



Faculty of Resource Science and Technology

**Synthesis, Characterisation and Antibacterial Properties of
Nitrobenzoylthiourea Compounds and their Silver(I) and Copper(II)
Complexes**

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Synthesis, Characterisation and Antibacterial Properties of Nitrobenzoylthiourea Compounds and their Silver(I) and Copper(II) Complexes

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A thesis submitted

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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ABSTRACT

Demand for highly resistant antibiotics has increased due to occurrence of multidrug-resistant microbial pathogens that reduced the efficiency of drugs. This study aims to synthesise and evaluate nitrobenzoylthiourea derivatives and their complexes for antibacterial activity, elucidate their mechanisms and optimize the properties of these compounds alongside their silver(I) and copper(II) thiourea metals through meticulous characterisation and molecular docking studies. The nitrobenzoylthiourea derivatives (**1–5**) and their complexes (**6–15**) were synthesised with yields ranging from moderate to excellent percentages (43–90%). Structural characterisation was conducted using Fourier Transform Infrared Spectroscopy (FTIR), ^1H and ^{13}C Nuclear Magnetic Resonance (NMR) Spectroscopy and CHNS elemental analysis. The FTIR spectra confirmed the formation of thiourea compounds, as indicated by the absence of the $\nu(\text{NCS})$ stretching band at $2000\text{--}2400\text{ cm}^{-1}$. The presence of absorption at $3100\text{--}3400\text{ cm}^{-1}$ attributed to the $\nu(\text{NH})$ band further supported this, indicating the complete reaction of all (NCS) with the amino acid. Moreover, the disappearance and shift of the $(\text{C}=\text{O})$ carboxylic peaks in the silver(I) and copper(II) thiourea complexes compared to the free ligand, suggest successful coordination of ligands to the metal ion through the carboxylate group. These compounds were later evaluated for their antibacterial activities against six different bacteria namely *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* via disc diffusion method. The complexes, particularly those with Ag^+ (**6–10**) and Cu^{2+} (**11–15**), exhibited enhanced antibacterial activity compared to the nitrobenzoylthiourea derivatives (**1–5**). Among all synthesised ligands (**1–5**), ((4-nitrobenzoyl)carbamoithioyl)alaninato (**4**) exhibited moderate antibacterial activity against

K. pneumoniae, with a 14 mm inhibition zone. ((4-nitrobenzoyl)carbamoithieryl)methioninato (2) exhibited mild antibacterial activity with 10 mm and 12 mm inhibition zones against *S. aureus* and *P. aeruginosa*, respectively. Comparatively, copper(II) thiourea complexes demonstrated better antibacterial activity than silver(I) thiourea complexes. Complex (11-15) revealed a mild to moderate antibacterial activity against *S. aureus* with an inhibition zone ranging from 10 mm to 15 mm. Additionally, di((4-nitrobenzoyl)carbamoithieryl)alaninatocopper(II) (14) displayed mild antibacterial activity against *B. cereus*, with 10 mm inhibition zone. Lastly, di((4-nitrobenzoyl)carbamoithieryl)valinatocopper(II) (13) showed moderate antibacterial activity with an inhibition zone of 16 mm against *K. pneumoniae*. Molecular docking analysis revealed promising binding energies for selected compounds with compounds (2) and (4) revealed superior binding energies of -7.7, -6.7, and -6.9 kcal/mol. Meanwhile, docking with copper(II) thiourea complexes demonstrates a higher binding energy of -10.4, -9.0, -9.4, -9.8 and -9.3 kcal/mol for complexes (11-15) against *S. aureus*. In contrast, complex (14) against *B. cereus* and complex (13) against *K. pneumoniae* demonstrated excellent binding at -8.3 and -9.0 kcal/mol, respectively. Due to limitations in the Autodock Vina program, the molecular docking analysis was restricted to the copper(II) complex. In summary, the antibacterial study on thiourea derivatives demonstrated their efficacy as antibacterial agents with enhanced activity from the integration of silver(I) and copper(II) thiourea complexes, render the potential of thiourea complexes as antibacterial agents.

Keywords: Thiourea, silver(I) complex, copper(II) complex, antibacterial activity, molecular docking

Sintesis, Perincian dan Sifat Antibakteria Sebatian Nitrobenzoilthiourea dan Kompleks Argentum(I) dan Kuprum(II)

ABSTRAK

Permintaan untuk antibiotik yang mempunyai ketahanan yang tinggi telah meningkat disebabkan kewujudan patogen mikrob perintang pelbagai ubat yang mengurangkan kecekapan ubat. Kajian ini bertujuan untuk mensintesis dan menilai terbitan nitrobenzoilthiourea dan kompleksnya untuk aktiviti antibakteria, mengkaji mekanisme mereka, dan mengoptimumkan sifat-sifat sebatian ini bersama-sama dengan argentum(I) dan kuprum(II) tioureaanya melalui pencirian yang teliti dan kajian dok molekul. Terbitan nitrobenzoilthiourea (1–5) dan kompleksnya (6–15) telah disintesis dengan hasil peratusan sederhana hingga tinggi (43–90%). Perincian struktur dijalankan menggunakan Spektroskopi Transformasian Fourier Inframerah (FTIR), ^1H dan ^{13}C Spektroskopi Resonans Magnet Nukleus (NMR) dan analisis unsur CHNS. Spektrum FTIR mengesahkan pembentukan sebatian tiourea, seperti yang ditunjukkan oleh ketiadaan jalur regangan $\nu(\text{NCS})$ pada $2000\text{--}2400\text{ cm}^{-1}$. Kehadiran penyerapan pada $3100\text{--}3400\text{ cm}^{-1}$ bagi jalur $\nu(\text{NH})$ membuktikan bahawa semua NCS telah bertindak balas dengan asid amino yang menjurus kepada pembentukan sebatian tiourea. Selain itu, kehilangan dan peralihan puncak karboksilik ($\text{C}=\text{O}$) dalam kompleks tiourea argentum(I) dan kuprum(II) berbanding dengan ligan bebas, memcadangkan tindak balas antara ligan dan ion logam melalui kumpulan berfungsi karboksilat telah berjaya. Kemudian, aktiviti antibakteria bagi semua sebatian tersebut dikaji ke atas enam bakteria berbeza iaitu Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes, Escherichia coli, Klebsiella pneumoniae, dan Pseudomonas aeruginosa melalui kaedah resapan agar. Hasil dapatan kajian menunjukkan

peningkatan aktiviti antibakteria bagi kompleks Ag^+ (6–10) dan Cu^{2+} (11–15), berbanding dengan ligan bebas, iaitu terbitan nitrobenzoiltiourea (1–5). Di antara semua ligan yang telah disintesis (1–5), ((4-nitrobenzoil)karbamotioil)alaninato (4) menunjukkan aktiviti antibakteria yang sederhana terhadap K. pneumoniae, dengan zon perencatan 14 mm. Manakala, ((4-nitrobenzoil)karbamotioil)methioninato (2) menunjukkan aktiviti antibakteria yang rendah dengan hanya zon perencatan sebanyak 10 mm dan 12 mm terhadap S. aureus dan P. aeruginosa. Secara perbandingan, kompleks tiourea kuprum(II) menunjukkan aktiviti antibakteria yang lebih tinggi daripada kompleks tiourea argentum(I). Kompleks (11–15) menunjukkan aktiviti antibakteria yang rendah hingga sederhana terhadap S. aureus dengan zon perencatan antara 10 mm hingga 15 mm. Tambahan pula, di((4-nitrobenzoil)karbamotioil)alaninatokuprum(II) (14) menunjukkan aktiviti antibakteria yang sederhana terhadap B. cereus, dengan zon perencatan 10 mm. Akhir sekali, di((4-nitrobenzoil)karbamotioil)valinatokuprum(II) (13) juga menunjukkan aktiviti antibakteria yang sederhana dengan zon perencatan 16 mm terhadap K. pneumoniae. Analisis pengedokan molekul menunjukkan tenaga ikatan yang memberansangkan bagi sebatian (2) dan (4) dengan tenaga pengikat yang tinggi sebanyak -7.7, -6.7, dan -6.9 kcal/mol. Sementara itu, pengedokan dengan kompleks thiourea kuprum(II) menunjukkan tenaga pengikat yang lebih tinggi sebanyak -10.4, -9.0, -9.4, -9.8, dan -9.3 kcal/mol untuk kompleks (11–15) terhadap S. aureus. Manakala, bagi pengedokan molekul kompleks (14) terhadap B. cereus dan kompleks (13) terhadap K. pneumoniae pula, tenaga pengikat yang ditunjukkan adalah sangat baik, masing-masing pada -8.3 dan -9.0 kcal/mol. Oleh kerana terdapat kekangan dalam program Autodock Vina, analisis pengedokan molekul hanya dijalankan untuk kompleks kuprum(II). Secara ringkasnya, kajian antibakteria terhadap terbitan tiourea menunjukkan keberkesanannya sebagai ejen antibakteria dengan peningkatan

aktiviti antibakteria yang ketara setelah digabungkan Bersama logam argentum(I) dan kuprum(II), menunjukkan potensi kompleks tiourea sebagai ejen antibakteria.

Kata kunci: *Tiourea, kompleks argentum(I), kompleks kuprum(II), aktiviti antibakteria, pengedokan molekul*

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LIST OF ABBREVIATIONS

Ag(I)	Silver(I)
CHNS	Carbon, Hydrogen, Nitrogen and Sulphur
Cu(II)	Copper(II)
^{13}C NMR	Carbon Nuclear Magnetic Resonance
DMSO-d ₆	Dimethyl Sulfoxide-d ₆
DNA	Deoxyribonucleic Acid
DMSO	Dimethyl Sulfoxide
EMA	European Medicines Agency
FDA	Food and Drug Administration
FTIR	Fourier Transform Infrared
^1H NMR	Hydrogen Nuclear Magnetic Resonance
KOH	Potassium Hydroxide
MIC	Minimum Inhibitory Concentration
NCS	N-Chlorosuccinimide
RNA	Ribonucleic acid

CHAPTER 1

INTRODUCTION

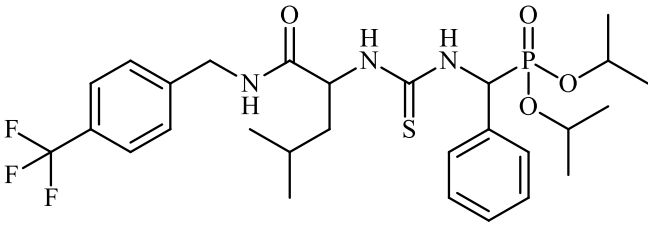
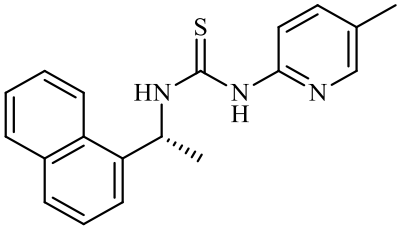
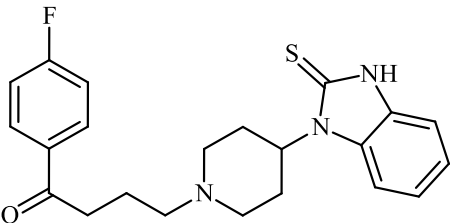
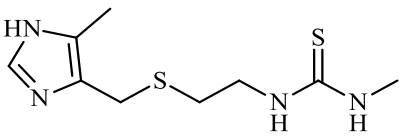
1.1 Antibacterial Properties of Thiourea

In recent years, there has been an increase interest in researching and developing novel antibacterial agents from diverse sources to combat bacterial resistance. Methods for screening and evaluating antibacterial activity have thus received increased focus. Current clinical pipeline and conventional organic antimicrobials primarily target specific biochemical processes are insufficient to combat the growing emergence and spread of antimicrobial resistance (Claudel et al., 2020). Therefore, it is highly necessary to design strategies for the development of new antibacterial agents that increase their efficacy and can be utilized by modern researchers.

Thiourea, also known as thiocarbamide, has been extensively utilised by researchers due to its diverse and promising applications. Due to the presence of reactive primary amine groups, thiourea is a suitable precursor for the synthesis of a vast array of derivatives. Thiourea and its derivatives have been reported to display a broad range of applications in the pharmaceutical industry owing to their biological activities (Halim et al., 2016) (Table 1.1). Additionally, thiourea which employed as an active ingredient in pharmaceuticals, has increases the usability of their derivatives as they pose no adverse risk on natural living bodies (Ohammad, 2018). The thiourea linkage has also played a significant role in the observed enhancements in various other fields, such as in herbicide study (Li et al., 2021), insecticidal (Lambert et al., 2017), rodenticidal study (Madabhushi et al., 2014) and as antiproliferative agent (Ghorbani et al., 2021). Building blocks found in established antimicrobial agents, such as functional groups bearing fluorine

atoms that can increase drug potency via metabolic stability and liprotein-ligand interaction, and quinoline cycles that have a structure similar to a well-known antimicrobial agent (Dolan et al., 2016), can both be considered as useful active units in thiourea-based compounds. The accessibility and efficiency of thiourea derivatives, as well as their potential antibacterial applications have made them highly desirable for antibacterial drug design.

Table 1.1: Example of biological properties of thiourea derivatives (Ronchetti et al., 2021; Shakeel et al., 2016)

Biological activity	Bioactive thiourea derivatives
 diisopropyl((3-(4-methyl-1-oxo-1-((4-(trifluoromethyl)benzyl)amino)pentan-2-yl)thioureido)(phenyl)methyl)phosphonate	Anti-viral
 (S)-1-(5-methylpyridin-2-yl)-3-(1-(naphthalen-1-yl)ethyl)thiourea	Anti-allergic
 1-(4-fluorophenyl)-4-[4-(2-sulfanylidene-3H-benzimidazol-1-yl)piperidin-1-yl]butan-1-one	Antipsychotic
 N-Methyl-N'-(2-[[5-methyl-1H-imidazol-4-yl)methyl]sulfany]ethyl)thiourea	Antihistamine

There are many commercial examples of thiourea containing drug as these building structure serve as excellent candidates for therapeutic applications. One worth mention is the antitubercular agent (thiocarlide and thioacetazone), anticancer agent (Enzalutamide) and antithyroid drugs (methimazole and carbimazole) (Doğan et al., 2020; Ronchetti et al., 2021; Choi & Jee, 2015) (Figure 1.1). Several reports and survey of publications have mentioned these drugs containing-thiourea compound exhibit their properties at cellular and enzymatic level thus indicates the ligand efficiency of thiourea compound. Thioureas are frequently used commercially in photographic film, plastics, pigments, elastomers, and textiles (Naz et al., 2020). With such mention applications, thiourea ligand have represent itself as moieties constitute a common framework of variety of drugs with wide range of properties.

Apart from these properties, thiourea also displayed significant antibacterial activity. Thiourea derivatives are versatile building blocks for the synthesis of numerous compounds and exhibit a broad range of biological activities, including antimicrobial properties (Soni et al., 2014). Due to their simple production, minimal toxicity, and favourable safety profile, thiourea derivatives have been the subject of numerous previous efforts to develop antibacterial agents. Encouraged by thiourea derivative's potential antimicrobial activity, evaluation on newly synthesised derivatives of their antibacterial action should be emphasised.

