum*)
HSAB	:	Hard Soft Acid Base
HT-29	:	Human colorectal adenocarcinoma cell line
IC_{50}	:	half maximal inhibitory concentration
MCF-7	:	Michigan Cancer Foundation-7 (breast cancer cell)
MDA-MB-231	:	Triple negative breast cancer cell line
MDBK	:	Madin-Darby Bovine Kidney Cells
MDR	:	Multiple Drug Resistance
NMR	:	Nuclear Magnetic Resonance
ppm	:	Parts per million
PXRD	:	Powder X-ray Diffraction
RBC	:	Red blood cell
SI	:	Selectivity index
SRB	:	Sulfate-reducing bacteria
TCA	:	Tricarboxylic Acid Cycle
UV-VIS	:	Ultra Violet -Visible
WHO	:	World Health Organization

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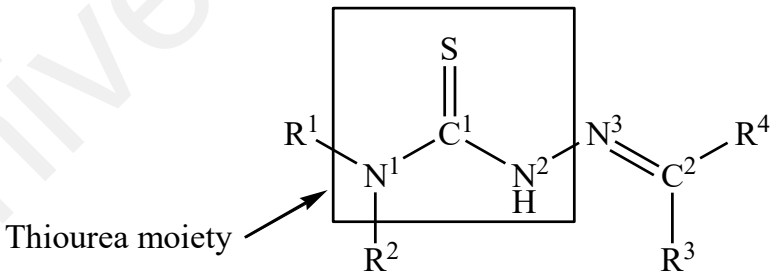
Appendix A: NMR Spectra

Appendix B: FTIR Spectra

Appendix C: PXRD Spectra

CHAPTER 1: INTRODUCTION

This research was conducted to study the synthesis and characterisation Ag(I) and Au(I) complexes comprising of a mixed ligand; thiosemicarbazone and phosphine. Nowadays, immeasurable numbers of organic group have been found and synthesise worldwide in the pursuit of finding pharmacologically active compound. One of an important class of organic compound was the thiosemicarbazone due to its significance in their chemical properties [1, 2] and biological activities [3-5]. Other than these significances, the thiosemicarbazone was also an interesting compound to be studied due to the ease of synthesis, production of high yields, requirement of minimal cost and diversity of possible derivatives obtained by tuning the starting materials used. The basic structure of thiosemicarbazone was shown in **Figure 1.1**. One of the fascinating chemical properties of thiosemicarbazone was their ability to chelate with various transition metal forming diverse binding modes due to the availability of sulphur atom and imine nitrogen atoms in its structure.



R^1 = H, aryl, alkyl group
R^2, R^3, R^4 = H, alkyl, aryl, heterocyclic group

Figure 1.1: Basic structure of thiosemicarbazone

Some of the biological properties reported on the thiosemicarbazone includes antimalarial [6-8], anticancer [9, 10], and anti-protozoan through the inhibition of cysteine proteases and other targets [11-14], which have been of great interest. In the late 1940s, *p*-acetamidobenzaldehyde thiosemicarbazone (thioacetazone) was introduced and later approved as the first clinical drug of this compound class for the treatment of multidrug-resistant tuberculosis which still being used until now [15]. Another earliest biological study was conducted by Brockman *et al.* in 1956 on the antineoplastic properties of pyridine-2-carboxaldehyde thiosemicarbazone and it was discovered that this compound derivative was potent in prolonging the life span of mice bearing L1210 leukemia [16]. Based on some literature surveys, it was revealed that the wide range of biological activities on these type of compounds was due to their property to diffuse through the cell lines' semi permeable membrane [17-19].

Since then, tremendous numbers of biological studies were being conducted on the thiosemicarbazone derives compounds as well as their transition metal complexes. One of the study that inspired the synthesise metal complexes of thiosemicarbazone in attaining bioactive compounds comes from the report presented by Taylor in 1966 on the cupric complex of 2-keto-3-ethoxybutyraldehyde *bis*(thiosemicarbazone) [20]. This study examined the metabolic effects of the complex in Sarcoma 180 ascites tumor cell and it was disclosed that the role for inhibition of neoplastic cell proliferation majorly played by the Cu ion. Another example of an excellent biological activity showed by the thiosemicarbazone metal complex was revealed by Casas *et al.* in 2006 where they had synthesised a linear gold complex utilizing the vitamin K3 thiosemicarbazone derivative using 2:1 molar ratio [21]. The cytotoxicity of their antiproliferative activity was found to be similar to the cisplatin against the cisplatin sensitive cell line A2780; while at the same time, it was observed to be 10 times higher than the cisplatin against the cisplatin-resistance cell line A2780cis.

Another type of ligand that was also adopted in the preparation of the Ag(I) and Au(I) complexes was phosphine. The mixed ligand system of thiosemicarbazone and phosphine was used instead of single ligands as we expect them to be more stable. Moreover, phosphines were also one of an important class of compounds due to their widespread use as ligands for the transition metal complexation and their biological properties [22]. Phosphorus was a unique element and it can be found around us in many different forms. Its chemistry was of great importance which displayed a spectacular expansion for the last twentieth century in the field of biochemistry and material chemistry. The term phosphine was used to described PH_3 as well as its organo-substituted derivatives of PH_2R, PHR_2 and PR_3. The usage of phosphine ligands in the organic and inorganic synthesis of complex chemicals had expended dramatically in the past decades. The R group of these phosphines could be substituted by either organic or inorganic group which will then alter the nucleophilic or electrophilic properties of the phosphorus or sterically constrain of the occurred reaction. One of the examples of study done on the synthesis of phosphine ligand was conducted by Tinnermann *et al.* in 2014. They synthesised a new family of cationic phosphine, pyridiniophosphines which when were used as ligands for complexation reaction. These compounds showed brilliant π-acceptor properties, thus enhanced the Lewis acidity of the coordinated metals [23].

The phosphine ligands as well as their complexes were also found to show good potential in the biological as well as catalytic activities [24-26]. Some of the biological studies carried out on these phosphine complexes include anticancer [27-29], anti-malarial [30, 31] and antibacterial [32, 33]. One of the commercialised phosphine metal complex available in the market is the auranofin which was constructed from transition metal gold with mixed ligand of triethylphosphine and thiols. Based on the excellent activities of this complex, a lot of studies were performed on this type of compounds by

tailoring their structure. In a report written by Krikavova *et al.* in 2014, they prepared a series of gold(I) complexes composed from triphenylphosphine and *N*-donor ligands that were derived from deprotonated mono- or disubstituted hypoxanthine [34]. The synthesised complexes also had similar or better activity as auspicious anti-inflammatory agents as compared to the clinical antiarthritic drug, auranofin. In another example, Abbehausen and her co-authors had synthesised a series of linear triphenylphosphine gold(I) complexes with substituted N-heterocycle ligands and all of these compounds were discovered to display micromolar range of cytotoxicity activity upon 96 hours of continuous exposure to the human tumor cell [35].

The transition metals used in this project were silver and gold in the form of Ag(I) and Au(I). Silver metal belong to the group 11 of transition metal block in the periodic table. Pure silver metal had the lowest contact resistance and the highest electrical as well as thermal conductivity as compared the other metal [36]. This metal usually exists in the form of inorganic salt and complexes such as silver nitrate and silver sulfonamide. Over the centuries, this metal had been widely used for several medical condition (e.g. treatment of wound) as well as in medical devices, textiles, cosmetics and home appliances [37]. Some of the advantages of silver was that it shows no effect on the mammalian cell membrane, thus relatively had limited toxicity to the humans [38]. However, the mechanism of action for the silver based pharmaceutical compounds have not been fully clarified. One of the possible deductions to the mechanism was that the Ag(I) was released to enter the cell membrane which therefore, disrupts its function [39]. Thus, it is important to have a ligand that can strongly coordinate to the active Ag(I) ions.

Meanwhile, the transition metal gold belongs to the same group as the silver, having electron configuration of $[Xe]4f^{14}5d^{10}6s^{1}$. The chemistry of gold was unique and

interesting due to the consequence of its important electronic properties. Furthermore, the electrochemical potential of this metal was very low as compared to the other transition metal, which makes it to readily accept electron from virtually any reducing agents in their cationic state to form metallic gold. This metal can exist in several oxidation state in its complex form and the most common state are +1 (aurous compound) and +3 (auric compound) [40, 41]. As the gold complexes exist in the oxidation state of +1, the Au(I) ($[Xe]4f^{14}5d^{10}$) behaves as soft Lewis acid which therefore, prefers to form complexation with ligands containing soft donor atoms such as sulphur and phosphorus in accordance to the Hard-Soft Acid Base (HSAB) theory. However, if the gold complexes exist in the oxidation state of +3, the Au(III) ($[Xe]4f^{14}5d^{8}$) would favour to form complexation with the hard Lewis base ligands consisting of nitrogen and/or oxygen atom. This type of complexes had been intensively studied for their potential application such as catalysis [42], biological activities [43] and nanotechnology [44].

These two transition metals also had the ability to form metal to metal interaction. This was essential for our study as we also envision to synthesis a metal clusters by chemical reduction of the synthesised complexes. The chemistry of metal cluster is an interesting subject to be studied for fundamental research [45-47] and practical application [48-50]. The fascination of the clusters is not only due to their small size, but also their resemblance to a molecule. It comprises of atomically precise metal cores, surrounds by shell of capping ligands. These clusters could be considered as a middle ground between small molecular and nanoparticle regimes. A small cluster could be regard as large molecule, and a large cluster could be regard as small nanoparticle. This low-valent transition metal cluster is of an increasing importance due to their potential application in various fields which include catalysis [51], electrochemistry [52], biomedicine [53] and quantum electronics [44]. Furthermore, among the transition

metal available, gold was one of the most research metal in the preparation of molecular cluster due to its properties and application versatility [54].

One of the biological studies done in our project was the evaluation of the anticancer activities of the synthesised compounds. The term anticancer could also be replaced as antiproliferative as well as antineoplastic. Cancer had become the main life-threatening disease around the globe. According to World Health Organization (WHO), there are more than 100 types of cancer exist in which each required a unique diagnosis and treatment. Furthermore, the number of new cancer cases were expected to increase by 70% in the next two decades [55]. The uncontrolled growth and rapid spreading of infected cells making the treatment of cancer becomes almost impossible [56]. Many synthetic drugs that were being used in the chemotherapy of cancer patient was neither not selective towards the infected cells nor showing slow inhibition of the growth of the tumor cells [57]. The treatments of the cancer cells have remained a challenge due to the development of multiple drug resistance (MDR) which displayed broad spectrum of changes in the biochemistry and cytogeny such as increased level of glutathione related enzyme, increment in the *p*-glycoprotein as well as decreased regulation of monooxygenase [58]. Hence, the discovery of new discerning anticancer agents had now become an essential research worldwide.

Another biological evaluation of the synthesised compounds was the anti-plasmodial or could also be referred as antimalarial activities. Malaria was the vector-borne parasitic tropical disease that caused by *Plasmodium* parasites. Although there were more than 120 *Plasmodium* species infecting mammals, birds and reptiles, only six species were known to infect the human beings which were *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malaria*, *Plasmodium ovale curtisi*, *Plasmodium ovale wallikeri* and *Plasmodium knowlesi* [59]. Its eradication had been a global health

challenge especially in Asian and African countries. Almost half of the world's population which is about 3.2 billion people was at risk of malaria. Statistically, in the year of 2015 alone, there were 95 countries and territories reported to have an ongoing malaria transmission [60]. Despite the preventable measures that could be taken, the curative actions in treating the infected patients were indeed an important task. Nonetheless, the commercially exploited antimalarial drugs were facing great resistance challenge [61, 62] that emphasized the need to the discovery of new antimalarial drugs.

The last biological studies done by our project was the evaluation of antibacterial activities towards gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli*. The emergence of antimicrobial resistance (AMR) had become alarming and posing threat to the human health. This was a natural phenomenon which caused by the abuse and overuse of antimicrobials [63]. According to the news release by WHO's new Global Antimicrobial Surveillance System (GLASS) in 2018, it was revealed that across 22 countries, there had been a widespread occurrence of antibiotic resistance among 500000 people which were suspected with bacterial infection [64]. Therefore, it is important for the constant development of new alternative compounds to prevent this problem.

1.1 Problem statement

The development of new drug has become very important subject to be studies nowadays as the existing drugs are now of an increasing resistance against diseases and some of their agents has been reduced in the bioavailability (the extent and rate of drug enters systemic circulation, thus accessing the site of action). Even though the thiosemicarbazones had been studied for a long period of time, a lot of researchers still extensively conducting their investigation on this type of compounds due to their excellent potential as biological agents. Moreover, phosphines were also found to

show brilliant biological properties in the form of their metal complexes. The incorporation of both biologically active ligands to the metal complex system of Ag and Au are what we aim to do for this project as these transition metals are found to be used in the clinically available drug. We envision that by combining all these active components, we can produce a biologically active compounds which can benefit the pharmaceutical industry. Often, an inorganic synthesis has mono-functionality of the complexes prepared. Therefore, aside from evaluating their biological properties, we also aim to prepare a metal clusters from the synthesised complexes. These metal clusters are an interesting field to be studied as they could be used in tailoring the formation of nanoparticles that we hope would benefit in various applications.

1.2 Objectives of study

The aims of this study are as follows:

1. To prepare a series of thiosemicarbazone ligand derivatives as well as their silver and gold complexes with phosphine ligands.
2. To characterise the synthesised compounds by various spectroscopic techniques as well as the elemental analysis, PXRD and X-ray crystallography.
3. To evaluate the in-vitro anti-plasmodial, antiproliferative and antibacterial activities of the synthesised compounds.
4. To synthesise and characterise metal clusters using the prepared complexes.

CHAPTER 2: LITERATURE REVIEW

2.1 Ligands

The preparation method of thiosemicarbazone ligands had been reported by quite a number of researchers. Based on most literature study, the thiosemicarbazone ligand was prepared by the condensation reaction of thiosemicarbazide with an aldehyde or a ketone in the presences of acid catalysed [65]. Although the thiosemicarbazone was often observed as thione, it could also appear in the thiol form depending on the environment of the compound owning to the intramolecular proton transfer. The synthesis of the thiosemicarbazone was done by reacting a thiosemicarbazide with an aldehyde derivative according to the reaction mechanism as illustrated in **Figure 2.1**. The most suitable pH for the formation of thiosemicarbazone to take place was *ca.* 4 – 5 [66]. Based on the proposed mechanism, the acidic environment was necessary to provide hydronium ion which assists the protonation of aminoalcohol to lose a molecule of water becoming iminium ion. The hydronium ion converts the poor leaving group (an –OH group) into a good one (an $–OH_2^+$ group). Its reaction rate was crucially dictated by the pH environment. When the reaction proceeds at low pH (*ca.* 3 and below), the concentration of the hydronium ion is expected to be too high. This will decrease the concentration of nucleophile needed in the reaction as the protonation of amine itself takes place to a substantial extent. Meanwhile, if the reaction proceeds at a relatively higher pH, the concentration of hydronium ion was most likely to be too low. This would lower the concentration of protonated aminoalcohol, thus lead to low yield of the desired product.

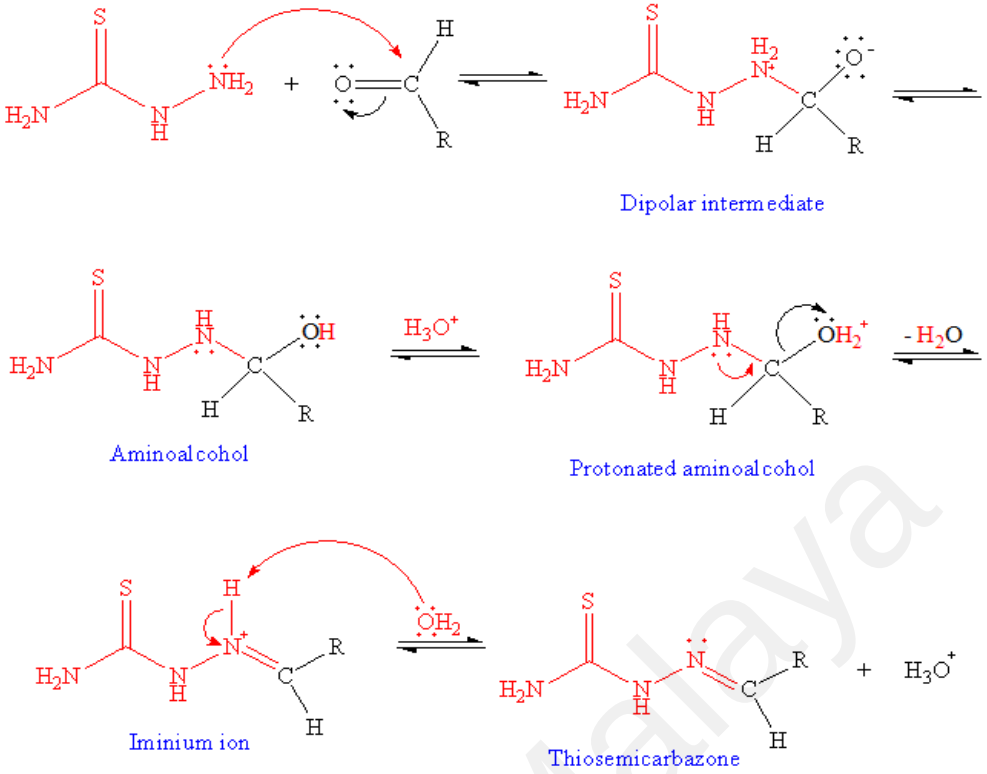


Figure 2.1: Reaction mechanism of thiosemicarbazone

The thiosemicarbazone had emerged to become one of an important sulphur donor ligand in the inorganic chemistry field, other than dithiocarbamate [67], dithiophosphate [68], dithiolate [69] and dithiooxamides [70]. Due to the presence of several donor atom in the thiosemicarbazone structure (N, N and S), this ligand was able to chelate with various transition metals such as vanadium [71], zinc [72], cobalt [73], iron [74], nickel [75], copper [76], silver [77] and gold [78] to form coordination complex. Due to the ability of the thiosemicarbazone to chelate with metal, it was widely used in the analytical application. Recently, a study conducted by Chen *et al.* in 2019, synthesised a new 9,9′-bianthracene-based thiosemicarbazone to be utilized as chemosensor for the detection of Hg^{+} and Ag^{+} [79]. Meanwhile, in 2015, an investigation was done by Park & Chang on the calorimetric sensor utilizing thiosemicarbazone derivative of 3-

hydroxynapthalimide. It was found to be selective towards the Cu^{2+} and was unaffected by the presence of other common metal ions and anions except Hg^{2+} [80].

