SYNTHESIS, STRUCTURAL CHARACTERIZATION AND BIOLOGICAL ACTIVITIES OF Ag(I) COMPLEXES WITH MIXED LIGANDS OF THIOSEMICARBAZONE AND TRIPHENYLPHOSPHINE.

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FACULTY OF SCIENCE
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DISSERTATION SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

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ABSTRACT

Six Ag(I) complexes containing mixed ligand of thiosemicarbazone derivatives and triphenylphosphine were synthesized. The structural information of the ligands and its silver complexes were assembled using various spectroscopic techniques such as CHN elemental analysis, Fourier Transformed Infrared (FTIR), ^{1}H, ^{13}C, $^{31}P\{^{1}H\}$and 2D-NMR. The spectroscopic data suggested that the 4-phenyl-3-thiosemicarbazone ligands were bound to the silver centre via the neutral sulfur donor atom. The complexes were known to be in tetrahedral arrangement with the thiosemicarbazone moiety existed as a thione tautomer. The biological properties of the complexes were investigated for their antiplasmodial and antiproliferative activities. The *in vitro* antiproliferative activities of these complexes were investigated towards the MDA-MB-231 and MCF-7 breast cancer cell line as well as HT-29 colon cancer cell line, yielding IC_{50} values in the moderate micromolar range for all the cell lines. These complexes were also tested for their antiplasmodial activity against chloroquine resistant *P. falciparum parasite*; HRP2 assay, along with cytotoxicity test on MDBK cells and possess good to modest activity.

Keywords: silver complex, thiosemicarbazone, antiplasmodial, antiproliferative, phosphine

SINTESIS, PENCIRIAN FIZIKAL DAN AKTIVITI BIOLOGI BAGI KOMPLEKS AG(I) BERSAMA CAMPURAN LIGAN TIOSEMIKARBAZON DAN TRIFENILFOSFINA

ABSTRAK

Enam kompleks Ag(I) yang mengandungi campuran ligan fosfina dan tiosemikarbazon telah berjaya disintesis. Struktur ligan dan kompleks dikenalpasti melalui pelbagai teknik spektroskopi antaranya analisis unsur CHN, spektroskopi FTIR, ^{1}H, ^{13}C, $^{31}P\{1H\}$ dan 2D-NMR. Maklumat spektroskopi mencadangkan 4-fenil-3-tiosemikarbazon bercantum pada logam argentum melalui atom penderma sulfur yang neutral. Kompleks itu bercantum di dalam susunan tetrahedron dengan moieti tiosemikarbazon wujud sebagai tautomer *thione*. Kompleks tersebut telah diuji keupayaan biologinya untuk aktiviti antiplasmodial dan antiproliferatif. Kajian aktiviti antiproliferative secara *in vitro* dilakukan terhadap sel barah payudara MDA-MB-231, MCF-7 dan sel barah kolon HT-29, mencapai nilai IC_{50} dalam linkungan mikromolar yang sederhana. Kompleks ini turut diuji untuk aktiviti antiplasmodial menentang klorokuina terhadap parasit *P. falciparum*; cerakin HRP2 serta ujian sitotoksisiti pada sel MDBK dan didapati mempunyai aktiviti yang baik dan sederhana.

Kata kunci: sebatian argentum, tiosemikarbazon, antiproliferatif, antiplasmodial

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Anal.Calc	:	Analytical calculation
EC_{50}	:	Concentration of a drug
EDX	:	Energy-dispersive X-ray spectroscopy
FTIR	:	Fourier Transformed Infrared
GI_{50}	:	Growth inhibition
IC_{50}	:	Concentration of an inhibitor
m.p.	:	Melting point
NMR	:	Nuclear Magnetic Resonance
O.D.	:	Optical density
PPh_3	:	Triphenylphosphine
RBC	:	Red blood cell
TSC	:	Thiosemicarbazone

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1 Research Background

Coordination compound has been an encounter to the inorganic chemist from its first recognition in the nineteenth century. Since the research by Alfred Werner, inorganic chemistry seen a countless study of coordination compounds, with a unique structural characteristic and possessed various applications(Constable & Housecroft, 2013). The coordination chemistry of silver(I) complexes with sulfur and phosphorus donor ligands has drawn massive attention due to the biological importance of silver-sulfur interaction in the living systems and the promising use of silver-phosphine complexes as antitumor and antimalarial agents (Muhammad Altaf *et al.*, 2013; A. A. Isab *et al.*, 2010; Rowan *et al.*, 2006). The coordination compounds can have diversity of structures relying on its coordination number, denticity of the ligand and the metals involved. The existence of more electronegative oxygen, nitrogen or sulfur atoms in the ligand frame could improve the coordination potentials of the ligand.

The employment of transition metal in drug discovery has become a favorite approach, especially when it is attached to the compound of common therapeutic value such as thiosemicarbazones (Adams *et al.*, 2016). Thiosemicarbazones (TSCs) are thioureas which generally possesed S-donor atom which can bind to metal and results in diverse chemical and structural characteristics (Du *et al.*, 2002). Thiosemicarbazones and semicarbazones as ligand, relatively have better selectivity and coordination tendency thus can form more stable complexes. They also may form macrocyclic ligands and also owned capability to form new and unique complexes with greater biological and analytical applications. A study done by Sharma et al., reported a significant anticancer

and antibacterial activity of mononuclear copper (II) macrocyclic thiosemicarbazone complexes (Sharma *et al.*, 2015).

The thiosemicarbazone acts as chelating ligand and usually binds as bidentate ligand to metallic cations giving complexes through the sulphur and hydrazine nitrogen atom. They are flexible ligands in both anionic and neutral forms. The ligands can also coordinate in a tridentate manner when other coordination group is present in the vicinity of donating centers. Due to the presence of NH-C=S group, the thiosemicarbazone can display thione – thiol tautomerism (**Figure 1.1**). They exist as thione form in solid but exist as an equilibrium mixture of thione and thiol form in liquid.

thione thiol

Figure 1.1: Thione and thiol form of thiosemicarbazone

The coordinating ability of the thiosemicarbazone to a wide range of metallic ions is due to the extended delocalisation of electron density over the NH-CS-NH-N system (Latheef *et al.*, 2007). The interest on this compounds is also derived from their wide spectrum of biological activity which is evidenced from wide range of antibacterial (Vinuelas-Zahinos *et al.*, 2011) , antiviral (Ainscough *et al.*, 1998), antituberculor (Netalkar *et al.*, 2014) and antitumor (Ashfield *et al.*, 2004) activities. Some thiosemicarbazones are somewhat specific inhibitors of ribonucleotide reductase, which is a significant metabolic mark for the progress of chemotherapeutic agents against cancer (Sandercock *et al.*, 2002).

Many research involving thiosemicarbazones and silver complexes have been done and their biological properties have been studied. Amongst the uses of thiosemicarbazones or silver complexes that have been reported are as antimalarial and in cancer treatment. Malaria is an infectious parasitic disease with an estimated 216 million cases of malaria in 91 countries in 2016, an increase of 5 million cases over 2015 (WHO, 2016). Malaria is triggered by parasites that infected people over the bites of an infested female mosquito. Human malaria is caused by four different species of *Plasmodium*: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and Plasmodium malariae. The most severe form is caused by P. falciparum which leads the death of about 1% of the infected patients (de Oliveira *et al.*, 2008). The commercial antimalarial drug, chloroquinine did show some ineffectiveness due to parasite resistance (Chinappi *et al.*, 2010) and some also cause color vision loss to the treated patients(Ventura *et al.*, 2003).

On the other hand, cancer is the second leading cause of death globally, and was responsible for 8.8 million deaths in 2015 with 1 in 6 deaths were due to cancer. (L. *et al.*, 2017). About 1.7 million new cancer cases are expected to be diagnosed in 2018 (L. *et al.*, 2018). Cancer is a group of diseases characterized by the uncontrolled growth and spread of abnormal cells (Cooper., 2000). With regard to the metal-based anticancer drugs, there are a great accomplishment of a well-known chemotherapeutic drug, cisplatin but the use of cisplatin were limited due to the side effects and toxicity (Oun *et al.*, 2018). Cisplatin (cisplatinum, also called cis-diamminedichloroplatinum(II), is a metallic (platinum) coordination compound with a square planar geometry. Its mode of action has been linked to its ability to crosslink with the purine bases on the DNA; interfering with DNA repair mechanisms, causing DNA damage, and subsequently inducing apoptosis in cancer cells(Dasari & Tchounwou, 2014).

Consequently, to overcome the limitations of the existence drugs, we synthesized a metal based drugs which aim to be less harm and more effective by using silver complexes with thiosemicarbazone and phosphine ligand.

1.2 Aim & scope

Silver complexes and their therapeutic potentials are known to be an interesting topics among various research groups, with the availability of early assessments regarding its chemical advancement (Rowan *et al.*, 2006). However, to the ability of our knowledge, there is a lacking of recent report on the anticancer potential and the antiplasmodial properties of the silver(I) complexes with mixed ligand system of thiosemicarbazone or triphenylphosphine ligands.

Hence, as a part of continuing interest in this area of research, we have synthesized a series of thiosemicarbazone derivatives ligands that are then attached to the silver center together with the triphenylphosphine ligand. The silver(I) complexes are envisioned to enhance the biological properties own by both ligands and silver center in a synergistic manner. Spectral data are also discussed to understand the coordination of silver to the sulfur atom from the ligand.

This thesis contains five chapters. Chapter 1 details the introduction to the research, Chapter 2 covers the literature study on the related topic of the research that includes both part of synthesis and the biological significance of the ligand and metal complexes. Next, Chapter 3 presents the experimental procedure to synthesis the compounds and next preparing it for biological study. Chapter 4 explains the results and discussions of the research and finally Chapter 5 concludes the findings and recommend the future work that can be done.

1.3 Research objectives

a) To synthesis a series of 4-phenyl-3-thiosemicarbazone ligands

b) To synthesis a series of silver complex with a mixed ligands of 4-phenyl-3-thiosemicarbazone and triphenylphosphine

c) To characterize thiosemicarbazone ligands and silver complexes using few spectroscopic techniques like ^{1}H, ^{13}C, ^{31}P $\{^{1}H\}$ and 2D-NMR and FTIR, and also few elemental analysis like CHNS, and EDX.

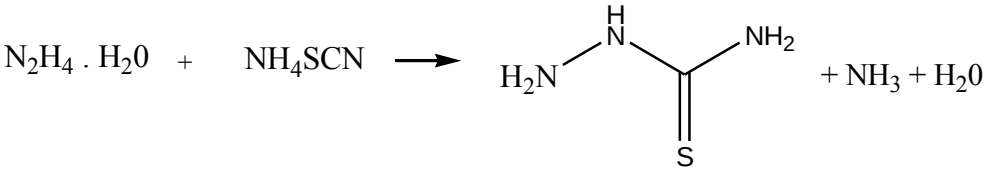
d) To study the antimalarial and anticancer activities on the synthesized silver complexes.

CHAPTER 2: LITERATURE REVIEW

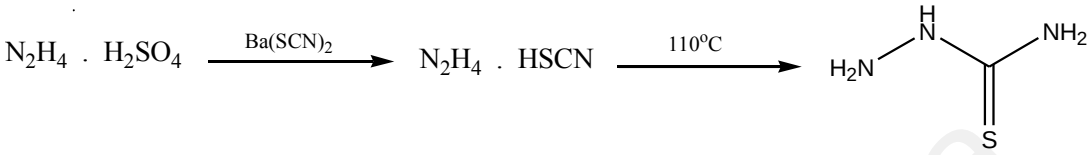
2.1 Thiosemicarbazone, the synthesis and its biological importance

Hydrazine is an inorganic compound with the chemical formula N_2H_4 (also written as H_2NNH_2), called diamidogen is the simplest diamine and is unique in its class because of the presence of N-N bond. Hydrazine is also of academic interest due to the presence of four replaceable hydrogen atoms and two free electron pairs. Also, in addition to this it has one weak N-N bond. However, hydrazine need to be handled in solution due to its dangerously unstable properties. Hydrazine is used in the synthesis of thiosemicarbazide which is also a precursor for the pharmaceuticals industry (Sardari *et al.*, 2017)

Commercially, there are few ways to produce thiosemicarbazide involving hydrazine hydrate as raw material. One of them is from the reflux reaction under nitrogen atmosphere of ammonium thiocyanate and hydrazine hydrate as shown in **Scheme 2.1**. This involves nucleophilic addition reactions and rearrangement reactions, and the product is subjected to a post treatment to obtain the thiosemicarbazide (Scott *et al.*, 1954). Another way to prepare thiosemicarbazide is by the reaction of hydrazine hydrate with sulfuric acid and barium thiocyanate 2-hydrate which digested and refluxed to form thiosemicarbazide as shown in **Scheme 2.2** (Dieke, 1949). While another study later on revealed a synthesis of 4-methyl-4-phenyl-3-thiosemicarbazide using hydrazine hydrate and dithiocarbamate derivatives (Scovill, 1991).



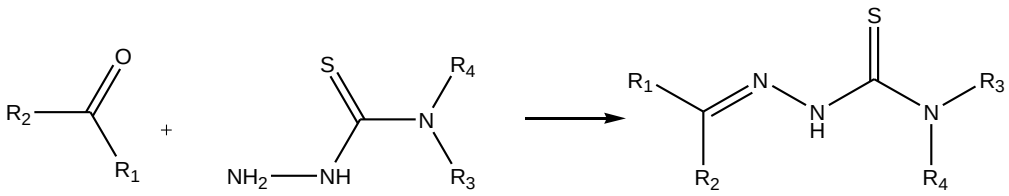
Scheme 2.1: Preparation of thiosemicarbazide using ammonium thiocyanate and hydrazine hydrate



Scheme 2.2: Preparation of thiosemicarbazide using hydrazine hydrate and sulfuric acid

Thiosemicarbazides are potent intermediates for the synthesis of pharmaceutical and bioactive materials and thus, they are used extensively in the field of medicinal chemistry. The imine bond (-N=CH-) in this compound is useful in organic synthesis, in particular for the preparation of heterocycles and non-natural β-aminoacids (Sardari *et al.*, 2017). Thiosemicarbazides present a variety of biological activities ranging from antimicrobial (Plech *et al.*, 2011), antiviral (Quenelle *et al.*, 2006), anticancer (Afrasiabi *et al.*, 2004) and antitumor (Singh *et al.*, 2000; T. Yousef *et al.*, 2010) activities. As for example, 1-amino-N-(pyridin-3yl)methanethiol-4-(pyridin-2-yl) thiosemicarbazide (H_2PPY) shows good in-vitro and in-vivo activity against tumor cell line (T. A. Yousef *et al.*, 2010).