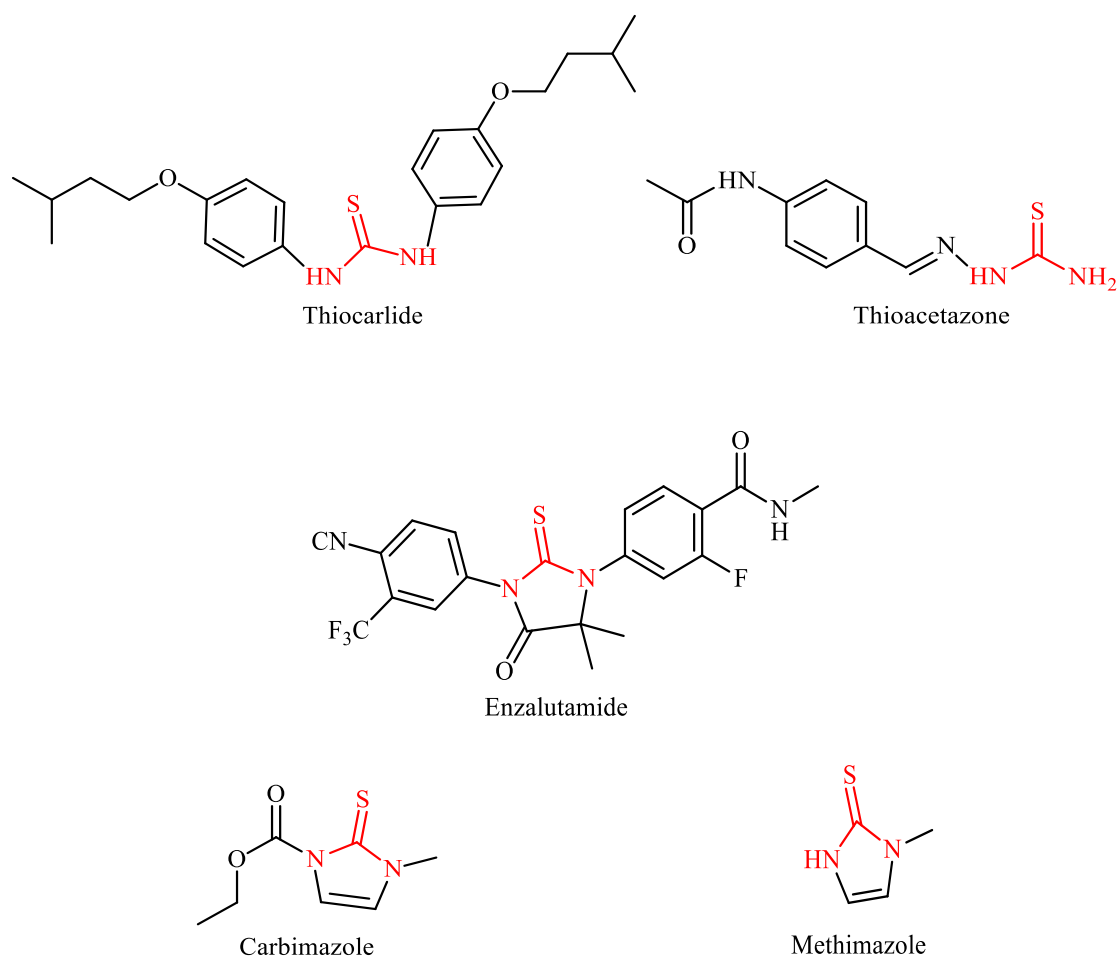


Figure 1.1: Chemical structures of thiourea-containing drugs (Doğan et al., 2020; Ronchetti et al., 2021; Choi & Jee, 2015)

Antibacterial properties of thiourea and its derivatives depended significantly on the presence of multifunctional sulphur and nitrogen atom donors. The heteroatoms of sulphur and nitrogen function as nucleophilic centres and allow the formation of intermolecular and intramolecular hydrogen bonds (Khan et al., 2020). Specifically, these hydrogen bonding interactions play a crucial role in determining the molecular structure, stability, and reactivity of the synthesised compounds (Pace et al., 2014; Fresch & Collini, 2023). The formation of hydrogen bonds contributes to the specific recognition and interaction of the compounds with

biological targets, including bacterial cell membranes and is essential for the of molecules with tailored functionalities and desired properties, including applications as antibacterial agent. Additionally, the presence of C=S groups on the same molecule makes it desirable for further chemistry (Rahman et al., 2021). The two main segments (C=S and NH) in thiourea provide interesting coordination modes for candidate of several applications. For instance, the C=S groups present in thiourea molecules offer unique reactivity that makes them suitable for various synthetic transformations. One example is their role as versatile ligands in coordination chemistry, where the sulfur atom can coordinate with transition metal ions to form stable metal complexes (Marakina et al., 2021). Due to their capacity to serve as hydrogen-bond donors, thiourea derivatives were also frequently employed as anion-binding receptors (Fakhar et al., 2017). The NH function is mainly responsible for the establishment of H-bonding (Shahzad et al., 2021). The presence of hydrogen bonding NH moieties boosts their solubility in aqueous media by facilitating interactions with water molecules through hydrogen bonding. The increased solubility promotes improved cellular uptake and transforming them into a biological agent with a wide range of biological characteristics (Canudo-barreras et al., 2021). The presence of a sulphur group as an electron-rich centre can also enhance lipophilicity and modulate the electron density of a compound. Sulphur's ability to donate electron pairs can increase its affinity for lipophilic environments, such as cell membranes, thus enhancing the molecule's ability to permeate through lipid barriers. This will eventually influence the organism's transmembrane diffusion capability and interaction with hydrogen bond donors (Kazeminejad et al., 2019), thereby influencing their biological activity and cellular interactions. Thiourea moieties proposing a better antibacterial activity is also because the C=S and NH groups of thiourea are readily protonated under acidic conditions (Ngaini et al., 2017). This

protonation renders these groups more reactive, allowing them to form stronger interactions with the carboxyl and phosphate groups on the bacterial surface, enhancing their activity (Bonomo et al., 2021), thus interfere with essential cellular processes, or facilitate the delivery of antibacterial agents into bacterial cells. With the mechanism of antibacterial action exhibited by thiourea, it could serve as a candidate for a potent antibacterial-inducing compound.

Furthermore, thiourea and its derivatives were also used as ligands in transition-metal-catalysed organic synthesis (Liu et al., 2014). The presence of the donor heteroatom in the ligand, such as oxygen, nitrogen, or sulphur, provides multibonding capacity to the thiourea molecule. They can be considered as a useful chelating agent due to their ability to encapsulate into their coordinating moiety metal ions (Binzet et al., 2013) especially with the presence of C=O and C=S functional group. Due to its capacity to coordinate with various metal ions as a monodentate or bidentate ligand, thiourea compound has attracted considerable interest as a versatile ligand with numerous applications (Yusof et al., 2010) to form stable complex (Figure 1.2).

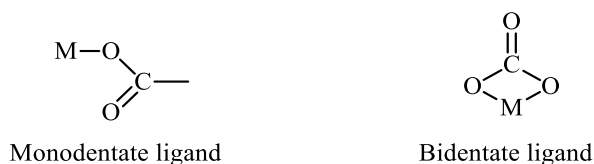


Figure 1.2: Example of thiourea- metal coordination

Thiourea compounds are exceedingly flexible as ligands, with the ability to bind to metal centers as neutral, mono-anion, or dianion. Due to the various coordination mode, thiourea metal complexes has gained growing interest in field such as metal extraction, synthetic precursor for nanomaterials, growth regulators for plant and drug synthesis (Yu et al., 2019). Sulphur atom

excellent donor properties make it possible for thiourea compound to form stable complex as it binds with metal ion. Therefore, this common strategies in modification thiourea derivatives into a stable complex with developed antibacterial properties is worth for further research.

1.2 Thiourea-Transition Metal Complexes

In recent years, a variety of metal complexes having thiourea ligands have been extensively discussed, particularly in the biological field, due to their antibacterial, antifungal, antimalarial, antitubercular and many other properties. The flexibility of thiourea due to their tautomeric form (thione and thiol) makes them easy to coordinate in different modes (Figure 1.3). Thiourea derivatives have the potential to be extremely versatile ligands due to their donor sites, which can facilitate the bonding between thiourea free ligand and metal ions. The donor atoms of oxygen, nitrogen, and sulphur in thiourea derivatives offer a variety of bonding opportunities as they are able coordinate through one or more of wide range of metal centres.

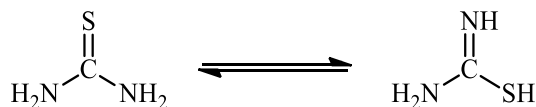


Figure 1.3: Tautomeric form of thiourea

Transition metal complexes exist in a diversity of oxidation states, allowing them to accommodate a wide range of organic molecules or ligands surround them (Khan et al., 2020). The remarkable capacity of thiourea to coordinate with transition metals give rise to highly stable complex structure. Some substituted thioureas have been structurally characterised as forming stable, neutral coordination compounds with various transition metal ions (Samarasinghe, 2018). Thiourea ligand having multi donor atom in their structure can contribute

as very good complexants. The enhanced functionality of thiourea provides accessibility and diversity of newly coordinated modes, ease of modification, and has piqued interest in the compatibility of thiourea-transition complex with the biological system.

The dynamic properties of transition metal compounds such as stability, or coordination have been of interest, especially for multi-drug-resistant bacteria (Tigineh et al., 2021). This is because the complexes formed when thiourea derivatives combine with soft metal ions such as late transition metals are highly thermodynamically stable (Khan et al., 2020). The thiourea-transition metal complex had also played a significant role in various other fields in which substituted thiourea and its Co(III) complexes have been used to combat infections that cause significant plant damage (Yang et al., 2012), Au(I) complex of thiourea was reported to autophagy or apoptosis by regulation of Reactive oxygen species (ROS) causes the damage of proteins, lipids and DNA (Stefa, 2021), and a diversity of metal complexes having thiourea ligands have been synthesised and analysed as useful intermediates in environmental control and as ionophores in ion-selective electrodes (ISEs) (Saeed et al., 2014).

Furthermore, thiourea derivatives and their transition metal complexes have been known for over a century and can be synthesised with a high yield (Mishra et al., 2012). The wide variety of metal–ligand combinations makes it possible to design new entities by varying the metal and its oxidation state, as well as the coordination geometries and coordination numbers, which give them the ability to tune the bond strengths and conformations of metal–ligand complexes (Zhang & Sadler, 2017). This tremendous enhanced-ligating properties might result in complexes with potential application as alternative drug for drug-resistant pathogens.

1.3 Antibacterial Properties of Thiourea Complexes

The antibacterial properties of thiourea metal complex in particular have been the subject of a significant amount of research, which has resulted in the abundance of detailed literatures. Both thiourea ligands and their metal complexes display important biological properties including antibacterial activity (Samarasinghe, 2018). It is known that metal complexes of ligands with sulphur donor atoms possess antibacterial properties (Abdullah & Salh, 2010). Thus, numerous biological applications for metal complexes with thiourea derivatives have been reported. Numerous metal complexes of thiourea derivatives with outstanding biological applications such as DNA binding, cytotoxicity (Khan et al., 2020), antimicrobial activity (Ikoko et al., 2015), and anticancer activity (Shahzad et al., 2021) have been studied to date. Most relevant examples in the field of medicinal chemistry are the platinum-based anticancer drugs cisplatin, oxaliplatin and carboplatin with nearly about 50% of all patients are treated with these compounds (Ghosh, 2019; Claudel et al., 2020). They are indeed characterised by a diverse set of properties that are applicable to a variety of biological areas. Thiourea complex activity as an antibacterial agent is particularly worth studying due to the possibility that their application could be a comprehensive approach to treating multi-drug resistant bacteria.

Thiourea complexes can have additional advantageous properties, such as enhanced lipophilic character of the central metal atom (Arslan et al., 2009), which results in a higher capability to penetrate the microorganisms through the lipid layer of the cell membrane. Increase in the bioavailability of the complex will facilitate the transportation of complex into bacterial cells and alter the thermodynamic properties of the complexes towards the targeted receptor. Metal complexes are thought to exert their effect through inhibition of enzymes, interaction with intracellular biomolecules, increased lipophilicity and modification of cell membrane functions

(Malik et al., 2018). Metal complex considerably improved the results given by thiourea ligand alone. The degree of lipophilicity of a molecule is a crucial factor in determining the magnitude of antimicrobial activity, as it directly influences the molecule's ability to penetrate microbial cell membranes. Therefore, the delocalisation of the electrons from the ligands to the central metal atom enhances the lipophilicity of the complex (Claudel et al., 2020). Whereas, the possibility of quick penetration in the cell membrane or strong coordination to the DNA strand also enhances the efficiency of metal-based drugs (Tigineh et al., 2021). With such benefits, it is anticipated that increasing the potency of thiourea complex in the modification of any desired direction through in-depth study and research will result in the production of a compound with superior antibacterial capabilities.

Therefore, thiourea ligand with outstanding criteria of having good physical and chemical stability, high selectivity and facile synthesis was able to accommodate the metal ions resulting in a wide variety of biological features. Nevertheless, the influence of metal ions towards structural properties of thiourea derivatives has also receive upsurge interest, resulting in the modification of physical and chemical properties of thiourea complexes that can be presented as a basis for future development. Thus, thiourea complex which can be afforded with stable coordination, easy accessibility, and minimal toxicity has the potential to be an effective antibacterial agent.

1.4 Problem Statement

The discovery of new medications has accelerated in recent decades due to the alarming increase in germs resistant to antibiotics (Fair & Tor, 2014). This is due to negligent antibiotic use, which resulted in the emergence of multidrug resistance bacteria (Möhler et al., 2017), limiting the efficacy of existing treatments and threatening public health. Microorganisms develop a variety of mechanisms to limit antimicrobial efficiency, including altering target sites, decreasing cellular permeability to allow for drug penetration, enzymatic destruction of antimicrobial substances, and biofilm development (Idrees et al., 2020). While the rise of antibiotic-resistant bacterial infections and the emergence of new resistance strains are concerning, the lack of development of new antibacterial classes must also be addressed (Dolan et al., 2016).

The pressing issue of antibiotic resistance necessitates the development of innovative antibacterial agents. Thiourea compounds, while demonstrating antibacterial activity, often suffer from limitations such as toxicity to human cells, a limited spectrum of activity, and the rapid development of bacterial resistance (Mancuso et al., 2021), hindering their effectiveness in real-world applications. Thus, understanding their efficacy against multidrug-resistant bacteria and their potential to overcome the limitations of existing treatments is essential for advancing the field of antimicrobial research. However, beyond these challenges lies the potential of nitrobenzoylthiourea derivatives and their metal complexes. While existing research has demonstrated their antibacterial properties (Saeed et al., 2010), there remains a need for more specific information about their current information and potential applications. Further investigation is warranted to elucidate their mechanisms of action and optimize their properties. By delving into the synthesis and characterisation of these compounds and their metal complexes, this research seeks to elucidate their potential as effective agents against multidrug-resistant microbial pathogens through meticulous

investigation and molecular docking studies. To address these challenges, this research focuses on the synthesis of nitrobenzoylthiourea derivatives and their metal complexes, namely with silver(I) and copper(II), as potential solutions. Nitrobenzoylthiourea compounds offer promising antibacterial properties, with the nitro group playing a significant role in enhancing antibacterial efficacy by interfering with essential bacterial processes (Noriega et al., 2022). Moreover, forming complexes with silver(I) and copper(II) ions further enhances their antimicrobial properties through synergistic effects (Garza-Cervantes et al., 2017). Therefore, this study aims to elucidate the mechanisms of action and optimize the properties of nitrobenzoylthiourea compounds and their metal complexes, addressing the critical need for effective and stable antibacterial agents against multidrug-resistant microbial pathogens.