Most of the synthesis procedure for the transition metal complexes was not as fuss as the synthesis of organic compounds. Generally, majority of the metal complexes (e.g. Mn^{II}, Fe^{II}, Co^{II}, Ni^{II}, Cu^{II} and Zn^{II}) were prepared by the reaction of either a metal halide, nitrate, sulfate, acetate or perchlorate with a thiosemicarbazone in an organic solvent by stirring at room temperature or refluxing [81, 82]. Other than these regular metal salt, other metal starting material could also be utilized for the synthesis; e.g. $VO(acetylacetonate)_2$ [83] and NH_4VO_3 [84]. Although the most common method for synthesising metal complexes was as mentioned above, modification of the synthesis route may be needed depending on the metal used in the reaction. For instance, in the preparation of chromium(III) and cobalt(III) complexes with 2-ketobutyric acid thiosemicarbazone, $NaHCO_3$ was added to the reaction to provide basic environment for the complexation to occur [85]. Another study conducted by Khanye *et al.*, gold complexes were prepared by a reflux reaction of Au(I) tetrahydrothiophene chloride with the thiosemicarbazone ligands under nitrogen for two hours in 1:2 molar ratio [86].

These thiosemicarbazone ligands could either act as monodentate, bidentate or multidentate depending on the transition metal employed to the system. Moreover, presence of multiple functional group other in a suitable position to the aldehyde or ketone of the thiosemicarbazone derivatives, further enhanced its coordination ability during the complexation reaction. Some of the plausible coordination mode of thiosemicarbazone to the metal were shown in **Figure 2.2 (a)** – **(g)**. The binding of the metal to the thiosemicarbazone can occur *via* the S atom of η^1–S as shown in **(a)** or μ_2–S shown in **(b)** [87-90]. Other than the S atom, the metal can also coordinate to the N atom through the binding modes of either η^2–N^3, S–chelation **(c)** or η^3–N^3, S–chelation and S–bridging **(d)** [91-93]. Moreover, if the C^2 substituents consist of donor atom (e.g.

X = N, O), additional binding modes are available, thus the metal can also bind to the ligand *via* η^3–X, N^3, S–chelation **(e)**, η^4–X, N^3, S–chelation and S–bridging **(f)**, and η^4–X, N^3, S–chelation and X–bridging **(g)** [94-96]. A study conducted by Pedrido *et al.* in 2009 found that addition of pyridine provides another N donor atom to the thiosemicarbazone frame, resulting in the N, N, S tridentate coordination mode depending on the nature of concerned metal ion [97].

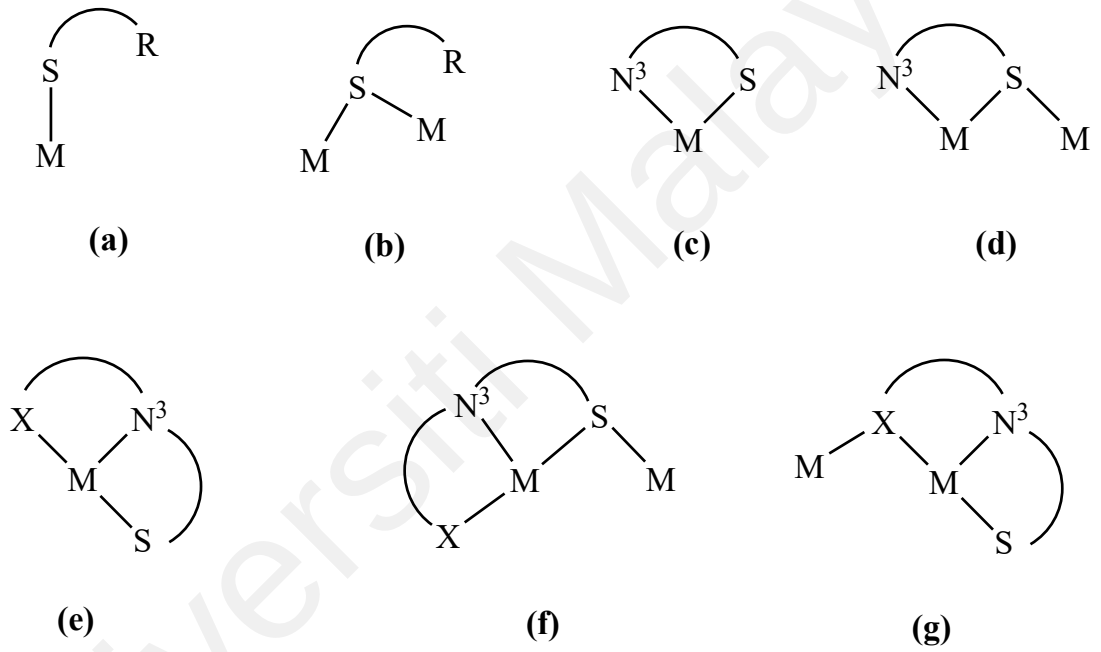


Figure 2.2: Coordination possibilities of thiosemicarbazone [98]

Among the most encountered ligands in the complexation of transition metal complexes were tertiary phosphine. Some of the metals that had been used in the preparation of phosphine complexes includes nickel [99, 100], copper [101, 102], palladium [103, 104], gold [105, 106] and silver [107, 108]. These phosphines were also often adopted in mixed ligands system of complexes where they were usually act as spectator rather than actor ligand. One of the studies conducted on the mixed ligands system of metal complex was done by Malecki *et al.* in 2014 where they synthesised

copper complex utilizing 5,7-dinitro-2-methylquinolin-8-ol and PPh_3, in which its fluorescence properties was compared to the free ligand [109]. These phosphine ligands could also provide stability for the mixed ligands system of variety metal complexes. In a study reported by Keter *et al.* in 2014, they synthesised phosphinogold(I) thiocarbamate complexes where the stability of these complexes were found to depend on the nature of phosphine ligand used [110]. Other than that, the studies on metal complexes with mixed ligands system of thiosemicarbazone and phosphine were also in the interest of many researcher. Some of the examples include the preparation of a new nickel triphenylphosphine complex with 5-methyl-2-hydroxy-*S*-methyl acetophenone thiosemicarbazone that forms a square-planar geometry as reported by Guveli *et al.* in 2018 [111]. Another example was presented by Arancibia *et al.* in 2015, which they synthesise palladium (II) and platinum (II) consisting of ferrocenyl and cyrhetrenyl thiosemicarbazone ligands as well as PPh_3 ligand, including the evaluation of antitubercular activities of the complexes [112].

2.2 Silver complex

The silver transition metal was particularly interesting due to the variety in its coordination number and geometry depending on the type of ligand used for the reaction. Some of the donor ligand that possessed high affinity towards the Ag(I) includes sulphur, nitrogen and phosphorus. Numerous monophosphine and diphosphine derived ligands had been investigated for their coordination behavior towards the silver ions [113-116]. The first Ag(I) phosphine complexes were prepared by Mann *et al.* in 1937 by utilizing tertiary phosphine forming $AgXL_n$ (L = PR_3 where R is either alkyl, aryl or H; n = 1 – 4; X = coordinating or non-coordinating anion) [117]. The general preparation method of these complexes comprises the reaction of phosphine ligands with Ag(I) salt in suitable solvents using appropriate molar ratio by stirring at room temperature or reflux. The structure of the synthesised Ag phosphine complex depends

on the type of phosphine used in the reaction as well as the applied stoichiometric ratio. For example, when an Ag(I) salt was reacted with a monodentate tertiary phosphine using 1:2 molar ratio, it generally resulted in the formation of either a monomeric complex $[AgX(PR_3)_2]$ or dimeric complex $[\{AgX(PR_3)_2\}_2]$, contingent upon the bulkiness of the ligand, donor properties of the phosphine ligand, the substituents and donor properties of the anion, X [118-121].

The investigations of the Ag(I) complex with thiosemicarbazone ligands had remain in the interest of researcher. In 2007, a study was performed by Lobana and his group on the novel neutral tetrameric Ag(I) cluster $[Ag(mtsc)]_4$ which was attained from the reaction of several Ag(I) sources (contain only Ag–O bonds) with tridentate ^{4}N-morpholyl 2-acetylpyridine thiosemicarbazone ligand [122]. Another study on the complexation of thiosemicarbazone ligand with Ag(I) revealed the formation of a hexanuclear cluster as the $AgNO_3$ reacted with 2-thiophene N(4)-methylthiosemicarbazone (**L**) in 1:1 molar ratio resulting in $[Ag_6(\mathbf{L})_6 \cdot 4DMF]$ [123]. Most of the studies of the Ag(I) complexes with thiosemicarbazone ligands were accompanied by the presence of phosphine ligands. These mixed ligands complexes were found to exhibit variety of coordination complexes as shown in **Figure 2.3 (a)** – **(f)**. The reaction of Ag(I) halide with the thiosemicarbazone and monodentate phosphine ligands were found to yield complexes of monomers **(a)**, halogen-bridge dimer **(b)** and sulphur-bridge dimer **(c)** [77, 124]. Meanwhile, the reaction of Ag(I) nitrate with these mixed ligands forms a sulphur-bridge dimer of **(d)** and **(e)** [124]. However, when a bidentate phosphine was used for the synthesis, a study by Lobana *et al.* discovered that the Ag(I) complex produced a triply hetero-bridge dimer with a short Ag···Ag contact of 2.9939(7) Å **(f)** [125].

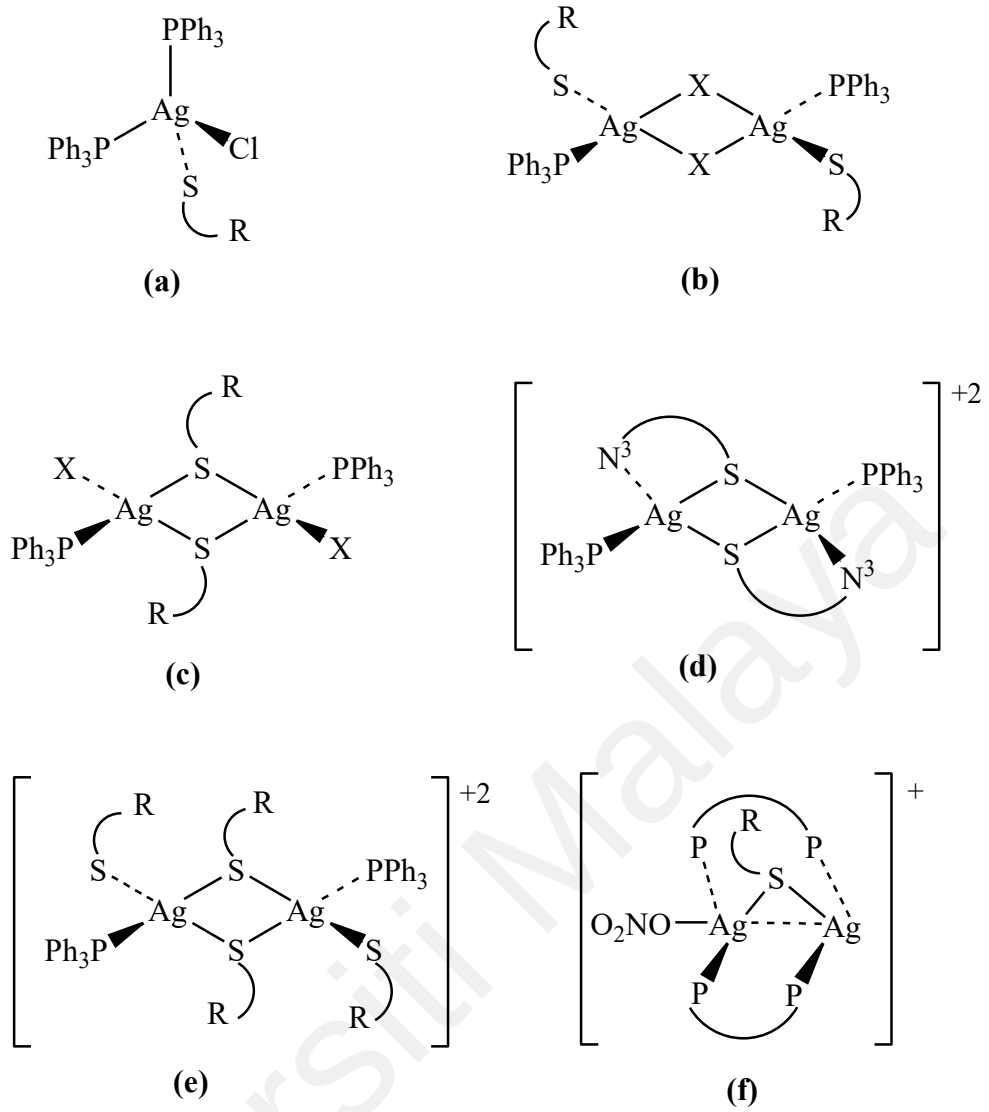


Figure 2.3: Coordination possibilities of Ag(I) complex with mixed ligands thiosemicarbazone and phosphine [126]

Although the synthesis of silver phosphine complexes had been studied for centuries, the investigation of their bioactivities only started in 1997. The biological activity for tetrahedral complexes of Ag(I) phosphine complexes were tested by Papathanasiou *et al.* against three mouse tumor cell lines (*P815 mastocytoma, B16 melanoma* and *P388 leukimia*) and the result showed their cytotoxicity were comparable to that of cisplatin [127]. Another study in 2011 conducted by Santini *et al.* on the synthesis, characterisation and cytotoxic activity of three Ag(I) complexes of phosphine against several human tumor cell lines (A549, MCF–7, HCT–15 of colon cancer and HeLa, A–

375 of human ovarian carcinoma) and the finding showed better or comparable activities than cisplatin [128]. Recently in 2018, Engelbrecht *et al.* investigates the synthesis, structural characterisation and also anticancer activity of mixed ligands system of silver(I) thiocyanate-4-methoxyphenyl phosphine complex [129]. It was revealed that this complex showed about 14 times lower IC_{50} value as compared to the cisplatin towards the malignant SNO oesophageal cancer cell lines.

The number of studies conducted on the biological activity of the Ag(I) thiosemicarbazone phosphine complexes was still sparse. The biological studies were mostly focus on the antimicrobial activities due to excellent antibacterial properties of the Ag metal. In 2013, Altef *et al.* reported the antimicrobial, antifungal and antituberculosis of these type of mixed ligand Ag(I) complexes [130]. These complexes revealed a promising potential activity against selective strains of Gram-positive and Gram-negative bacteria, fungous and *Mycrobaterium tuberculosis H37Rv*. Meanwhile, a sulfur-bridged dinuclear Ag(I) thiosemicarbazone complex $[Ag_2(PPh_3)_2(\mu\text{-S-Brcatsc})_2(\eta^1\text{-S-Brcatsc})_2](NO_3)_2$ (Brcatsc:2-bromo-3-phenylpropenalthiosemicarbazone) was found to exhibit inhibitory effect only on gram-positive bacteria (*S. aureus* and *E. faecalis*) but not gram-negative bacteria [131].

2.3 Gold complex

Among the oxidation number available for the gold complexes, the Au(I) was by far the most studied subject among researcher due to its interesting properties. Several types of ligands had been investigated for the complexation with the Au(I) which generates mononuclear, dinuclear or polynuclear derivatives. The chemistry of mononuclear Au(I) complexes was generally dominated by two-coordinate, linear complexes forming [AuXL]. Although these linear complexes had been known for a long time, it become more noticeable due to the discovery of intermolecular gold-gold

contact (aurophilic interaction) that associate the monomeric units into dimers, oligomers or even uni- and multidimensional polymers. Along with the aurophilic interactions, secondary bonds such as hydrogen bonding also plays an important role in the determination of the Au(I) complexes solid state arrangement [132]. This interaction usually occurred in the neutral complexes of [AuXL], which were synthesised using variety of donor ligands such as phosphine, carbine and amine; while the anionic moiety can possibly be a halide, alkyl, aryl and so on. Some of the examples of an early studies found that the complexes of [AuCl(CO)] [133] and [Au(CN)(NMe)] [134] formed two dimensional layers, while complexes of [AuCl(PMe_3)] [135] and [Au(CN)(PMe_3)] [136] were chains. An example of a trimer was found from complex of [Au($OCOCF_3$)(PMe_3)], while a dimer was discovered from complexes of [AuX(PR_3)] mainly derived from medium-sized ligands [137].

The aurophilic interaction of the gold complexes makes it very interesting subject to be studied. Great variety of thiolate ligands had been used to synthesise the complexes of Au(I) in the form of [Au(SR)(PR_3)]. The thiolate functional group consist of sulphur donating atom which would bind to the gold centre. It was observed that many of these complexes serve as a building block to obtained polynuclear species [138]. Furthermore, this type of complexes was also found to have potential application in several fields. For example, auranofin [triethylphosphine-(2,3,4,6-tetra-*O*-acetyl-β-1-D-thiopyranosato-S)-gold(I)] was commercialise as drug for the treatment of antiarthritic patients [139]. Although the Au(I) had high tendency to readily bind to the thio-ligands in the presence of tertiary phosphine, the reports of the complexation of Au with thiosemicarbazone are quite sparse. As compared to the Au(I) thiosemicarbazone complex, more reports were available for the complexation of thiosemicarbazone with Au(III) [78, 140, 141].

One of the earliest investigation on the of Au(I) thiosemicarbazone complex was described in 2006 by Casas *et al.* on the synthesis of neutral complex, [AuCl(η^1-S-L)(PEt_3)] (HL = Vit. K_3 thiosemicarbazone) [21]. Following this, in 2008, Lobana and his group studied the synthesis and characterisation of a fluorescent Au(I) ionic complex, $[Au_2(3\text{-}NO_2\text{-}Hbtsc)_4]Cl_2 \cdot 2CH_3CN$ formed by the reaction of Au(I) chloride with 3-nitrobenzaldehyde thiosemicarbazone (3-NO_2-Hbtsc) in the presence of PPh_3 using 1:1:1 molar ratio with acetonitrile as the reaction solvent [142]. It was found that, although the triphenylphosphine was present in the reaction, it did not bind to the Au(I) thiosemicarbazone complex, instead separated as [AuCl(PPh_3)]. In a recent study conducted by Tavares *et al.* in 2017, novel Au(I) complexes were synthesised through the reaction of aryl-thiosemicarbazone ligands with Au(PPh_3)Cl [143]. It was discovered that the complexation of Au(I) aryl-thiosemicarbazone phosphine complexes were obtained by reacting Au(PPh_3)Cl in dichloromethane with the respective ligand that was dissolved in acetone using 1:1 molar ratio, at room temperature, in the dark for 6 hours. In another report by Molter *et al.* in 2011, a different approach was used to prepared the Au(I) thiosemicarbazone phosphine complexes [144]. They prepare the Au(I) complexes by an overnight reaction of Au(PPh_3)Cl with thiosemicarbazone ligand in basic environment provided by sodium methoxide at room temperature in the absence of light. Hence, to ensure the attachment of phosphine group to the Au(I) thiosemicarbazone complex, gold phosphine complex should be first prepared before reacted with the ligand.

2.4 Metal cluster

The intense exploration of the metal cluster begins as a wide range of new cluster core frameworks were discovered throughout the 1970s and 1980s. The discovery of chemical synthesis for colloidal gold particle was reported in 1857 by Michael Faraday [145]. He synthesise a deep red colloidal gold by reduction of $AuCl_4^-$ solution using

phosphorus in carbon disulfide and deduced that the color was attributed to the size of Au particles, although he was lacked with evidence to support this hypothesis. However, with the advancement of technology, in 1951 Turkevich *et al.* proved the prediction made by Faraday as they observed Au NP size of ~ 3 – 10 nm using electron microscopy [146]. During 1990s, literature report on the cluster chemistry showed a decline in volume. Nevertheless, over the past decades, gradual and slow resurgence on the topic was observed which predominantly due to increasing interest of nanotechnology, as well as the realisation of large molecular cluster was in the cusp of nano domain [147].