Upon condensation reaction of thiosemicarbazide or substituted thiosemicarbazide with aldehyde or ketones, a derivatives of imines called thiosemicarbazone is formed (**Scheme 2.3**). Thiosemicarbazone (**Figure 2.1(a)**) is a referent of a semicarbazone (**Figure 2.1(b)**) which contains sulfur in position of oxygen atom. In this research, 4-phenyl-3-thiosemicarbazone is used as ligand as shown in **Figure 2.1(c)**.



Scheme 2.3: Formation of thiosemicarbazones

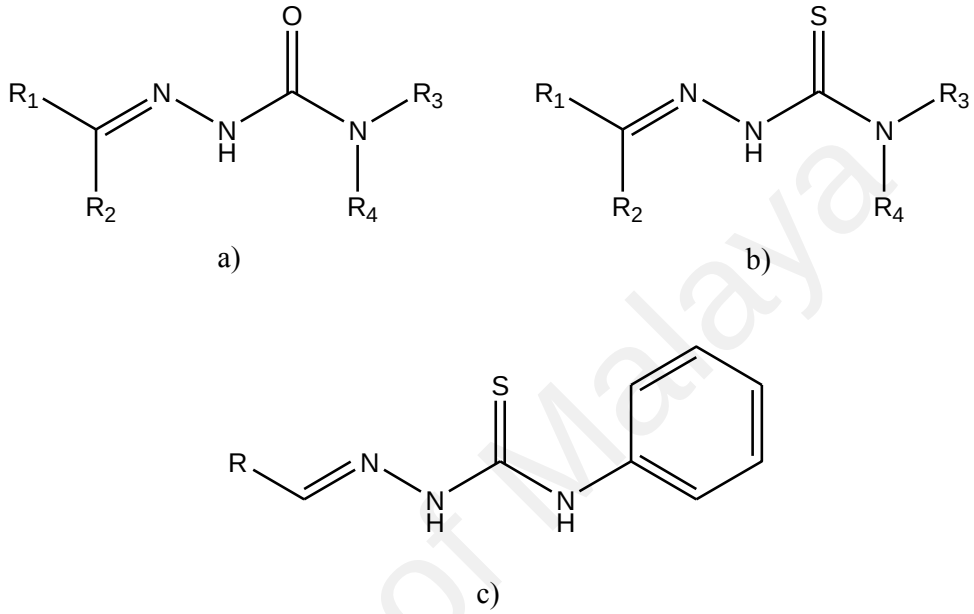


Figure 2.1: a) Semicarbazone, b)thiosemicarbazone and c)4-phenyl-3-thiosemicarbazone

The –C=S group in thiosemicarbazone offers the opportunity for the electron delocalization thus, increasing the denticity of these compounds which is the number of donor groups in a single ligand that bind to the central atom in a coordination complex. The azomethine nitrogen and thioamide sulfur are the vacant donor sites found in the thiosemicarbazone compounds. In addition, if heterocyclic rings are introduced to the thiosemicarbazone framework, the hetero atoms can also perform as the donor sites.

Thiosemicarbazones are very flexible ligand. They can attach to metal as neutral molecule or after deprotonation as anionic ligand. Thiosemicarbazones offers extensive series of bioactivities, and their pharmacological and chemistry relevance have been comprehensively investigated. Bits of interest date back to the starting of the 20th century but the earliest reports on their medical applications started to appear in the fifties as drugs

against tuberculosis and leprosy (Bavin *et al.*, 1950; Jacobsen, 1950). In 1950, Hamre *et al*., who reported the antiviral activity of derivatives of benzaldehyde thiosemicarbazone which were active against neurovaccinal contamination in mice when given orally (Hamre *et al.*, 1950). Later in 1951, Domagk stated that some thiosemicarbazones of cyclic aldehydes and ketones shows antitubercular activity *in vitro(Domagk, 1951).* The anti-leukemic effect of 2-formylpyridine thiosemicarbazone was first described by Brockman *et al*. in 1956 (Brockman *et al.*, 1956). Later in 1965, French *et al*. formulated hypotheses about the mode of action of the α(N)-heterocyclic thiosemicarbazones; the active molecules shared a tridentate nature, which permits them to be effective chelators, and better carcinostatic activity was obtained by varying the aromatic system (French & Blanz, 1965).

Far along, 2- formylpyridine thiosemicarbazones and their VO(IV) complexes displayed potent *in vitro* antibacterial activity towards *E. Coli (Maiti et al., 1988)*. The capability to constrain ribonucleotide reductase (RR), a crucial enzyme for DNA synthesis promotes the antitumor activity of such thio compounds (Moore *et al.*, 1970). Through a conjugated N-N-S tridentate ligand system, the thiosemicarbazone side chain at a position α to the heterocyclic nitrogen, is vital for anticancer activity (French & Blanz, 1966). In human phase II trials, 3- aminopyridine-2-carboxylaldehyde thiosemicarbazone, Triapine, was identified as a cancer chemotherapeutic agent (Jiang *et al.*, 2006). Anticancer activities of a number of α-(N)-heterocyclic carboxaldehyde thiosemicarbazones were also reported (Pingaew *et al.*, 2010).

An extensive series of thiosemicarbazones obtained from 2-acetylpyridine was tested by Klayman et al for antimalarial activity against plasmodium berghi in mice (Klayman *et al.*, 1979). Back in 1995, The Ru (III) chelates derived from 2-acetylpyridine-(4-methylthiosemicarbazone) seem to be the most efficient inhibitors for malarial activity

(Offiong & Martelli, 1995). The 2-benzoylpyridine thiosemicarbazones also exerted good antimalarial activity (Pingaew *et al.*, 2010). In the same year, the synthesis of ferrocenylthiosemicarbazone gold complexes exhibit moderate anti-plasmodial activity. The substituted thiosemicarbazone ligand binds in a monodentate fashion *via* the thione sulfur atom to Au(I), and in a chelating N,S-mode for the Au(III) complexes (Khanye *et al.*, 2010)

2.2 Silver Complexes with thiosemicarbazone and mixed ligands system.

Silver exists in the human body at very low concentrations (<2.3 μgl^{-1}) and is absorbed through the mucus membranes, lungs, skin and the gastrointestinal tract (Wan *et al.*, 1991) in the form of silver protein complexes (Lansdown, 2007). Silver does not appear to be aggregating poison and can be endured at high concentrations within the body, later eradicated from the body through the faeces and urine (Wan *et al.*, 1991). For centuries, elemental silver and silver salts have been used in curative and preventive health care acting as antimicrobial agents. It was used as disinfectant in ancient Greece and Rome, while the Macedonians used it to boost the healing of wounds (Spear, 2010) . It is thought that the antimicrobial properties of silver complexes reliant on the discharge of biologically active silver(I) ions (Lyutakov *et al.*, 2015). Amongst the few silver(I) compounds that are commonly prescribed for their topical anti-bacterial properties are silver-sulfadiazine for the treatment of burns (Chung & Herbert, 2001), a dilute solution of $AgNO_3$ which is used for bacterial contagions and conjunctivitis in infants (Klasen, 2000) and silver fusidate as antimicrobial agent (Hamilton-Miller & Shah, 1996).

As a matter of fact, thiosemicarbazones themselves possess a great biological activity, but the involvement of metal to its structure helps to promote a greater anticancer, antimicrobial, antifungal, antimalarial, and antiviral activities compared to

free ligands (Jungwirth *et al.*, 2011; Kovacevic *et al.*, 2010; Sartorelli *et al.*, 1977; Shim *et al.*, 2013). Coordination to metal increases the lipophilicity of the compound, increasing the speed of compound entrance into the cell. Thus, the metal complex can be more active than the free ligand (Ejidike & Ajibade, 2015). The mechanism can include coordinating to a metal *in vivo* or the metal complex may be a transport for activation of the ligand as the cytotoxic agent (Farrell, 2002).

On the other hand, phosphorus as ligand is also useful in organometallic chemistry and biological studies. Cyclopalladated complexes containing phosphorus and thiosemicarbazone shows a moderate antiplasmodial activity against *Plasmodium falciparum* strains has been reported (Chellan *et al.*, 2010) . Earlier this year, a study reported a silver(I) complexes containing a mixed ligand system of phosphine and thiazolidine showing a highly potent antimalarial and anticancer agents(Mohd Sofyan *et al.*, 2018). In a same year, a study on silver(I) thiocyanate 4-methoxyphenyl phosphine complex also known as *UJ3* is shown to be as effective against human esophageal cancer cells which known to become resistant to current forms of chemotherapy (Engelbrecht *et al.*, 2018). The *UJ3* complex is found to be as effective as Cisplatin in killing cancer cells. It requires a 10 times lower dose and focuses narrowly on cancer cells, so fewer healthy cells are killed.

Tertiary phosphines, PR3, are an important class of ligands because of their electronic and steric properties which can be altered in a systematic and predictable way over a very wide range by varying the R groups. Phosphine ligands play an important role in determining the metal complex framework (Chadwick A. Tolman, 1977; C. A. Tolman *et al.*, 1974). Tertiary phosphines also stabilize an exceptionally wide variety of metal complexes of interest to the organometallic chemist as their phosphine complexes. Phosphines are more commonly spectator rather than actor ligands (Troev, 2018).

Tertiary phosphine complexes of silver(I) of the type [AgXLn], where L = tertiary phosphine; n= 1–4; X = coordinating or non-coordinating anion, were first prepared in 1937 (Mann *et al.*, 1937). The common preparation involves the reaction of stoichiometric amounts of the phosphine ligand with the appropriate silver(I) salt. The reaction of silver(I) salts with monodentate tertiary phosphines in a 1:2 stoichiometric ratio usually results in the formation of either monomeric or dimeric complexes depending on the the bulkiness of the ligand, the substituents, and the donor properties of the phosphine ligand and the anion (Barron *et al.*, 1986). Phosphorus is mainly regarded as σ-donors. The lone pair on phosphorus can donate electron density to a metal. Increasing the electron density on phosphorus through the use of electron-donating substituents (R) increases the strength of σ-donation. Phosphines can also act as π-acceptors, with the σ*-orbital of the P-R bonds playing the role of the acceptor. π-Bonding arises through the donation of electron density from the metal into an empty orbital of the ligand, which exhibits phosphorus 3p character(Mitoraj & Michalak, 2010).

There is yet no study findings on silver complexes with thiosemicarbazone and phosphorus ligands reported for anticancer or antimalarial activities, but there are study done involving other biological studies. In 2010, Isab *et al*., reported the silver complexes with mixed ligands of triphenylphosphine and thiourea exhibits significant antimicrobial properties (A. A. Isab *et al.*, 2010). Later, silver complex $[Ag_2(PPh_3)_2(NH_2CSNHNH_2)_4](NO_3)_2$ showed striking activities against some strains of Gram-negative bacteria and one strain of Gram-positive bacterium (Muhammad Altaf *et al.*, 2013). The antifungal activity is also favorable for the compound. It is remarkably due to the tendency to undergo further ligand replacement reactions with the biological ligands such as proteins and DNA.

In this project, we have synthesized a series of thiosemicarbazone derivatives ligands that are then attached to the silver center together with the triphenylphosphine

ligand. The silver(I) complexes with the mixed ligands system are envisioned to enhance the biological properties own by both ligands and silver center in a synergistic manner. The synergistic properties of both ligands hypothetically enhanced towards discovering new and novel anti-plasmodial and/or anti-cancer compounds.

CHAPTER 3: METHODOLOGY

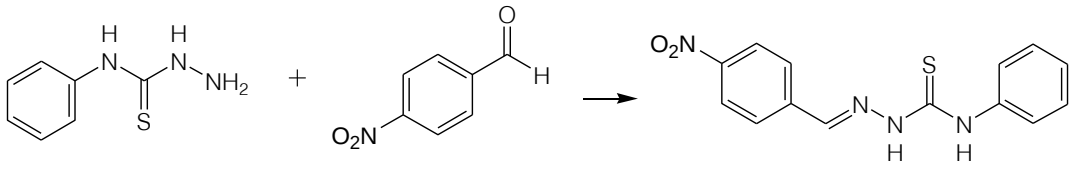
3.1 Materials & methods

All reagents and solvents were reagent grade and used without prior purification unless stated otherwise. The progress of all reactions were monitored by thin-layer chromatography which was performed on 2.00 x 6.00 cm aluminium sheets pre-coated with silica gel 60 (HF-254, Merck) to a thickness of 0.25 mm. The FTIR spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrophotometer (ATR) in the frequency range of 450-4000 cm^{-1}. The ^{1}H, ^{13}C, $^{31}P\{^{1}H\}$ NMR spectra were recorded on a JEOL FT-NMR ECX 400 (ECX 400) spectrometer using deuterated DMSO and chloroform as solvent. The CHNS analysis were obtained using Perkin Elmer CHNS/O 2400 Series II.

3.2 General procedures on the syntheses of the thiosemicarbazone ligands (L1-L6)

All thiosemicarbazone ligands (**L1**-**L6**) were prepared according to the procedure described in the literature (Benmohammed *et al.*, 2014) with slight modifications. Generally, the 4-phenyl-3-thiosemicarbazide (0.17 g, 1.00 mmol) was dissolved in 10.00 mL of ethanol and stirred. Next, the corresponding aldehyde which was pre-dissolved in 10.00 mL of ethanol was then added to the solution followed by four drops of glacial acetic acid. The mixture was then refluxed for 3-4 hours and the progress of the reaction was monitored using TLC. Product was obtained by filtration and later dried in vacuum pump.