1.5 Objectives

This research embarked on the following objectives:

- i. To synthesise thiourea derivatives derived from 4-nitrobenzoyl chloride and their Ag(I) and Cu(II) complexes.
- ii. To characterise all the synthesised compounds using FTIR, CHNS elemental analysis and ^1H NMR and ^{13}C NMR spectroscopy.
- iii. To evaluate the antibacterial activity of 4-nitrobenzoyl thiourea ligands and their Ag(I) and Cu(II) complexes against six different bacteria namely *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.
- iv. To elucidate the molecular interaction of ligand and their Ag(I) and Cu(II) complexes at the binding site of protein.

CHAPTER 2

LITERATURE REVIEW

2.1 Thiourea a Strong Compound for Antimicrobial Agent

2.1.1 Thiourea History

Thiourea is an organo-sulphur compound with formula $\text{CH}_4\text{N}_2\text{S}$. It is structurally similar to urea except that oxygen atom is replaced by a sulphur atom. Thiourea was first synthesised in 1873 by Polish chemist Marcell Nencki as the first urea analogue distinguished by the substitution of an oxygen atom for a sulphur atom (Ronchetti et al., 2021). Thioureas have important applications in organic synthesis and are intermediates in a number of organic synthesis reactions (Naz et al., 2020). The thiourea pharmacophore has specific binding locations known as the hydrogen binding region (NH), the complementary region (S), and the auxiliary binding region (1,3-substituents) (Pingaew et al., 2018). As a result, it is not surprising that a huge number of thiourea derivatives are used in a wide variety of novel structure coordination that can improve medicinal applications.

Thiourea functionality is important in medicinal chemistry because it allows for the formation of stable hydrogen bonds with biological target recognition elements such as proteins, enzymes, and receptors. The stabilisation of ligand-receptor interactions and the identification of bioactive sites are made possible in large part by a hydrogen bond network, which is further explained in section 2.5. Thiourea was described as an important pharmacophore in numerous pharmacologically active compounds, such as anti-inflammatory, anticancer, and antimicrobial agents (Nedeljković et al., 2023). Thiourea moieties constitute a common framework for a wide

range of medicines and bioactive compounds, giving it advantage in representing privileged structures in medicinal chemistry. It is interesting to note that numerous derivatives of thiourea are already in use in clinical settings, such as isoxyl which is used for tuberculosis treatment, while trovirdine is used for the treatment of human immunodeficiency virus (HIV) (Arafa et al., 2022). According to Ngaini and coworkers (2017), numerous techniques have been reported as being capable of producing this versatile group of thiourea derivatives since it is conveniently accessible and produces great yields. As a result of recent advances in the discovery of new disease targets, there is a growing interest in the design and development of thiourea-based therapies.

2.1.2 Antimicrobial Resistance and Its Global Concern

Emergence of resistance among bacterial pathogens is acknowledged as a significant threat to global public health. Since the beginning of the antibiotic era, people have known that some bacteria are resistant to antibiotics. However, it has only been in the last twenty years that dangerous, antibiotic-resistant strains have been showing up with a disturbing regularity. Some have asserted that a post-antibiotic era is imminent as a result of the escalating evolution of antibiotic resistance and the diminishing supply of antibiotics (Fair & Tor, 2014). The World Health Organization (WHO) has ranked antimicrobial resistance (AMR) among the top ten global public health and development hazards to humanity. Therefore, AMR has emerged as one of the most significant public health concerns of the 21st century (Murray et al., 2022). Antimicrobial resistance (AMR) is defined as the ability of microorganisms to survive and proliferate in the presence of antimicrobial agents (Abushaheen et al., 2020). It typically caused by harmful pathogen such as parasites, fungi and bacteria that develop mutation to resist the

drugs designed to kill them, making infections more difficult to treat and increasing the risk of disease transmission, severe illness, and mortality.

Antibiotics have become one of the most important medical treatments needed for the growth of more complex medical approaches (Munita & Arias, 2016). However, the improper use of antibiotics accelerates the rate at which microorganisms develop resistance. According to the World Health Organization, misuse and exploitation of antimicrobials are the primary causes of the emergence of drug-resistant pathogens. In 2015, 30% of outpatient antibiotic prescriptions were unnecessary, with 50% of antibiotics prescribed for acute respiratory infections being unnecessary (Habboush et al., 2023). An increase in the use of antibiotics, whether through increase consumption or overuse, is one of the many factors that could contribute to bacterial resistance. For example, penicillin which is a popular commercial antibiotic introduced in the early 1940s was significantly being used to combat many diseases (Dodds, 2017). However, the excessive use of antibiotics in humans has lead to the emergence of resistant organisms with various mechanisms of resistance (Reygaert, 2018). In addition, a relatively common occurrence of antibiotic resistance, multidrug-resistance, and extensively drug-resistance is occurring as a result of decreased development of novel-acting antibiotics (Alam et al., 2019). The antimicrobial resistance mechanism (Figure 2.1) were divided into four categories (Uddin et al., 2021; Reygaert, 2018). As antibiotics lose their efficacy, even the most common infectious diseases have become more difficult to treat. Therefore, the persistent emergence of AMR diminishes the effectiveness of treating infectious diseases (Bonomo et al., 2021). As a consequence of drug resistance, antimicrobial medications become ineffective, and treating infections becomes increasingly challenging.

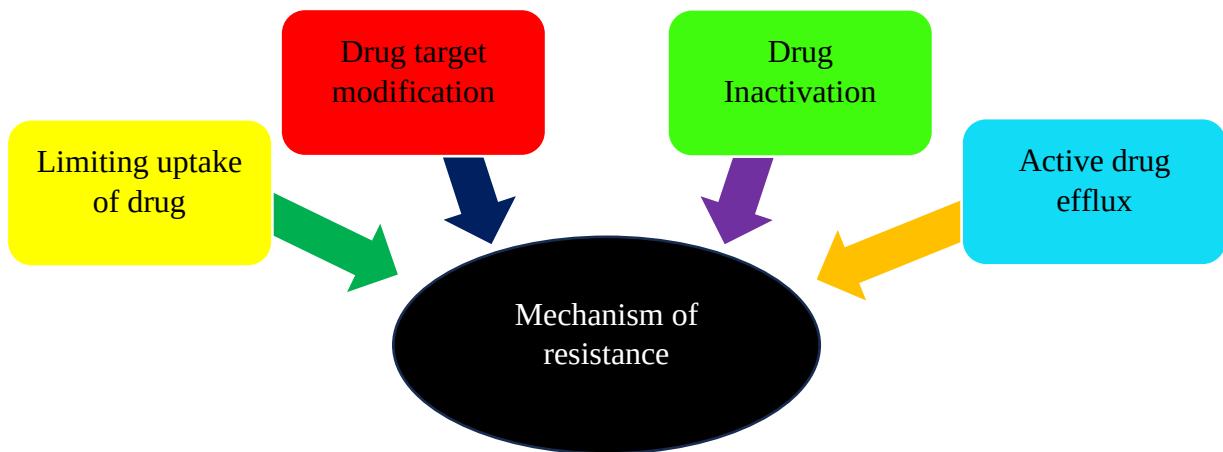


Figure 2.1: Antimicrobial resistance mechanism.

According to a 2019 report by the World Health Organization (WHO), AMR is responsible for the fatalities of 700,000 people, with the number expected to rise to 20 million by 2050 (Uddin et al., 2021). Whereas, according to the 2019 Antibiotic Resistance (AR) Threats Report from the Centers for Disease Control and Prevention (CDC), more than 35,000 people die as a consequence, with at least 1.27 million deaths in 2019 alone. Displayed in Figure 2.2 are global fatalities (counts) attributable to and associated with bacterial antimicrobial resistance by infectious syndrome (Murray et al., 2022).

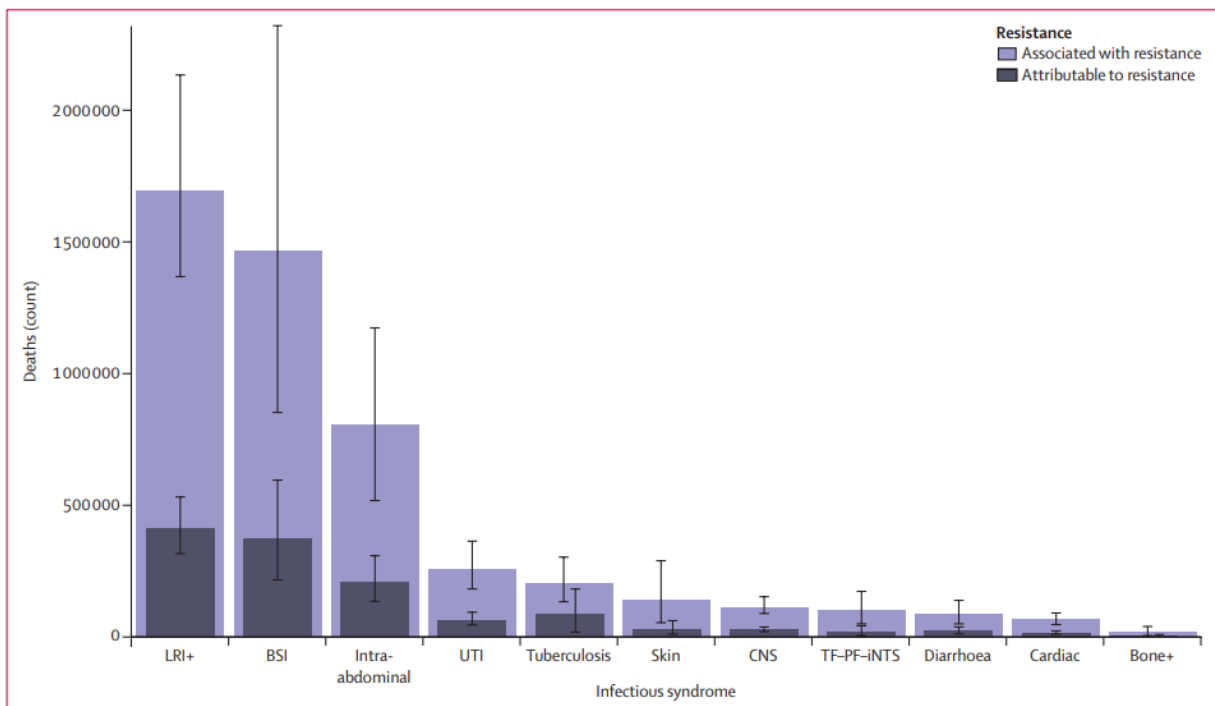


Figure 2.2: Chart of global deaths (counts) attributable bacterial antimicrobial resistance. Graph obtained from study reported by Murray and co-worker (2022)

Though the scenario is grim and can be regarded as natural process, lack of awareness and knowledge can accelerate the emergence and spread of antimicrobial resistance. In the 21st century, natural genetic evolution to resist antibiotics has reached paradoxical proportions, making AMR a worldwide health threat that requires early intervention (Abushaheen et al., 2020). Therefore, there is a need for an increased number of scientific advancements that have the potential to aid in the discovery and development of new drug-resistant pathogens.

2.1.3 Thiourea Exhibiting Microbial Resistance Properties

Significant research is required to establish a new paradigm of an effective antibiotic. Novel chemical scaffold-based antibacterial compounds are a significant development in the fight against antibiotic resistance (Hou et al., 2023). The ability of substances to interact with receptors and trigger particular cell responses, as well as the biochemical mechanisms of pathogen cross-resistance, must be incorporated into the development. Thiourea is a sulphur-bearing scaffolds, which are present in many biologically active agents with antibacterial activities (Hashem et al., 2020). Increasing evidence demonstrates that thiourea derivatives play a crucial role in medicinal chemistry and drug discovery (Canudo-Barreras et al., 2021). Due to its notable importance, regulatory bodies such as the Food and Drug Administration (FDA) and European Medicines Agency (EMA) have granted approval for several thiourea-containing compounds to be marketed as medicinal products for the treatment of various human diseases (Ronchetti et al., 2021).

Recently in the search for novel antimicrobial agents, it was found that thiourea derivatives have been reported for biological activities. For instance, 1-(2'-furanyl) acyl-3-(2'-aminophenyl) thiourea possesses anti-intestinal nematode activity and it is also a prodrug (Özgeri, 2021), nicotinoyl thiourea 3 (ACH-806) which has been use as an inhibitor of hepatitis C virus (HCV) (Özgeri, 2021; Yang et al., 2013) and several analogues of thiourea derivatives inhibit the catalytic site of bacterial type II topoisomerases, specifically DNA gyrase and topoisomerase IV (Bielenica et al., 2015).

Thiourea moieties which consist of C=S and NH group play important role in antibacterial activity. This is primarily because the C=S and NH groups of thiourea are readily protonable in acidic conditions and can react with the carboxyl and phosphate groups on the

bacterial surface, thereby enhancing the activity (Bonomo et al., 2021). Thiourea-derived compounds have been shown to be beneficial as antibacterial agents with various structural alterations that may improve a compound's biological activity. Several other studies have demonstrated the effectiveness of simple thiourea molecules as bioactive compounds following certain modifications (Mital et al., 2015). Thiourea derivatives, including cyclohexyl thiourea and phenylthiourea, are considered promising model compounds in the study of solid-state chemistry. This is mostly owing to their ability to form intramolecular and intermolecular hydrogen bonds, which involve N-H proton-donor groups and predominantly sulphur atoms (Rahman et al., 2021). While naphthylthiourea, phenylthiourea, and 1,3-diphenylthiourea were extremely cytotoxic to rat hepatocytes, the addition of short-chain alkyl substituents to the phenylthiourea core decreased its toxicity (Bielenica et al., 2015). In contrast, modification of isoxyl, the symmetrical diphenylthiourea derivative, yielded a library of antimycobacterial compound (Maalik et al., 2019). Because thiourea compounds have so many diverse uses and qualities, their antimicrobial efficacy must be investigated further. Before delving into the significance of thiourea as an antimicrobial agent, it is necessary to comprehend the mechanism of action of antibiotic antibacterial compounds in inducing specific bacterial cell responses. Antibacterial activity can be categorised according to one of the five mechanisms listed in Table 2.1. Meanwhile, Table 2.2 depicted few examples of thiourea exhibit antibacterial mechanisms. By elucidating the mechanisms through which these derivatives exert their antibacterial effects, valuable insights into novel therapeutic approaches that may effectively combat multidrug-resistant microbial pathogens are gained. This underscores the significance of understanding microbial resistance mechanisms in developing more effective antibacterial agents.