This metal clusters were usually compromised of an inorganic core with passivating supporting ligands shell, which provide both stability and solubility. These ligands offer challenges as well as opportunities. In recent years, atomically precise synthesis of metal cluster protected with organic ligand [148] and polymer [149] was undergoing a dramatic advancement. From the early reports, the production of molecular cluster could be tracked to Brust-Schiffrin method, where metal complexes were utilized as a universal metal precursor, then reduced to form the cluster [150]. In those early days, the creation of highly monodispersed nanoparticles was found to be a great challenge. At that time, phosphine type ligand was adopted to protect the gold core along with thiolate ligand. Nowadays, one of the major problems faced by most cluster chemist was finding a simple yet high yielding procedure to synthesise a homo- and heterometallic clusters of specific nuclearities.

The synthesis of metal clusters can be done using bottom up approach by reducing metal ion precursors in the presence of suitable ligands. One of the earliest reports on this bottom up approach was written by Brust *et al.* in 1994 [150] where they used two-phase synthetic protocol utilizing water and organic solvent (toluene). The aqueous

solution was employed to dissolve the metal precursors and then phase transferred to an organic solvent using tetraoctylammonium bromide as phase transfer reagent. In the last step of this method, protecting ligands as well as reducing agents were added to the mixture to obtain the desired products. This Brust method was then revised by Goulet and Lennox using ^{1}H NMR data analysis [151]. It was suggested that in one-phase method, the Au(I) thiolate was more likely to be the precursor, while in two-phase Brust method, the main precursor was the Au(I)-tetraoctylammonium halide complex instead of the $[Au(I)SR]_n$ polymer. This suggestion was supported by the reported work of Li *et al.* through their detailed investigation utilizing Raman and NMR spectroscopy [152]. The solvent used for the one-phase Brust-Schiffrin method was commonly a polar solvent such as methanol and THF. Meanwhile, the reducing agent that was used for most studies was sodium borohydride ($NaBH_4$) [153].

In order to get highly monodispersed nanoclusters, several modifications have been adopted to the Brust-Shiffrin method, which mainly includes manipulating the parameters such as solvents, temperature, concentration of reactant and reducing agents. For example, in a study conducted by Zhu *et al.* synthesised gold cluster of $Au_{25}(SR)_{18}$ by adjusting the stirring rate and control the temperature used during the reaction [154]. Other than this method, several other routes were also implied in the synthesis of these metal clusters. These include photoreduction synthesis [155], electrochemical synthesis [156], microwave assisted synthesis [157], slow reduction method [158] and solid-state route [159, 160]. One of the examples was reported by Rao and his co-author where they had synthesised Ag_9 cluster by grinding a mixture of $AgNO_3$ with mercaptosuccinic acid in 1:5 molar ratio, followed by reduction using $NaBH_4$ (5-fold molar excess with respect to $AgNO_3$) [161]. Chakraborty *et al.* had also synthesised Pt_{11} cluster utilizing solid state route by pastel and mortar grinding of chloroplatinic acid with 4-(tert-butyl) benzyl mercaptan in molar ratio of 1:10 before the addition of $NaBH_4$

and the product was purified using high performance liquid chromatography (HPLC) [162].

CHAPTER 3: METHODOLOGY

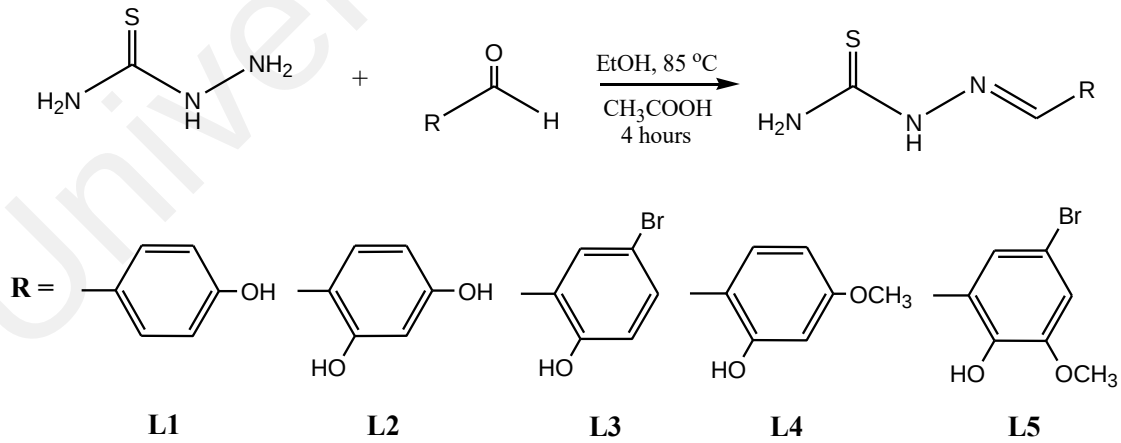
3.1 Chemicals

All solvents and reagents in this study were of analytical grade and commercially purchased from Sigma Aldrich Ltd., unless stated otherwise. The thiosemicarbazide, 4-hydroxy benzaldehyde, 2,4-dihydroxy benzaldehyde, 5-bromo-2-hydroxy benzaldehyde, 2-hydroxy-4-methoxy benzaldehyde, 5-bromo-2-hydroxy-3-methoxy benzaldehyde, silver nitrate, chloroauric acid trihydrate, PPh_3, (*p*-tolyl)PPh_2, sodium hydroxide, sodium borohydride, acetic acid, ethanol, methanol and acetonitrile were used without further purification.

3.2 Experimental

3.2.1 Synthesis of thiosemicarbazone ligands

The synthesis of thiosemicarbazone ligands was carried out as described in the published literature [65], with minor modifications. The general procedure was as illustrated in **Scheme 3.1**.



Scheme 3.1: Synthesis of thiosemicarbazone ligands

3.2.1.1 4-hydroxy benzaldehyde thiosemicarbazone [L1]

Thiosemicarbazide (6.0 mmol, 0.55 g) and 4-hydroxy benzaldehyde (6.3 mmol, 0.77 g) were dissolved in appropriate amount of ethanol. The thiosemicarbazide was stirred at high temperature to make sure all the solids were dissolved. After that, the 4-hydroxy benzaldehyde solution was added dropwise into the thiosemicarbazide solution followed by few drop acetic acid. This mixture was then refluxed for four hours. No precipitate was observed until the end of reaction. Thus, it was left for few days at room temperature for slow evaporation. The yield was 47% and its melting point was 230 °C.

3.2.1.2 2,4-dihydroxy benzaldehyde thiosemicarbazone [L2]

Thiosemicarbazide (6.0 mmol, 0.55 g) and 2,4-dihydroxy benzaldehyde (6.3 mmol, 0.87 g) were dissolved in appropriate amount of ethanol. The thiosemicarbazide was stirred at high temperature to make sure all the solids were dissolved. After that, the 2,4-hydroxy benzaldehyde solution was added dropwise into the thiosemicarbazide solution followed by few drop acetic acid. This mixture was then refluxed for four hours and precipitate starts to form after few minutes of reaction. The precipitate was filtered and washed with cold ethanol. The yield was 56% and its melting point was 238 °C.

3.2.1.3 5-bromo-2-hydroxy benzaldehyde thiosemicarbazone [L3]

Thiosemicarbazide (6.0 mmol, 0.55 g) and 5-bromo-2-hydroxy benzaldehyde (6.3 mmol, 1.27 g) were dissolved in appropriate amount of ethanol. The thiosemicarbazide was stirred at high temperature to make sure all the solids were dissolved. The 5-bromo-2-hydroxy benzaldehyde solution cannot be fully dissolved in ethanol. However, the partially dissolved solution was added dropwise into the thiosemicarbazide solution followed by few drop acetic acid. This mixture was then refluxed for four hours and precipitate starts to form after few minutes of reaction. The precipitate was filtered and washed with cold ethanol. The yield was 85% and its melting point was 250 °C.

3.2.1.4 2-hydroxy-4-methoxy benzaldehyde thiosemicarbazone [L4]

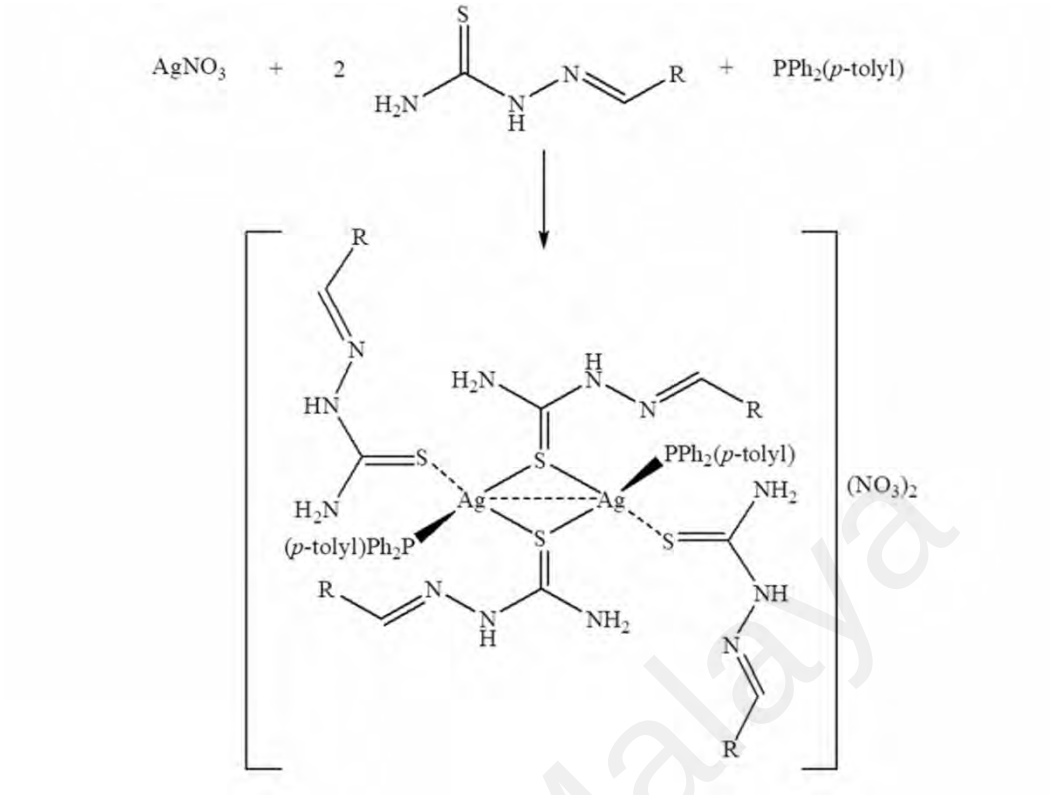
Thiosemicarbazide (6.0 mmol, 0.55 g) and 2-hydroxy-4-methoxy benzaldehyde (6.3 mmol, 0.96 g) were dissolved in appropriate amount of ethanol. The thiosemicarbazide was stirred at high temperature to make sure all the solids were dissolved. After that, the 2-hydroxy-4-methoxy benzaldehyde solution was added dropwise into the thiosemicarbazide solution followed by few drop acetic acid. This mixture was then refluxed for four hours and precipitate starts to form after few minutes of reaction. The precipitate was filter and washed with cold ethanol. The yield was 69% and its melting point was 212 °C.

3.2.1.5 5-bromo-2-hydroxy-3-methoxy benzaldehyde thiosemicarbazone [L5]

Thiosemicarbazide (6.0 mmol, 0.55 g) and 5-bromo-2-hydroxy-3-methoxy benzaldehyde (6.3 mmol, 1.45 g) were dissolved in appropriate amount of ethanol. The thiosemicarbazide was stirred at high temperature to make sure all the solids were dissolved. The 5-bromo-2-hydroxy-3-methoxy benzaldehyde solution cannot be fully dissolves in ethanol. However, the partially dissolved solution was added dropwise followed by few drop acetic acid. This mixture was then refluxed for four hours and precipitate starts to form after few minutes of reaction. The precipitate was filtered and washed with cold ethanol. The yield was 95% and its melting point was > 250 °C.

3.2.2 Synthesis of Ag(I) complexes

The outline of the reaction as shown in **Scheme 3.2** and the structure of the product was proposed referring to the previous study [130] as well as spectroscopic data attained since no single crystal data could be obtained to date.



Scheme 3.2: Synthesis of Ag(I) complex

3.2.2.1 $[Ag_2(PPh_2(p\text{-tolyl}))_2(L1)_4].(NO_3)_2$ [Ag1]

$AgNO_3$ (0.1 mmol, 0.02 g) and 4-hydroxy benzaldehyde thiosemicarbazone, **L1** (0.2 mmol, 0.04 g) were dissolved in a solvent mixture (5 mL) of acetonitrile and methanol (ratio of 2:3). Then, the thiosemicarbazone solution was added dropwise to the Ag solution. Following that, the reaction mixture was stirred for three hours at 50°C. No precipitate was observed. The reaction mixture was further treated with (*p*-tolyl)PPh_2 (0.1 mmol, 0.03 g) in the same solvent mixture (5 mL) and the stirring continued for another two hours. The solution was then filtered to remove any impurities and dried *in vacuo*. The yield was 26% and its melting point is 130 °C.

3.2.2.2 $[Ag_2(PPh_2(p\text{-tolyl}))_2(L2)_4].(NO_3)_2$ [Ag2]

$AgNO_3$ (0.1 mmol, 0.02 g) and 2,4-dihydroxy benzaldehyde thiosemicarbazone, **L2** (0.2 mmol, 0.04 g) were dissolved in a solvent mixture (5 mL) of acetonitrile and

methanol (ratio of 2:3). Then, the thiosemicarbazone solution was added dropwise to the Ag solution. Following that, the reaction mixture was stirred for three hours at 50°C. No precipitate was observed. The reaction mixture was further treated with (*p*-tolyl)PPh_2 (0.1 mmol, 0.03 g) in the same solvent mixture (5 mL) and the stirring continued for another two hours. The solution was then filtered to remove any impurities and dried *in vacuo*. The yield was 40% and its melting point is 136 °C.

3.2.2.3 $[Ag_2(PPh_2(p\text{-tolyl}))_2(L3)_4].(NO_3)_2$ [Ag3]

$AgNO_3$ (0.1 mmol, 0.02 g) as well as 5-bromo-2-hydroxy benzaldehyde thiosemicarbazone, **L3** (0.2 mmol, 0.05 g) were dissolved in a solvent mixture (5 mL) of acetonitrile and methanol (ratio of 2:3). Then, the thiosemicarbazone solution was added dropwise to the Ag solution. Following that, the reaction mixture was stirred for three hours at 50°C. Precipitate started to form after a while. These reaction mixture was further treated with (*p*-tolyl)PPh_2 (0.1 mmol, 0.03 g) in the same solvent mixture (5 mL) and the stirring continued for another two hours. The precipitate started to lessen a few minutes after the addition. The solution was then filtered to remove unreacted precipitate and dried *in vacuo*. The yield was 29% and its melting point is 120 °C.

3.2.2.4 $[Ag_2(PPh_2(p\text{-tolyl}))_2(L4)_4].(NO_3)_2$ [Ag4]

$AgNO_3$ (0.1 mmol, 0.02 g) as well as 2-hydroxy-4-methoxy benzaldehyde thiosemicarbazone, **L4** (0.2 mmol, 0.05 g) were dissolved in a solvent mixture (5 mL) of acetonitrile and methanol (ratio of 2:3). Then, the thiosemicarbazone solution was added dropwise to the Ag solution. Following that, the reaction mixture was stirred for three hours at 50°C. Precipitate started to form after a while. These reaction mixture was further treated with (*p*-tolyl)PPh_2 (0.1 mmol, 0.03 g) in the same solvent mixture (5 mL) and the stirring continued for another two hours. The precipitate started to lessen a

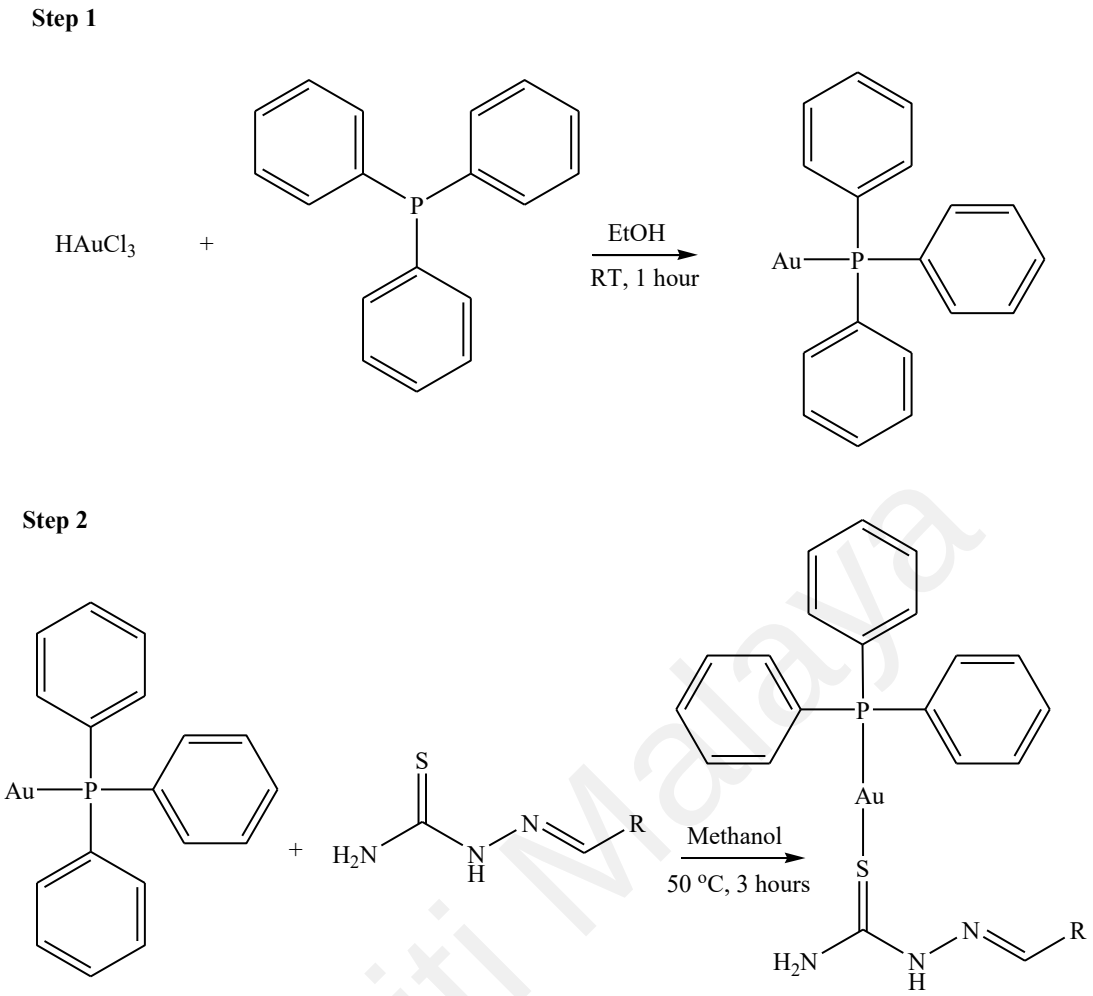
few minutes after the addition. The solution was then filtered to remove unreacted precipitate and dried *in vacuo*. The yield was 39% and its melting point is 122 °C.