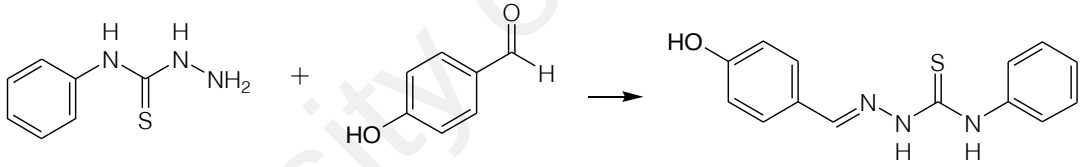
3.2.1 Synthesis of L1: 4-nitrobenzaldehyde- N-phenyl-thiosemicarbazone



Scheme 3.1: Synthesis of ligand L1

For **L1**, the aldehyde used was 4-nitrobenzaldehyde (0.15 g. 1.00 mmol). The reaction follows the general procedure stated (**Figure 3.1**). Yield: 70 %, M.p. 260 ^{o}C. IR data ν(cm^{-1}) : (N-H) 3103.08, 2980.20, (C=N) 1595.02, (C=S) 846.76, ^{1}H-NMR (DMSO, δ ppm): 12.06(s,1H)(N-H); 10.30(s,1H)(N-H) ; 8.20(overlap with aromatic C-H, 1H)(CH=N), ^{13}C-NMR (DMSO, δ ppm): 177.10 (C=S) ; 140.70 (C-Ph) ; Anal. Calc. for $C_{14}H_{12}N_4O_2S$ C: 55.99; H, 4.03; N, 18.65. Found: C, 55.20; H, 3.73; N, 18.59

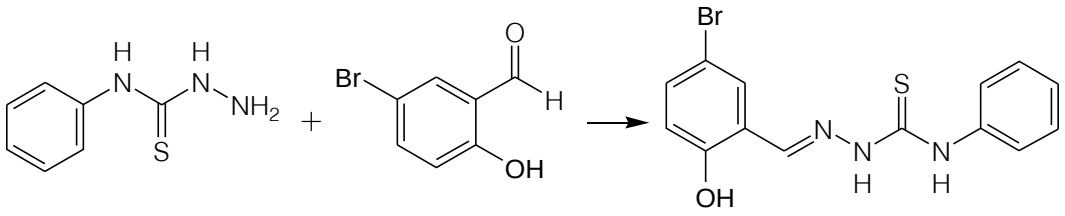
3.2.2 Synthesis of L2: 4-hydroxybenzaldehyde-N-phenyl-thiosemicarbazone



Scheme 3.2: Synthesis of ligand L2

For **L2**, the aldehyde used was 4-hydroxybenzaldehyde (0.12 g, 1.00 mmol). The reaction follows the general procedure stated (**Figure 3.2**)**.** Yield: 60 %, M.p. 240 ^{o}C. IR data ν(cm^{-1}) : (N-H) 3158.08, 2977.11 (C=N) 1604.28 (C=S) 851.49, ^{1}H NMR (DMSO, δ ppm): 11.62(s,1H)(N-H) ; 9.94(s,1H)(N-H) ; 9.88 (s,1H)(C-OH) ; 8.04(s,1H)(CH=N), ^{13}C NMR (DMSO , δ ppm): 175.90(C=S) ; 144.00 (C-Ph) ; Anal. Calc. for $C_{14}H_{13}N_3OS$: C, 61.97; H, 4.83; N, 15.49. Found: C, 61.24; H, 4.92; N, 14.98

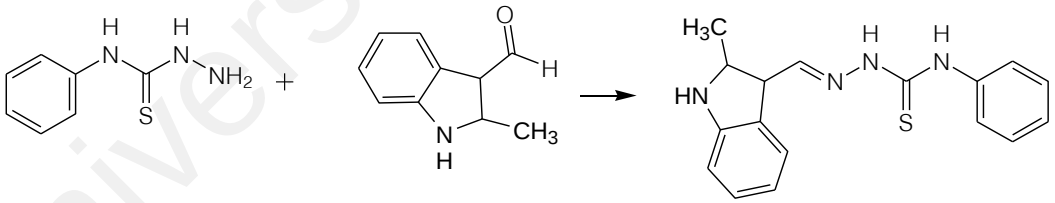
3.2.3 Synthesis of L3: 5-bromo-2-hydroxybenzaldehyde N-phenylthiosemicarbazone



Scheme 3.3: Synthesis of ligand L3

For **L3**, the aldehyde used was 5-bromo-2-hydroxybenzaldehyde (0.20 g, 1.00 mmol). The reaction follows the general procedure stated (**Figure 3.3**). Yield: 65 %, M.p. 250 ^{o}C IR data ν(cm^{-1}) : (N-H) 3141.28, 2984.50, (C=N) 1604.57, (C=S) 829.04, ^{1}H NMR (DMSO, δ ppm): 11.78(s,1H)(N-H); 10.14(s,1H) (N-H) ; 10.28(s,1H)(C-OH) ; 8.38(s,1H)(CH=N), ^{13}C NMR (DMSO, δ ppm): 176.60 (C=S) ; 139.50 (C-Ph) ; Anal. Calc. for $C_{14}H_{12}BrN_3OS$: C, 48.01; H, 3.45; N, 12.00. Found: C, 48.24; H, 3.44; N, 12.12

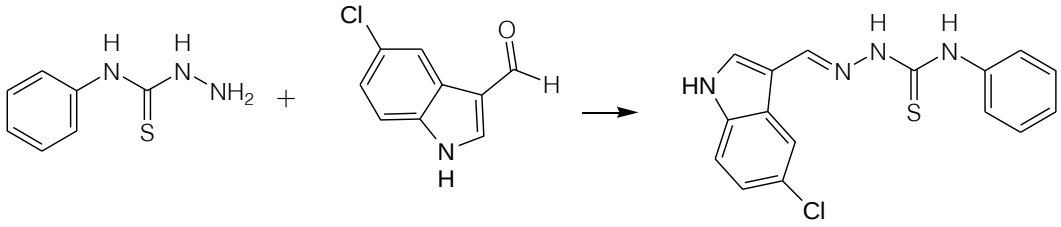
3.2.4 Synthesis of L4: 2-methylindole-3-carboxaldehyde N-phenylthiosemicarbazone



Scheme 3.4: Synthesis of ligand L4

For **L4**, the aldehyde used was 2-methylindole-3-carboxaldehyde (0.16 g, 1.00 mmol). The reaction follows the general procedure stated (**Figure 3.4**). Yield: 80 %, M.p. 230 ^{o}C. IR data ν(cm^{-1}): (N-H) 3394.64, 3285.30. 3209.93, (C=N) 1589.25 (C=S) 839.31, ^{1}H NMR (DMSO, δ ppm): 11.55(s,1H)(N-H_{indole}); 11.40(s,1H)(N-H); 9.50(s,1H)(N-H) ; 8.50 (s,1H)(CH=N), ^{13}C NMR (DMSO, δ ppm): 174.50 (C=S) ; 141.70 (C-Ph) ; Anal. Calc. for $C_{17}H_{16}N_4S$: C, 65.78; H, 5.84; N, 18.05. Found: C, 66.01; H, 5.19; N, 17.32

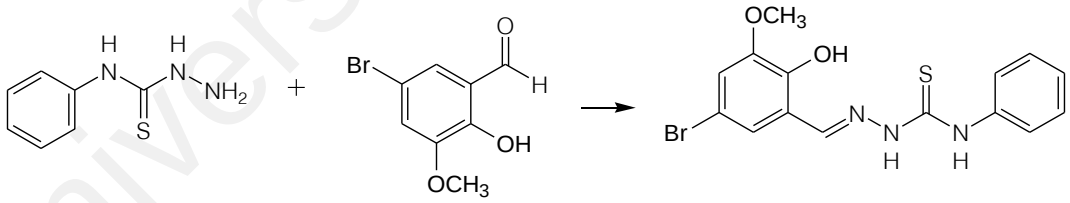
3.2.5 Synthesis of L5: 5-chloroindole-3-carboxaldehde N-phenyl-thiosemicarbazone



Scheme 3.5: Synthesis of ligand L5

For **L5**, the aldehyde used was 5-chloroindole-3-carboxaldehyde (0.18g, 1.0 mmol). The reaction follows the general procedure stated (**Figure 3.5**). Yield: 45%, M.p. 210 ^{o}C, IR data ν(cm^{-1}) : (N-H) 3338.98, 3132.20, 2973.42 (C=N) 1609.25 (C=S) 866.50, ^{1}H NMR (DMSO, δ ppm): 11.83(s,1H)(N-H); 11.58(s,1H)(N-H_{indole}) ; 9.72(s,1H)(N-H) ; 8.35 (s,1H)(CH=N), ^{13}C NMR (DMSO, δ ppm): 175.10 (C=S) ; 141.20 (C-Ph) ; Anal. Calc. for $C_{16}H_{15}ClN_4S$: C, 58.09; H, 4.57; N, 16.93. Found: C, 57.65; H, 3.95; N, 16.20

3.2.6 Synthesis of L6: 5-bromo-2-hydroxy-3-methoxybenzaldehyde N-phenylthiosemicarbazone



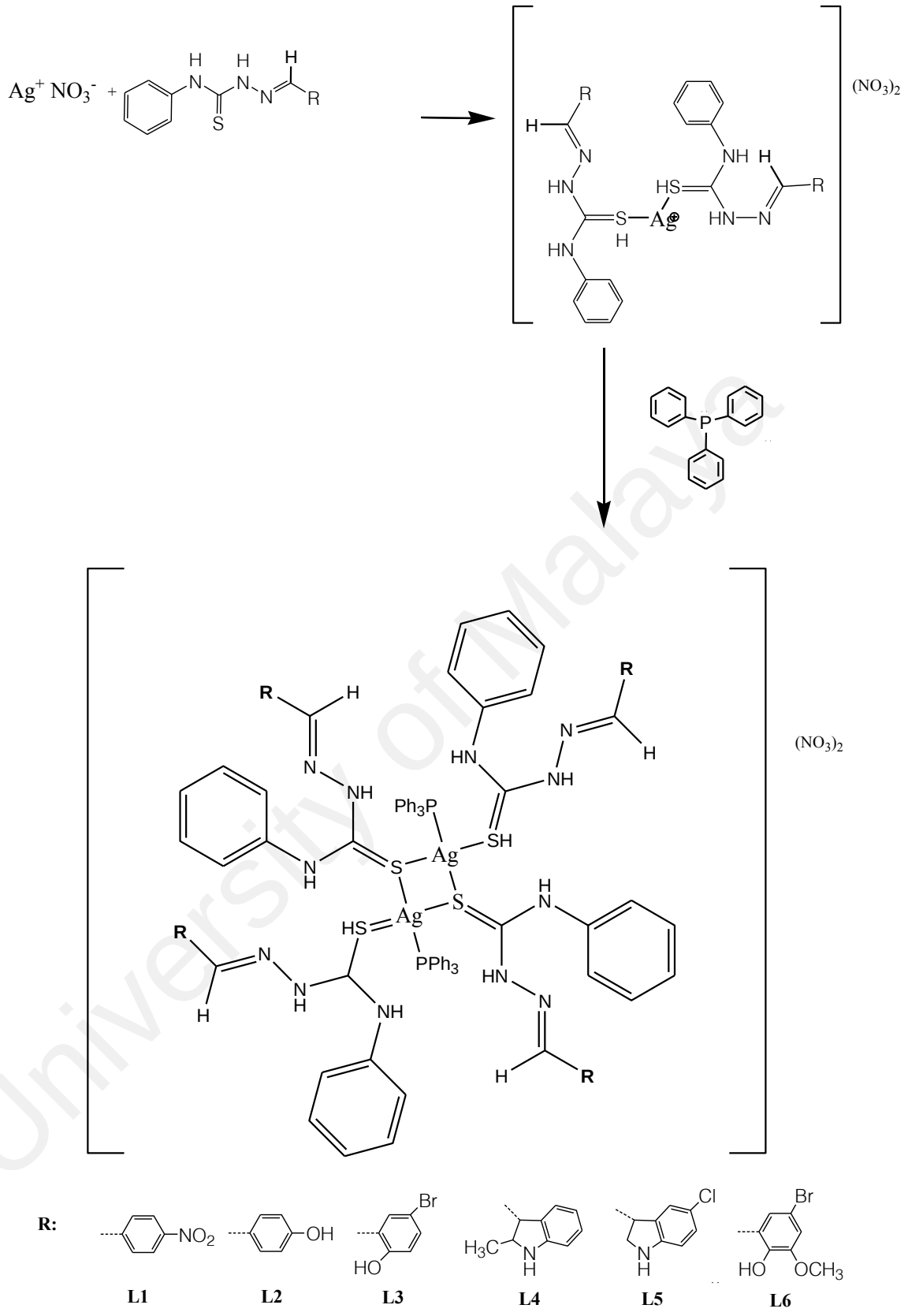
Scheme 3.6: Synthesis of ligand L6

For **L6**, the aldehyde used was 5-bromo-2-hydroxy-3-methoxybenzaldehyde (0.23 g, 1.0 mmol). The reaction follows the general procedure stated (**Figure 3.6**). Yield: 70 %, M.p. 190 ^{o}C. IR data ν(cm^{-1}): (N-H) 3300.41, 2973.79 (C=N) 1604.00, (C=S) 858.86. 1H NMR (DMSO , δ ppm): 11.80(s,1H)(N-H) ; 10.10 (s,1H)(N-H) ; 9.52 (s,1H)(OH) ; 8.43(s,1H)(CH=N) ; 3.81 (s,1H)(OCH_3), 13C NMR (DMSO , δ ppm): 176.60 (C=S) ;

140.00 (C-Ph) ; Anal. Calc. for $C_{15}H_{14}BrN_3O_2S$: C, 47.38; H, 3.71; N, 11.05. Found: C, 47.89; H, 3.75; N, 11.08

3.3 Syntheses of silver complexes (P1-P6)

$AgNO_3$ (0.17 g, 1 mmol) was dissolved in a in a mixture of acetonitrile/methanol (2:3) (20 mL) and the respective thiosemicarbazone ligands (**L1-L6**) (2 mmol) was added. The mixture was refluxed at 55 °C for 3-4 hours. The reaction mixture was further treated with triphenyl phosphine (0.262 g, 1 mmol) in 5 ml acetonitrile/methanol (2:3) and let it continue refluxed for 2-3 more hours. The latter step might results in solubilisation of the precipitates obtained in the first step (if any). The progress of the reaction was observed by using thin layer chromatography. The solution obtained was filtered and later evaporated to dryness and further purified for analysis. The silver complexes were brown and black powders which were soluble either in DMSO or a mixture of warm methanol and acetonitrile. Synthesis route towards silver complexes with thiosemicarbazone(**L1-L6**) and triphenylphosphine ligands were shown in **Figure 3.7** .