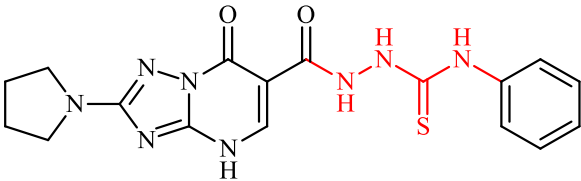
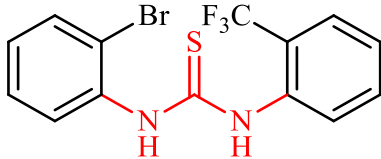
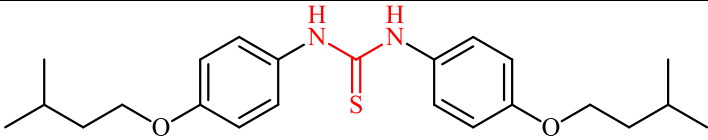
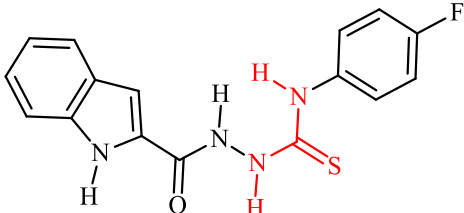
Table 2.1: Mechanism of antibacterial activities

Mode of action	Background	Drugs example and their mechanism of action
Interfering with bacteria cell wall synthesis	Specific antibiotics interfere with the synthesis of peptidoglycans, rendering the cell vulnerable to osmotic pressure and autolysis which result the bactericidal antibiotic to inhibit the synthesis of cell wall (Uddin et al., 2021). Microorganism are then killed due to the inhibition of peptidoglycans biosynthesis by these cell wall inhibitor (Kirmusaolu, 2017).	Bacterial cell was made by a cross-linked peptidoglycan is essential to the integrity of cell wall structure (Rani & Patri, 2019). β -lactam antibiotics (such as penicillin and its derivatives) bind with the target site of penicillin-binding proteins (PBPs) to prevent the cross-linking of the glycan chains in bacterial cell wall from occurring (Abushaheen et al., 2020).
Inhibition of bacteria protein biosynthesis	Protein synthesis is a complex process involving various of enzyme. The complex 70S units ribosome bacteria consist of 30S and 50S subunits (Krause et al., 2016). Specific antibiotics interfere with the subunits of the 70S bacterial ribosome to terminate protein translation.	Protein is translated from mRNA by tRNA in ribosome (Sahra et al., 2019). Tetracycline antibiotics diffuse passively through porin channels in the bacterial cell membrane and bind to the 30S ribosomal subunit, inhibiting tRNA binding to the mRNA-ribosome complex by obstructing the A (aminoacyl) site where aminoacyl-tRNA docks onto the 30S subunit (Grossman, 2016).
Interference with bacterial nucleic acid synthesis	Antibiotics can inhibit replication, transcription, and folate synthesis of microorganisms which then interfere with the nucleic acid synthesis of bacteria (Sahra et al., 2019).	Quinolone drugs interfere with DNA synthesis of bacteria by disrupting bacterial topoisomerase type II (Pham et al., 2019) an enzyme involved in DNA replication. It targets two essential enzymes of bacteria, DNA gyrase and DNA topoisomerase IV, by binding reversibly to the DNA-protein complexes containing gyrase and topoisomerase IV. Quinolones also inhibit enzyme function by blocking the resealing of the DNA double-strand break thereby functioning as topoisomerase poisons (Hooper & Jacoby, 2016).

Table 2.1 continued

Mode of action	Background	Drugs example and their mechanism of action
Inhibition of metabolic pathway	Antibiotics function as anti-metabolites, or competitive inhibitors of bacterial metabolic enzymes, to control bacterial infections (Uddin et al., 2021). Specific antibiotic target bacterial metabolic enzyme by inhibit the enzyme involve in the synthesis of bacterial Tetrahydro-folic Acid (TH4) which is a cofactor in many reactions, especially in the synthesis of amino acids and nucleic acids.	Folic acid metabolism is essential to ensure the proper functioning of all living cells, where it participates in the reactions of synthesis of some amino acids (Fernández-Villa et al., 2019). Trimethoprim is a synthetic antibiotic, in which it exerts antimicrobial activity by prevents reduction of dihydrofolate to tetrahydrofolate (active form of folic acid) by inhibiting the enzyme dihydrofolate reductase (Waller & Sampson, 2018). Thus, prevent folate synthesis by bacteria decreases production of folic acid to a level where bacteriostatic inhibition of growth occurs.
Inhibition of bacterial membrane function	Several antimicrobial drugs interfere with several targets by interacting with the bacterial membrane via a lipophilic moiety, resulting in membrane structural degradation and functional impairment (Hurdle et al., 2011)	Polymyxin B and colistin primarily membrane-targeting antibiotic cause cell damage leading to cell death by disrupting the membrane integrity (Mohapatra et al., 2021). Whereas polymyxin B treatment induces lipid exchange between the outer and inner membranes of Gram-negative bacteria, resulting in membrane disruption overall (Moffatt et al., 2019).

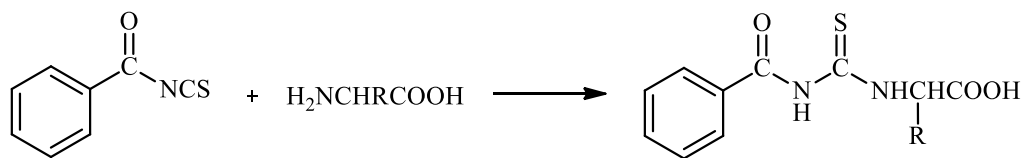
Table 2.2: Examples of thiourea derivatives exhibit antibacterial mechanism

Mode of action	Structure	Action
Inhibition of bacteria protein biosynthesis	 2-(7-oxo-2-(pyrrolidin-1-yl)-4,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine-6-carbonyl)-N-phenylhydrazinecarbothioamide	The activity against both gram-positive and gram-negative bacteria is enhanced by the disruption of protein synthesis (Hashem et al., 2020)
Interference with bacterial nucleic acid synthesis	 1-(2-bromophenyl)-3-(2-(trifluoromethyl)phenyl)thiourea	By exerting its inhibitory effect on topoisomerase IV, this compound demonstrates the greatest efficacy against both standard and methicillin-resistant strains of <i>Staphylococcus aureus</i> and <i>Staphylococcus epidermidis</i> (Bielenica et al., 2018)
Interference with mycolic acid biosynthesis	 1,3-bis(4-(isopentyloxy)phenyl)thiourea	Affect mycolic acid production, a long-chain fatty acid in mycobacterial cell envelopes that makes the cell envelope complex and impermeable, making the bacteria less susceptible to antibiotics (Tapera et al., 2022).
Interference with bacterial nucleic acid synthesis	 N-(4-fluorophenyl)-2-(1H-indole-2-carbonyl)hydrazinecarbothioamide	The preliminary models of a novel category of gyrase and topoisomerase IV inhibitors (Kowalczyk et al., 2021).

2.2 Synthesis of Thiourea

Thiourea compounds have recently gained considerable interest for its potential use in preparation of wide variety of biological active compounds (Alkherraz et al., 2014). The versatility of the thiourea backbone enables the development of numerous new compounds. It has successfully served as the basis for the development of several compounds with a broad range of biological activity which is useful as insecticides, bactericides, and plant growth regulators (Zhao et al., 2013). There are numerous ways for synthesising thiourea derivatives, all of which are simple and yield-enhancing. In the majority of preparations, C=S-containing reactants such as isothiocyanates, dithiocarbamates, carbon disulfide, thiophosgene, and their equivalents are utilised (Steppeler et al., 2020).

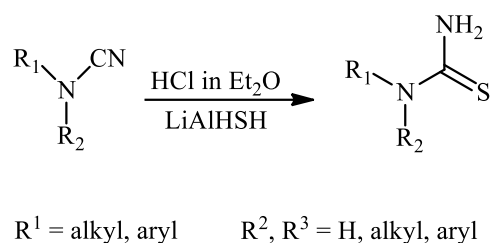
A review of the literature reveals that isothiocyanate is a key intermediate in synthesising thioureas (Maddani & Prabhu, 2010). Isothiocyanate forms di- or trisubstituted thiourea derivatives when react with amino acids and amines molecule. Numerous methods have been published for producing isothiocyanates, which are needed to synthesise thiourea derivatives. For instance, the reaction of an acyl chloride with ammonium thiocyanate in the presence of phase transfer catalysis (PEG-400) to produce acyl isothiocyanate (Wu et al., 2012). Acyl isothiocyanate reacts with appropriate substituted anilines to create acylthiourea, with reaction proceeding readily as aniline is both a nucleophile and a base catalyst (Li et al., 2010). General synthesis pathway of thiourea from isothiocyanate and amino acid is shown in Scheme 2.1.



Scheme 2.1: Preparation of thiourea from isothiocyanate and amino acid (Li et al., 2010)

Preparation of thiourea from isothiocyanate and amino acid usually used to obtain monodentate thiourea derivatives. Isothiocyanate is a valuable building block in natural product and synthetic access to isothiocyanate are convenient, obtained principally by reacting primarily through the reaction of primary amides with highly electrophilic C=S sources (Scattolin et al., 2017). When alcohols are used as solvents, the limited long-term stability of isothiocyanate reactants and the prospect of side product formation are frequently cited as disadvantages (Steppeler et al., 2020).

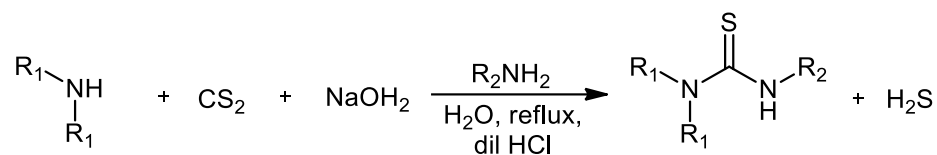
Alternatively, the synthesis pathway involving the reaction of ammonia with carbon dioxide to form cyanamide (H_2CN_2) and subsequent reaction with hydrogen sulfide (H_2S) to form thiourea has been explored (Odularu et al., 2021). General synthesis pathway of thiourea derivatives from cyanamide is shown in Scheme 2.2.



Scheme 2.2: General method for synthesis of Thiourea derivatives from Cyanamide (Shakeel, 2016)

It was also utilized to obtain monosubstituted thiourea (Shakeel, 2016). The most prominent characteristic of this method including low energy consumption, low pollution source, rich raw material origin, less product of impurity and green cleaner process (Wang & Lv, 2011). However the reaction is difficult to carry out due to carbon dioxide's inertness and the reaction yield is typically low, resulting in a high production cost (Dai et al., 2019).

Additionally, in another approach, the synthesis of dithiocarbamate is pivotal. This synthesis process involved the utilization of carbon disulfide and ammonia as the primary reactants to produce dithiocarbamate, which is then decomposed to generate ammonium thiocyanate which is then isomerized to form thiourea (Dai et al., 2019). The synthesis pathway of thiourea derivatives from carbon disulphide was depicted in Scheme 2.3.



Where $\text{R}_1 = \text{R}_2 = \text{alkyl}$

Scheme 2.3: General method of synthesis of thioureas from Carbon disulphide (Steppeler et al., 2020)

This method is utilised to synthesise symmetrical and asymmetric thioureas, yielding a variety of di- and trisubstituted thiourea derivatives (Maddani & Prabhu, 2010). It is also a simple method for preparing substituted thioureas because the starting materials are readily available and no additional synthetic stages are necessary (Ding et al., 2019). However there are few disadvantages, including its flammability and volatility, necessitate its use in excess, and the atomic economy suffers from the formation of sulphur-containing by-product, typically hydrogen sulphide (Steppeler et al., 2020).

The preparation process of the desired compounds is determined primarily by its structure's functionality and the availability of necessary starting materials. This is due to the importance of developing a high yielding, gentle, and straightforward preparation process for the synthesis of thiourea. The potential disadvantages associated with the use of particular

reactants, such as their limited stability, toxicity, flammability, and production cost, should not be overlooked.

2.3 Thiourea Bearing Amino Acid Side Chain

Amino acids are important primary building blocks of protein which are linked *via* peptide bond forming predefined sequences to constitute the backbone of all known protein. Amino acids not only serve as building elements for protein synthesis in microorganisms, but also play significant roles in their metabolism, survival, and play a crucial role in microbial proliferation (Dai et al., 2022). Along with other efforts for medication modification, amino acids may be a promising choice in the fight against microbial resistance. Antimicrobial resistance has had a significant impact on the global health system, as microorganisms have developed the ability to resist antimicrobial drugs, either by lowering their efficiency or by rendering them completely inactive (Idrees et al., 2020). Thus, the synthesis of biologically active amino acid-coupled derivatives has lately gained significant attention due to the fact that the coordination of thiourea-amino acid derivatives to multiple transition metal ions forms stable complexes that could be applied as potential antibacterial agents (Fakhar et al., 2017).

According to the study, when biologically active chemicals are chelated, they exhibit increase bacteriostatic properties. This arises from the interaction between transition metal ions and amino acids, resulting in the immersion of biological significant molecules (Chohan et al., 2006). For instance, Kadir and co-worker (2016) has reported that by combining thiourea-metal complex with phenylalanine enhances the antibacterial activity of the compound. This is contributed by the delocalised electrons in the phenyl moiety which increased the compound's

lipophilicity, for better penetration of the bacteria's cell wall. In another study conducted by Wan Zullkiplee and coworker (2016), tris-thiourea derivatives with amino acid side chains have shown antibacterial action against *E. coli* as concentration increases, owing to the amino acid substituent's capacity to boost the selectivity toward targeted cations. Furthermore, amino acids have been utilised in conjunction with antimicrobial medications (*in vitro*) and have been demonstrated to improve the efficiency of the drugs against planktonic bacteria (Idrees et al., 2020). The biological activity of transition metal-amino acid complexes has been extensively documented (Al-haidery, 2012), with the amino acid side chain acting as a linker in the complex formation. Each amino acid had a unique antibacterial effect (Yoshihara et al., 2021) while, understanding the role and mechanism of amino acids in boosting antibiotic activity will allow for the practical development of novel antibiotic active ingredients.

2.4 Biological Activities Of Silver(I) and Copper(II) Complexes

2.4.1 Silver and Copper as Transition Metal

Transition metal is the elements found at the middle portion of periodic table specifically elements found in the group 3 through 12 of periodic table. The majority of transition metals share similar physical characteristics, including being excellent heat and electricity conductors, hard and ductile, and having high boiling and melting points. Compounds with biological activity that are conjugated to a transition metal may exhibit enhanced or distinct biological activities, since transition metal complexes have demonstrated higher biological activities than their non-complex counterparts (Ikoko et al., 2015). Additionally, metal ions, which are positively charged and electrophiles, tend to bind and interact with biological components like DNA and protein because they are electron-rich (Boros et al., 2020). Therefore, it is established that the coordination of a drug with particular transition metals affects the drug's antibacterial and biological activities (Möhler et al., 2017).

Silver and copper have been incorporated into wide variety of commercial products due to their enhanced antibacterial qualities, particularly in light of their lengthy history as antibiotics in human health care. Their usage trace all the way back to 1832, when workers worked in copper mines appeared to be immune to cholera during outbreaks in Paris, France (Grass et al., 2011). While silver, in the form of 0.5 % silver nitrate aqueous has been extensively used to treat severe burns since 1960 as an alternative to antibiotics (Nherera et al., 2017), and their efficacy in the wound dressing sector has continued to improve. In fact, the development and use of silver and copper as antibacterial agents has been steadily enhanced over the years. Surprisingly, silver remains an effective alternative for preventing and treating topical

infections, as Ag^+ ions may likely enhance antibacterial activity when combined with certain antibiotics, which have increased their action against Gram-negative bacteria and appear to contain more resistance determinants than antiseptics (Dissemond et al., 2020). Copper's antimicrobial range has also been proved in variety of fields, most notably clinical health care. In 2008, it was discovered that microbes become inactive upon contact with copper, and the US Environmental Protection Agency (EPA) later supported the use of copper surfaces as an anti-microbe agent, which eventually resulted in the adoption of copper vessels as a low-cost potable water alternative (Tian et al., 2012). Copper surfaces have been demonstrated to reduce total microbial counts and the prevalence of infections in real-world investigations conducted in hospitals (Inkinen et al., 2017).