3.2.2.5 $[Ag_2(PPh_2(p\text{-tolyl}))_2(L5)_4].(NO_3)_2$ [Ag5]

$AgNO_3$ (0.1 mmol, 0.02 g) as well as 5-bromo-2-hydroxy-3-methoxy benzaldehyde thiosemicarbazone, **L5** (0.2 mmol, 0.06 g) were dissolved in a solvent mixture (5 mL) of acetonitrile and methanol (ratio of 2:3). Then, the thiosemicarbazone solution was added dropwise to the Ag solution. Following that, the reaction mixture was stirred for three hours at 50°C. Precipitate started to form after some times. These reaction mixture was further treated with (*p*-tolyl)PPh_2 (0.1 mmol, 0.03 g) in the same solvent mixture (5 mL) and the stirring continued for another two hours. The precipitate started to lessen a few minutes after the addition. The solution was then filtered to remove unreacted precipitate and dried *in vacuo*. The yield was 46% and its melting point is 126 °C.

3.2.3 Synthesis of Au(I) complexes

The synthesis of Au(I) phosphine thiosemicarbazone complexes were done in two steps reaction. The first step involves the synthesis of precursor which is Au(I) phosphine complex according to the published procedure [163]. The outline of the procedure was illustrated in **Scheme 3.3**.



Scheme 3.3: Synthesis of gold(I) complex

3.2.3.1 [Au(L3)(PPh$_3$)] [Au3]

NaOH (0.10 mmol, 0.004 g) was dissolved in 1 mL methanol and then added to the suspension of the synthesised gold phosphine complex (0.10 mmol, 0.05 g) in methanol (4 mL). Separately, the 5-bromo-2-hydroxy benzaldehyde thiosemicarbazone, **L3** (0.10 mmol, 0.03 g) was dissolved in 4 mL of methanol then added dropwise to the reaction suspension. The mixture was stirred for 3 hours at 50 °C. The precipitation formed was separated by filtration and washed with methanol. This precipitate was then crystallizes using acetonitrile at room temperature. The yield was 43% and its melting point is 166 °C.

3.2.3.2 [Au(L4)(PPh_3)] [Au4]

NaOH (0.10 mmol, 0.004 g) was dissolved in 1 mL methanol and then added to the suspension of the synthesised gold phosphine complex (0.10 mmol, 0.05 g) in methanol (4 mL). Separately, the 2-hydroxy-4-methoxy benzaldehyde thiosemicarbazone, **L4** (0.10 mmol, 0.02 g) was dissolved in 4 mL of methanol then added dropwise to the reaction suspension. The mixture was stirred for 3 hours at 50 °C. The precipitation formed was separated by filtration and washed with methanol. This precipitate was then crystallizes using acetonitrile at room temperature. The yield was 40% and its melting point is 184 °C.

3.2.4 Biological assay

The Ag(I) and Au(I) complex with mixed ligands thiosemicarbazone and phosphine were subjected for biological evaluation on their activities towards the antiproliferative assay and antimalarial assay. The work was a collaboration between groups. The antimalarial assays were carried out at the Bioassay Unit, Herbal Medicine Research Centre, Institute for Medical Research lead by Dr. Mohd Ridzuan Mohd Abd Razak while, the anticancer assay was carried out at the Faculty of Health Sciences, Universiti Kebangsaan Malaysia by Mrs. Fariza Juliana Nordin. As for the antibacterial study, it was conducted by Dr. Khomaizon Bt Abdul Kadir Pahirul Zaman from University Malaysia Kelantan. The detail procedures of both biological activities are as described further in this chapter.

3.2.4.1 Antiproliferative assay

A sulforhodamine B (SRB) assay was performed to determine the inhibition concentration (IC_{50}) of all Ag(I) complexes, as described in previous studies [164]. Each cell was treated at varied concentration: 5.053-161.686 μM (for **Ag1**), 4.843-154.960 μM (for **Ag2**), 4.189-134.055 μM (for **Ag3**), 4.680-149.752 μM (for **Ag4**), and 3.936-

125.944 μM (for **Ag5**). After 48 hours, each cell was fixed in the plate with 50 μL of 50% (w/v) trichloroacetic acid (TCA) solution and incubated for an hour at 4°C. Following that, these plates were washed with tap water for five times then air dried. Then, the cells were stained with 100 μL of 0.4% (w/v) SRB staining solution and incubated for 10 minutes at room temperature. Subsequently, the plates were washed with 1% (v/v) acetic acid for three times (to remove unbound stains) and air dried. Then, 200 μL of 10 mM Trizma base was added into each well then were shaken well for 10 minutes. The absorbance was read at 490 nm using a microplate reader. Meanwhile, the IC_{50} values were calculated based on the following formula: IC_{50} = (OD sample/OD control) × 100. It should be noted that these experiments were triplicated.

3.2.4.2 *P. falciparum* HRPII assay

All Ag(I) complexes were evaluated in vitro for antiplasmodial activity using HRPII assay [165, 166] as described elsewhere, with certain modifications [167]. Briefly, ring-infected red blood cells (RBCs) with 0.05% parasitemia and 1.5% haematocrit were exposed with serially diluted compounds in a candle jar for 72 hours at 37ºC. The final tested concentrations ranged between 0.25 μg/ml to 15.7 μg/ml. The negative control was the infected RBC without compounds or replaced with sterile H_2O. After 72 hours of incubation, the test plates were kept overnight at -80 ºC. The test plates were thawed to lyse the infected RBCs at room temperature. The parasite-compound exposure activity (end point) was measured using HRPII assay, as described by Noedl *et al.* [166, 168]. The collected data was then transferred to HN-NonLin software (malaria.farch.net) to get 50% effective concentration (EC_{50}) values directly from the graph.

3.2.4.3 Cytotoxicity assay

The MDBK cells were maintained in complete DMEM culture medium containing 25 mM HEPES, 0.4% sodium bicarbonate ($NaHCO_3$), 100U of Penstrep (100U penicillin and 100U streptomycin) supplemented with 10% fetal bovine serum (FBS). The cytotoxicity of the Ag(I) complexes were measured using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [169]. Briefly, the MDBK cells (1×10^3 cells per well) were exposed with serially diluted (two-fold serial dilutions) compounds with the range of final concentration between 0.78 µg/ml to 25 µg/ml. in triplicates. The positive control for cell growth was the cell suspension without test substance while the negative control was the cell suspension with 0.05% of Triton X100. The culture was incubated for 72 hours in CO_2 incubator (CO_2 concentration of 5% at 37 ºC). Subsequently, 50 µL of MTT solution (5 mg MTT in 1 mL PBS and 2.5 mL DMEM media) was added into each well. Likewise, the plates were further incubated for four hours in CO_2 incubator (CO_2 concentration of 5% at 37 ºC). The medium was then removed and replaced with 200 µL of DMSO to dissolve the MTT formazan product. The solution was subsequently mixed for 15 minutes and once for 30 seconds before the measurement of absorbance at 540 nm was taken with microplate reader (FLUOstar Omega, Germany). Both growth inhibition and 50% cytotoxic concentration (CC_{50}) values were estimated in percentage based on dose response curve. A selectivity index (SI) – which corresponded to the ratio between the antiplasmodial and cytotoxic activities – was calculated according to the following formula:

$$SI_{Plasmodium} = \frac{CC_{50\ normal\ cell\ lines}}{EC_{50\ Plasmodium}}$$

3.2.4.4 Antibacterial assay

The antibacterial activities **L3**, **L4**, **Au3** and **Au4** were tested against gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli* using paper disc diffusion

assay. Bacterial cultures were freshly prepared by inoculating a single colony of test organisms in 20 mL nutrient broth (NB) at 37 °C for 24 hours. 0.2 mL of the bacterial inoculums was spread evenly on Mueller-Hinton agar (MHA) medium. The synthetic compounds were dissolved in methanol and each pipetted onto a sterile 6 mm paper disc. Paper discs were dried in a laminar air-flow prior to transfer onto the MHA containing bacterial inoculums. Trimethoprim (50 μg/mL) and methanol were used as positive and negative controls, respectively. Plates were incubated at 37 °C for 24 hours. The presence or absence of inhibition zone was observed and recorded during the incubation period.

3.2.5 Synthesis of metal cluster

The metal cluster was prepared using liquid assisted mechanochemical grinding technique in accordance to the study conducted by Rao *et al*. with little modification [161]. 1 mmol of synthesised complex was reacted with 5mmol of sodium borohydride ($NaBH_4$) by grinding for 30 minutes. The liquid used to assist this reaction was MeOH which was added gradually during the reaction. The product was left to dry at room temperature for a day before it was scrapped out. The preliminary result of the prepared metal cluster will be discussed in latter chapter.

3.3 Instrumentations

Several instruments and equipment have been utilised in this study for the characterisation all the synthesised ligand and metal complexes to determine its structure. These includes CHN analyser, Infra-red spectroscopy, UV-visible spectroscopy, NMR spectroscopy, energy-dispersive X-ray spectroscopy, powder X-ray diffraction and single crystal X-ray Diffractometer. All of these instruments will be discussed further in the latter section.

3.3.1 CHN analyser

The CHN analyses were performed in this study by using Perkin Elmer CHNS/O 2400 Series II to rapidly determine the percent composition of the carbon, hydrogen and oxygen present in the synthesised compounds. The obtained value from the analysis will be compared to the calculated value in order to confirm the proposed structure as well as the purity of the analysed compounds. This elemental analyser is based on the classical Pregl-Dumas method which requires high combustion temperature in an oxygen-rich environment. The temperature of the combustion column can go up to 950 °C, while the temperature of the reduction column can go up to 640 °C. The samples were weighed and packed in a tin capsule to be analysed and the instrument was calibrated using acetanilide as the standard.

3.3.2 FTIR spectroscopy

The Infra-red (IR) spectra were determined using a Perkin Elmer Spectrum One FT-IR spectrophotometer (ATR). The acronym ATR stands for Attenuated Total Reflection which is one of the four major sampling techniques used in FTIR analysis. Currently, this technique has grown to become one of the most utilized by researcher due to its ease of use. This instrument requires no sample pre-treatment either it is a powder, liquid or gel. The most important part is that, the samples must be in contact with the active surface of the crystal for them to be analysed. Practically, any organic or inorganic compound consist of covalent bonds that absorbs various frequencies of electromagnetic radiation in the infrared region of the electromagnetic spectrum. Therefore, all the synthesised ligands and its complexes were analysed using FTIR spectroscopy in the wavelength range of 4000 – 450 cm^{-1} to validate the functional group present in the compounds.

3.3.3 UV-visible spectroscopy

The UV-visible spectra were recorded with an Agilent Technologies Cary60 UV-VIS spectrophotometer in the region of 400 – 800 nm. In this study, this instrument was used to analyse the synthesised clusters in comparison to the complex in terms of changes in the d-d electronic transition state of the metal. This electronic transition give rise to the absorption in the visible region which is responsible for the color of the transition metal. The samples were dissolved in suitable solvents and placed in a cuvette for them to be measured. The light source from the spectrophotometer will emits light waves of visible and ultraviolet wavelengths that will passes through the sample, resulting in the measurement of the light transmittance (T) by the sample. The absorbance (A) was then calculated by formula of A = –log(T) and the spectrum will be presented by absorbance *vs* wavelength.

3.3.4 NMR spectroscopy

JEOL FT-NMR ECX 400 (ECX 400) was employed for the NMR analysis at 400 MHz in deuterated solvents without internal reference. The NMR spectroscopy were utilized in this study to investigate the proton (^{1}H), carbon (^{13}C) and deprotonated phosphorus (^{31}P{^{1}H}) of the synthesised thiosemicarbazone ligands, their complexes as well as metal clusters. While the infrared (IR) spectroscopy discloses the functional group present in the compound, this spectroscopy provides the information about the number of magnetically distinct atoms of the type being studied. Therefore, through the combination of the IR and NMR data, the proposed structure of the synthesised compounds could be confirmed. Furthermore, this spectroscopy could also help to determine the purity of the compounds that being analysed.

3.3.5 EDX spectroscopy

The Energy-dispersive X-ray spectroscopy (EDX), can also be referred as EDS or EDAS is an X-ray technique that is used for identifying elemental composition of materials. This spectroscopy detects the X-ray emitted by the sample during bombardment by an electron beam for the elemental composition characterisation of the analysed volume. The generated data of EDX analysis will be presented by the spectra consist of peaks corresponding to the elements that makes up the true composition of the sample being examined.

3.3.6 Powder X-ray diffraction

The powder X-ray diffraction (PXRD) was recorded on X-ray diffractometer (PANalytical, Netherlands) with Cu Kα characteristic radiation (wavelength λ=0.154 nm) at the voltage of 40kV and current of 40mA and the scanning rate was 4.25°/min and the scanning of 2θ was from 0-40° at room temperature (25 °C). Most materials have their own unique diffraction patterns like a "fingerprint" which therefore can help to identify the compounds by using a database of diffraction pattern. In powder X-ray diffraction, these patterns are obtained from powder of the material instead of an individual crystal. This technique helped to provide information about the phase as well as the crystallinity of the material. It is a non-destructive characterisation method that can be used for the study of minerals, zeolite, metal-organic framework (MOF) and other extended solids. The PXRD is also utilized for establishment of molecular species bulk purity.

3.3.7 Single crystal X-ray diffraction

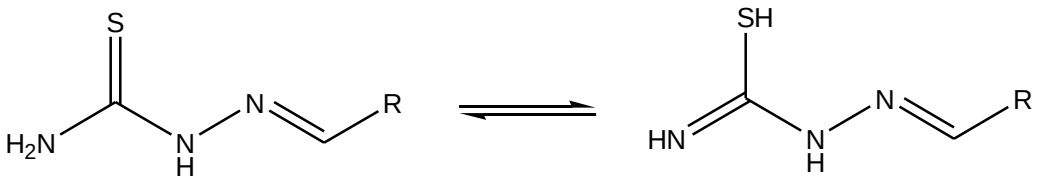
The single crystal was analysed using Agilent Oxford Supernova Single Crystal Dual Wavelength X-ray Diffractometer and XtaLAB Synergy, Dualflex, AtlasS2 Rigaku Oxford Diffractometer using CuK_α (λ = 1.54184 Å) radiation. The single crystal X-ray

diffraction is also a non-destructive technique that provides detailed information on the internal lattice of crystalline substances. This includes unit cell dimensions, bond lengths, bond angles and also details of side ordering. Data processing and absorption correction were performed using a multi-scan method within CrysAlis PRO. The structures were solved by a direct method using SHELXS. The data was processed using Olex2 [170], PLATON, [171] and Mercury [172] programs. As compared to the powder XRD, this technique is much more time consuming and data intensive.

CHAPTER 4: RESULTS AND DISCUSSION

In this study, we synthesised and investigated an inorganic compound of Ag and Au metal ions. A total of five thiosemicarbazone derivatives ligands were synthesised, which was the 4-hydroxy benzaldehyde thiosemicarbazone (**L1**), 2,4-dihydroxy benzaldehyde thiosemicarbazone (**L2**), 5-bromo-2-hydroxy benzaldehyde thiosemicarbazone (**L3**), 2-hydroxy-4-methoxy benzaldehyde thiosemicarbazone (**L4**) and 5-bromo-2-hydroxy-3-methoxy benzaldehyde thiosemicarbazone (**L5**). These thiosemicarbazone ligands were derivatize on the substituents of the aromatic ring by varying the attached functional group. The phosphine ligands used in the preparation of these complexes were (*p*-tolyl) diphenylphosphine and triphenylphosphine. Altogether, seven complexes were successfully synthesised, which five of them were Ag(I) complexes and the other two were Au(I) complexes.

The synthesis procedure of the ligands results in precipitation except for **L1**. Crystalline solid starts to form at room temperature after three days. The solubility of the synthesised ligands merely were good in organic solvents namely dimethyl sulfoxide and dimethylformamide. Other than these solvents, the synthesised ligands was also soluble in acetonitrile except for **L4** and **L5**. The thiosemicarbazone was non-hygroscopic and can be stored at room temperature for long period of time. This was confirmed by periodically monitoring their ^{1}H NMR spectra. These ligands also exhibit thione-thiol tautomerism, as shown in **Figure 4.1**. Depending on the reaction condition such as the pH, this reversible reaction can occurs in numerous thioamide-type derivatives as majority give rise to *S*-alkyl and *N*-alkyl derivatives [173].



Thione Thiol

Figure 4.1: Thione – thiol tautomerism of thiosemicarbazone ligand

In the preparation of the Ag(I) complexes, one pot synthesis was utilized by reacting $AgNO_3$ with thiosemicarbazone followed by addition of (*p*-tolyl)PPh_2 after several hours of stirring. During the synthesis, complexes **Ag1** and **Ag2** produced a clear solution from the beginning of experiment towards the end. However, complexes **Ag3 – Ag5** produced precipitate a few minutes after the addition of the thio-ligand. As the reaction progressed, the precipitate started to lessen upon addition of phosphine ligand, but the solution did not become fully clear. Hence, presumably the existence of another substituent (bromine and methoxy) other than the hydroxyl group reflects the observed situation.

In this study, we expect that the Ag metal will coordinate to the sulphur of thiosemicarbazone displaying variable binding modes, namely, η^1-S and μ-S [98]. The previous study conducted on the Ag complex of sulphur consisting ligand and phosphine ligand using similar molar ratio as we did in the study, revealed that the silver atom was found binding to one terminal P atom of phosphine, one S atom of η^1-S thiosemicarbazone and two S atom of two μ-S thiosemicarbazone [130, 131].

Up to this point, it was revealed that the synthesised silver(I) complexes were non-hygroscopic which it would not decompose after being stored for an extended duration. It was reaffirmed by periodically monitored of its NMR spectrum. The solubility of the synthesised silver(I) complexes is good in organic solvents such as dimethyl sulfoxide,

dimethylformamide, tetrahydrofuran, acetonitrile and methanol. However, in the aqueous state, the decomposition of the complex to colloidal silver take place over period of time. This process prevents the growth of single crystals as it restrains the thermodynamic stability of the redox-active silver(I) complexes with phenolic ligands in solution [174, 175].

Unlike Ag(I) complex, the preparation of the Au(I) complex was done in a two steps reaction. The mixed ligands gold complexes were synthesised by the formation of gold phosphine complex to act as a precursor, followed by a reaction with the synthesised thiosemicarbazone ligands. However, only two complexes were successfully synthesised which were **Au3** and **Au4** using the thiosemicarbazone derivatives **L3** and **L4**. Although the same method was applied to prepare the other gold complexes of **L1**, **L2** and **L5,** unfortunately these thiosemicarbazone derivatives did not bind to the synthesised Au(I) phosphine. Several other attempts had been done to bind the gold phosphine complex to the thiosemicarbazone ligand **L1**, **L2** and **L5** by modifications of the used method. Some of the modification includes stirring at room temperature, extending the stirring period and reaction without the basic condition. Solid-solid interaction by mechanochemical grinding was also employed to synthesis the gold complexes. Regrettably, none of these methods were able to bind these three ligands to the gold phosphine complex.