Scheme 3.7: Synthesis route towards silver complexes with thiosemicarbazone(L1-L6) and triphenylphosphine ligands

3.3.1 Synthesis of P1: $[Ag_2(PPh_3)_2(L1)_4].(NO_3)_2.H_2O$

To synthesis complex **P1**, a thiosemicarbazone ligand **L1** (0.60 g, 2.00 mmol) was used. The reaction follows the general procedures outlined in section **3.3**. The complex was in dark yellow colour and soluble either in DMSO or a mixture of warm methanol and acetonitrile. Yield: 50%, M.p. 155-160 °C IR data ν(cm^{-1}): (N-H) 2983.12, 2888.57, (C=N) 1589.21 (C=S) 840.88. ^{1}H NMR (DMSO, δ ppm): 12.33(s,4H)(N-H), 10.72(s,4H)(N-H); 8.18 (overlap with aromatic C-H,4H)(CH=N), ^{13}C NMR (DMSO , δ ppm): 175.80 (C=S) ; 143.00 (C-Ph) ; Anal. Calc. for $C_{92}H_{78}Ag_2N_{16}O_8P_2S_4$ C, 56.91; H, 4.05; N, 11.54; S, 6.61.Found: C, 56.30; H, 3.57; N, 11.65; S, 6.61.

3.3.2 Synthesis of P2: $[Ag_2(PPh_3)_2(L2)_4].(NO_3)_2.H_2O$

To synthesis complex **P2**, thiosemicarbazone ligand **L2** (0.56 g, 2.00 mmol) was used. The reaction follows the general procedures outlined in section **3.3**. The complex was brown in colour and soluble either in DMSO or a mixture of warm methanol and acetonitrile. Yield: 45%, M.p. 125-138 °C. IR data ν(cm^{-1}): (N-H) 2981.06, 2889.37 (C=N) 1595.67, (C=S) 834.24. ^{1}H NMR (DMSO, δ ppm): 11.88(s,4H)(N-H), 10.22(s,4H)(N-H) ; 7.99 (s,4H)(CH=N), 9.98(s,4H)(O-H) ^{13}C NMR (DMSO, δ ppm): 173.90 (C=S) ; 146.60 (C-Ph); Anal. Calc. for $C_{92}H_{82}Ag_2N_{12}O_4P_2S_4$ C, 60.53; H, 4.53; N, 9.21; S, 7.03. Found: C, 58.90; H, 3.82; N, 9.26; S, 6.48

3.3.3 Synthesis of P3: $[Ag_2(PPh_3)_2(L3)_4].(NO_3)_2.H_2O$

To synthesis complex **P3**, thiosemicarbazone ligand **L3** (0.70 g, 2.00 mmol) was used. The reaction follows the general procedures outlined in section **3.3**. The silver complexes was black in colour and soluble either in DMSO or a mixture of warm methanol and acetonitrile. Yield: 40%. M.p. 128-143 ° IR data ν(cm^{-1}): (N-H) 3141.28, 2984.50 (C=N) 1604.57, (C=S) 824.43. ^{1}H NMR (DMSO ,δ ppm): 12.02(s,4H)(N-H), 10.46(s,4H)(N-H) ; 8.42 (s,4H)(CH=N), 10.58(s,4H)(O-H) ^{13}C NMR (DMSO , δ ppm): 174.00 (C=S) ;

141.50 (C-Ph) ; C Anal. Calc. for: $C_{92}H_{78}Ag_2Br_4N_{12}O_4P_2S_4$ C, 51.60; H, 3.67; N, 7.85; S, 5.99. Found: C, 49.65; H, 3.17; N, 7.81; S, 5.31.

3.3.4 Synthesis of P4: $[Ag_2(PPh_3)_2(L4)_4].(NO_3)_2.H_2O$

To synthesis complex **P4**, thiosemicarbazone ligand **L4** (0.62 g, 2.00 mmol) was used. The reaction follows the general procedures outlined in section **3.3**. The silver complexes was black in colour and soluble either in DMSO or a mixture of warm methanol and acetonitrile. Yield: 45%. M.p. 122-130 °C IR data ν(cm^{-1}): (N-H) 3012.28, 2880.97, 2649.02 (C=N) 1600.00 (C=S) 830.01. ^{1}H NMR (DMSO, δ ppm): 11.63(s,1H)(N-H_{indole}), 11.60(s,4H)(N-H), 9.73(s-4H)(N-H); 8.41 (s,4H)(CH=N), ^{13}C NMR (DMSO, δ ppm): 172.90 (C=S) ;144.00 (C-Ph) ; Anal. Calc. for $C_{104}H_{94}Ag_2N_{16}P_2S_4$ C, 63.28; H, 4.80; N, 11.35; S, 6.50. Found: C, 59.62; H, 4.34; N, 10.98; S, 5.89.

3.3.5 Synthesis of P5: $[Ag_2(PPh_3)_2(L5)_4].(NO_3)_2.H_2O$

To synthesis complex **P5**, thiosemicarbazone ligand **L5** (0.66 g, 2.00 mmol) was used. The reaction follows the general procedures outlined in section **3.3**. The silver complexes was shiny black in colour and soluble either in DMSO or a mixture of warm methanol and acetonitrile. Yield: 40%. M.p 131-134 °C. IR data ν(cm^{-1}) : (N-H) 3000.96, 2883.01, 2733.51 ,(C=N) 1598.27, (C=S) 834.04, ^{1}H NMR (DMSO , δ ppm): 11.88(s,1H)(N-H_{indole}); 10.35(s,4H)(N-H), 10.01(s,4H)(N-H) ; 8.00 (s,4H)(CH=N), ^{13}C NMR (DMSO , δ ppm): 173.70 (C=S) ;133.80 (C-Ph) ; Anal. Calc for $C_{100}H_{86}Ag_2N_{16}P_2S_4$ C, 62.63; H, 4.52; N, 11.69; S, 6.69 Found: C, 59.89; H, 3.89; N, 10.91; S, 6.53.

3.3.6 Synthesis of P6: $[Ag_2(PPh_3)_2(L6)_4].(NO_3)_2.H_2O$

To synthesis complex **P6**, thiosemicarbazone ligand **L6** (0.76 g, 2.00 mmol) was used. The reaction follows the general procedures outlined in section **3.3**. The silver complexes was shiny brown in colour and soluble either in DMSO or a mixture of warm methanol and acetonitrile. Yield: 40%. M.p 134-140 °C. IR data ν(cm^{-1}) : (N-H) 3049.11, 2879.23,

(C=N) 1599.12 (C=S) 742.69, ^{1}H NMR (DMSO , δ ppm): 12.00(s,4H)(N-H), 10.53(s,4H)(N-H) ; 8.46(s,4H)(CH=N), 9.52(s,4H)(O-H) ^{13}C NMR (DMSO , δ ppm): 174.40 (C=S) ; 836.84 (C-Ph) ; Anal. Calc for $C_{96}H_{86}Ag_2Br_4N_{12}O_8P_2S_4$ C, 50.99; H, 3.83; N, 7.43; S, 5.67. Found: C, 48.09; H, 3.38; N, 7.72; S, 6.02

3.4 Biological procedures

The synthesized complexes were tested for their antimalarial properties done by our collaborator Dr Mohd Ridzuan bin Mohd Abdul Razak from Bioassay Unit, Herbal Medicine Research Centre, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia. The details procedure of the study are as described below.

The antiproliferative activities were carried out by Dr Nur Fadilah Rajab and her research team from Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Kuala Lumpur Campus, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur.

3.4.1 Antimalarial assay

(a) ***In vitro culture and synchronization of P. falciparum***

The CQ resistant *P. falciparum* were grown by candle jar technique (3% CO_2 and 17% O_2) (Trager & Jensen, 1977). The culture was set up in a 25 cm^3 culture flask with filtered vent and maintained in complete RPMI 1640 culture medium (Invitrogen, USA). The *P. falciparum* was grown in 'O' type fresh red blood cell (RBC) with the initial culture started with 1% parasitemia at 2.5% hematocrit. The parasite density was monitored daily by making thin blood smears stained with 10% Giemsa solution and observed under the microscope at 1000 times magnification. When the parasitemia of the parasite culture reached approximately 5 to 7%, the parasites were synchronized using 5% sorbitol (Lambros & Vanderberg, 1979) and cultured for one complete cycle prior to be used in *in vitro P. falciparum* HRP2 assay.

(b) ***In vitro P. falciparum HRP2 assay***

All compounds were evaluated *in vitro* for their antiplasmodial activities by HRP2 assay (Noedl *et al.*, 2005; Noedl *et al.*, 2002) as described elsewhere (Mohd Abd Razak *et al.*, 2014) with some modifications. Briefly, the compounds were solubilised in 100% dimethyl sulphoxide (DMSO) to get 50 µM stocks. In preparation of compound or drug stock plates, the compounds (50 µM) were serially diluted (2 fold dilution) to 8 point concentrations (ranging from 25 µM to 0.39 µM) in DMSO from well A1 to A7 in a 96 well plate. Fifteen microliters of serially diluted stock extracts were transferred correspondingly into watery plates containing 225 µl of sterile H_2O. An aliquote of watery plates will be used in HRP2 assay.

Ring-infected red blood cells (RBCs) of 5% parasitemia were tuned so that the parasitemia and hematocrit were 0.05% and 1.5% respectively. A 10 µL of each serially-diluted extract was moved to a test plate containing parasitized RBCs, and later incubated in a candle jar at 37^{o}C for 72 hours. The final test concentrations ranged from 0.156 µM to 0.002 µM, while that of DMSO was 0.3%. artemisinin (Art) (Sigma, USA) , chloroquine (CQ) (Sigma, USA), mefloquine (Mef) (Sigma, USA) and quinine (Q) (Sigma, USA) – whose final test concentrations were 1772.6 – 27.7 nM for CQ, 3495 – 54.6 nM for Q, 601.3 – 9.4 nM for Mef, and 51.2 – 0.8 nM for Art – were the standard controls used to validate the test. Meanwhile, the negative controls comprised infected RBCs without extracts or with sterile H_2O only. Following incubation for 72 hours, the test plates were then kept overnight in -80^{o}C. Then, to haemolyse the infected RBCs, it were kept at room temperature. Subsequently, the activities of the compounds against the parasite were measured via HRP2 assays.

One day prior to the assay, 100 µl of immunoglobulin M (IgM) capture antibody (MPFM-55A, ICL, Inc, Newberg, OR, USA) specific for *P. falciparum* HRP2 (1 µg/ml

in phosphate-buffered saline (PBS)) were added to each well of a 96-well ELISA plates (Microlon 600, Greiner, Germany). The plates were covered and incubated at 4^{o}C overnight. Following incubation, the contents of the wells were removed and the plates were washed three times with 0.05% PBS-Tween 20 (PBST). The non-binding sites of the ELISA plates were blocked with 200 µl/well of 2% bovine serum albumin in PBS for 2 hours at room temperature. Following the blocking step, the ELISA plates were washed 3 times with 200 µl of 0.05% PBST. Hundred microliters of the *P. falciparum* infected RBC lysates (freeze thawed) were transferred from the test plates into ELISA plates and incubated in humidity chamber for 1 hour at room temperature. The ELISA plates were washed as described above. Hundred microliters of the detector antibody (MPFG-55P, ICL, Inc, Newberg, OR, USA) conjugated with horseradish peroxidase (0.2 µg/ml in PBS) were added to each well, and incubated in humid chamber for 1 hour at room temperature. Following a subsequent washing step similar to the above, 100 µl of 3,3',5,5'-tetramethyl benzidine (TMB) chromogen (Zymed Lab., Inc., San Francisco, CA, USA) was added to each well and incubated for 10 min in dark, followed by the addition of 50 µl of 1M sulphuric acid. The absorbance was determined by using ELISA plate reader at a wavelength of 450 nm (FLUOstar Omega, Germany). The collected data were transferred to HN-nonLin software (malaria.farch.net) to get a 50% Inhibitory Concentration (IC_{50}) value directly from the graph.

3.4.2 In vitro cytotoxicity assay

The MDBK cells were maintained in complete DMEM culture medium containing 25 mM HEPES, 0.4% sodium bicarbonate ($NaHCO_3$), 100U of Pen-Strep (100U penicillin and 100U streptomycin) supplemented with 10% fetal bovine serum (FBS). The cytotoxicity of the extracts were measured by 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay (Mosmann, 1983). Prior to the day of test, the stock plates were prepared by serially diluting (2 fold dilution) the stock extracts (50

μM) to 7 point concentration (ranging from 25 μM to 0.39 μM) with DMSO. Then, 6 μl of serially diluted stocks were transferred into 96 well plates containing 294 μl of complete DMEM media (Medium plates).

On the day of the test, MDBK cells were harvested and adjusted to 1×10^4 cell per ml. A hundred microliter of cell suspension was seeded into each well of a 96-well plate and allowed to grow overnight. Then, 100 μl of test compounds taken from medium plate (as prepared above) were added to each well accordingly ranging final concentration of 0.25 μM to 0.004 μM. The final concentration of DMSO in all test was less than 1%. All tests were performed in duplicate. The positive control for cell growth is the cell suspension without test substance while the negative control is the cell suspension with 0.05% Triton X 100. The culture was incubated at 37°C in 5% CO_2 incubator for 72 hours. Fifty microliters of MTT solution (5 mg MTT in 1ml PBS and 2.5 ml DMEM media) were added to each well. The plates were further incubated for 4 hours at 37°C in 5% CO_2 incubator. The medium was removed and replaced with 200 μl of DMSO to solubilise the MTT formazan product. The solution was mixed for 15 min and once for 30 sec before measuring the absorbance at 540 nm with a micro plate reader (FLUOstar Omega, Germany). The percentage of growth inhibition and the IC_{50} were estimated from a dose response curve.