Silver and copper's antimicrobial qualities may be highly dependent on their concentration or availability, as well as their antibacterial mechanism of action against microorganisms. For example, silver has little germicidal activity in its inert metallic form but, once silver binds to bacteria, its activated ionised Ag^+ exhibits antimicrobial activity via intracellular absorption. The activated Ag^+ ion bound to electron donor receptors such as disulphide, amino, imidazole, carbonyl, and protein phosphate residues on bacteria's cell membrane, resulting in intracellular absorption *via* endocytic vacuoles and phagocytosis (Tri Handok et al., 2018). This could result in the inactivation of the microbial enzyme system, hence degrading the cell's functionality. In another study, Sim and coworker (2018) has identified three mechanisms: silver cations reacting with peptidoglycan components, forming pores and puncturing the bacterial cell wall; silver ions entering the cell and inhibiting respiration and disrupting metabolic pathways; and bacterial DNA and replication cycle deterioration. Once inside the cell, silver ions bind to DNA and RNA molecules, condensing them and making it

harder for ribosomes to transcribe or read DNA and RNA, which is an essential process for protein synthesis and cell division (Mohamed et al., 2020). Silver-induced degenerative alterations in bacterial RNA, DNA, mitochondrial respiration, and protein cells have been shown to result in cell death, although the mechanism by which silver ion Ag^+ interacts with bacteria is unknown (Curran & McCann, 2009).

The similar principle might be applied to copper, as copper exposure has been shown to impair the integrity of the bacterial cell wall, resulting in the microorganism's mortality (Montero et al., 2019), while also increasing their susceptibility to copper ions, which cause metabolic dysfunction. According to Arendsen and coworkers (2019), when copper ions reach a critical intracellular concentration, the plasma membrane becomes increasingly permeable, allowing important cell content including nucleotides, amino acids, and potassium. Copper ions are able to donate and accept electrons and oxidise from Cu^+ to Cu^{2+} via the formation of reactive oxygen species (ROS), thereby causing damage to the cell membrane. This catalyses the formation of ROS, with major consequences of oxidative stress to membrane lipids, amino acids in proteins, and nucleic acids, ultimately induce variety of cellular response and resulting in enzyme deactivation due to oxidative stress (Kurutas, 2016; Arendsen et al., 2019). Other conditions that may need to be addressed for copper to exhibit antimicrobial action include copper concentration, antimicrobial element size, copper shape, type of contact, and chemical environment (Vincent et al., 2018). Moreover, biocidal metals, such as silver and copper, exhibit the additional attribute of prolonged activity, gradually releasing their cations at amounts that are detrimental to bacteria (Wakshlak et al., 2015).

Silver and copper's caustic and irritant properties toward microorganisms, as well as their vast variety of applications, deserve additional research since it will build a new pathway

for understanding the chemical activity of the compound and its biological application. A thorough understanding of the toxicity of silver and copper toward bacteria should be subjected to comparative studies, particularly in metal-ligand coordination, as this can provide a better understanding of the resistance mechanism prior to clinical trials involving the development of new drugs with increased microorganism resistance.

2.4.2 Silver(I) Complexes

Due to the extensive range of biological properties of silver(I), research on its coordination with ligands to generate silver complexes has advanced. Extensive research has been conducted on silver complexes, which have been shown to be even more powerful against bacteria than silver salts (Abarca et al., 2012). Silver ion interacts with a broad spectrum of bacterial and fungal cell components, resulting in microbial cell suppression at extremely low concentrations (Alasmary et al., 2015). These biological capabilities are primarily due to the silver's ion ability to bind to bacterial enzymes. Silver binds to thiol groups on proteins and enzymes, inhibits cellular respiration, destroys the cell membrane, and interference to nucleic acids (Jimenez et al., 2017). A growing amount of research has been undertaken on the application of silver(I) complexes, as the complexes are leading candidates for the treatment of a variety of infectious disorders (Rahimah et al., 2018).

Numerous ligands have been investigated for their capacity to bind with silver achieve the desired biological features. The choice of ligand is critical in the creation of new metallotherapeutic drugs and in the discovery of new pathways for the biological features of silver complexes since it must give both physical and chemical qualities necessary for the desired properties. Additionally, the ligands utilised in silver(I) complexes operate as a carrier

for the silver(I) ion into the biological system, and the magnitude of silver complex's antibacterial capabilities may be connected to their ease of participation in ligand exchange processes (Azócar et al., 2014).

Active Ag^+ ions generate strong silver-ligand coordination, which is essential because the silver complex action results in the release of Ag^+ , that can penetrate the cell membrane and cause cell death. Silver complexes exhibit a broad spectrum of antibacterial action, eradicating both Gram-positive and Gram-negative bacteria, demonstrating that silver is a more harmful metal to microbes than a number of other metals (Rakhshani, 2019). While silver compounds are well known to have good antimicrobial activities, it is worth to mention that ligands coordinate to the metal complex play crucial role in enhancing the bioactivity of compound. Combination of N-Heterocyclic carbenes (NHCs), pyridine derivatives and mixed ligand derivatives employed in antibacterial research boosted their biological capabilities. This is due to their antimicrobial, anticancer, wide structural variety and diverse physical, photophysical as ligand complex (Ali et al., 2013; Altaf & Stoeckli-Evans, 2010).

The activities of NHC-silver complexes are influenced by the NHC ligand structures, which include factors such as hydrophobic substitution and steric bulk on the imidazole ring, which can slow the rate of silver ion release (Prencipe et al., 2021). Imidazole ligand is an excellent ligand due to present of electron rich atom in the imidazole ring. In 2017, report by Johnson and coworker reported that the lipophilic characteristic of Ag(I) -NHC acetate complexes (Figure 2.3) exhibited enhanced antibacterial activity against Gram-positive and Gram-negative bacteria (10-12 mm areas of clearance) allow better penetration through the lipid membrane.

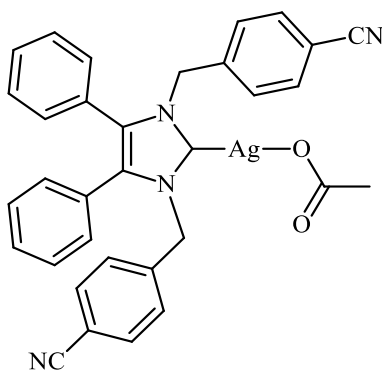


Figure 2.3: Example structure of NHC-silver complexes (Johnson et al., 2017)

NHC-silver complex is a cyclic compounds that are typically formed by deprotonating salts of ligands such as imidazolium, benzimidazolium, diazepinium, and pyrimidium (Gök et al., 2014). It has been used to create more stable silver(I) compounds due to the strong silver-carbon bond and the NHC core's capacity to be modified for a wide variety of applications (Liang et al., 2018). Results indicated that silver carbene complexes exhibited a range of antibacterial activity against bacteria and fungus and the activity was depending on the nature of the ligand utilised (Özdemir et al., 2010).

Furthermore, the presence of some pyridine derivatives, such as nicotinic acid and picolinic acid, has a significant effect on the physiological function (Kalinowska-Lis et al., 2014). In 2016, Felczak and coworker have successfully synthesised a silver(I) complex of 4-(hydroxymethyl)pyridine (Figure 2.4), which demonstrated excellent cytotoxicity against tumour cells, the silver(I) complexes also demonstrated selectivity toward various types of tumour cells, which was likely due to the type of ligand attached to the silver(I) ions, as it likely depicted the complex's stability and hydrophilicity-lipophilicity of the complex formed by the ligand.

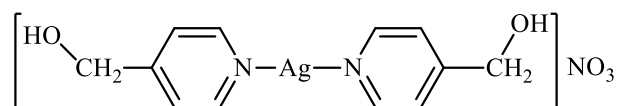


Figure 2.4: Example structure of pyridine-silver complex (Felczak et al., 2016)

Pyridine-silver complex was obtained when metal-pyridine integrates pyridine and its derivatives, which can act as bidentate or tridentate ligands in the production of metal complexes. Pyridine's Lewis basic character, which is based on its nitrogen lone pair, qualifies it as a ligand for transition metals, as well as its potential to form a range of metal complexes (Pal, 2018). Some pyridine-based ligands have demonstrated substantial cytotoxicity and have been shown to be antitumor agents when combined with metals (Altaf & Stoeckli-Evans, 2010).

Furthermore, ligand system including both phosphine and nitrogen-based donor ligands is an example of silver-mixed ligand complex (Kharat et al., 2011). The phosphine ligand appears to be critical in determining the complex characteristics as their presence boosts the lipophilicity and membrane permeability of metal-based complexes which makes them as an active compound (Keter et al., 2014). Liang and co-workers (2018) explained on the antibacterial activity of new silver(I) complexes 5,5-diethylbarbiturate (barb) complexes with a series of bis(diphenylphosphino) alkanes exhibited antibacterial activity against both gram-positive and gram-negative strains being comparable to AgNO_3 and silver sulfadiazine, while displayed an effective cell growth inhibition by inducing apoptosis via mitochondrial pathways (Figure 2.5).

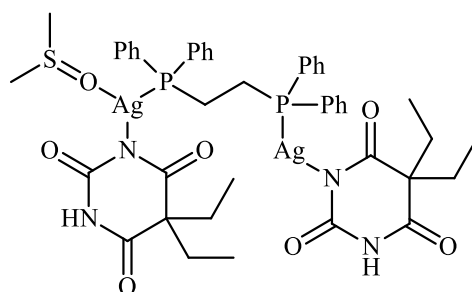


Figure 2.5: Example structure of mixed ligand-silver complex (Liang et al., 2018)

Mixed ligand-silver complex is a silver(I) complexes containing a variety of N, S, O, P, and C donor ligands. Mixed ligand complexes contain at least two distinct categories of ligands bound to the same metal ion, and the presence of more than one type of ligand in a complex increases the possibility of variance in the complex's predicted features (Wankhede et al., 2014). Silver(I) complexes comprising mono- or bidentate phosphine ligands and thiol or thione moieties have well established applications as anticancer and antimalarial agents (Rahimah et al., 2018).

The concern of silver metal and complexes in overcoming this enduring microbial resistance crisis should be a focal point of attention in the coming years. Simultaneously, it can be concluded that complexation of bioactive ligands with silver boosted their biological capabilities when compared to their free ligands.

2.4.3 Copper(II) Complexes

Copper ion have shown a significant important of its properties in combating microorganism. The relative contribution towards the damage of membrane cell wall would be due to the final mechanism of the release of copper ions, Cu^+ and Cu^{2+} (Salah et al., 2021). Additionally, copper ions can interact with DNA via surface association and bind to DNA

residues, resulting in considerable propagation of reactive oxygen species (ROS) and consequent DNA breakage (Lian et al., 2016). As the broad spectrum of copper complexes exhibiting biological features may be dependent on a variety of factors, it is wise to assess the complexity of the compound's structure. On the other hand, the addition of coligands may alter the planarity, hydrophobicity, and coordination geometry of copper complexes, hence increasing the affinity of copper-based agents for DNA and enhancing their biological activity (Lian et al., 2016). A study conducted by Cabiddu (2022) has reported a Cu(II) having auxiliary carboxylate ligand with general formula of $[\text{Cu}(\text{phen})_2(\text{CAn})](\text{NO}_3)$ and $[\text{Cu}(\text{phen})_2(4\text{-Mecdoa})]$ (Figure 2.6). It is revealed that the introduction of an auxiliary carboxylate moiety increased the affinity for DNA, DNA-cleaving activity, and DNA damage in complexes.

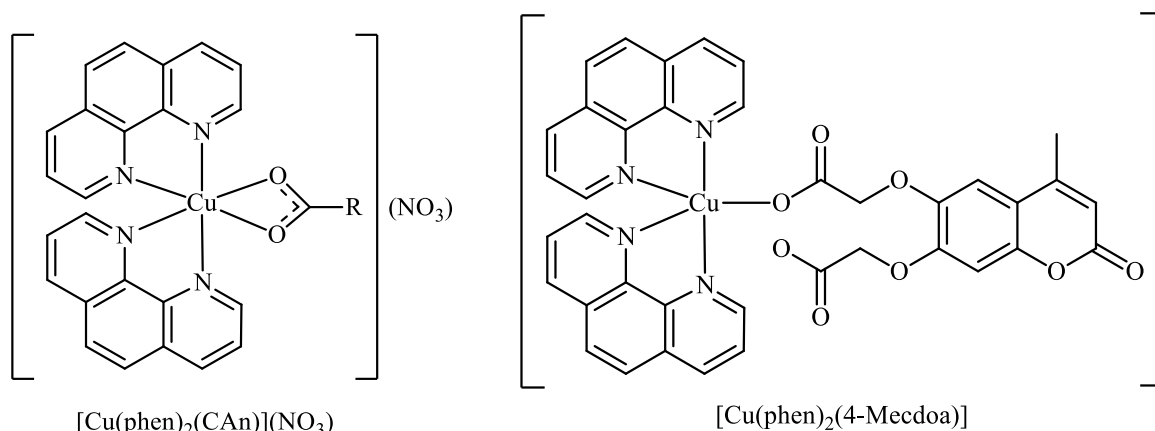


Figure 2.6: Example structure of copper-auxiliary carboxylate ligand (Cabiddu, 2022)

The $[\text{Cu}(\text{phen})_2(4\text{-Mecdoa})]$ appears to interfere with DNA synthesis without directly interacting with DNA, the induction of cell death is dose-dependent and switchable between apoptosis and necrosis (Cabiddu, 2022). Essentially, the metal ion covalently bonds to critical biomolecules, impairing their function and ultimately resulting in cell death via several cellular processes such as apoptosis and necrosis (Boros et al., 2020).

In addition, another research investigation on copper complex coordination aroyl hydrazone derivatives involved the synthesis of several derivatives with varying complex-to-ligand ratios, $[\text{Cu}(\text{L})(\text{NO}_3)(\text{H}_2\text{O})]\text{H}_2\text{O}$ (Figure 2.7). It was found that a 1:1 ratio of copper to ligand induces apoptosis reactive oxygen species (ROS), elucidated its potent anticancer activity (Deng et al., 2016).

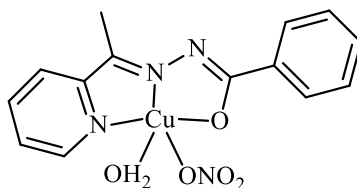


Figure 2.7: Copper complex of aroyl hydrazone derivatives (Deng et al., 2016)

Pathway of reactive oxygen species (ROS)-mediated mitochondrial dysfunction, which kills cancer cells without significantly harming normal cells, whereas the coordination numbers of the copper ion probably have a much more direct effect on the cytotoxicity (Deng et al., 2016). Copper(II) complex antimicrobial and anticancer activity is mediated through intercalative interactions with DNA and DNA degradation caused by the complex's metallonuclease activity (Rostas et al., 2020).

Furthermore, copper(II) coordination with a variety of N, S, P, and O donor ligands was also extensively reviewed due to the copper-containing coordination compound's potential application (Figure 2.8).

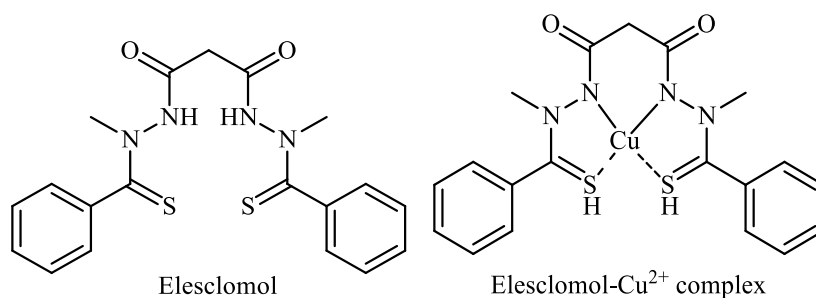


Figure 2.8: Example of mixed ligand-copper complex (Krasnovskaya et al., 2020)

Elesclomol is a chemotherapeutic agent ligand for leukaemia. It has been shown to exert antitumor activity by forming Cu(II) coordination, which results in oxidative stress within the malignant cell, disrupts cellular energy metabolism, and initiates the path of mitochondrial apoptosis in tumour cells, ultimately resulting in their death (Krasnovskaya et al., 2020).