Similar to the Ag complexes, the coordination of Au phosphine to the binding site of thiosemicarbazone was also determined by the HSAB theory. Therefore, it was deduced that the coordination of the Au^{+} to the thiosemicarbazone ligand was through the sulphur atom instead of the nitrogen atom. We also expect that the synthesised Au(I) complexes would form a linear geometry as was revealed by the previous studies on these type of Au(I) complexes [144].

The mixed ligands gold complexes were found to be non-hygroscopic and could be stored at room temperature for long period of time. This was proven from the NMR analysis done to the complexes from time to time. These complexes were found to be soluble in chloroform, dichloromethane, acetonitrile and dimethyl sulfoxide. The crystal of **Au3** and **Au4** were obtained from acetonitrile after being left at room temperature for a day and a week respectively.

4.1 General characterisation

The physical properties, percent yield and elemental analysis of the synthesised thiosemicarbazone ligands as well as silver and gold complexes were recorded as shown in **Table 4.1**. All the calculated value of percent yield was in good agreement with the data obtained from the CHN analysis.

From the table, it could be seen that the percent yield of all the thiosemicarbazone ligands were relatively high except for **L1**. This observation could be due to the slow evaporation of the product at room temperature, which could lead to incomplete crystallization of the **L1**. Meanwhile, all the silver and gold complexes were found to have relatively low percentage yield. This low yield could be caused by incomplete complexation reaction or experimental errors during the filtration step or amid the purification phase. In order to overcome this, we try to resynthesize the complexes and we also try to extend the time of the synthesis. Unfortunately, their percentage yield did not change much from the previous calculated value.

Table 4.1: CHN elemental analysis data and physical properties of synthesised compounds

Compound	Color	Yield (%)	Experimental (Calculated)		
			C	H	N
L1	Yellow	47	49.11 (49.21)	4.63 (4.65)	20.83 (21.52)
L2	Yellow	56	45.38 (45.49)	4.22 (4.29)	19.81 (19.89)
L3	White	85	35.01 (35.05)	2.49 (2.94)	14.91(15.33)
L4	White	69	47.51 (47.99)	5.24 (4.92)	18.76 (18.65)
L5	White	95	35.54 (35.54)	3.11 (3.31)	12.81 (13.81)
Ag1	Yellow	26	53.64 (54.27)	4.14 (4.55)	11.37 (10.85)
Ag2	Yellow	40	51.85 (52.11)	4.32 (4.37)	10.29 (10.42)
Ag3	Yellow	29	44.88 (45.08)	3.56 (3.57)	8.61(9.01)
Ag4	White	39	52.97 (53.24)	4.26 (4.71)	9.73 (10.07)
Ag5	Yellow	46	45.06 (44.78)	3.42 (3.76)	7.84 (8.74)
Au3	Yellow	43	47.30 (47.37)	3.22 (3.83)	6.14 (6.11)
Au4	White	40	42.50 (42.58)	3.16 (2.64)	5.73 (5.69)

Based on the data from CHN elemental analysis, it was suggested that the stoichiometry of the synthesised compounds was indeed congenial with the proposed stoichiometry. In case for silver complexes, the thiosemicarbazone ligands were discovered to formed complexes with silver nitrate in the presence of (*p*-tolyl)PPh_2 in the molar ratio of 2:1:1 respectively. On the other hand, the stoichiometry of gold complexes synthesised by the reaction of auric acid with PPh_3 and thiosemicarbazone ligands were found in agreement with the molar ratio of 1:1:1 used in the experimental.

4.2 Spectroscopic characterisation

4.2.1 FTIR analysis

All the important vibrational modes of bonding; bending and stretching of the synthesised compounds were tabulated in **Table 4.2**. Based on the obtained FTIR spectra, all of the important band for the thiosemicarbazone ligands were observed indicating a successful synthesis of these ligands.

The spectra of the Ag(I) complexes indicated the coordination of Ag to thiosemicarbazone ligand, which revealed the presence of ν(C=N) vibrational modes in all the complexes (**Ag1** – **Ag5**). Nevertheless, a very weak band around 2800-2500 cm^{-1} of ν(S-H) was observed in **L1**, **L3**, **L4**, and **L5** spectra indicates that the ligands exist as thiols. However, this band was not present in the spectrum of their corresponding complexes. A study by Lobana *et al.* (2009) showed the presence of intramolecular and intermolecular hydrogen bonding of hydroxyl and amino groups in silver thiosemicarbazone complex contributes to band broadening at 3600–3000 cm^{-1} region, which causing the overlapping of the bands around this region; thus this may be the plausible rationalization for the disappearance of the ν(S-H) in the complex [176]. Therefore, from the FTIR spectra, the tautomerism of the thiosemicarbazone ligands could not be confirmed and it would be further analyzed by the NMR spectroscopy in the latter section. Meanwhile, ν(S-H) was not present in **L2** or its corresponding complex (**Ag2**), which suggested that it appears in thione form.

Table 4.2: Selected frequency (cm^{-1}) of FTIR spectra data of the synthesised compounds

	ν (N1-H_2)	ν (N2-H)	ν (N1-H)	ν (S-H)	ν (C=N)	ν (NO_3^-)	ν (P-C_{Ar})	ν (C=S)
L1	-	3472.18	3359.32	2750.81	1578.19	-	-	-
L2	3348.34, 3254.01	3478.78	-	-	1584.03	-	-	875.60
L3	-	3468.58	3356.45	2833.38	1594.67	-	-	-
L4	-	3454.90	3245.15	2814.90	1543.57	-	-	-
L5	-	3450.84	3352.54	2812.67	1529.07	-	-	-
Ag1	-	*	*	*	1591.40	1305.19	1094.92	-
Ag2	*	*	-	-	1599.63	1372.04	1095.63	848.87
Ag3	-	*	*	*	1601.79	1316.04	1094.06	-
Ag4	-	*	*	*	1627.32	1324.09	1095.40	-
Ag5	-	*	*	*	1596.83	1310.54	1093.83	-
Au3	3338.98, 3267.79	3138.98	-	-	1595.62	-	1099.71	905.63
Au4	3345.76, 3271.18	3132.20	-	-	1597.81	-	1101.19	959.89

The * indicates overlapping of bands around 3600 – 3000 cm^{-1} due to the band broadening

The coordination of the Ag to the sulphur of thiosemicarbazone ligand was confirmed by the thioamide band, ν(C=S) was located at 848.87 cm^{-1} for **Ag2**, which demonstrated the shift to lower energy region (875.60 cm^{-1}) as compared to that of **L2**. This could be due to the weakening of C=S bond with the coordination of silver *via* sulphur atom. Furthermore, the coordination of phosphorus to silver center was observed from the presence of characteristic peak ν(P-C_{ph}) in the region of 1091–1095 cm^{-1}. Other than that, the sharp band around 1300 cm^{-1}, attributed to NO_3^- bend peak demonstrated the presence of non-coordinated NO_3^- in all five complexes in this study. Both the ν(P-C_{ph}) and ν(NO_3^-) were not observed from the FTIR spectra of the thiosemicarbazone ligands but present in the spectra of the silver complexes. This observation was found to be coinciding with the previous studies [176].

Meanwhile, for the Au(I) complexes, the coordination of thiosemicarbazone ligand to the gold phosphine was confirmed by the presence of ν(C=N) band located at 1595.62 cm^{-1} and 1597.81 cm^{-1} for **Au3** and **Au4** respectively. Moreover, the attachment of the phosphine group to the mixed ligand Au(I) complexes was proven by the observation of characteristic peak ν(P-C_{ph}) in the region of 1099.71 cm^{-1} and 1101.19 cm^{-1} for both **Au3** and **Au4** correspondingly. Although the ligands **L3** and **L4** were found to exist in thiol tautomerization, its Au(I) complexes was speculated to exist in the thione tautomer due to the presence of ν(N1-H_2) as well as ν(C=S) band that was observed at 909.89 cm^{-1} and 959.89 cm^{-1} for **Au3** and **Au4** spectrum respectively.

4.2.2 NMR spectroscopy

4.2.2.1 ^{1}H NMR spectroscopy

The thiosemicarbazone ligands and its silver complexes were analyzed using NMR spectroscopy by dissolving them in the deuterated DMSO while, the gold complexes **Au3** and **Au4** were dissolved in deuterated chloroform. The assigned ^{1}H NMR data of

significant chemical shifts for the ligands and their complexes were tabulated in **Table 4.3**.

Table 4.3: Important ^{1}H NMR chemical shift (ppm) of synthesised compounds

	δN1-H_2	δN1-H	δN2-H	δS-H
L1	-	9.82	11.20	8.01
L2	9.70	-	11.13	-
L3	-	10.18	11.37	Overlap at 8.16–8.11
L4	-	9.90	11.21	7.97
L5	-	9.42	11.39	Overlap at 8.09
Ag1	-	9.98	11.64	8.55
Ag2	9.86	-	11.51	-
Ag3	-	10.34	11.71	8.57
Ag4	-	10.02	11.51	8.39
Ag5	-	9.59	11.73	8.58
Au3	5.10	-	5.45	-
Au4	-	5.37	4.97	-

The thione–thiol tautomerism of the thiosemicarbazone ligands as shown in **Figure 4.1** could be deduced from the ^{1}H NMR data. Based on the obtained ^{1}H NMR spectra, it was indicated that all the thiosemicarbazone ligands exist as thiols except for ligand **L2**. One of the indication of ligands exist as thiol tautomer was the absence of N1H_2 signal from the spectra. Instead of N1-H_2 peak, the signal of N1-H was observed around δ 10.18–9.42 ppm for **L1**, **L3**, **L4**, and **L5**. Furthermore, the presence of S-H signal

around δ 7.97–8.11 ppm for the **L1**, **L3**, **L4**, and **L5** also helps to indicate that these ligands exist in thiols tautomerism. Meanwhile, the thiosemicarbazone ligand **L2** was deduced as thione by the presence of N1-H_2 signal that was perceived at δ 9.70 ppm. Accordingly, the absence of S-H peak from the spectra of **L2** also suggested that it appear as thiones instead of thiols. **Figure 4.2** reveals the comparison of ^{1}H NMR thione-thiol tautomerism between **L1** and **L2**.

The ^{1}H NMR spectra of the Ag mixed ligands complexes were then analysed and compared to its thiosemicarbazone ligand. It was found that all spectra of the synthesised Ag complexes were closely resembling their respective free ligands in the addition of the phosphine peaks. Henceforth, all the complexes were presumed to exist as thiols tautomers except for complex **Ag2**. The S-H signal for the thiol complexes were observed to be majorly shifted downfield *ca.* δ 0.48 ppm, thus reaffirmed the coordination of the thiosemicarbazone ligands to silver ions was through the sulphur atom without being deprotonated. Meanwhile, minor downfield shifting of protons attached to N1, N2 of thiosemicarbazone and hydroxyl group were observed in the spectra of these Ag complexes. This was resulted upon their participation in hydrogen bonding either intermolecular, intramolecular or both. Furthermore, the bonding of phosphine was proved by the presence of extra aromatic protons in the region of δ 7.15–7.50 ppm in these complexes, which were absent in the ligands of thiosemicarbazone. The latter conformation of the complex formation in agreement with Altaf *et al.* [177].

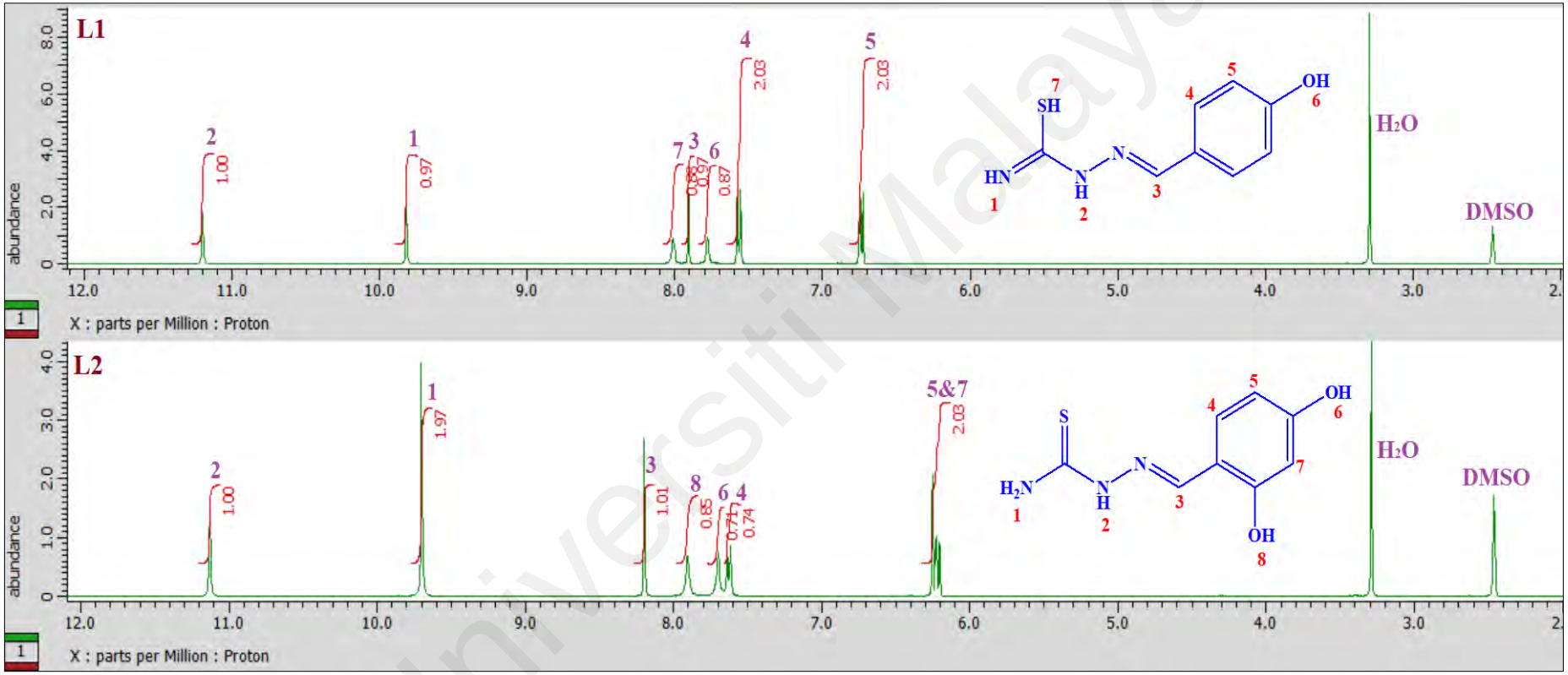


Figure 4.2: Comparison of ^{1}H NMR between L1 and L2

As for the mixed ligand Au(I) complexes, it was observed that ^{1}H NMR spectra of both **Au3** and **Au4** were quite different from their respective ligand **L3** and **L4**. The proton peaks of N1 and N2 for the Au(I) complexes were found to be shifted further upfield *ca.* δ 5.44 ppm as compared to their free ligands. This could be due to the different type of deuterated solvents used for analysing these compounds. The **L3** and **L4** ligands were examined using deuterated DMSO while their complexes **Au3** and **Au4** were examined using deuterated chloroform. The difference between these two solvents was that their ability to form hydrogen bonding where the DMSO was coordinating solvent while the chloroform was non-coordinating solvent. As the chemical shift of N-H was strongly affected by the hydrogen bonding, it will move further downfield when coordinating solvent was used. Therefore, the N-H peak of the ligands appears more downfield around 11 ppm as compared to their Au complexes which was around 5 ppm. No comparison of this observation to previous studies could be made as the literature report on the Au(I) thiosemicarbazone phosphine complex was very limited. However, a review paper written by Lobana *et al.* reported the observation of peak for hydrogen at the N1 position around δ 5.00 ppm in several studies, although different metal was adopted into the synthesise complexes [17]. Moreover, from the ^{1}H NMR spectra, the tautomerization of Au(I) complexes in solution was also observed. It was discovered that the **Au3** exist as thione even though its ligand, **L3** occur as thiols based on the presence of N1-H_2 peak at δ 5.10 ppm instead of N1-H peak. Nonetheless, in the liquid state, the complex **Au4** remained to exist as thiols, similar to its ligand **L4**.

4.2.2.2 ^{13}C NMR spectroscopy

The ^{13}C NMR spectroscopy for all the thiosemicarbazone ligands and its complexes were analyzed using the same deuterated solvent as the ^{1}H NMR spectroscopy. All the expected ^{13}C NMR signal of the thiosemicarbazone ligands were observed from the NMR spectra. The most important signal to be observed from both the ligands and the

complexes were the C–S and the C=S signals. This peak appears to be the most downfield in both the ligands and complexes. Compared to its free ligands, there was an upfield shift in ^{13}C spectra of these complexes for thiocarbonyl carbon signal with the coordination of metal to the ligand through sulphur atom as shown in **Figure 4.3** and **Figure 4.4** for Ag(I) and Au(I) complex correspondingly. As the Ag and Au atom coordinated to the sulphur atom, the carbon attached to the sulphur atom become more shielded. This observation was indeed in agreement with the previous conducted studies related to the silver thiosemicarbazone phosphine complex [177] and gold thiosemicarbazone phosphine complex [144].

As for the silver(I) complexes, (*p*-tolyl)PPh_2 was utilize as the phosphine ligand. Therefore, the presence of *p*-tolyl methyl resonance in the region of δ 21.26–21.42 ppm distinguished the coordination of phosphine ligand to these complexes. Meanwhile, PPh_3 was adopted to the gold(I) complexes as the phosphine ligand used in this study. Hence, no other peak than the addition of aromatic could be observed to distinguish the presence of phosphine in these Au(I) complexes. The ^{13}C NMR signal for aromatic group of the phosphine for both the silver and gold complexes were discovered in the form of doublet, specifically for C(–P), C–2, and C–3. Nonetheless, C–4 appeared as singlet. These results are indeed proven to be observed in previous related study [178].