A selectivity index (SI), corresponding to the ratio between antiplasmodial and cytotoxic activities was calculated according to the following formula:

$$SI_{\textit{Plasmodium}} = IC_{50\ \text{normal cell lines}} / IC_{50\ \textit{Plasmodium}}$$

3.4.3 Antiproliferative assay

Sulforhodamine B (SRB) assays was carried out to determine the half maximal inhibitory concentration (IC_{50}) of the compounds (Vichai & Kirtikara, 2006). Briefly, the cells were treated with 5.053 – 161.686 μM, 4.843 – 154.960 μM, 4.189 – 134.055 μM, 4.680 – 149.752 μM, or 3.936 – 125.944 μM sets of concentrations for all the synthesised complexes **P1, P2, P3, P4, P5** and **P6** respectively. Forty-eight hours later, using 50 μL of 50% (w/v) trichloroacetic acid (TCA), the cells were secured to the plates, and kept further incubated at 4°C for an hour. Using tap water, the plates were then washed five times, air-dried, stained with 100 μL of 0.4% (w/v) SRB staining solution, and further incubated for another 10 min at room temperature. Consequently, the plates were washed three times with 1% (v/v) acetic acid to remove the unbound stains. Following air-drying, 200 μL of 10 mM Trizma base was added into the wells, shaken for 10 min. The reading of the absorbance is taken using microplate reader at 490 nm, and the IC_{50} calculated using the formulae; IC_{50} = (OD sample/OD control) × 100. All experiments were performed in triplicates.

CHAPTER 4: RESULTS & DISCUSSION

4.1 Introduction

Six thiosemicarbazone ligands and its corresponding silver complexes with triphenylphosphine were synthesized. The thiosemicarbazone ligands were prepared based on the literature (Benmohammed *et al.*, 2014) with slight modifications starting from the 4-phenyl-3-thiosemicarbazide that was added to the aromatic aldehyde. The precipitate formed which was white or yellow powders were soluble in most common organic solvents.

Each of the six synthesized derivatives of 4-phenyl-3-thiosemicarbazone was then reacted with silver and triphenylphosphine in a mixture of methanol/acetonitrile as a solvent to obtain their corresponding silver complexes. All of the syntheses gave a clear final solution except for **P1** and **P5** which gave precipitate upon addition of the corresponding thiosemicarbazone ligands. However, in the latter case, the precipitate lessened once the triphenylphosphine is added. The complexes were brown and black in color later structurally characterized by NMR , FTIR, EDX and elemental analysis.

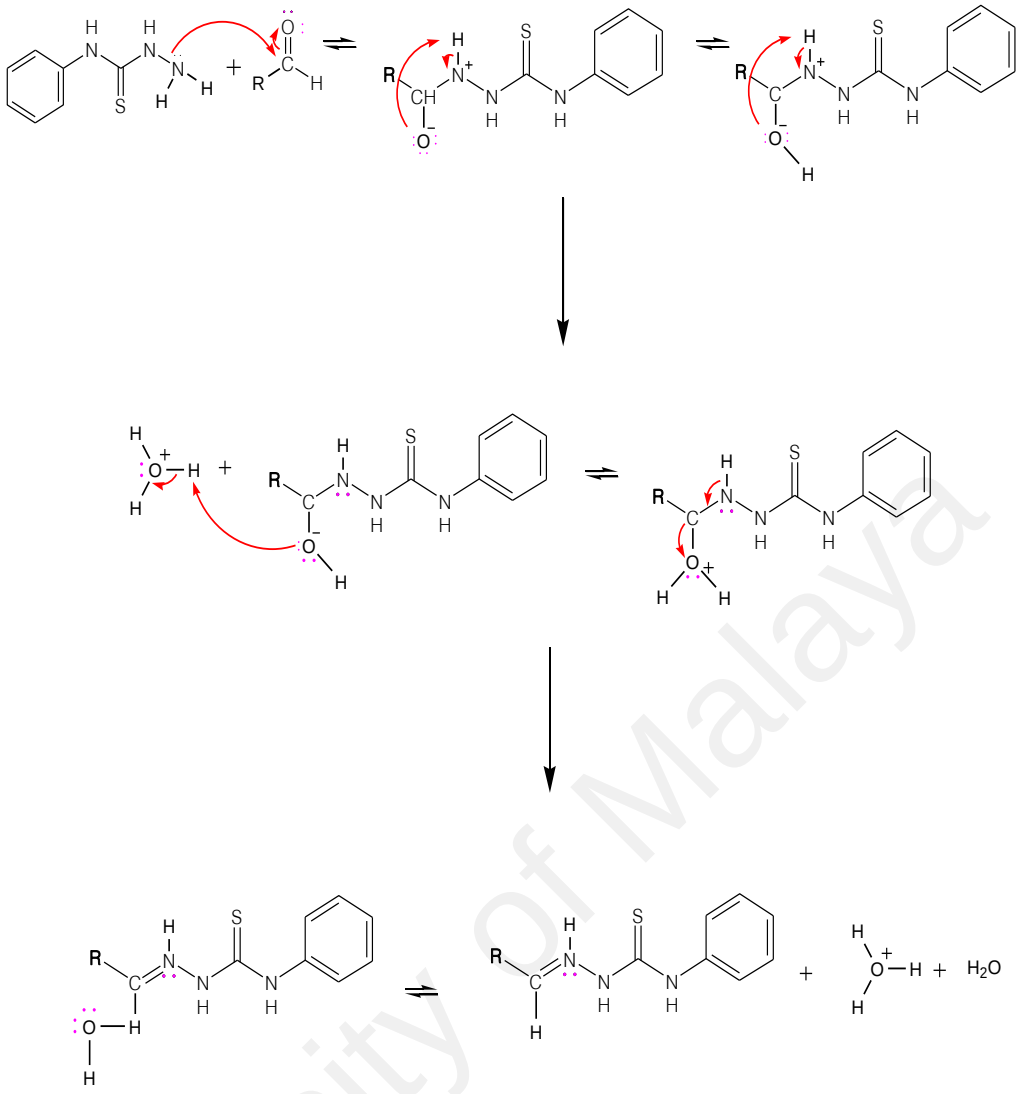
The solubility of the synthesized silver complexes were good in organic solvents such as acetonitrile, dimethyl sulfoxide (DMSO) or dimethylformamide. Several techniques were done to crystallize the complexes including methods mentioned by the previous studies which managed to obtain single crystals (Muhammad Altaf *et al.*, 2013; Lobana, Kumari*, et al.*, 2008). Among the methods were slow evaporation at room temperature and low temperature (refrigerator) for several days and also recrystallization of the scraped product from organic solvent (DMF, DMSO, acetonitrile, methanol). However, none of the methods used were able to generate crystals suitable for X-ray diffraction studies to date. Altaf et al., reported silver compounds containing nitrogen and sulfur as

ligands are hard to crystallise as this type of compounds may appeared to be polymeric(M. Altaf *et al.*, 2013).

4.2 Reaction mechanisms

4.2.1 Reaction mechanism for the synthesis of 4-phenyl-3-thiosemicarbazones

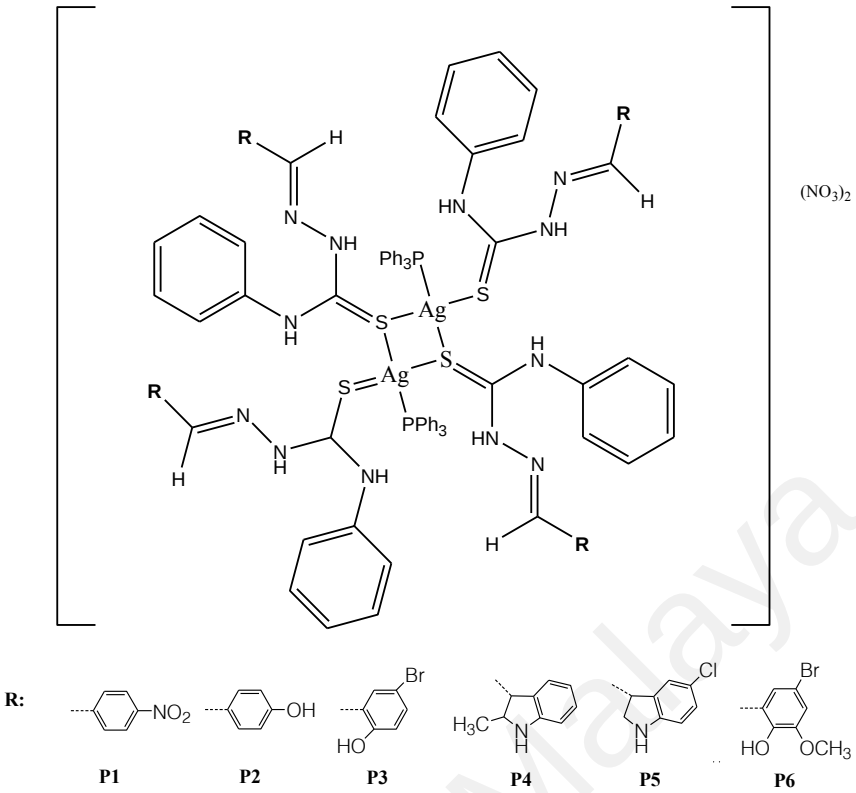
The 4-phenyl-3-thiosemicarbazone ligands were formed *via* the condensation reaction of 4-phenyl-3-thiosemicarbazide and aldehyde using ethanol as solvent and acetic acid as catalyst. Typically, as shown in **Scheme 4.1**, the Schiff based formation of an imine is a sequence of two types of reactions; addition followed by elimination. First, the amine nitrogen acts as nucleophile, attacking the carbonyl carbon of the aldehyde. Then it forms an unstable addition compound called carbinolamine. Since carbinoamine is an alcohol, it undergoes acid catalyzed dehydration. The dehydration of carbinolamine is the rate determining steps of Schiff based formation and that is why the reaction is catalyzed by acid. The nitrogen is then deprotonated, and the electrons from N-H bond push off the oxygen from the carbon leaving a C=N double bond (imine) and displaced water molecules.



Scheme 4.1: Reaction mechanism of formation of 4 phenyl-3-thiosemicarbazone

4.2.2 Reaction mechanism for the syntheses of silver complexes

The formation of silver complexes occurred when silver receive lone pair of electrons from sulphur and phosphorus donor atom. The complexes were suggested to bind tightly to the metal centre through M-S bridging bonds as they were synthesised in 1:1:2 silver to phosphine to thiosemicarbazone(Muhammad Altaf *et al.*, 2013). In agreement with the previous study(Muhammad Altaf *et al.*, 2013; Sultana *et al.*, 2010), the silver complexes were dinuclear, with each silver atom tetrahedrally coordinated to one P atom of PPh_3, one sulfur atom of a terminal thiosemicarbazone and two S atoms of bridging thiosemicarbazone groups as shown in **Figure 4.1**.



R:

P1 P2 P3 P4 P5 P6

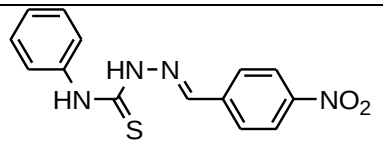
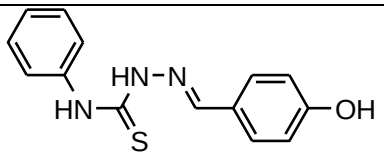
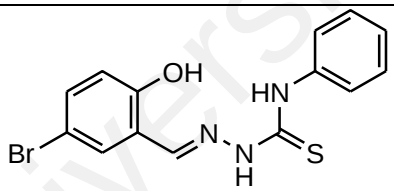
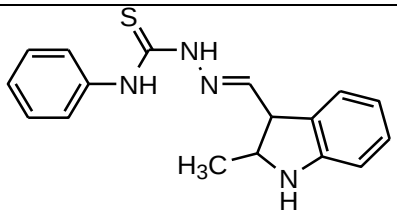
Figure 4.1: Expected structures of silver complexes

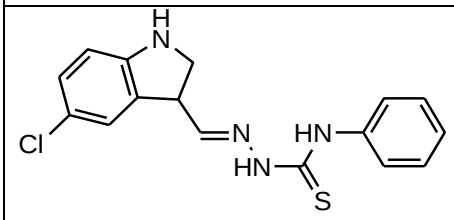
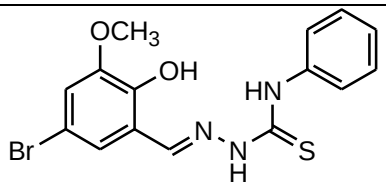
4.3 Chemical Characterization

4.3.1 CHN Elemental Analysis

Table 4.1 shows the physical properties and elemental analysis of yhe synthesized thiosemicarbazone ligands (L1-L6) and its silver complexes (P1-P6). The experimental data was found to be in a good agreement with the calculated value of proposed structure.

Table 4.1: Analytical data and physical properties of the ligands and silver complexes

Compound	Colour	Yield (%)	Calculated (%) / Found (%)		
			C	H	N
	Yellow	70	55.99 55.20	4.03 3.73	18.65 18.59
P1	Dark-yellow	50	56.91 56.30	4.05 3.57	11.54 11.65
	Brown	60	61.97 61.24	4.83 4.92	15.49 14.98
P2	Brown	45	60.53 58.90	4.53 3.82	9.21 9.26
	Dark brown	65	48.01 48.24	3.45 3.44	12.00 12.12
P3	Black	40	51.60 59.65	3.67 3.17	7.85 7.81
	Dark brown	80	65.78 66.01	5.84 5.19	18.05 17.32

P4	Black	45	63.28 59.62	4.80 4.34	11.35 10.98
	Black	45	58.09 57.65	4.57 3.95	16.93 16.20
P5	Shiny black	40	62.63 59.89	4.52 3.89	11.69 10.91
	Brown	70	47.38 47.89	3.71 3.75	11.05 11.08
P6	Shiny brown	40	50.99 48.09	3.83 3.38	7.43 7.72

The percentage yield for complex P3, P5 and P6 were found to be quite low as compared to others. This might due to some experimental errors during the filtering and purification steps of the compounds. Losses occur in the separation and purification of the desired product from the reaction mixture. There are also the possibility of incomplete reaction which the reactants are not completely converted to product. If a reverse reaction occurs, the final state contains both reactants and products might be in a state of chemical equilibrium.