It was also reported that several copper coordination compounds based on 1,10-phenanthroline (Figure 2.9) exhibited greater antimicrobial activity than their free ligand (Krasnovskaya et al., 2020). The author suggests that the coordination compound's interactions with the bacterial chromosome resulted in a decrease in bacterial reproduction, whereas copper ion's redox activity, is dependent on the presence of reducing agents. This implies in the imbalance between oxidant and antioxidant in favour of the oxidant causing interruption of the redox equilibrium (Dumanović et al., 2021).

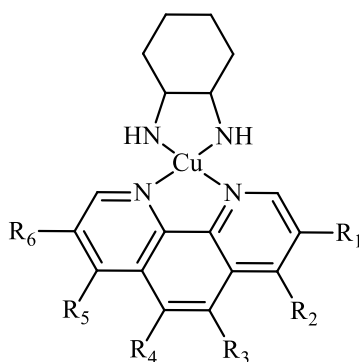


Figure 2.9: Structure of 1, 10-phenanthroline (Krasnovskaya et al., 2020)

The continuous and quick development of copper's broad-spectrum biocidal characteristics, together with the advancement of research toward clinical trials, has resulted in an increasing awareness of copper's antimicrobial application in health care (Arendsen et al., 2019). Moreover, copper-based medications are anticipated to be less toxic than other metallodrugs owing to copper's essentiality in the body (Khan et al., 2020). Copper(II) complexes have demonstrated a promising antibacterial and therapeutic impact, and the stability of the copper coordination structure interaction suggests that it may be a useful coordination chemical for clinical usage.

2.4.4 Thiourea Silver(I) and Copper(II) Complexes: Carboxyl Interaction & Biological Activity

Thiourea also known as thioureaorgano catalyst is a useful catalyst for organic synthesis. The nitrogen and sulphur donor atoms contribute significantly to the structural variety and bonding modes of thiourea derivatives in coordination chemistry. Due to the availability of hard and soft donor sites, thiourea ligands are well-known as selective metal binders (Ghazal et al.,

2018). Thiourea derivatives are excellent ligands for metal ions due to their presence of S and N atoms that act as donor sites for transition metals such as Ni^{2+} , Zn^{2+} , Pb^{2+} , Fe^{3+} , Hg^{2+} , Pb^{2+} , Ag^+ , and Cu^{2+} (Fakhar, Yamin, et al., 2018). Therefore, owing to the existence of active functional groups C=O, C=S, and NH, thiourea exhibits a broad spectrum of microbiological activity, as they can provide suitable sites for microbial interaction. Report supported by Singh and coworkers (2017) discussed several factors that may influence the rate of inhibition of microbial growth by thiourea derivatives, including the number of thiourea units in the thiourea backbone, the lipophilic characteristic, the structure-activity relationship of thiourea derivatives and their complexes, and the thiourea substitution. According to Wan Zullkiplee and coworkers (2016), thiourea derivatives exhibit antibacterial activity resulted from their functional group reactivity with the carboxyl and phosphate groups on the bacterial surface. The potential nucleophilic properties of the sulphur and nitrogen atoms inside thiourea complexes, along with the presence of charge on the molecules, could have potentially facilitated their ability to permeate the bacterial cell wall, thereby inhibiting bacterial development (Ikokoh et al., 2015).

The complexation of thiourea with silver(I) and copper (II) has been extensively studied due to their structural diversity and potential biological effects. Thiourea, as a soft base, exhibits an affinity to bind with soft acids such as mercury, copper, silver, and cadmium ions (Fakhar et al., 2017). Thiourea and thiocarbonyl compounds in general are soft ligands, because they possess a relatively low charge density, making them preferentially interact with soft metal ions that are also low in charge density, via the sulphur atom (De Marco, 2013). This coordination occurs as a result of the desolvation of soft acid Ag^+ or the acceptor of Ag(I) with the donor atoms of thiourea ligands such as S and N, at the point where the hydrogen atoms of the ligand's amide group interact with the solvent's oxygen atom, facilitating the coordination process.

Furthermore, the primary source of activity exhibited by silver compounds is attributed to the release of Ag^+ ions, which readily bind to thiol and imidazole functional groups. Although Ag^+ ions can undergo precipitation, nucleophilic attack, and reduction in physiological environments, their stability and activity can be modulated by employing appropriate auxiliary ligands (Lin et al., 2013).

According to Aldabaldetrecu and co-workers (2018), the nature of the Ag-OOC bond of the ligand in antibacterial complexes is important, as it features a compound of silver ions coordinated with the ligand carboxylic acid groups, as illustrated below (Figure 2.10):

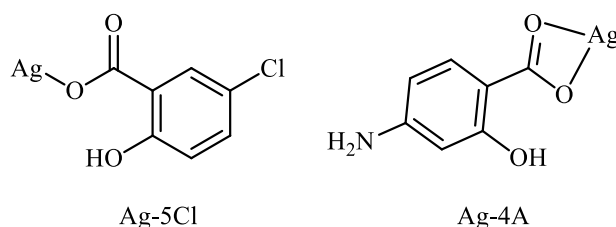


Figure 2.10: Compound of silver(I)-carboxylate ligand coordination (Aldabaldetrecu et al., 2018)

Apart from that, phosphine involvement in silver-ligand coordination were also being applied widely. Silver(I) complexes with monodentate or bidentate phosphine ligands and thiol or thione moieties, in particular, have displayed a long history of use as anticancer and antimalarial drugs (Rahimah et al., 2018). The rate of silver ion Ag^+ release from carboxylic compounds may be the determining factor in stability and antibacterial activity. The study also revealed that compounds with more covalent bonds would exhibit a delayed release of silver ions, as Ag-4A is more effective over time due to its higher silver content compared to Ag-5Cl, which has stronger covalent bond between the silver ion and the carboxylate group of the ligand (Aldabaldetrecu et al., 2018). Moreover, structure-activity relationship of thiourea carboxylate

silver(I) coordination suggested increase antimicrobial activity due to the weak Ag-O bond which easily replaced with O-, N- or S- donors of biological ligand in comparison to the Ag-N bond, thus strengthen the ionic character of the interaction (Azócar et al., 2014). The ligand-exchange reactions have suggested the relative order of Ag⁺ bond strength are from large affinity for S- and P-donors to relatively small affinity for O- donors (Tsendzughul & Ogwu, 2020).

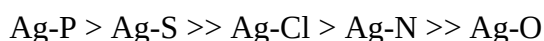


Figure 2.11: Relative order of Ag⁺ bond strength (Tsendzughul & Ogwu, 2020)

In other study, minimum inhibitory concentration (MIC) value shown broader antimicrobial activity spectra for silver complexes with Ag-O and Ag-N bonds compared with the bonds with Ag-P and Ag-S (Manuel, 2012). For instance, 1,10-phenanthroline (phen) and its derivatives are advantageous N-donor ligands for the synthesis of new active silver(I) complexes due to their ability to act on a wide range of biological functions (Segura et al., 2014).

Furthermore, the diverse range of biological activities exhibited by Cu-thiourea complexes can be ascribed to the capacity of copper to engage in redox reactions and coordinate ligands through various mechanisms including acting as monodentate ligands through the sulphur atom in mononuclear complexes, as well as forming S-bridges with metal ions in dinuclear complexes (Drzewiecka-Antonik et al., 2020). Copper's capability in living organism to alternate between Cu(I) and Cu(II) oxidation levels also makes it an ideal biological cofactor, notably in electron transfer pathways (Ghazal et al., 2018). As for copper-thiourea complex,

some reports demonstrated that the antibacterial activity of some compounds was enhanced when they are reacted to with copper (Halim et al., 2016).

In another study, Cu(II) complex having Cu-OOC were synthesised and distorted octahedral structures observed and the complex was screened for antibacterial activity (Figure 2.12) (Ramesh et al., 2016). The study also revealed that $[\text{Cu}(\text{PCBCH})_2(\text{H}_2\text{O})_2]$ were found to be more effective than their antibacterial drugs, streptomycin, against Gram positive (*B. subtilis*, *S. aureus*) and Gram negative (*E. coli*, *K. pneumonia*) bacteria (Ramesh et al., 2016).

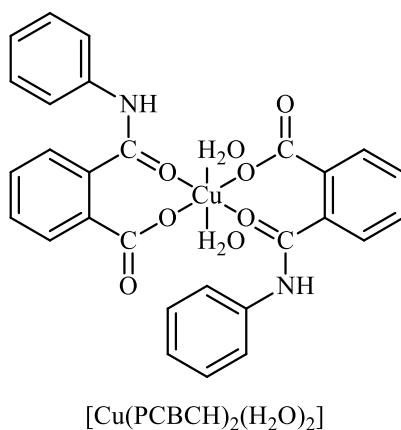


Figure 2.12: Compound of copper(II)-carboxylate ligand coordination (Ramesh et al., 2016))

Additionally, the high interest in copper complexes with sulphur and additional nitrogen ligands originate from their use in biological and pharmaceutical research, as well as their ability to form a variety of structural motifs in which these ligands can coordinate metal atoms via either sulphur or nitrogen atoms, or both (Herres-pawlis et al., 2017).

Furthermore, it have been reported that thiourea complexes containing a copper(II) ion (Figure 2.13) were more effective at combating hospital bacteria from growing than standard bacterial rods, with their efficiency being dependent on the type of electronegative

functionalities attached to the benzene ring (Stefa, 2021). For instance, 3-chloro-4-fluorophenylthiourea and 4-iodophenylthiourea appeared to have the most effect on microbial strains with MIC values of 4 g/mL (Stefa, 2021).

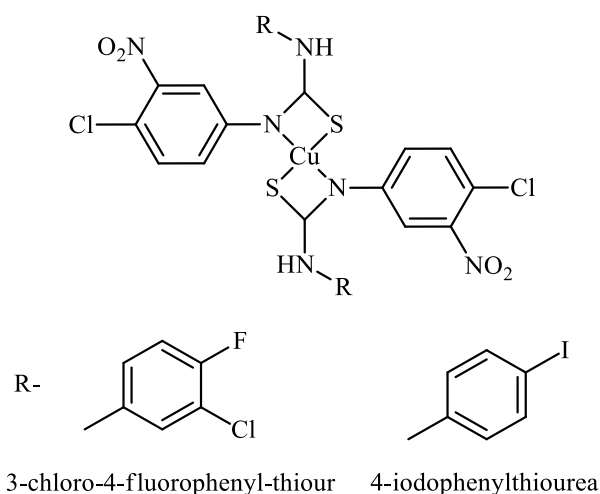


Figure 2.13: Thiourea complex exhibit inhibitory growth property (Stefa, 2021)

The enhanced activity of a complex is most likely due to the ligand's chelation effect, which increases the delocalisation of p-electrons across the entire chelate ring, thereby increasing the lipophilicity of complexes, which is responsible for their penetration into lipid membranes, interferes with enzyme activity, and may result in cell apoptosis (Khan et al., 2020). This behaviour could explain why chelation tends to make the ligand act as a more powerful and potent antibacterial agent, as it reduces the polarity of the metal atom by partially sharing the positive charge of its metal ion with a donor group, thereby enabling the possible delocalisation of electron over the entire chelate ring (Knittl et al., 2018).

The biological properties of metal complexes depend upon the type of metal and its oxidation state and nature of the ligand (Khan et al., 2020). Thus, the enhanced donor atom functionality and strong coordination ability of thiourea derivatives contribute significantly to

their ability to display metal-ligand biological characteristics. Due to the same reason, when thiourea-derived ligands are coordinated with transition metal ions, their biological potency increases, implying that complexation can be used as a dose reduction strategy, as the coordination process reduces the polarity of the metal cation through partial charge sharing with donor atoms and electron delocalisation over the chelate (Bielenica et al., 2018). This results in increased of the metal atom's lipophilicity and its capacity to penetrate microorganisms through the lipid layer of the cell membrane.

As a result, it is reasonable to believe that the metal complex can greatly enhance the capabilities of the thiourea ligand alone, allowing for the development of a novel thiourea drug by modifying the thiourea ligand-metal coordination. Coordination of thiourea derivatives with transition metals may result in the formation of stable complexes capable of a broad range of biological functions.

2.5 Molecular docking

Docking of small molecule compounds into the binding site of a receptor to predict the interaction occur within the complex is an important part of the structure-based drug design process. The first molecular docking algorithm was develop by Kuntz et al. in the year of 1982 through the estimation of the released binding energy (Azad., 2023). Molecular docking is a computer method that tries to figure out virtually how two biological macromolecules (protein, DNA/RNA, peptide) or small molecules (endogenous ligands, drugs) bind to each other (Pantsar & Poso, 2018). Molecular docking can be used to distinguish between putative binders and non-binders in large chemical databases at the atomic level, enabling us to characterise the behaviour of small molecules in the binding site of target proteins and to elucidate fundamental

biochemical processes (Seeliger et al., 2010; Meng et al., 2012). Report by Tao and co-workers (2020) describe the standard flow chart of molecular docking process which mainly involves spatial matching and energy matching between ligand and receptor for optimal conformation and focuses on agreeability (Figure 2.14).

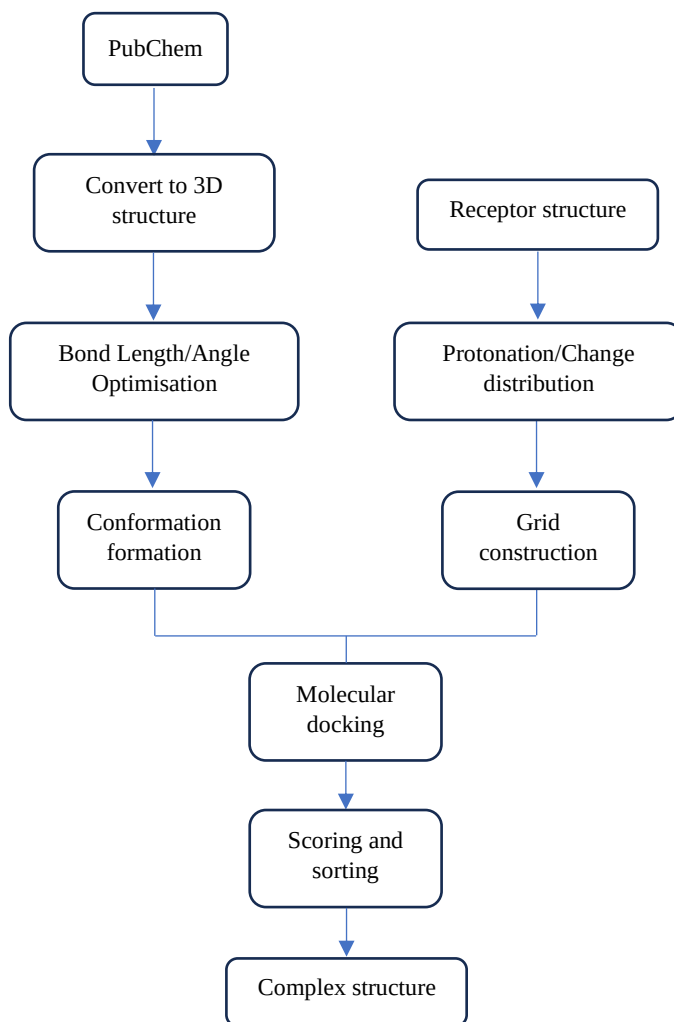


Figure 2.14: Flow chart of molecular docking process (Tao et al., 2020)

2.5.1 Role of Molecular Docking in Antibacterial Study

Molecular docking has become the most popular and effective structure-based method for predicting the interactions between molecules and their biological targets at atomic level. In antibacterial study, the knowledge of ligand target binding within the protein binding site help in understanding the complex process occur on the cellular level to develop improvement for the structure-based drug design. Molecular docking techniques seek to predict the best binding mode of a ligand to a macromolecular partner (considered to be the protein), in order to better comprehend the biological perspective, as ligand-protein interactions are involved in numerous biological processes with consequential effects (Salmaso & Moro, 2018). This study was performed to examine the structural and functional factors that influence the mode of action in antibacterial activity (Echeverría et al., 2017). By analysing the binding interactions between the synthesised compounds and their target proteins, such as ionic interactions, hydrogen bonds, and van der Waals interactions, we can assess the affinity of the compounds for their targets. This information allows us to predict the binding site of the target protein and the synthesised compound (referred as pose), which was assessed as their affinity score, thereby providing insights into how the compound may exert its antibacterial effects (Pantsar & Poso, 2018). Some of the crucial docking information for antibacterial study may pertain to the fundamental interaction involving ligand function in biologically active compounds, which justifies the use of molecular docking. (Figure 2.15). Molecular docking is typically performed by first predicting the molecular orientation of a ligand within a receptor, and then estimating their complementarity through the application of a scoring function. (Pinzi & Rastelli, 2019).