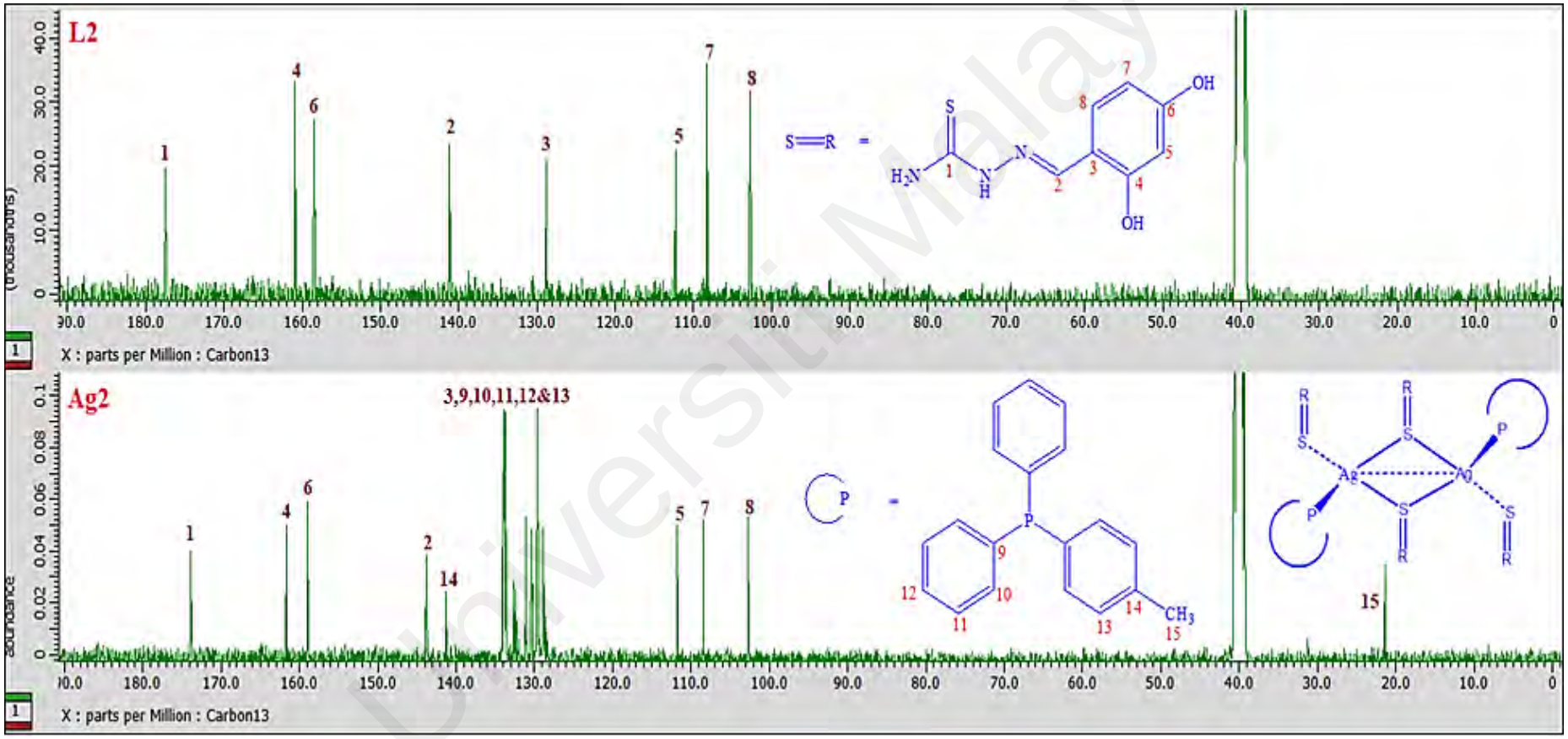


Figure 4.3: Comparison of ^{13}C NMR spectra for L2 and Ag2

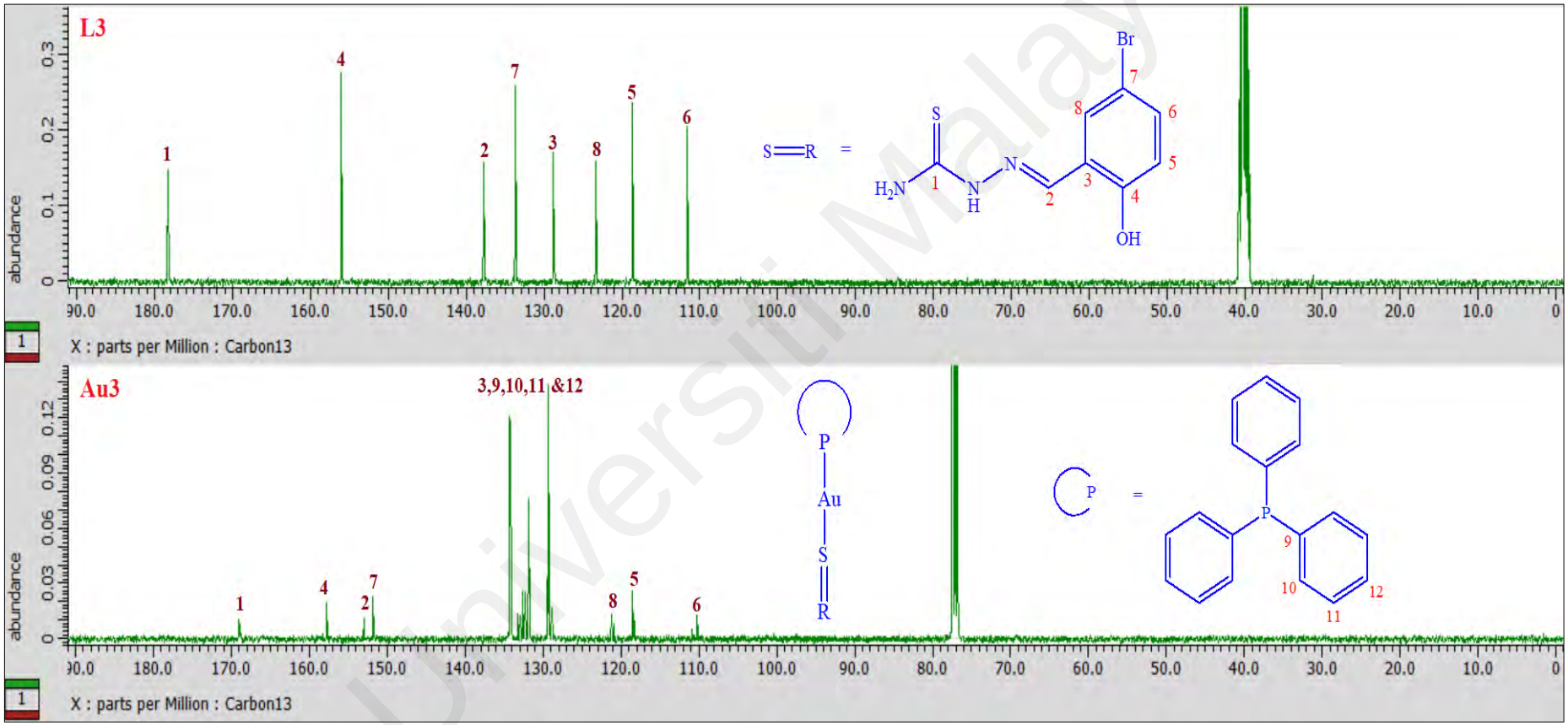


Figure 4.4: Comparison of ^{13}C NMR spectra for L3 and Au3

4.2.2.3 ^{31}P NMR spectroscopy

The $^{31}P\{^{1}H\}$ NMR spectroscopy were performed to verify the bonding of the phosphine to the gold(I) and silver(I) complexes. Free phosphine ligands and the complexes were analyzed for comparison. The chemical shifts in $^{31}P\{^{1}H\}$ NMR for all silver(I) complexes displayed one sharp peak, which were attributed to the P atom of the diphenyl(*p*-tolyl)phosphine. This confirmed the chemical environment of phosphorus atoms is chemically equivalent, hence the geometry around silver(I) ion should be tetrahedral and not square planar [179]. Compared to its free phosphine ligand, the resonance of phosphorus in these complexes shifted downfield, which confirmed the coordination of metal to ligand. Considering the limited studies on Ag(I) diphenyl(*p*-tolyl)phosphine complex, the obtained chemical shifts of $^{31}P\{^{1}H\}$ for these complexes were compared to the observed PPh_3 values [177]. It should be noted that the observed values were rather similar and conclusively in agreement with the previous study.

As for the Au(I) complexes, the $^{31}P\{^{1}H\}$ NMR spectra revealed a sharp singlet peak for both Au(I) phosphine chloride complex and Au(I) thiosemicarbazone phosphine complexes. The peak was observed at δ 33.80 ppm in the spectra of Au(I) PPh_3 complex. Meanwhile, the mixed ligand Au(I) complexes were found at δ 38.41 ppm and δ 38.59 ppm for **Au3** and **Au4** respectively. The ^{31}P NMR signal showed a downfield shift upon the coordination of thiosemicarbazone ligand to the Au PPh_3 complex as the phosphorus of phosphine become more deshielded. Furthermore, the observed value of the synthesized **Au3** and **Au4** were discovered congruent with previous studies [144].

4.2.3 EDX spectroscopy

The obtained Ag(I) complexes with mixed ligands of thiosemicarbazone derivatives and phosphine were subjected to EDX analysis to validate the presence of metal in the compound. The spectra of EDX analysis obtained were as shown in **Figure 4.5**.

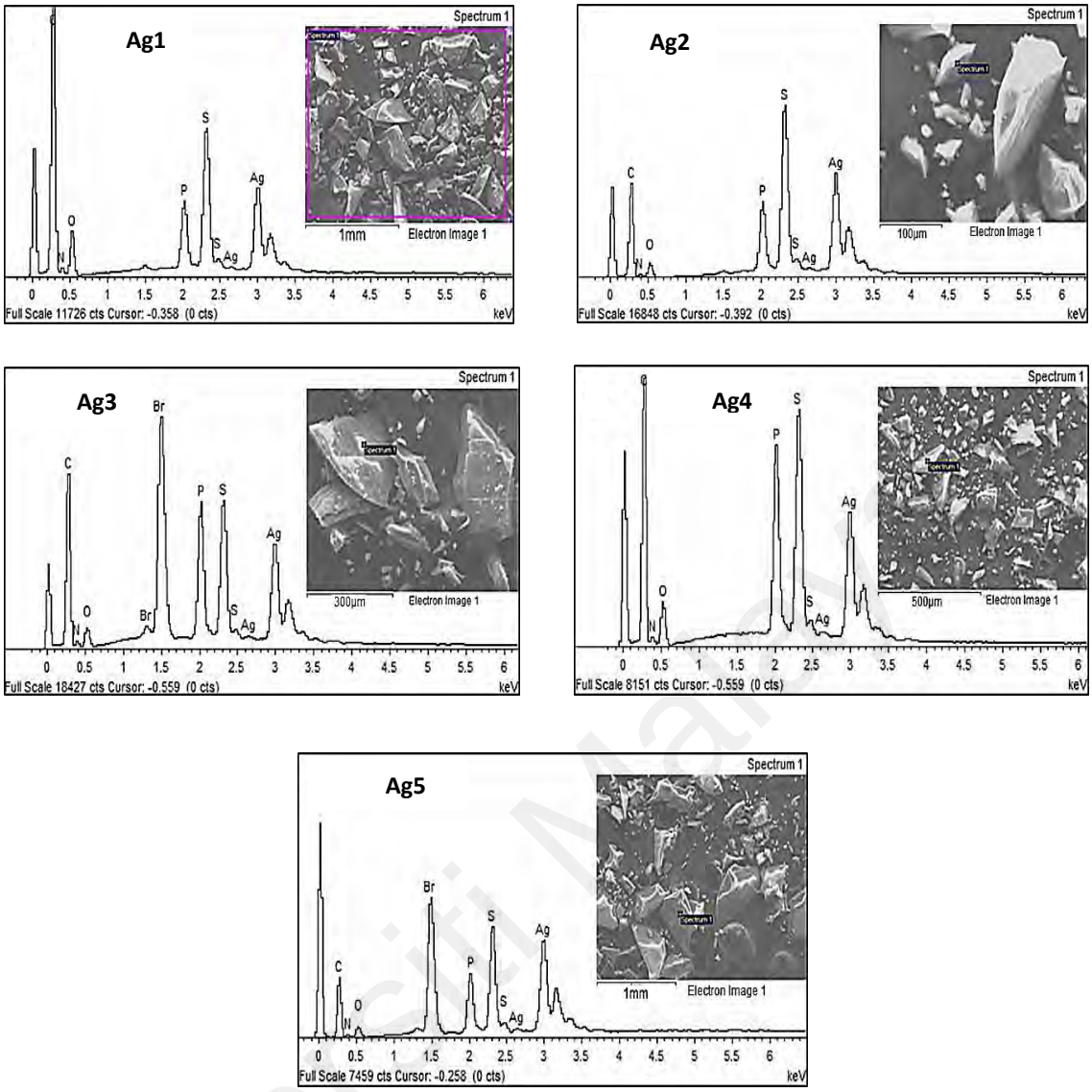
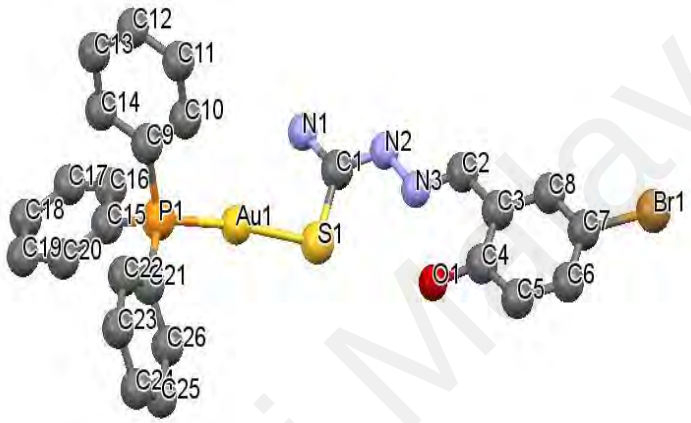


Figure 4.5: EDX spectra of complexes Ag1 – Ag5

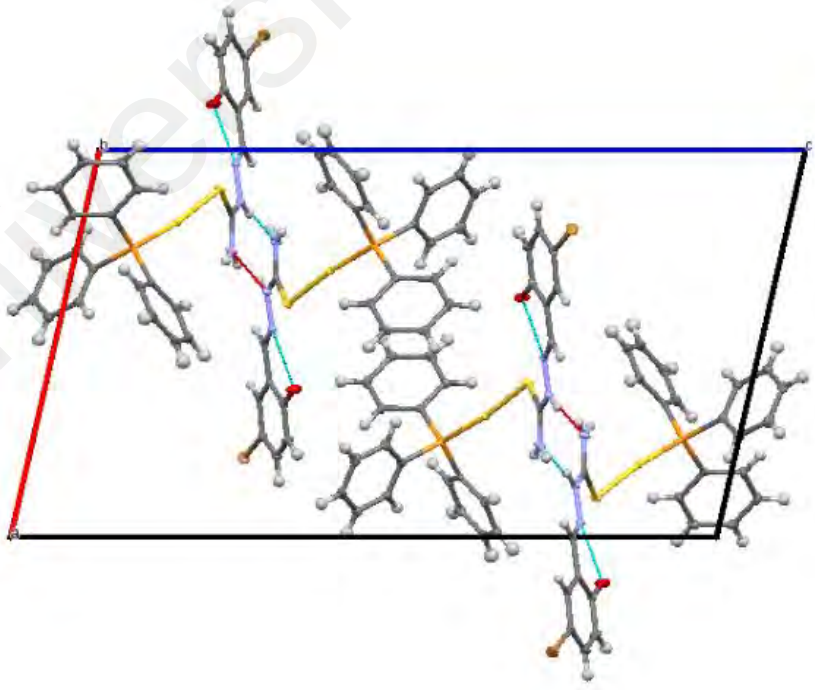
Based on the result obtained, it was indicated that the silver metal as well as other chemical components such as sulphur and phosphorus of the synthesised complexes were present. Hence, this give an insight of the metal-ligand complexation. Furthermore, no presence of other component than what made up the complexes was observed, which means that no impurity was detected.

4.2.4 Single Crystal X-Ray diffraction

The proposed structure of gold complexes with mixed ligands phosphine and thiosemicarbazone was further confirmed by the single crystal X-ray diffraction studies. The molecular structures of **Au3** and **Au4** were shown in **Figure 4.6** and **Figure 4.7** respectively. The gold atom was found to be linearly coordinated (170° and 172°) to the phosphine of PPh_3 and sulphur of thiosemicarbazone derivatives for both **Au3** and **Au4**.

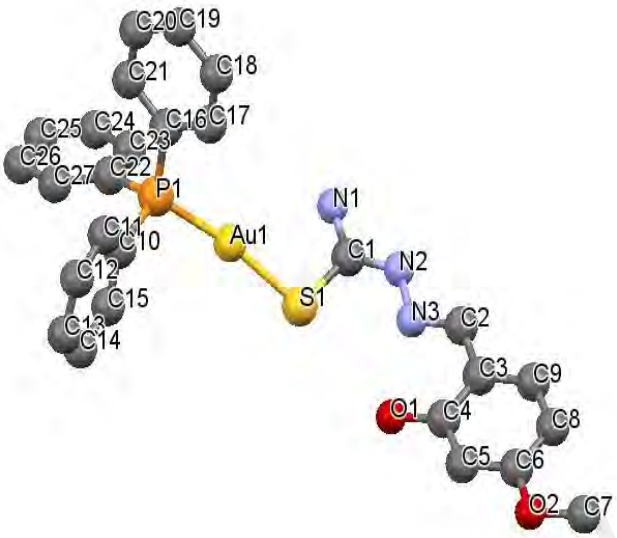


(i)

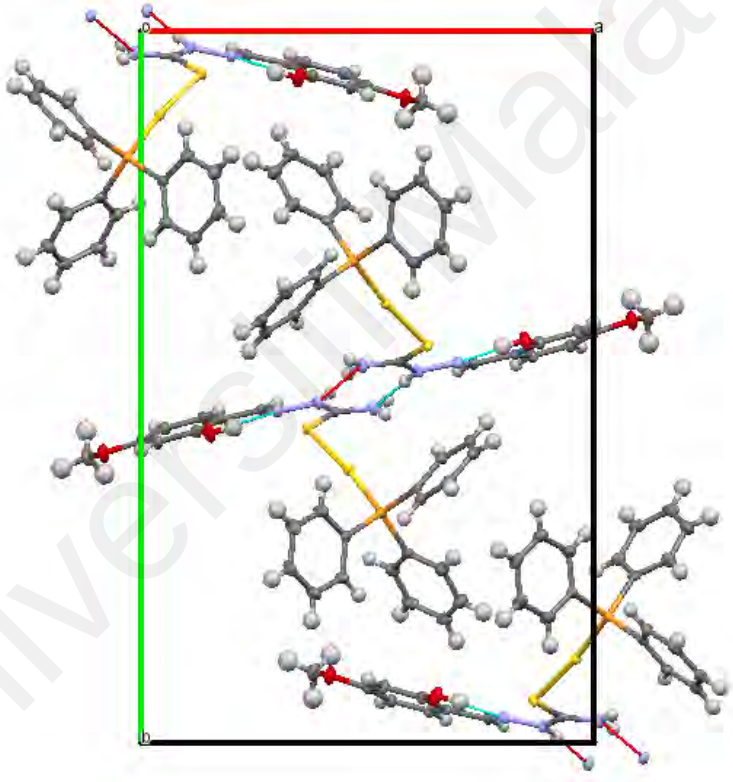


(ii)

Figure 4.6: **(i) Molecular structure of Au3. Displacement ellipsoid are shown at the 50% probability level. Hydrogen atom have been omitted for clarity (ii) Crystal packing of Au3.**



(i)



(ii)

Figure 4.7: (i) Molecular structure of Au4. Displacement ellipsoid are shown at the 50% probability level. Hydrogen atom have been omitted for clarity. (ii) Crystal packing of Au4.