4.3.2 Fourier-Transform Infrared Spectroscopy

Thiosemicarbazones are known to exhibit characteristic bands in specific energy regions corresponding to various functional groups. FT-IR spectral bands are used to confirm the existence of various functional groups observed in the spectra. The FT-IR

spectra of the complexes were compared to those of their ligands in order to investigate the point of attachment of the ligand to the metal center. Selected FT-IR spectroscopy vibration bands for the free ligands and its corresponding complexes are given in **Table 4.2**.

Table 4.2: Selected FT-IR absorptions for the free ligands and their silver (I) complexes.

Compound	ν(C=S)	ν(P-C_{Ar})	ν(NH)	C=N
L1	846.76	-	3103.08, 2980.20	1595.02
P1	840.88	1091.20	2983.12, 2888.57	1589.21
L2	851.49	-	3158.08, 2977.11	1604.28
P2	834.24	1076.26	2981.06, 2889.37	1595.67
L3	829.04	-	3141.28, 2984.50	1604.57
P3	824.43	1093.85	2927.27, 2980.00	1622.67
L4	839.31	-	3394.64, 3285.30, 3209.93	1589.25
P4	830.01	1094.63	3012.28, 2880.97, 2649.02	1600.00
L5	866.50	-	3338.98, 3132.20, 2973.42	1609.25
P5	834.04	1093.70	3000.96, 2883.01, 2733.51	1598.27
L6	858.86	-	3300.41, 2973.79	1604.00
P6	836.84	1094.47	3049.11, 2879.23	1599.12

The stretching frequencies of the (N-H), (C-N), and (C=S) functional groups are very useful when determining the mode of coordination of the thiosemicarbazone ligands. The corresponding spectral bands occur in the regions 3100 - 3500 cm^{-1}, 1580 - 1630 cm^{-1} and 820 - 900 cm^{-1}, respectively. Thiosemicarbazones have been known to exhibit thione-thiol tautomerism due to the presence of a thioamide -NH-C=S functionality. It has been reported that thiosemicarbazones can coordinate to metal centers either as a neutral

(thione) or as a mono-anionic (thiolate) ligand. The question of thione-thiol tautometism in the ligands were ruled out due to the absence of a characteristic (S-H) spectral band (in the region 2600 to 2500 cm^{-1}) in the respective FTIR spectra which confirm its existence in thione formation.

The FT-IR spectra of compounds in our case also exhibited two stretching frequencies in the (N-H) region, confirming that the ligands were coordinated as the thione form. When evaluating the nature of the bonding in thiosemicarbazone complexes, the (N-H) stretching frequency is known to play a very important role. The bands assigned to (N-H) can be split into two regions: (i) 3450 to 3210 cm^{-1} due to the - N^1H_2 group, (ii) 3180 to 3150 cm^{-1} due to the -N^2H group (Lobana, Sharma, *et al.*, 2009). The presence of a band corresponding to N^2H group, suggests the coordination of a thiosemicarbazone to the metal center in a neutral form, while its absence, suggest the deprotonation of hydrazinic N^2H proton in the complexes.

Coordination of the respective free thiosemicarbazone ligands to the silver(I) metal center results in a shift in the the (N-H) stretching frequencies to lower energy in the range of 2880 cm^{-1}-3049 cm^{-1}. A decrease was also observed in the (N-H) stretching frequencies of the –N^2H group in the range of 2649 cm^{-1} -2889 cm^{-1}.The FT IR spectra of complexes P4 and P5 exhibited indolic (N-H) stretching frequencies at 3012 cm^{-1} and 3000 cm^{-1} respectively. A comparison between the indolic (N-H) stretching frequency observed in the FT IR spectra of the ligands and complexes illustrated a shift to lower energy upon coordination of the free thiosemicarbazone ligand to the metal center.

The involvement of the thiocarbonyl moiety of the thiosemicarbazone ligands in the complexation process was inferred based on the decrease in the (C=S) stretching frequencies upon coordination. Medium intensity spectral bands in the region 866-824 cm^{-1} were earlier assigned to (C=S) stretching frequency. Upon coordination, a decrease

in the range of 6-32 cm^{-1} was observed in the (C=S) stretching frequencies of each complex as compared to its corresponding ligand. The magnitude of the shift in the (C=S) stretching frequencies confirmed that the thiosemicarbazone ligands are also coordinated of to the metal center via the neutral thioamide sulphur(Lobana, Khanna, *et al.*, 2009). Based on the results presented above, it is evident that the thiosemicarbazone ligands are coordinated to the silver(I) metal center via the sulphur atom of the thiocarbonyl group.

The infra-red spectra of the complexes also confirm the coordination of the ligand as they revealed the presence of ν(C=N) vibrational modes in the region of 1630- 1580 cm^{-1} in all of the ligands and complexes. There is also a presence of intra- and intermolecular hydrogen bonding of the hydroxyl and amino groups which cause band broadening in the region of 3600 – 3000 cm^{-1}. A characteristic ν(P-C_{Ar}) peak around 1090 cm^{-1} indicated the presence of the triphenylphosphine that is coordinated to the silver center. This finding was supported by the research done by Lobana et al., who also got ν(P-C_{Ar}) peak at the similar value (Lobana, Khanna, *et al.*, 2008). The FT-IR spectrum for the complex (**P5**) did not detect the peak for -C-Cl as compared to its ligand (**L5**) at 761.44 cm^{-1} that further proposed the detachment of the –Cl to form HCl in the reaction mixture. This is later proven then when observations made on the reaction mixture shows that there is an increase of the pH value from *ca.* 7.5 to 4.5.

4.3.3 Nuclear Magnetic Resonance Spectroscopy (NMR)

The thiosemicarbazone ligands were dissolved in deuterated chloroform solvent and its Ag(I) complexes were dissolved in deuterated dimethyl sulfoxide (DMSO-d_6) solvent. The ^{1}H NMR chemical shifts of the ligands and complexes are summarised in **Table 4.3**.

Table 4.3: Selected 1H-NMR chemical shifts of the ligand and their Ag complexes (in DMSO-d6)

Compound	δ N-H (*ppm*)	δCH=N (*ppm*)	δO-H (*ppm*)
L1	12.06, 10.30	8.20	-
P1	12.33, 10.72	8.18	-
L2	11.62, 9.94	8.04	9.88
P2	11.88, 10.32	7.99	9.98
L3	11.785, 10.14,	8.38	10.28
P3	12.02, 10.46	8.42	10.58
L4	11.55, 11.40, 9.50	8.50	-
P4	11.63, 11.60, 9.73	8.41	-
L5	11.83, 11.58, 9.72	8.35	-
P5	11.88, 10.35, 10.01	8.00	-
L6	11.80, 10.10	8.43	9.52
P6	12.00, 10.53	8.46	9.70

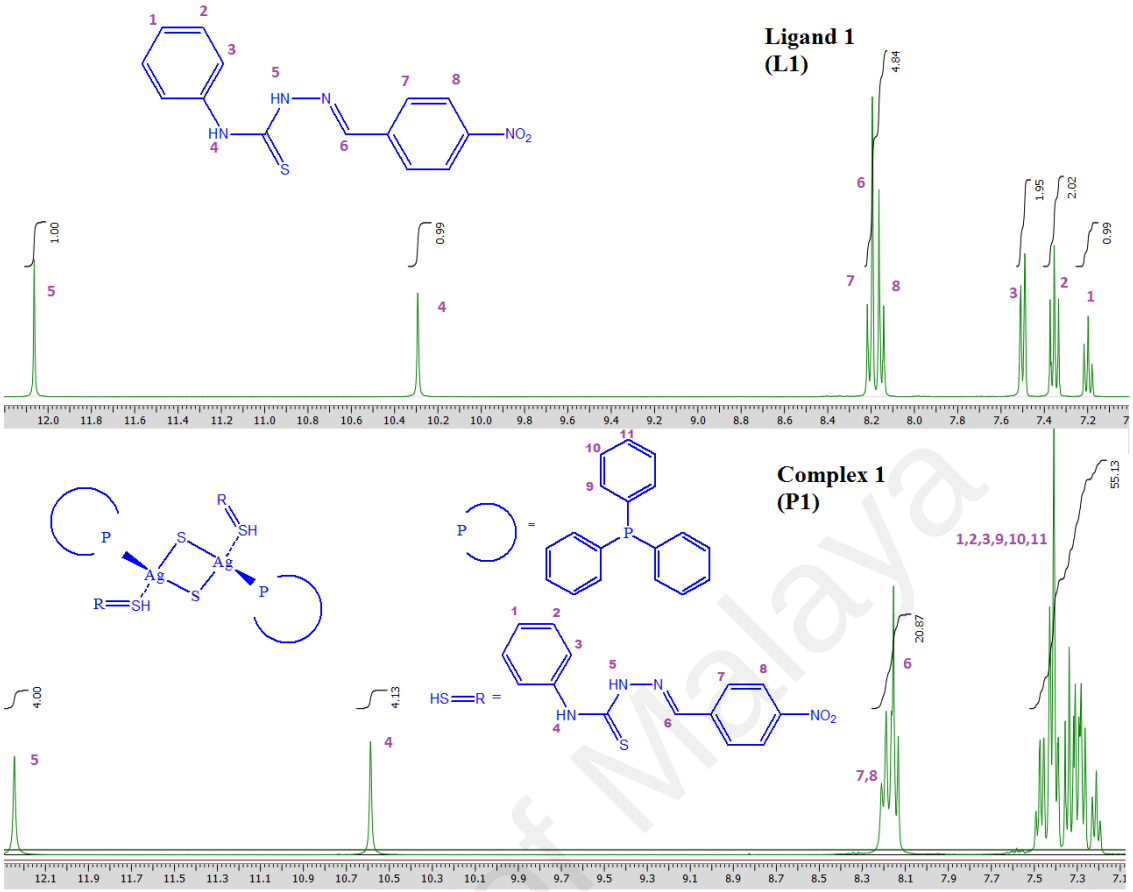


Figure 4.2: The assignments of proton for ^{1}H NMR of L1 and P1

Figure 4.2 shows the assignments of proton for ^{1}H NMR spectra of ligand **L1** and complex **P1**. From the spectrum, the most deshielded peak was found at δ 12.2 ppm which shows the signals of NH(4) and followed by another singlet peak for the other NH(5) at δ 10.6 ppm. The proton peak for the complex shifted more downfield as compared to the free ligand upon coordination to silver metal. The deshielding of the N-H proton is related to an increase of the p electron density in the C–N bond upon complexation (A. A. Isab *et al.*, 2010). Around δ 8.1-8.2 ppm, two pairs of doublet with *J*=31.14 and *J*=10.07 were found indicating the CH(7) & CH(8) ,and seen overlapping with the CH(6) singlet. While the other signals for aromatic protons were comfirmed by the multiplets peak observed at δ 7.2-7.5 ppm. The coordination of the triphenylphosphine ligand is proved by the additional aromatic proton observed at the region of δ 7.10–7.50 *ppm* in the complexes which was not present in their respecting ligands.

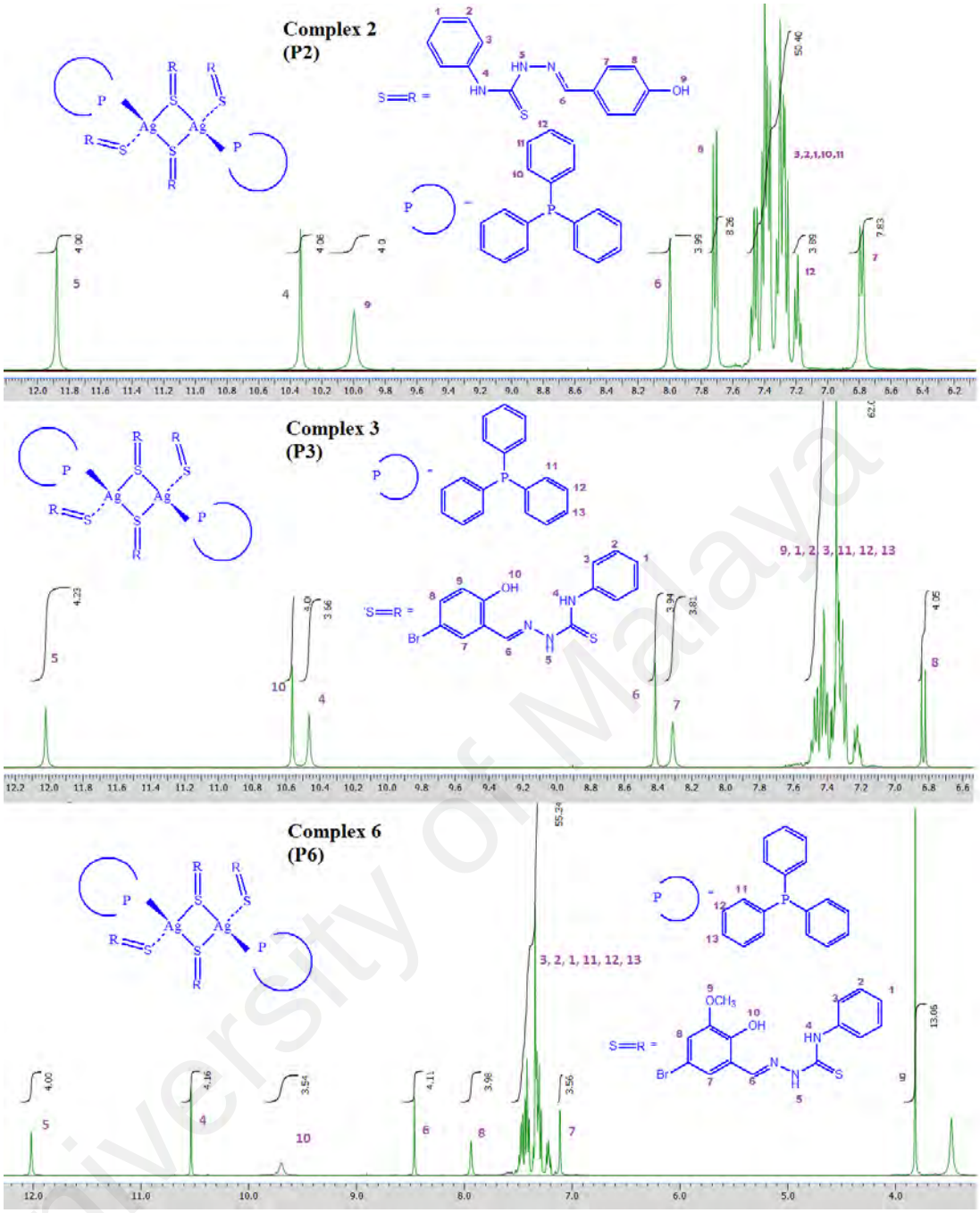


Figure 4.3: The assignments of proton for ^{1}H NMR spectra of complexes P2, P3 and P6