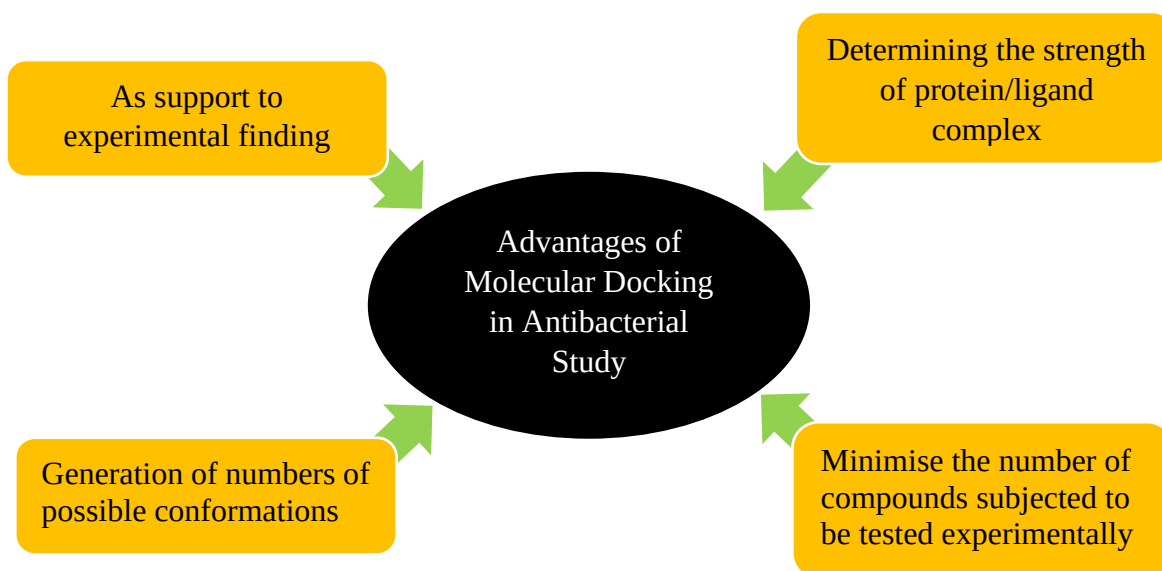


Figure 2.15: Advantages of molecular docking

The biologically important molecules play a significant role in bioactivity of drugs as it is governed by molecular recognition through drug and target protein interactions. Consequently, comprehending the technique to control various biochemical processes by analysing the effect and function of compounds was utilised frequently in drug design (Ahuja et al., 2022). Among the multiple forces that are involved in protein-ligand interactions, hydrogen bonds play a crucial role in protein-ligand interactions by effectively stabilising the interaction between a drug and its receptor (Ghosh & Brindisi, 2020). The capacity of substances to form hydrogen bonds between donor and acceptor groups is a crucial aspect of their molecular recognition and bioactivity. Meticulous analysis of hydrogen bonding patterns reveals the precise molecular interactions driving the binding between compounds and the target proteins involved in bacterial cell processes. This understanding facilitates the rational design of novel therapeutics with improved potency, optimized chemical structure, and enhanced selectivity against multidrug-resistant microbial pathogens. Moreover, other interactive processes in

molecular docking between protein and ligand include electrostatic interaction, van der Waals forces and hydrophobic interaction (Tao et al., 2020). Example of docking involving thiourea derivatives functionality in biological active compounds are presented in (Figure 2.16).

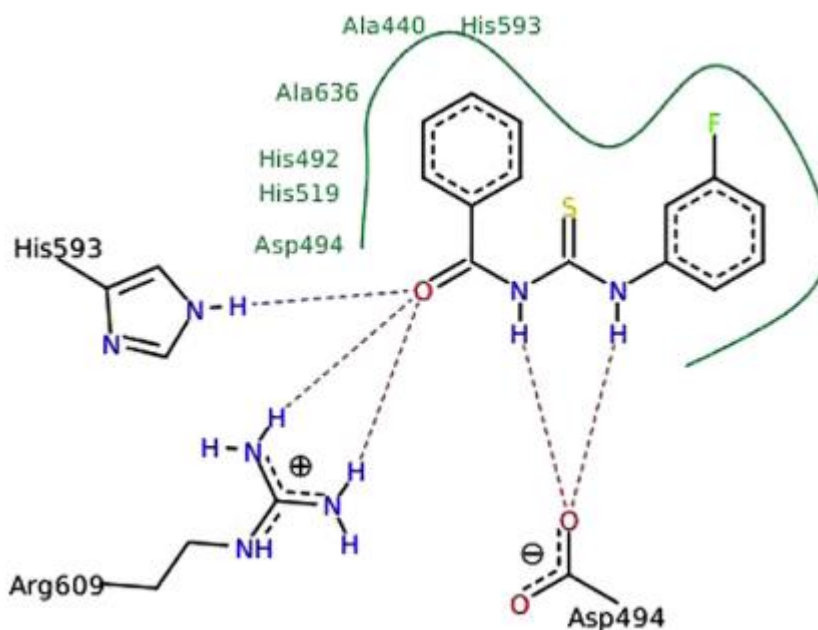


Figure 2.16: Illustration of interactions involve in molecular docking (Rauf et al., 2013)

An illustration of the X-ray crystallographic structure of a thiourea derivative is provided, in which a green curved line is employed to indicate hydrophobic interaction with the neighbouring residues, while the dotted lines represent hydrogen bonding interactions with the residues Asp494, His593, and Arg609 (Rauf et al., 2013). Thus, the molecular mechanism of intermolecular interaction at molecular level can be better characterised after identifying the interaction force together with information supported by the experiments. From the docking study, predicting and analysing the binding modes and affinity between potential drug candidates and their target proteins will aid in the rational design and optimization of novel

therapeutics. By understanding the precise interactions at the molecular level, including the binding modes of thiourea compounds and their metal complexes with target proteins involved in bacterial cell processes, key residues and structural features essential for antibacterial activity can be identified.

It has been shown that thiourea derivatives and their complexes show significant promise in wide range of applications and are not just limited to their antimicrobial activity. A lot of research has shown that they could be useful in the fields of molecular recognition, materials science, cultivating, pharmaceuticals, and other biological activities (Zahra et al., 2022). A comprehensive review on the methodologies of thiourea synthesis and coordination with transition metal help to better understand the broad relevance of compound's antibacterial functionality. As well as emphasising the key role of transition metals Ag(I) and Cu(II) in improving the quality of thiourea ligands in antibacterial-target interactions.

CHAPTER 3

METHODOLOGY

3.1 Materials

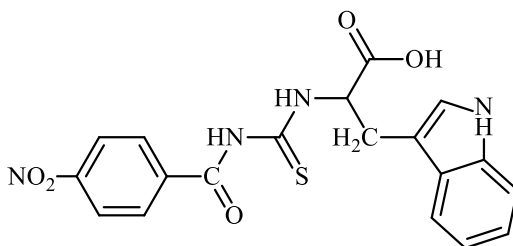
4-nitrobenzoyl chloride, potassium thiocyanate (KSCN) powder, tryptophan, methionine, serine, valine, L-alanine, cysteine, silver(I) nitrate, copper(II) chloride, potassium hydroxide (KOH), distilled acetone, ethanol, acetonitrile, ethyl acetate, propanol, methanol, hexane, dichloromethane, chloroform, cyclohexanol, nitromethane, tetrahydrofuran, diethyl ether, toluene, petroleum ether is of reagent grade and used without purification. Acetone was distilled over magnesium sulfate anhydrous. General method for synthesis of thiourea from isothiocyanate were referred from Shakeel and co-worker (2016).

3.2 Instrumentation and Analytical Characterisation of Thiourea Compounds

Characterisation of pure thiourea compounds were conducted using Fourier Transform Infrared Spectrometry (FTIR) Perkin-Elmer Spectrum (GX2000) spectrometer. The FTIR spectra (v/cm^{-1}) of all compounds were determine by using Attenuated Total Reflection (ATR) technique. The CHNS elemental analysis of thiourea ligands and complexes were performed using Thermo ScientificTM FlashSmartTM Elemental Analyzer. The ^1H and ^{13}C NMR spectroscopy were used to confirm the chemical structure of thiourea ligands and their complexes by identifying the H and C present in the synthesised compounds. The samples were analysed using the ^1H and ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy (JEOL ECA 500 operating at 500M Hz (^1H) and 125M Hz (^{13}C) spectrophotometer. All NMR spectra were recorded in DMSO- d_6 solutions and the chemical shifts were reported in δ (ppm).

3.3 Synthesis of Nitrobenzoylthiourea Ligands

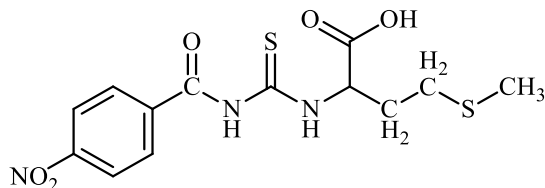
3.3.1 Synthesis of ((4-nitrobenzoyl)carbamoithiyl)tryptophanato (**1**)



4-nitrobenzoyl chloride (0.01 mol, 1.86 g) and potassium thiocyanate (0.01 mol, 0.97 g) (KSCN) were dissolved in 120 ml of distilled acetone. The solution was stirred for 1 hour until white precipitate formed. The white precipitate, potassium chloride (KCl) was removed while the filtrate formed was kept to be used in the next step. A 0.01 mol (2.04 g) of tryptophan was added to the filtrate solution and the solution mixture was heated to reflux for three days (72 hours) at 65 °C before being filtered, ultimately yielding only the targeted compounds while eliminating unwanted products. The mixture was allowed to cool at room temperature and the filtrate was left to evaporate at room temperature for several days. After several days, orange solid was formed and collected as ligand (**1**) (3.357 g, 81.40%). Compound was purified by recrystallisation using ethanol/dichloromethane (2:1) ratio. FTIR $\nu_{\max}$ 3412 (N-H), 1717 (C=O carboxylic), 1674 (C=O amide), 1514 (C=C aromatic), 854 (C=S), 1159 (C-N). Calcd. for (C₁₉H₁₆N₄O₅S); C: 55.33%, H: 3.91%, N: 13.58%, S: 7.77%, found: C: 55.72%, H: 4.18%, N: 13.87%, S: 6.51%. ¹H NMR (500 MHz; DMSO-d₆) δ 11.89 (1H, s, NH), 11.04 (1H, d, J = 7.6 Hz, NH), 10.92 (1H, d, NH), 8.28 (2H, d, J = 4.6 Hz, Ar-H), 8.03 (2H, d, J = 1.5 Hz, Ar-H), 7.49 (1H, d, J = 7.6 Hz, Ar-H), 7.29 (1H, d, J = 7.6 Hz, Ar-H), 7.14 (1H, d, J = 2.3 Hz, Ar-H), 7.01 (1H, t, J = 7.6 Hz, Ar-H), 6.90 (1H, t, J = 7.6 Hz, Ar-H), 2.47 (1H, q, J = 1.8 Hz, CH), 2.06

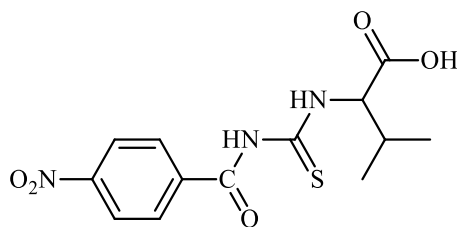
(2H, d, $J = 6.1$ Hz, CH₂). ¹³C-NMR (125 MHz; DMSO-d₆) δ 180.2 (C=SNH), 172.3 (C=OOH), 167.5 (C=ONH), 150.3 (Ar-C), 138.5 (Ar-C), 136.6 (Ar-C), 130.7 (Ar-C), 127.9-108.9 (Ar-C), 58.9 (CH), 26.7 (CH₂).

3.3.2 Synthesis of ((4-nitrobenzoyl)carbamothioyl)methioninato (2)



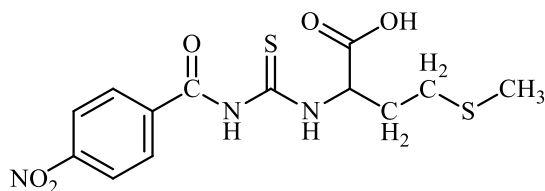
The procedure (3.3.1) was repeated using methionine (0.01 mmol, 1.49 g). After several days, yellow solid was formed and collected as ligand **(2)** (2.899 g, 81.11%). Compound was purified through washing with distilled water. FTIR $\nu_{\max}$ 3218 (N-H), 1722 (C=O carboxylic), 1508 (C=O amide), 1508 (C=C aromatic), 853 (C=S), 1156 (C-N). Calcd. for (C₁₃H₁₅N₃O₅S₂); C: 43.69%, H: 4.23%, N: 11.76%, S: 17.94%, found: C: 43.71%, H: 4.22%, N: 11.82%, S: 17.76%. ¹H NMR (500 MHz; DMSO-d₆) δ 11.88 (1H, s, NH), 11.09 (1H, d, $J = 7.4$ Hz, NH), 8.27-8.31 (2H, m, Ar-H), 8.09-8.12 (2H, m, Ar-H), 4.97 (1H, dd, $J = 12.6, 6.9$ Hz, CH), 2.51 (2H, q, $J = 7.5$ Hz, CH₂), 2.46 (2H, t, $J = 1.7$ Hz, CH₂), 2.03 (3H, s, CH₃). ¹³C-NMR (125 MHz; DMSO-d₆) δ 180.7 (C=SNH), 172.3 (C=OOH), 167.4 (C=ONH), 150.3 (Ar-C), 138.2 (Ar-C), 130.8 (Ar-C), 123.8 (Ar-C), 57.2 (CH), 30.9 (CH₂), 29.7 (CH₂), 15.2 (CH₃).