The important bond lengths and angles of the synthesised gold complexes along with their thiosemicarbazone derivatives **L3** and **L4** were tabulated in **Table 4.4**. In

comparison of the thiosemicarbazone ligand to the gold complex crystal structure, elongation of C1–S1 and C1–N1 bond distance were observed, illustrating the delocalization of the ligand's backbone. The C1–S1 bond distance increases from 1.682(3) Å to 1.762(4) Å for **Au3**, while the **Au4** bond increases from 1.685(2) Å to 1.770(3) Å. As for the C1–N1 the bond length increases from 1.315(5) Å to 1.342(5) Å and 1.321(2) Å to 1.334(5) Å for **Au3** and **Au4** respectively. Meanwhile, the gold complexes C1–N2 bond length was found to be shorten as compared to the free thiosemicarbazone derivatives and this observation was confirmed by the previous study [21]. Inquisitively, there was no intra- or intermolecular Au–Au bonding, although it was often observed in most gold(I) complex [180]. One of the reasons could probably be due to the bulkiness of the ligands used.

Table 4.4: Important bond length and bond angle of Au3 and Au4

	L3	**L4**	**Au3**	**Au4**
	Bond length [Å]			
Au – P	-	-	2.2564(9)	2.2561(9)
Au – S	-	-	2.3015(9)	2.3051(8)
C1 – N1	1.315(5)	1.321(2)	1.342(5)	1.334(5)
C1 – N2	1.337(4)	1.336(2)	1.315(5)	1.307(5)
C1 – S	1.682(3)	1.685(2)	1.762(4)	1.770(3)
	Bond angle [°]			
P – Au – S	-	-	172.18(3)	170.51(3)
C1 – S – Au	-	-	106.03(13)	106.15(12)
N1 – C1 – S	122.5(3)	122.11(15)	119.2(3)	119.1(2)
N2 – C1 – S	119.4(3)	120.31(14)	122.2(3)	122.4(3)

Although no aurophilic interaction was observed, intermolecular and intramolecular hydrogen bonding were perceived from both of the Au(I) complexes. It was found that the intramolecular bonding happened between the 2-hydroxy group with azomethine nitrogen atom (N3···O–H) for both **Au3** (2.592 Å) and **Au4** (2.608 Å). Meanwhile, the intermolecular hydrogen bonding occurred between the (N2–H···N1) of one Au(I) complex with another Au(I) complex forming an infinite one-dimensional chain. The hydrogen bonding of **Au3** and **Au4** were depicted in **Figure 4.8** and **Figure 4.9** respectively.

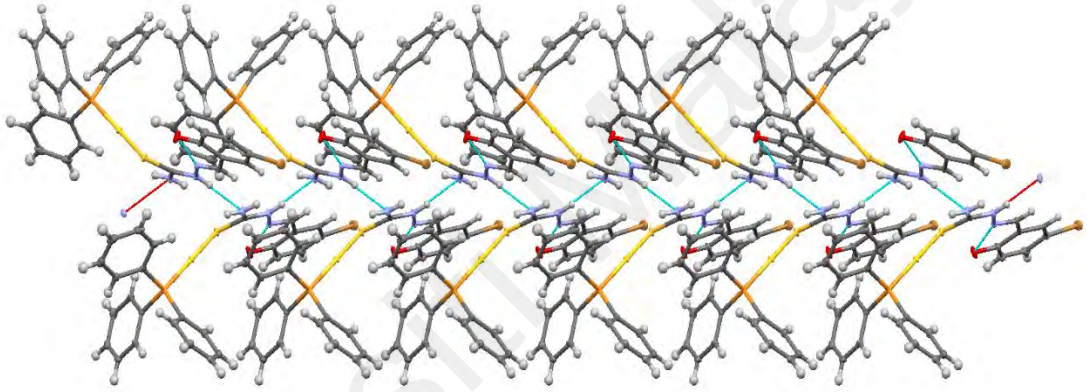


Figure 4.8: One-dimensional chain of Au3 via N2–H···N1 (2.167 Å)

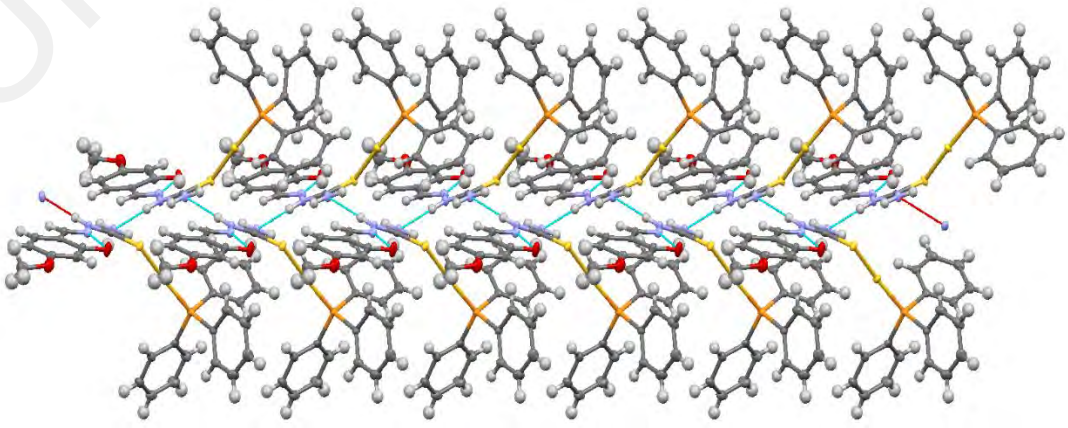


Figure 4.9: One-dimensional chain of Au4 via N2–H···N1 (2.159 Å)

4.2.5 Powder X-Ray diffraction

The Powder X-ray diffraction (PXRD) were also utilized for the characterisation of the synthesised compounds. The PXRD data of the thiosemicarbazone ligands were compared to their respective complexes as shown in **Figure 4.10**.

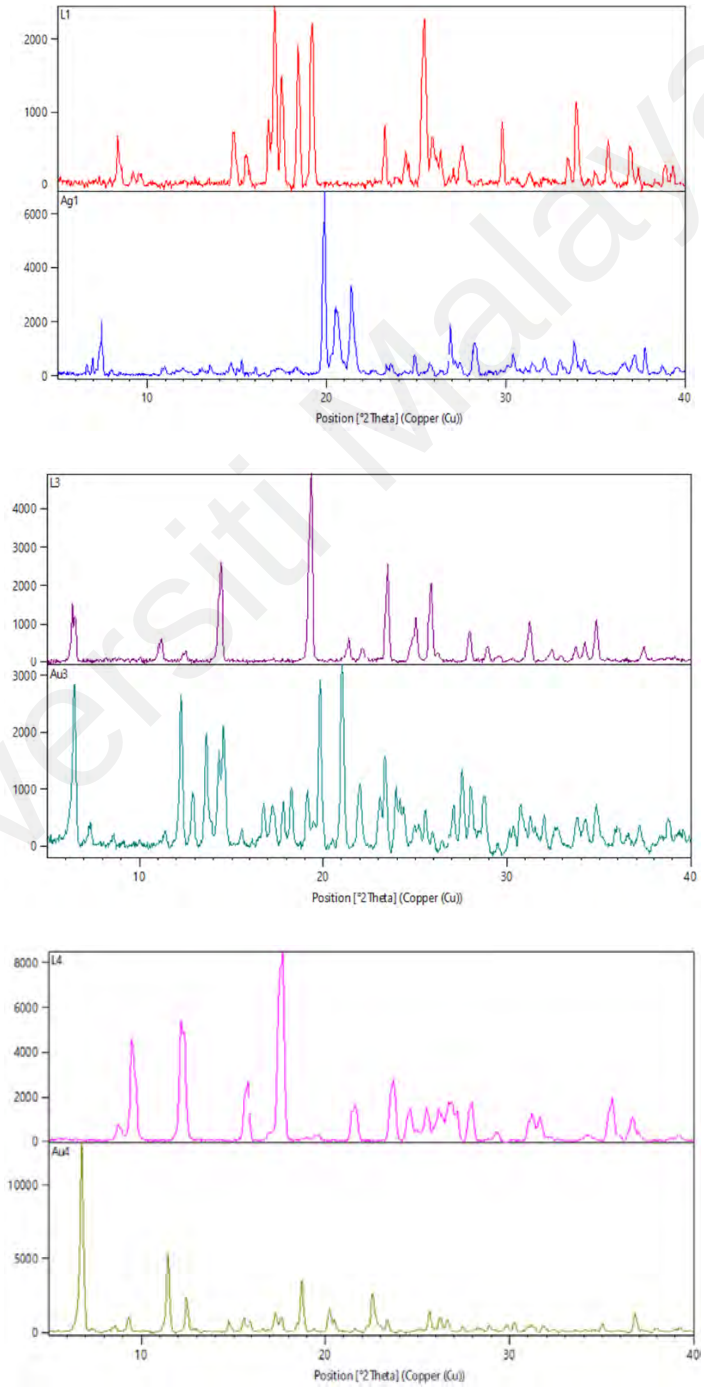


Figure 4.10: Comparison of PXRD pattern for thiosemicarbazone ligand and its corresponding complex

Regrettably, the synthesised Ag(I) complexes were non-crystalline (Appendix section C) except for complex **Ag1**. When we compare the result of the thiosemicarbazone ligand with their corresponding complexes, we could observe the changes in their PXRD pattern. This analysis helps to indicate that the ligand and its complex was not of the same compound, thus the coordination of the transition metals to the ligand may cause these changes.

The PXRD analysis also aid the confirmation of bulk materials to that of the crystallographic information file (CIF) obtained from single crystal X-ray crystallography (SCXRD) measurements of the synthesised gold(I) complexes. The powder X-ray diffraction of bulk samples of **Au3** and **Au4** were analyzed and the results were illustrated in **Figure 4.11**. It was discovered that the patterns of PXRD for both bulk gold(I) complexes were indeed in agreement with the pattern stimulated from CIF data.

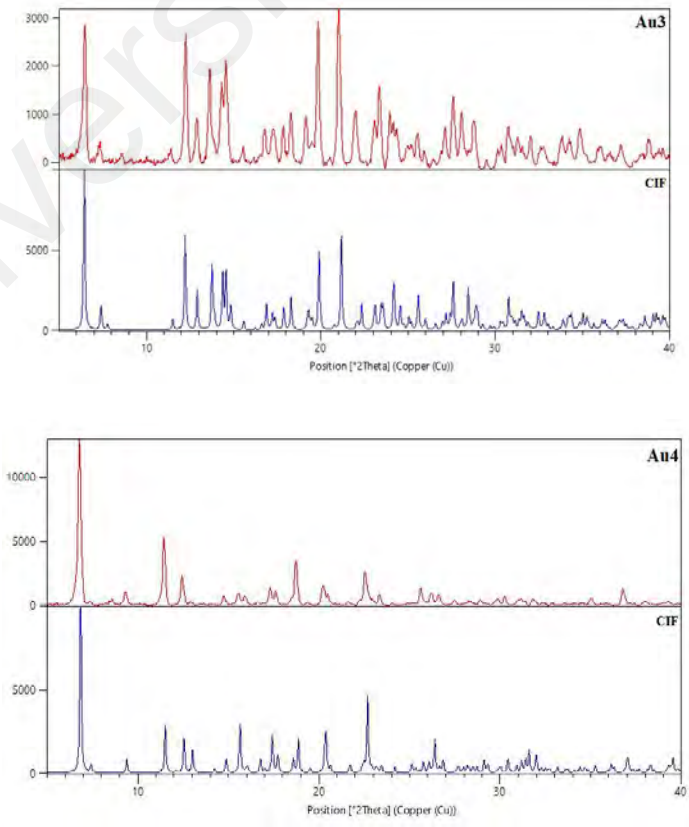


Figure 4.11: Comparison between PXRD and CIF for Au3 and Au4

4.3 Cluster synthesis

Although both Ag(I) and Au(I) complexes were used for the synthesis of the metal cluster, we only managed to successfully obtain the Au clusters using this method. The yield of the Ag cluster was too low that we can't further analysed them for the characterisation. The preparation of metal clusters was done by reducing the synthesised Au(I) complexes with $NaBH_4$ in 1:5 molar ratio by mechanochemical grinding. A few minutes after the reaction, the color of the solid start to show some changes. Then, MeOH was added to the reaction drop by drop occasionally during the reaction and the color of the compound turned darker. The color change of the compounds can be seen from **Figure 4.12**. This change of color was one of an early indication for the formation of metal cluster as it suggests changes in the oxidation state of the metal, which will be further analysed using the UV-Vis spectroscopy. As we were using the crystal of the complexes for the reaction, we expect no unreacted ligand present in the cluster formed.

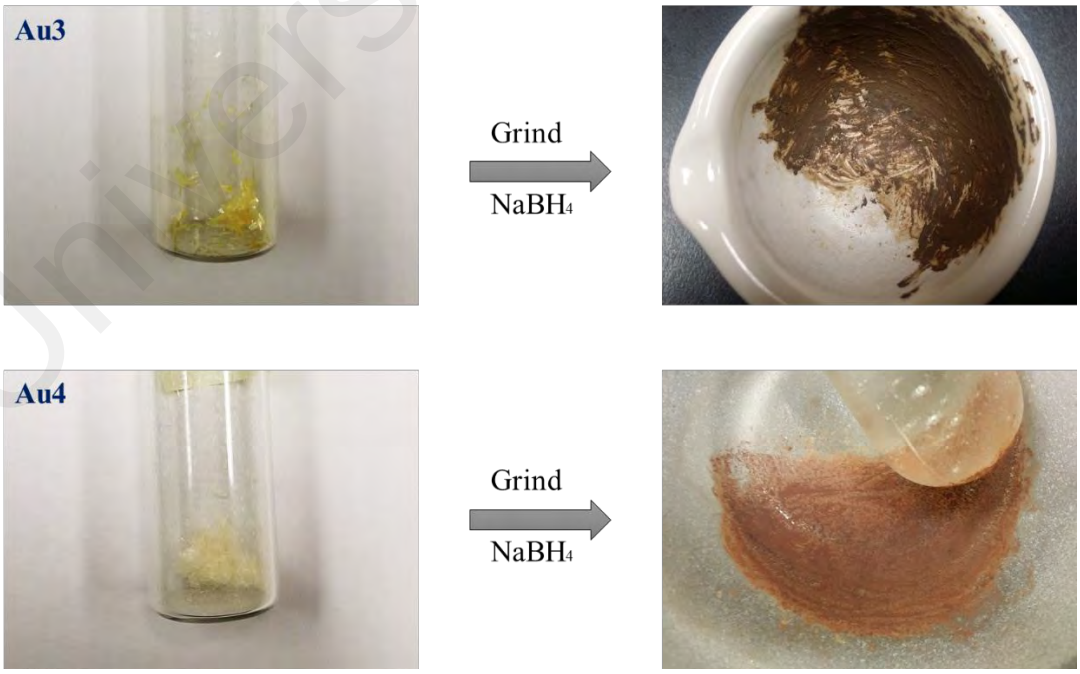


Figure 4.12: Color change of complex Au3 and Au4 to its cluster

These metal clusters were then subjected for UV-Vis analysis to study changes in the oxidation state of the Au(I). The comparison of the UV-Vis spectra for both **Au3** and **Au4** complex to its cluster was illustrated in **Figure 4.13**. Based on the obtained spectra, it could be seen that the maximum absorption of both clusters shifted to higher wavelength which were around 400 nm when compared to their corresponding complexes. This observation could be attributed to the change of their metal oxidation state.

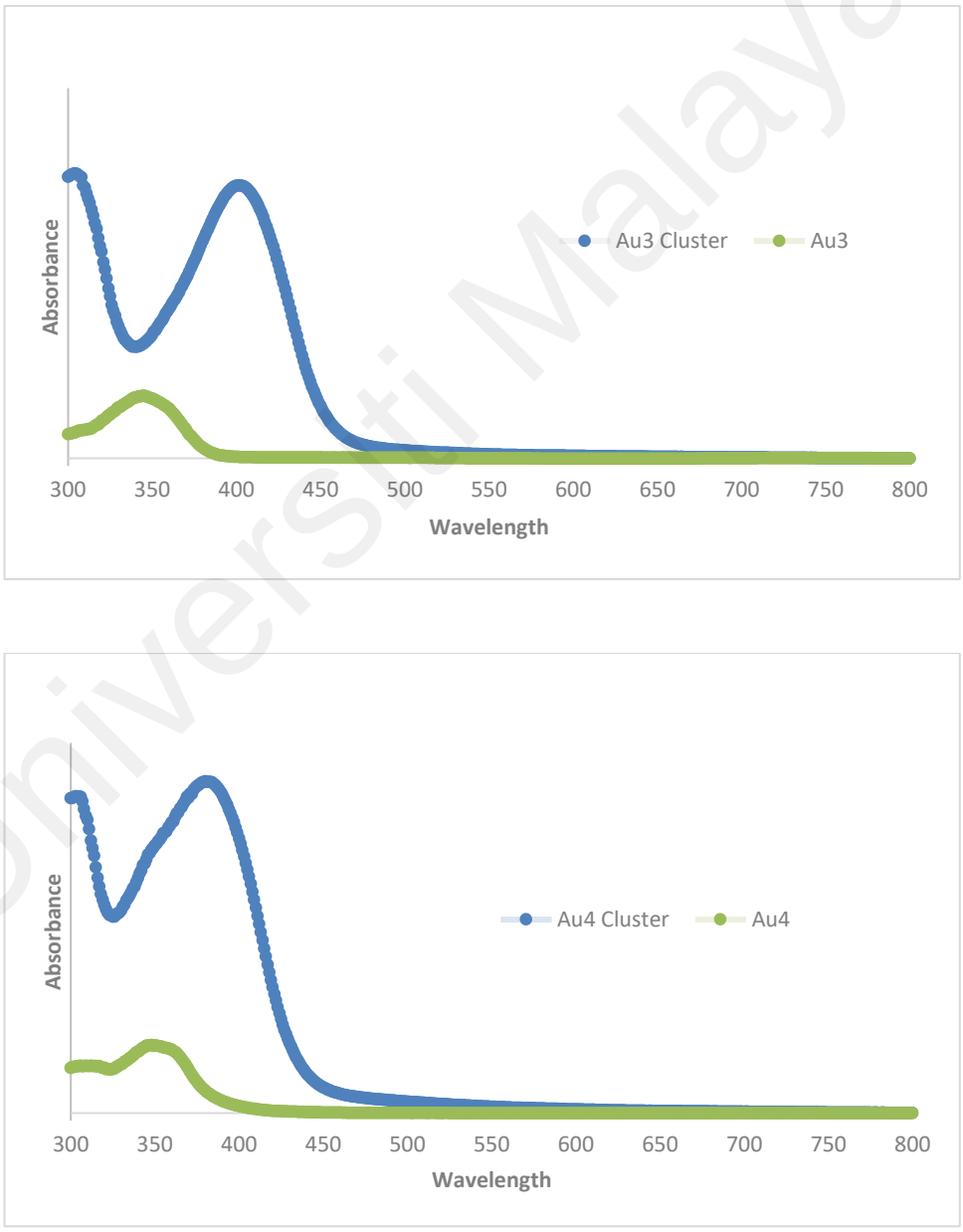


Figure 4.13: Comparison of UV-Visible spectra for Au3 and Au4 with its cluster

Other than that, these clusters were also analysed using $^{31}P\{H\}$ NMR to determine the chemical environment of the phosphorus atom as phosphine was one of the ligands present in the complex used for this reaction. The $^{31}P\{H\}$ NMR spectra for both **Au3** and **Au4** clusters were shown in **Figure 4.14**.