Figure 4.3 shows the assignments of proton for ^{1}H NMR spectra of complexes **P2**, **P3** and **P6**. For complex **P2**, the most downfield signal is the peak for NH(5) at δ 11.88 ppm followed by the NH(4) at δ 10.22 ppm. Later, a broad singlet of OH is found at δ 9.99 ppm. The hydroxyl singlet was also observed on **P3** and **P6** at 10.58 and 9.70 ppm. These hydroxyl peaks of complexes were found shifted downfield by δ 0.1 to 0.3 ppm from their

position in the free ligands upon coordination to metal. For CH(6) on all three complexes, the NMR spectra exhibits a singlet around δ 8- 8.5 ppm. Around δ 6 to 8 ppm, a group of mixed multiplets were found indicating the proton peak for all the aromatic protons including the aromatic PPh_3 protons. A methoxy-OCH_3(9) proton peak for compound **P6** was also detected at as triplet at δ 3.80 *ppm.*

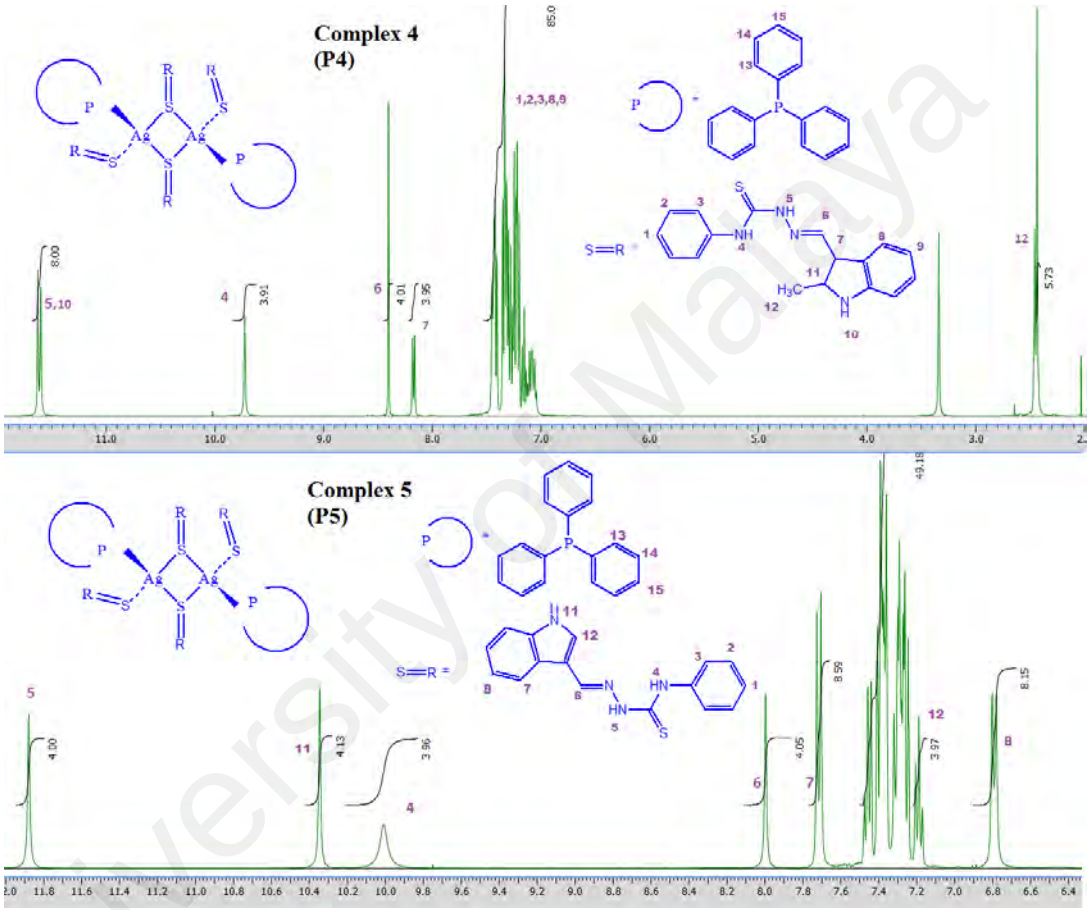


Figure 4.4: The assignments of proton for complex P4 and P5

Figure 4.4 shows the assignments of proton for ^{1}H NMR spectra of complexes **P4** and **P5**. For **P4** and **P5** complexes, there was a presence of indole group and there was a few additional proton peaks like indolic NH peak, and a CH_3 proton peak for complex **P4**. From complex **P4** spectrum, from the most downfield positions were the overlapping singlets of NH(5) and NH(10) at δ 11.60 ppm. It was then followed by another NH(4) peak at δ 9.73 ppm. Later, a singlet CH(6) and a doublet CH(7) peaks were found adjacent

to each other at δ 8.15 and 8.40 ppm. The most shielded peak would be the CH3(12) peak but was seen overlapping with the deuterated DMSO water peak at δ 2.5 ppm. For complex P5, the doublets which were found on CH(7) and CH(8) at δ 7.70 ppm (*J*= 8.70)and 6.60 ppm (*J*=8.24) ppm further comfirm the missing of Cl at CH(8) during complexation reaction which was stated in the discussions earlier.

The appearance of NH, CH, OH peak as a single signal integrated for four protons shows that there were four thiosemicarbazone ligands in the complex. The integration for protons in the aromatic regions later shows similar amount of four thiosemicarbazone groups and two triphenylphosphine groups in the complex which is 66 protons for **P1** and **P2**; 62 protons for **P3** and **P4**, 70 protons for **P5** and 58 protons for **P6**. This later indicates that the silver complexes were dinuclear and each silver atom was tetrahedrally coordinated to three thione sulfur of thiosemicarbazone and one phosphorus ligand.

^{13}C NMR experiment was also done in order to identify the various types of carbon atoms in the compounds. To further justify the structural characterization, we also run few DEPT experiments on selected compounds. Distortionless enhancement by polarization transfer (DEPT) is a NMR method used for determining the presence of primary, secondary and tertiary carbon atoms. All of the carbon peaks on the spectra were individually assign to each carbon and further justify the credibility of its assignment. The ^{13}C NMR chemical shifts of the ligands and complexes are summarised in **Table 4.4.**

Table 4.4: Selected ^{13}C and $^{31}P\{^{1}H\}$ NMR chemical shifts of the ligand and their Ag complexes (in DMSO-d6)

Compound	δ ^{31}P(*ppm*)	δC=S (*ppm*)	δ (N-C-Ph) (*ppm*)
L1	-	177.1	140.7
P1	10.1	175.8	143.0
L2	-	175.9	144.0
P2	10.0	173.9	146.6
L3	-	176.6	139.5
P3	10.2	174.0	141.5
L4	-	174.5	141.7
P4	10.0	172.9	144.0
L5	-	175.1	141.2
P5	10.0	173.8	133.9
L6	-	176.6	140.0
P6	10.1	174.5	141.5

The ^{31}C NMR spectrum of the complexes (**P1-P6**) showed signals due to the C=S resonance appears upfield compared to its ligands. Coordination of the respective free thiosemicarbazone ligands to the silver(I) metal center results in a shift in the (C=S) carbon peak from δ 177.1 to 175.8 ppm for compound **1**, δ 175.9 to 173.9 ppm for compound **2,** δ 176.6 to 174.0 ppm for compound **3**, δ 174.5 to 172.9 ppm for compound **4**, δ 175.1 to 173.8 ppm for compound **5** and δ 176.6 to 141.5 ppm for compound **6**. This was because of the lowering of the >C=S bond order upon coordination with metal(Anvarhusein A. Isab *et al.*, 2002) and a shift of the N→C electron density producing partial bond character in the C-N bond(Nawaz *et al.*, 2011).

For compounds **P2**, **P3** and **P6**, the C-hydroxyl resonance at the thiosemicarbazone ligand was observed at δ 139.0 *ppm.* In the spectrum for compound **P6**, the C-methoxy resonance discovered at δ 57.0 *ppm* whilst the C-methyl for compound **P4** resonance observed at δ 12.0 *ppm*. For all of the complexes, a significant upfield shift for the aromatic carbons resonances in the triphenylphosphine was observed at δ ranging from 131.0 to 132.0 *ppm* compared to free ligand at 137 *ppm.* This findings were supported by the research done by Altaf et al., which also found a similar value for aromatic triphenyl phosphines(Muhammad Altaf *et al.*, 2013). It appeared as doublet for the C(P), C(2) and C(3) while the C(4) appeared as singlet which further confirmed the attachment of the triphenylphosphine ligand to the silver centre (Nawaz *et al.*, 2011).

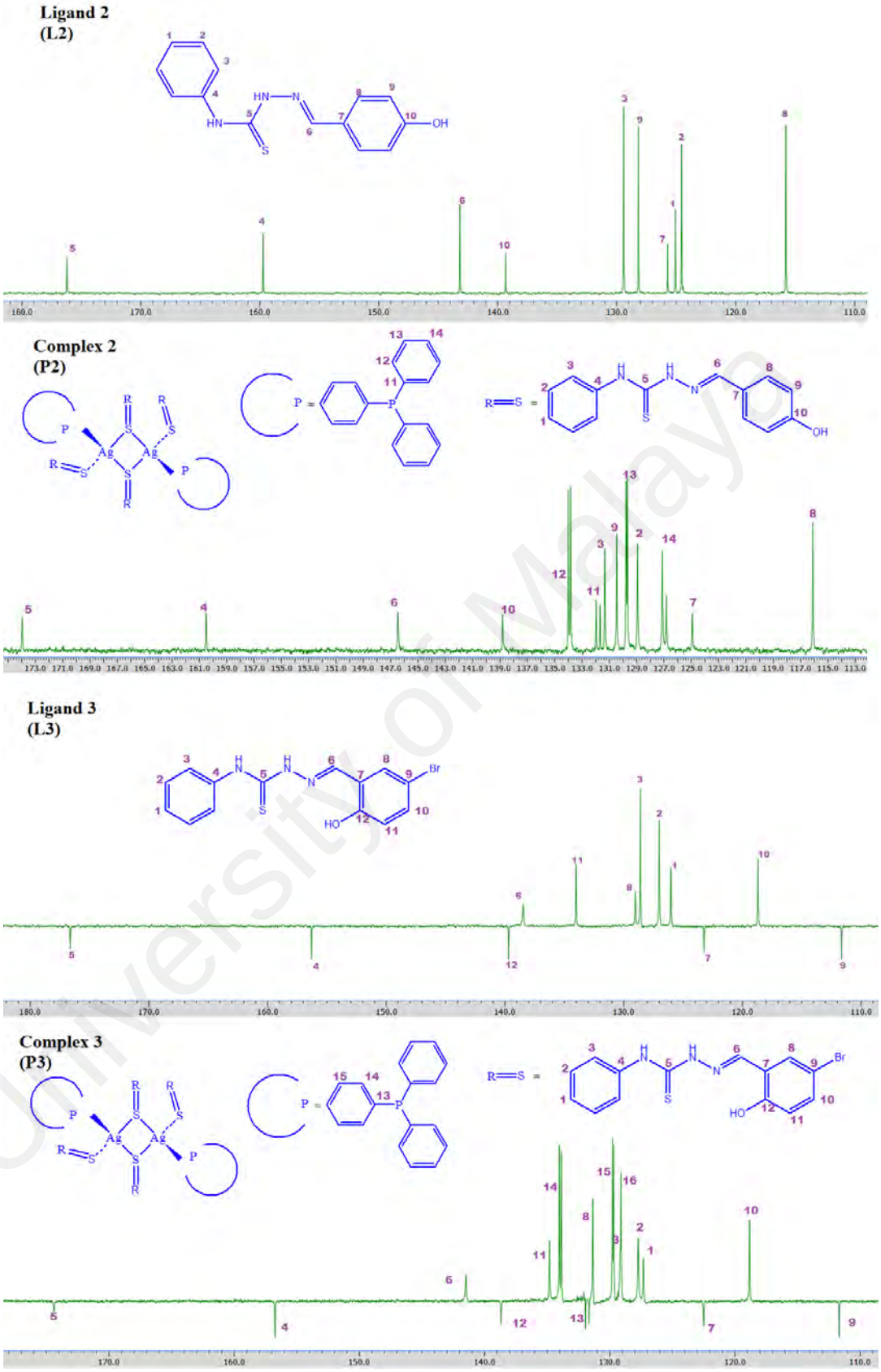


Figure 4.5: Comparison of ^{13}CMR spectrum of ligands and complexes 2 and 3

Figure 4.5 shows the ^{13}C NMR spectrum of ligand and complex **2** and ^{13}C DEPT NMR spectrum of ligand and complex **3**. The most deshielded carbon peak in ligand and complex **2** were the C=S peak at δ 177.1 and 175.8 ppm followed by C_{Ar}-NH at δ 159.5 and 160.5 ppm. Going upfield, NHC-Ph was found at δ 143.0 ppm for **L2** and δ 146.0 ppm was found for **P2** and C-hydroxry was found around δ 139 ppm for both **L2** and **P2**. All the aromatic peaks were also assigned at δ 135- 115 ppm with a presence of doublet peaks corresponding to the C_{Ar} (12)(13)(14)-P. From the ^{13}C DEPT NMR spectrum of **L3** and **P3**, the assignments of each carbon were made based on the class of carbon; primary and tertiary carbon on down side, and secondary carbon on the upper side. 12 carbon peaks were identified for **L3** and 15 carbon peaks were found on **P3** indicating the additional carbon peaks from PPh_3.