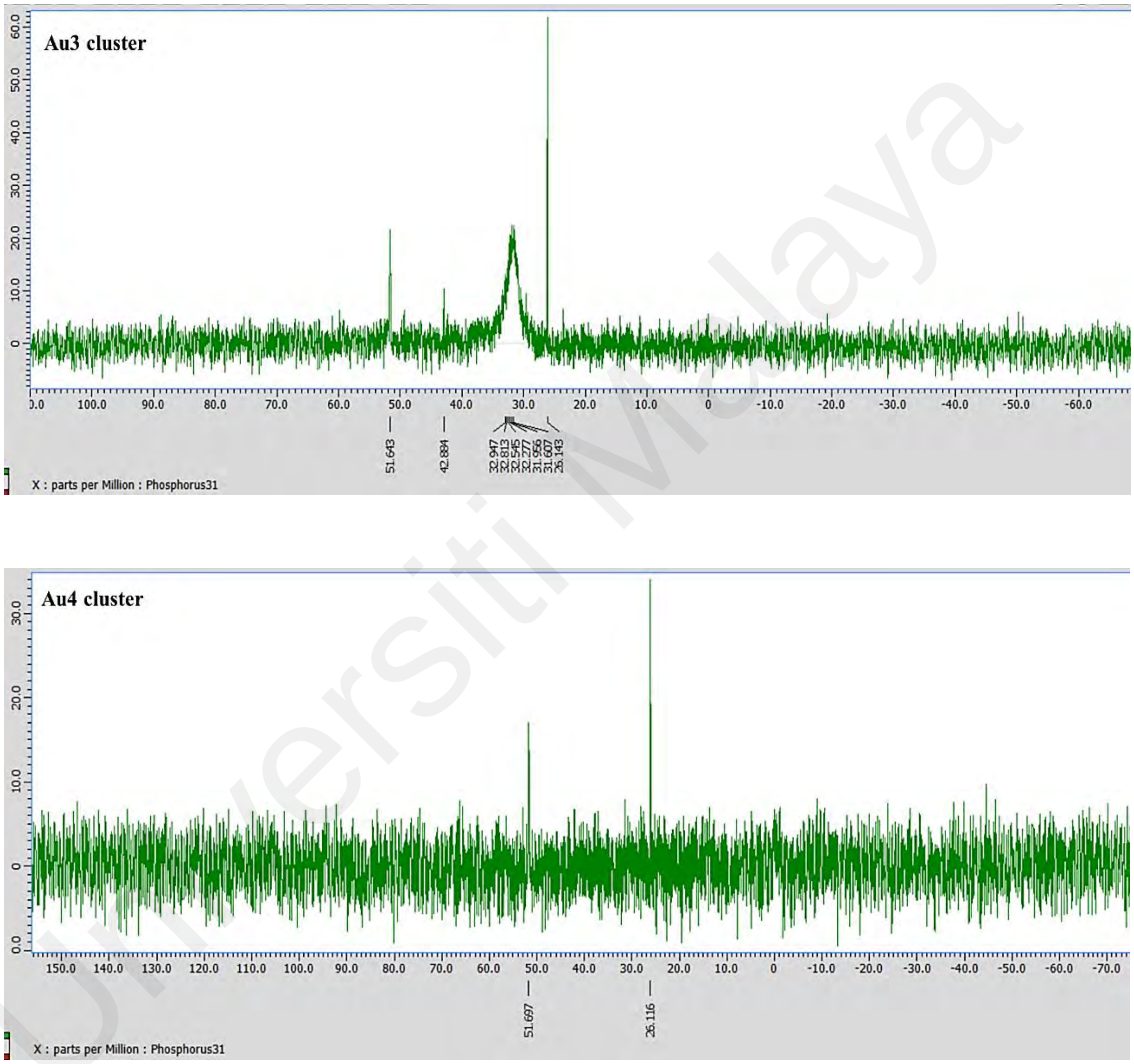


Figure 4.14: $^{31}P\{H\}$ NMR spectra of Au3 and Au4 cluster

From the result, it could be seen that the **Au3** cluster have four different phosphorus chemical environment while the **Au4** cluster only has two. This observation suggested that the synthesised compound was either made up of one single cluster having different

phosphorus chemical environment or it could be a mixture of clusters. Furthermore, two of the peaks for **Au4** cluster were found to be similar to two of the four peaks of **Au3** cluster, which were around 51 ppm and 26 ppm. None of these $^{31}P\{H\}$ NMR peak matched the peak of the Au(I) complexes which were around 38 ppm, indicating that all the complexes had been reacted.

4.4 Biological activity

The synthesised Ag(I) and Au(I) complexes were subjected to different type of biological evaluation. In this study, the Ag(I) complexes were tested for their antiproliferative and anti-plasmodial properties while the Au(I) complexes were evaluated for their antibacterial activities. As per discussed in Chapter 2, the previous biological studies of Ag(I) complexes were mostly investigating their antimicrobial and antibacterial activities. Therefore, for this study we would like to explore on the other biological aspect of these complexes to further improve our knowledge in them. Meanwhile, for the synthesised Au(I) complexes, we decided to investigate their antibacterial activities as the studies carried out on this biological property of Au complexes was still sparse although it has so much potential.

The antiproliferative activities were reported by their half maximum inhibition concentration (IC_{50}) which measure the effectiveness of the compounds in inhibiting a specific biological or biochemical function. It was a quantitative measure that indicates the amount of tested substance (inhibitor) needed to inhibit a given biological process by 50%. As for the anti-plasmodial activities, the half maximal effective concentration (EC_{50}) of the tested complexes were being measured. The EC_{50} refers to the concentration of a toxic compound/substance tested that indices 50% of mortality in cells after a specified exposure time. Both the IC50 and EC_{50} value should give lower value as an indication for a good result. These compounds were then further tested for

their selectivity index (SI) in order to determine the safety of the drug for *in-vivo* treatment for a given viral infection. This value gives an insight about the selectivity of the tested compounds towards the virus and not the host which was the normal cells. It needs to have higher value for an indication of better result.

4.4.1 Antiproliferative activity

Transition metals such as silver have been long regarded as antimicrobial agent but their potential in cancer therapeutics has been underexplored [181, 182]. The in-vitro anticancer activity of silver was previously reported [183]. Essentially, the advantage of silver is that it has lower toxicity compared to other metals such as platinum (e.g. cisplatin) [184]. Meanwhile, extensive antiproliferative activity of thiosemicarbazones was found on different tumor cell lines and display common features of other compounds with carcinogenic potency [185, 186]. On the other hand, it was demonstrated that silver phosphine compound exerted in-vitro antiproliferative effect [187, 188]. Based on IC_{50} values, all the synthesised compounds in this study displayed significantly high potential as antiproliferative agent for breast, MDA-MB-231, MCF-7, and colorectal HT-29 cell lines (**Table 4.5**).

Table 4.5: The antiproliferative activities of the silver complexes (IC_{50} in µM)

Silver complex	MDA-MB-231	HT-29	MCF-7
Ag1	4.45±1.88	2.84±0.27	3.89±2.11
Ag2	3.26±1.37	2.83±0.32	4.53±1.86
Ag3	4.66±1.53	3.11±0.56	3.74±1.29
Ag4	2.55±0.67	2.20±0.60	3.80±1.88
Ag5	3.68±1.63	2.52±0.68	3.20±1.55
Cisplatin	25.28	5.28	35

Complex **Ag4**, which consist of methoxy and hydroxyl group was found to have the best activity for colon cancer, HT-29 with IC_{50} = 2.20±0.60 and one of the breast cancer cell lines which is MDA-MB-231 with IC_{50} = 2.55±0.67 μM as compared to the other complexes. Meanwhile, breast cancer MCF-7 shows the best result with complex **Ag5** with IC_{50} = 3.20±1.55 μM in the presence of bromine, hydroxyl and methoxy moieties. In comparison with clinically used metal compounds such as cisplatin, these compounds showed greater antiproliferative effect towards MDA-MB-231 (IC_{50} = 25.28 μM) [189], MCF-7 (IC_{50} = 35 μM) [190], and HT-29 (IC_{50} = 5.28 μM) [191]. Thus, in-depth studies on these compounds as metallotherapeutic agents for cancer diseases are required.

4.4.2 Antiplasmodial activity

The obtained EC_{50} values of the silver complexes are shown in **Table 4.6**. All of the complexes (**Ag1** – **Ag5**) showed significantly promising antiplasmodial activity with the obtained EC_{50} values ranged between 0.75 and 2.02 μM. Among these silver(I) complexes, **Ag5** was found to be the most potent with the presence of hydroxyl, methoxy, and bromine moieties in the aromatic ring of thiosemicarbazone. On the contrary, the absence of bromine (**Ag3**) and methoxy (**Ag4**) in the aromatic ring demonstrated almost two-fold reduction in the antiplasmodial activity. However, the antiplasmodial activity was maintained for complexes **Ag1** and **Ag2** with the presence of either one or two hydroxyl group in the aromatic ring of thiosemicarbazone. In comparison with the commercialized antimalarials, the EC_{50} values of the silver complex **Ag1** to **Ag5** were higher as most of the antimalarials such as pyrimethamine (antifolate), chloroquine (4-aminoquinoline) and artemisinin derivatives (endoperoxides) scored EC_{50} values of less than 0.1 μM against the drug sensitive *P. falciparum* and more than 0.1 μM against the drug resistant *P. falciparum* [192]. Nevertheless, the importance of each functional group in enhancing the selectivity of silver complex was unclear, which should be promptly addressed in future studies.

Table 4.6: The antimalarial, cytotoxicity (EC_{50} in µM) and selectivity index (SI) of the silver complexes

Silver complex	HRPII	Normal MDBK	Selectivity index (SI)
Ag1	1.45±0.31	0.64±0.06	0.44
Ag2	1.16±0.32	0.58±0.08	0.50
Ag3	2.02±1.07	0.51±0.02	0.25
Ag4	1.69±1.54	0.54±0.02	0.32
Ag5	0.75±0.42	0.77±0.16	1.03

Furthermore, to know the safety of these complexes, the cytotoxicity against MDBK cells was further examined. The selectivity indices were calculated (**Table 4.6**) for all silver(I) complexes, which revealed low SI values (≤1). In other words, lack of selectivity was demonstrated as the inhibitory activity against the *P. falciparum* parasite was lesser than the normal mammalian cell line, MDBK cells. Studies on silver compounds against malaria have been limited. A related study [193] conducted in 1975 discovered that the splenectomized mice with the infection of *P. berghei* were cured when silver sulfadiazine was administered orally and subcutaneously in doses not exceeding 1050 mg/kg/day for five days to these CF-1 mice. Following in 2013, another study [194] reported that silver(I) complexes with *N*-heterocyclic carbine ligands showed promising in-vitro antiplasmodial activity but with further testing, these compounds exhibited strong haemolytic properties on parasite culture even with the weakest doses, which indicated toxic activity. In short, this study revealed that the toxicity of silver was debatable despite the recognition of silver as one of the least toxic metals [195].

4.4.3 Antibacterial activity

The antibacterial activity of the thiosemicarbazone ligands **L3** and **L4** as well as their Au complexes **Au3** and **Au4** were assessed according to the diameter of the zone of inhibition against *E. coli* and *S. aureus*. All the synthesised compounds showed antibacterial activities against both test bacteria, except for **Au3** (**Table 4.7**, **Figure 4.15**). This indicates the presence of a broad spectrum of antibacterial substances in the synthesised compounds.

Table 4.7: Inhibition zone (mm) of synthetic compounds against test microorganisms

Compounds	*E. coli*		*S. aureus*	
	Positive control	Test	Positive control	Test
L3	25	12	18	13
L4	22	14	18	14
Au3	25	-	19	-
Au4	23	10	18	8

Note: (-) indicates no zone of inhibition.

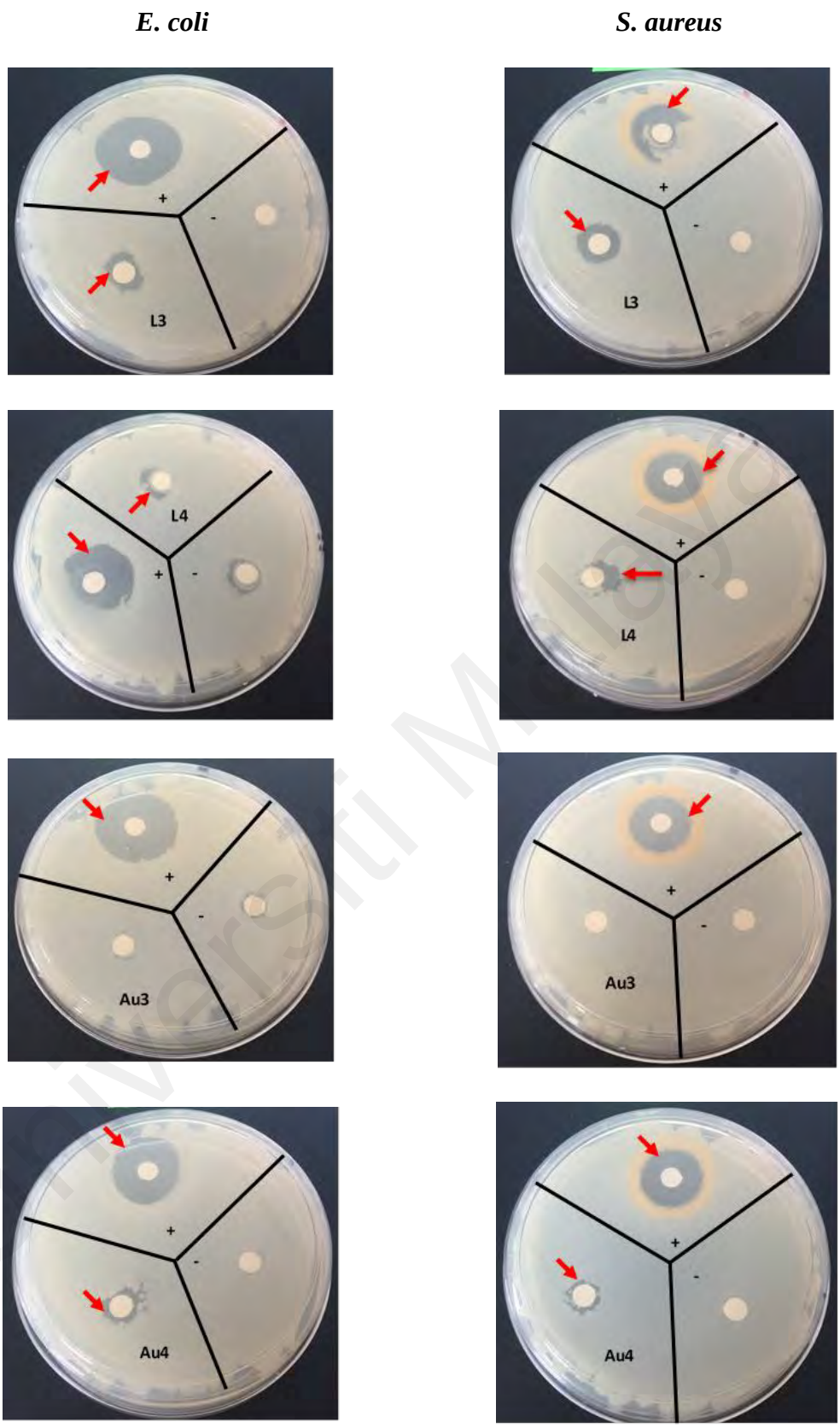


Figure 4.15: Inhibition zones (red arrow) observed on assay plates indicating the antibacterial activities of the synthetic compounds against E. coli and S. aureus. Note: '+' indicates positive control, '-' indicates negative control

Based on the data obtained from the experiment, it can be observed that the free ligand **L4** exhibited the most inhibition zone as compared to the other tested compounds for both bacterial strains. However, the differences of the inhibition zone between **L4** and **L3** were very small, which was 2 mm and 1 mm for *E. coli* and *S. aureus* bacterial strain respectively. This could be due to the derivatisation of the thiosemicarbazone on the benzaldehyde which was 5-bromo-2-hydroxyl for **L3** and 2-hydroxyl-4-methoxy for **L4**. Although the **L3** revealed a good result, its Au complex **Au3** did not exhibit any antibacterial activity against both strains. As for the Au complex of **L4**, the **Au4** shows reduction in the inhibition zone by 4 mm for *E. coli* strain and 6 mm for *S. aureus* strain when compared to its free ligand. From this result, it can be deduced that the coordination of the Au to the thiosemicarbazone only decreases the antibacterial activity of the compound, although some study shows an increase of activity upon coordination to transition metal [196].

CHAPTER 5: CONCLUSION

In conclusion, a series of thiosemicarbazone ligands were successfully synthesised and characterised. All the synthesised ligands were found to exist as thiols except for ligand **L2**, that shows tautomerisation of thione. Five silver(I) complexes were successfully synthesised and characterised using both thiosemicarbazone and diphenyl(*p*-tolyl) phosphine. The silver atom was coordinated to the sulphur atom of thiosemicarbazone and phosphorus atom of diphenyl(*p*-tolyl) phosphine having tetrahedral geometry. Based on the result and discussion on Chapter 4, the obtained spectroscopic data agreed with the suggested structure although no crystal was obtained. As for the gold(I) complex, only two were successfully synthesised and characterised, which were using **L3** and **L4** thiosemicarbazone ligands. The gold atom was found to be coordinated to the sulphur of thiosemicarbazone and phosphorus of triphenylphosphine, forming a linear complex. All the spectroscopic data was also found in agreement to the suggested structure and it was further proved by the single crystal X-Ray analysis. No Au-Au interaction was observed from the prepared complex although it was found in most studies of the gold complex. However, intermolecular and intramolecular hydrogen bonds were discovered for these Au(I) complexes among the thiosemicarbazone ligand. The preparation of metal cluster using synthesised complexes as the precursor was also found to be possible based on the preliminary data discussed in Subsection 4.3.

The evaluation of these complexes against three cancer cell lines revealed potential in the antiproliferative activity as these Ag(I) complexes yielded low IC_{50} values (2.2–4.6 μM) in all cell lines. The antiplasmodial activity of these silver complexes against chloroquine-resistant *P. falciparum* parasite were also examined, which demonstrated good activity, but unfortunately were not selective. Further modification on the

structural frame of thiosemicarbazone or phosphine may improve the SI value. As for the assessment of antibacterial activity for gold complexes and its thiosemicarbazone ligand, it was revealed that the ligands show promising activities but not their complexes.

5.1 Suggestion for future work

This study had given us an insight on the possibility of synthesising metal clusters from the prepared complexes. These synthesised cluster can be further characterised to determine their structure as well as their particle size. Furthermore, based on the results and discussion in Chapter 4, separation technique can also be done to investigate the possibility of having mixture. Other than that, we can also evaluate their potential as catalyst or biological agent.

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