In the $^{31}P\{^1H\}$ NMR spectra of the complexes **P1**-**P6** (**Table 4.4**), a sharp singlet was observed at δ 10 *ppm* reflecting the phosphorus resonance for triphenylphosphine. This signals has shifted downfield as compared to its free ligand which indicates the complexation had occurred between the silver center and the triphenylphosphine ligand. This findings were also supported by Isab et al., whom also receive similar $^{31}P\{^1H\}$ NMR spectrum peak of triphenylphosphine (A. A. Isab *et al.*, 2010). An example of $^{31}P\{^1H\}$ NMR spectra of the free (triphenylphosphine) ligand and the silver complex (**P2)** is being shown in the **Figure 4.6**.

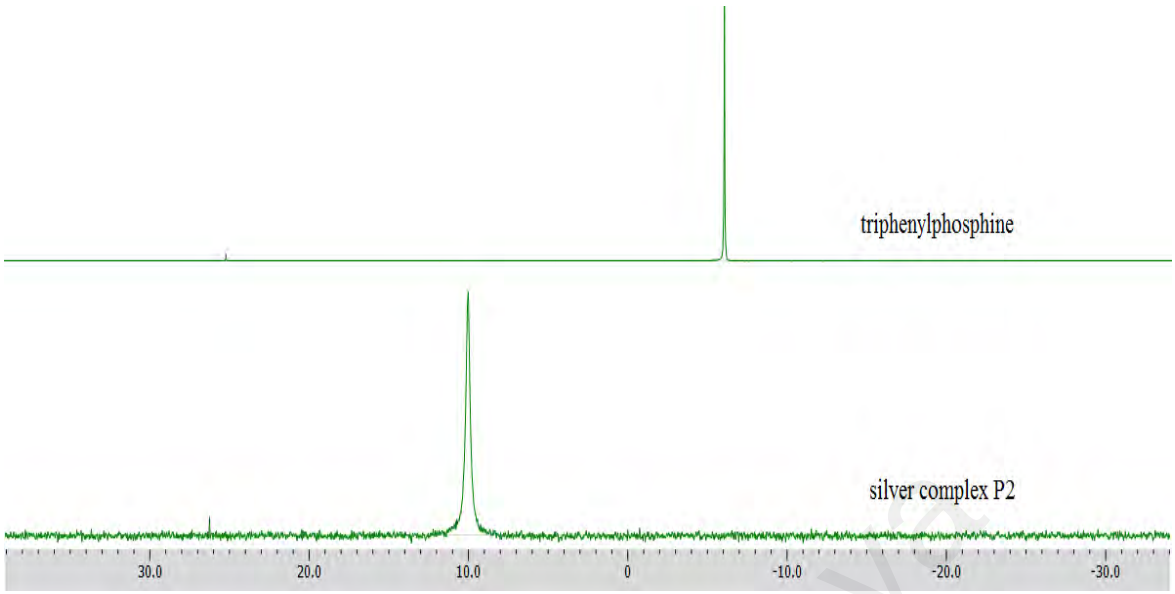


Figure 4.6: $^{31}P\{^{1}H\}$ NMR spectrum of free ligand (triphenylphosphine) with silver complex P2

Based on the results presented above, it is evident that both ligands; thiosemicarbazone and phosphorus have bind to the silver metal and thiosemicarbazone ligands are coordinated to the silver(I) metal center via the sulphur atom of the thiocarbonyl group. The spectroscopic data discussed showed that all of the ligands and their complexes exist as a thione tautomer. The silver(I) complex was dinuclear, having each silver atom coordinated to three thione sulfur atoms of thiosemicarbazone and to one phosphorus atom of PPh_3 in a nearly tetrahedral environment in agreement with the previous study (Muhammad Altaf *et al.*, 2013)

4.3.4 Energy Dispersive X-ray Spectroscopy (EDX)

An analysis using Energy Dispersive X-ray (EDX) Spectroscopy was also done to confirm the existence of silver metal in all complexes. From the analysis, the silver metal is presence in all six series of complexes which gives clear indication of the successful complexation. Other elements such as carbon, nitrogen, oxygen, sulfur, phosphorus and bromine that are expected to exist from the ligands also presence in the complexes. **Figure 4.7** shows the EDX data for silver complexes **P1-P6.**

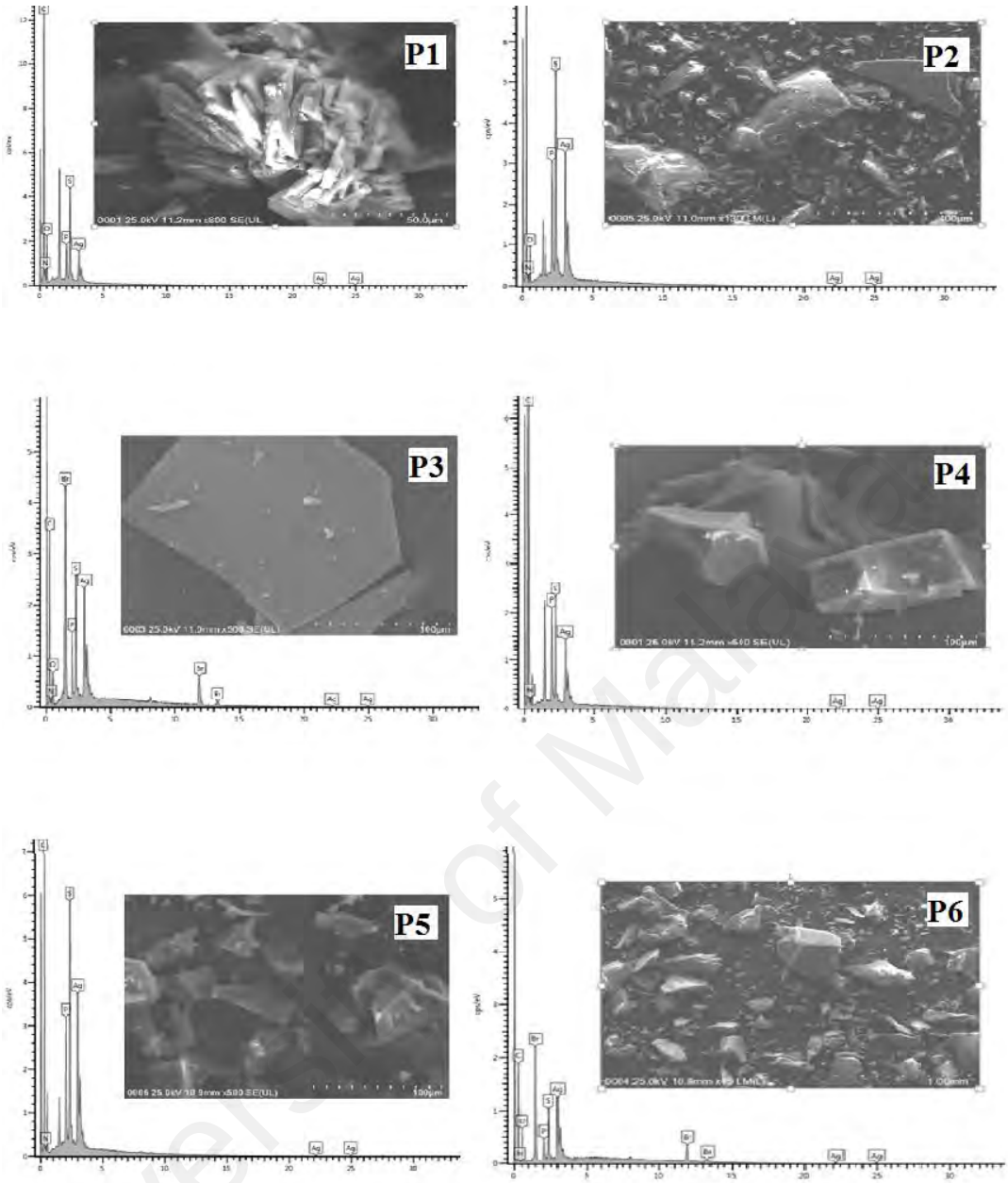


Figure 4.7: EDX data on complex P1-P6

4.4 Biological Studies

4.4.1 The antiplasmodial study.

The *P. falciparum* HRP2 assay showed a promising antiplasmodial activity for all of the complexes against the chloroquine resistant *P. falciparum* parasite asexual cycle in red blood cell *in vitro*. The EC_{50} value showed by the compounds were scored within the

acceptable cut-off values (<1-5 µM) for further *in vivo* antimalarial study (Fidock *et al.*, 2004) (**Table 4.5**).

Table 4.5: Antiplasmodial and cytotoxicity activity of complexes 2-6 (EC_{50} in µM).

Complexes	*P. falciparum* HRP2	Normal MDBK	Selectivity Index (SI)
P2	1.739 ± 0.243	1.215± 0.180	0.699
P3	4.000 ± 0.142	0.821 ± 0.068	0.205
P4	2.120 ± 1.184	0.945 ± 0.102	0.446
P5	3.776 ± 0.280	0.984 ± 0.046	0.261
P6	4.266 ± 0.311	1.675 ± 0.183	0.393

The cytotoxic effect of the compounds on MDBK cells was assessed in order to determine the ratio of cytotoxicity to biological activity (SI). The biological efficacy is not due to *in vitro* cytotoxicity when the index is ≥10 (Weniger *et al.*, 2001). However, the growth inhibitory effect of the compounds on normal cell line, MDBK, was more prominent than the *P. falciparum* parasite (**Table 4.5)** where, the SI value for each compound was lower than 10. The results suggested that the antiplasmodial activities showed by the compounds could be due to *in vitro* cytotoxicity effect.

The selectivity index of silver complexes showed the highest for compound **P2** (0.699) and the lowest SI value for compound **P3** (0.205). The highest SI value for the complex **P2** might be due to the presence of hydroxyl group at the *para* position as compared to -*ortho* position for **P3**. Adding to that, the complex P3 also consists of bromide group at *meta* position. The additional of methoxy group for complex **P6** at *meta* position, showed a slightly higher SI value of 0.393 as compared to complex **P3**. Based on the previous study, the insertion of methyl (Souza *et al.*, 2015) and hydroxyl (Smeijsters *et al.*, 1999; Sturm *et al.*, 2009) groups into the compounds did showed an improvement in the

antiplasmodial activity. Nevertheless, the importance of each functional groups in improving the selectivity of the silver complex remains uncertain.

4.4.2 Antiproliferative study

Transition metals like silver has long history as antimicrobial agent but its potential as cancer therapeutics has a lot to be discovered(Banti & Hadjikakou, 2013; DESOIZE, 2004). It has been reported that silver has anticancer activity *in-vitro*(Youngs *et al.*, 2009). The advantage of silver is that it has lower toxicity as compared with other metals like platinum (*e.g.* cisplatin)(Rafique *et al.*, 2010). The thiosemicarbazones have wide antiproliferative activity on different tumors cell lines and display common features of all compounds with carcinogenic potency(Arora *et al.*, 2014; Serda *et al.*, 2014). On the other hand, silver phosphine compound had shown to exert an *in-vitro* antiproliferative effect (Kyros *et al.*, 2010; Zartilas *et al.*, 2009).

Table 4.6: Antiproliferative activities of complexes 1- 6 (IC50 in µM).

Complexes	MDA-MB-231	HT-29	MCF-7
P1	15.11 ±4.24	-	19.62 ±4.87
P2	6.81 ±1.69	7.23 ±0.10	4.48 ±0.86
P3	-	6.86 ±0.17	3.51 ±1.13
P4	5.48 ±1.16	5.79 ±1.92	7.13 ±0.71
P5	5.27 ±1.44	6.03 ±0.76	4.34 ±1.69
P6	-	7.14 ±0.76	4.39 ±0.74

Based on the IC_{50} values, the antiproliferative effect on cancer cell lines MCF-7 and HT-29 for complexes **P2**, **P3**, **P4**, **P5** and **P6** displayed a modest potential (**Table 4.6)**. However, the complex **P1** was observed to own a weaker potential in all cancer cell lines tested. For the cancer cell line MDA-MB-231, the good antiproliferative effect was only

observed for complexes **P2**, **P4** and **P5**. Nonetheless, in comparison with clinically used metal compounds; cisplatin, this compounds showed better antiproliferative effect on MDA-MB-231 (IC_{50}=25.28 μM)(Wang *et al.*, 2016) and MCF-7 (IC_{50}= 35 μM)(Mansouri-Torshizi *et al.*, 2016) but not for the HT-29 (IC_{50}=5.28 μM)(Xu *et al.*, 2015). Thus, these compounds warrant an in-depth study as metallotherapeutic agents for cancer disease.

CHAPTER 5: CONCLUSIONS

5.1 Conclusion

A series of six silver complexes containing mixed ligands of 4-phenyl-3-thiosemicarbazone derivatives and triphenylphosphine were successfully synthesized and characterized. The spectroscopic data showed that all of the ligands and their complexes exist as a thione tautomer. The complexes were suggested to bind tightly to the metal centre through M-S bridging bonds in tetrahedral arrangements as they were synthesised in 1:1:2 silver to phosphine to thiosemicarbazone ratio. The silver(I) complex was dinuclear, having each silver atom coordinated to three thione sulfur atoms of thiosemicarbazone and to one phosphorus atom of PPh_3 in a nearly tetrahedral environment

The antiproliferative activity of these complexes were investigated towards the MDA-MB-231 and MCF-7 breast cancer cell line as well as HT-29 colon cancer cell lines. Compounds **P2**-**P5** shows good potential as metallotherapeutic agents. While for antimalarial study, the antiplasmodial activity against chloroquine resistant *P. falciparum* parasite shows good activity, however the compounds were found to be not as selective as the known drugs.

5.2 Recommendation for future work

In a future work, we attempt to grow crystal for further confirmation on the geometric arrangement and coordination of the silver complexes. In addition, the compounds **P2**-**P6** warrant an in-depth study as metallotherapeutic agents for cancer diseases. Besides that, the modification of the 4-phenyl-3-thiosemicarbazone structural frame and varying the substituents on the aromatic group in phosphine might also improve the SI value.

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LIST OF PUBLICATIONS AND PAPERS PRESENTED

Poster Presentation: Synthesis & Characterisation of Various Phosphone Stabilised Silver Complexes with 4-Phenyl-Thiosemicarbazones Ligands, 45th IUPAC World Chemistry Congress, 2015-08-09 to 2015-08-14, International Union of Pure and Applied Chemistry (IUPAC), (International).