3.3.3 Synthesis of ((4-nitrobenzoyl)carbamothioyl)valinato (3)



The procedure (3.3.1) was repeated using valine (0.01 mol, 1.17 g). After several days, milky white solid was formed and collected as ligand **(3)** (2.912 g, 89.51%). Compound was purified by recrystallisation using propanol/dichloromethane (2:1) ratio. FTIR $\nu_{\max}$ 3237 (N-H), 1716 (C=O carboxylic), 1672 (C=O amide), 1521 (C=C aromatic), 854 (C=S), 1163 (C-N). Calcd. for (C₁₃H₁₅N₃O₅S); C: 47.99%, H: 4.65%, N: 12.92%, S: 9.86%, found: C: 48.06%, H: 4.73%, N: 12.97%, S: 8.26%. ¹H NMR (500 MHz; DMSO-d₆) δ 11.93 (1H, s, NH), 11.17 (1H, d, J = 7.7 Hz, NH), 8.28 (2H, d, J = 8.9 Hz, Ar-H), 8.11 (2H, d, J = 8.9 Hz, Ar-H), 2.46 (1H, t, J = 1.7 Hz, CH), 2.27-2. (1H, m, CH), 0.91-0.98 (6H, m, 2CH₃). ¹³C-NMR (125 MHz; DMSO-d₆) δ 181.1 (C=SNH), 171.8 (C=OOH), 167.8 (C=ONH), 150.4 (Ar-C), 138.4 (Ar-C), 130.8 (Ar-C), 123.8 (Ar-C), 63.2, 30.7 (CH), 19.2, 18.6 (CH₃).

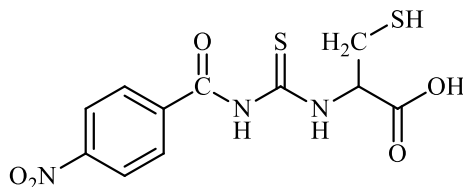
3.3.4 Synthesis of ((4-nitrobenzoyl)carbamothioyl)alaninato (**4**)



The procedure (3.3.1) was repeated using alanine (0.01 mol, 0.89 g). After several days, orange-brick solid was formed and collected as ligand **(4)** (2.363 g, 79.5%). Compound was purified by recrystallisation using ethanol/dichloromethane (2:1) ratio. FTIR $\nu_{\max}$ 3281 (N-H), 1709 (C=O carboxylic), 1659 (C=O amide), 1532 (C=C aromatic), 847 (C=S), 1167 (C-N). Calcd. for (C₁₁H₁₁N₃O₅S); C: 44.44%, H: 3.73%, N: 14.13%, S: 10.79%, found: C: 44.84%, H: 3.97%, N: 14.58%, S: 8.68%. ¹H-NMR (500 MHz, DMSO-d₆) δ 11.88 (1H, s, NH), 11.11 (1H, d, J = 6.9 Hz, NH), 8.28 (1H, d, J = 8.9 Hz, Ar-H), 8.09 (1H, d, J = 8.9 Hz, Ar-H), 4.83-4.77 (1H, m, CH),

1.46 (3H, d, $J = 7.2$ Hz, CH_3). ^{13}C -NMR (125 MHz, DMSO-d_6) δ 180.1 (C=SNH), 173.4 (C=OOH), 167.6 (C=ONH), 150.3 (Ar-C), 138.5 (Ar-C), 130.7 (Ar-C), 123.9 (Ar-C), 17.7 (CH_3).

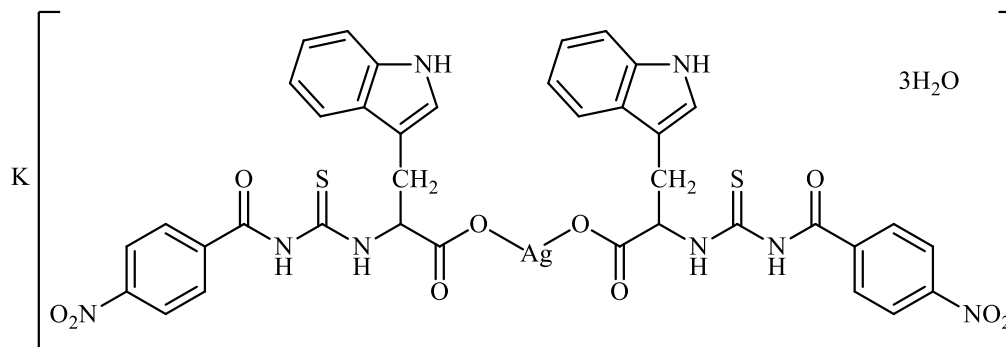
3.3.5 Synthesis of ((4-nitrobenzoyl)carbamothioyl)cysteinato (5)



The procedure (3.3.1) was repeated using cysteine (0.01 mol, 1.2116 g). After several days, orange solid was formed and collected as ligand **5** (2.706 g, 82.15%). Compound was purified by recrystallisation using ethanol/hexane (1:2). FTIR ν_{max} 3113 (N-H), 1716 (C=O carboxylic), 1668 (C=O amide), 1519 (C=C aromatic), 848 (C=S), 1157 (C-N). Calcd. for ($\text{C}_{11}\text{H}_{11}\text{N}_3\text{O}_5\text{S}_2$); C: 40.11%, H: 3.37%, N: 12.76%, S: 19.47%, found: C: 40.43%, H: 3.57%, N: 14.07%, S: 16.49%. ^1H -NMR (500 MHz, DMSO-d_6) δ 11.90 (1H, s, NH), 11.21 (1H, d, $J = 7.4$ Hz, NH), 8.27 (1H, d, $J = 5.2$ Hz, Ar-H), 8.09 (1H, d, $J = 8.9$ Hz, Ar-H), 3.57 (1H, dd, $J = 11.7, 9.5$ Hz, CH), 2.46 (2H, t, $J = 1.9$ Hz, CH_2). ^{13}C -NMR (125 MHz; DMSO-d_6) δ 172.4 (C=SNH), 166.3 (C=OOH), 149.9 (C=ONH), 142.3 (Ar-C), 136.9 (Ar-C), 130.6 (Ar-C), 124.0 (Ar-C), 57.2 (CH), 32.9 (CH_2).

3.4 Synthesis of Thiourea Complexes

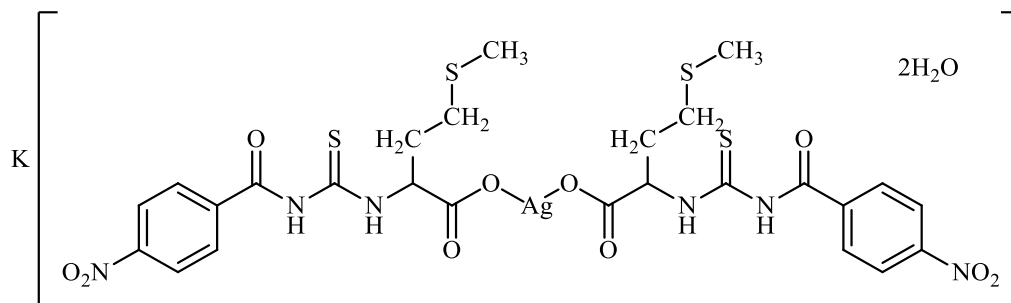
3.4.1 Synthesis of potassium di((4-nitrobenzoyl)carbamothioyl)tryptophanato)silver(I) (6)



Ligand **1** (0.412 g, 0.001 mol) was dissolved in 10 ml of ethanol followed by continuous stirring until all solid has dissolved. A (4.7 ml, 0.001 mol) of KOH was added into the solution and the mixture was stirred at room temperature for 30 minutes. The addition of KOH solution was done to deprotonate the hydrogen ion from the carboxylic acid functional group of the ligand. This will facilitate coordination of metal ions to the carboxylate group of the ligand, resulting in the formation of thiourea complexes. Round bottom flask used was wrapped in aluminium foil before the addition of metal solution to prevent any exposure to light. This measure was implemented because silver metal is susceptible to reactions triggered by exposure to light, which could reduce the silver(I) complexes into silver metal. A solution of AgNO₃ (0.085 g, 0.0005 mol) in 2 ml of distilled water was later added before the mixture was stirred for another 24 hours. Then, the mixture was filtered out and left to evaporate in a dark surrounding for few days to give out dark grey precipitate of complex **6** (0.409 g, 79.9%). FTIR ν_{max} 2974 (N-H), 1680 (C=O amide), 1520 (C=C aromatic), 850 (C=S), 1165 (C-N). Calcd. for (C₃₈H₄₀AgN₈O₁₅S₂); C: 44.58%, H: 3.54%, N: 10.94%, S: 6.26%, found: C: 44.92%, H: 3.54%, N: 11.08%, S: 6.72%. ¹H NMR (500 MHz; DMSO-d₆) δ 11.89 (2H, s, NH), 11.13 (2H, d, J =

15.2 Hz, NH), 10.91 (2H, d, $J = 4.0$ Hz, Ar-NH), 8.27 (2H, d, $J = 8.6$ Hz, Ar-H), 8.07 (2H, d, $J = 8.9$ Hz, Ar-H), 7.49 (2H, d, $J = 7.7$ Hz, Ar-H), 7.30 (2H, d, $J = 8.0$ Hz, Ar-H), 7.14 (2H, d, $J = 5.4$ Hz, Ar-H), 7.02 (2H, t, $J = 7.6$ Hz, Ar-H), 6.91 (2H, t, $J = 8.0$ Hz, Ar-H), 3.22 (2H, dd, $J = 14.7, 6.2$ Hz, CH), 2.45 (4H, d, $J = 14.9$ Hz, CH₂). ¹³C-NMR (125 MHz; DMSO-d₆) δ 173.3 (C=SNH), 167.4 (C=OO), 153.3 (C=ONH), 150.3, 136.6, 130.2, 124.0 (Ar-C), 121.5, 118.9, 111.9, 109.3 (Ar-C), 53.9.4 (CH), 27.7 (CH₂).

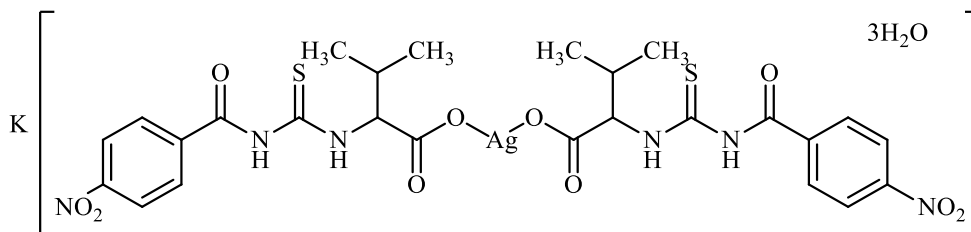
3.4.2 Synthesis of potassium di((4-nitrobenzoyl)carbamoithiyl)methioninato)silver(I) (7)



The procedure (3.4.1) was repeated using ligand 2 (0.357 g, 0.001 mol), KOH (4.7 ml 0.001 mol) and AgNO₃ (0.085 g, 0.0005 mol). Following several days of allowing the mixture to evaporate in a dark surrounding, a light grey precipitate of complex 7 (0.337 g, 75.22%) was obtained. FTIR $\nu_{\max}$ 2978 (N-H), 1682 (C=O amide), 1521 (C=C aromatic), 852 (C=S), 1166 (C-N). Calcd. for (C₂₆H₃₆AgN₆O₁₄S₄); C: 34.86%, H: 3.60%, N: 9.38%, S: 14.32%, found: C: 34.90%, H: 3.68%, N: 9.34%, S: 15.08%. ¹H NMR (500 MHz; DMSO-d₆) δ 11.88 (2H, s, NH), 11.09 (2H, d, $J = 7.4$ Hz, NH), 8.28 (2H, d, $J = 8.9$ Hz, Ar-H), 8.10 (2H, d, $J = 8.9$ Hz, Ar-H), 4.96 (2H, q, $J = 6.7$ Hz, CH), 2.53-2.49 (4H, m, CH₂), 2.43 (4H, t, $J = 14.9$ Hz, CH₂), 2.03 (6H, s, CH₃). ¹³C-NMR (125 MHz; DMSO-d₆) δ 180.6 (C=SNH), 172.3 (C=OO), 167.4 (C=ONH),

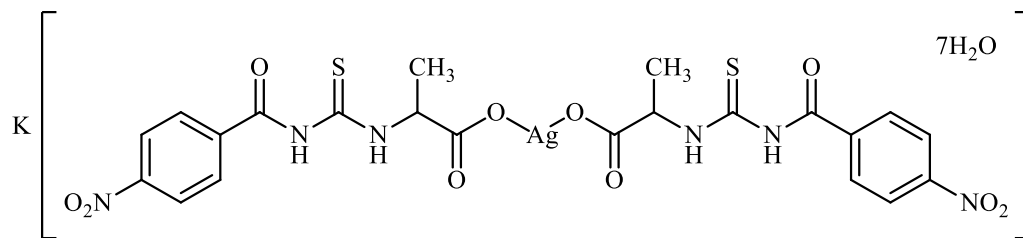
150.2 (Ar-C), 138.2 (Ar-C), 130.8 (Ar-C), 123.8 (Ar-C), 57.2 (CH), 30.9 (CH₂), 29.7 (CH₂), 15.2 (CH₃).

3.4.3 Synthesis of potassium di((4-nitrobenzoyl)carbamothioyl)valinato)silver(I) (**8**)



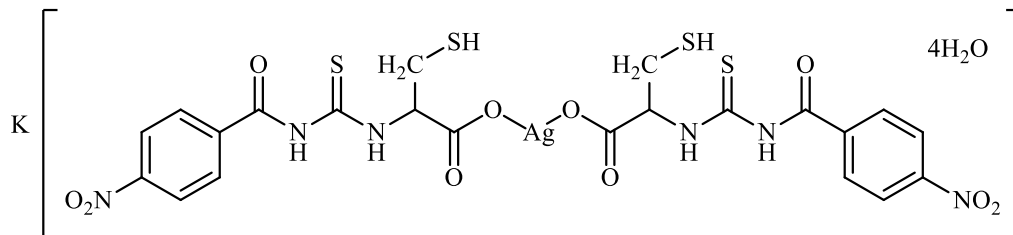
The procedure (3.4.1) was repeated using ligand **3** (0.325 g, 0.001 mol), KOH (4.7 ml 0.001 mol) and AgNO₃ (0.085 g, 0.0005 mol). Following several days of allowing the mixture to evaporate in a dark surrounding, a dark brown precipitate of complex **8** (0.321 g, 75.53%) was obtained. FTIR $\nu_{\max}$ 2968 (N-H), 1679 (C=O amide), 1518 (C=C aromatic), 849 (C=S), 1172 (C-N). Calcd. for (C₂₆H₄₀AgN₆O₁₆S₂); C: 36.75%, H: 4.03%, N: 9.89%, S: 7.55%, found: C: 36.38%, H: 3.54%, N: 9.51%, S: 8.48%. ¹H NMR (500 MHz; DMSO-d₆) δ 11.94 (2H, s, NH), 11.19 (2H, d, J = 15.2 Hz, NH), 8.28 (2H, dd, J = 14.9, 6.0 Hz, Ar-H), 8.14 (2H, dd, J = 14.9, 6.0 Hz, Ar-H), 2.46 (2H, t, J = 15.0 Hz, CH), 2.16 (2H, dq, J = 26.0, 7.2 Hz, CH), 0.96-0.88 (12H, m, CH₃). ¹³C-NMR (126 MHz; DMSO-d₆) δ 173.0 (C=SNH), 167.8 (C=OO), 153.6 (C=ONH), 150.5 (Ar-C), 138.7 (Ar-C), 130.4 (Ar-C), 124.0 (Ar-C), 58.0 (CH), 19.6-18.0 (CH₃).

3.4.4 Synthesis of potassium di((4-nitrobenzoyl)carbamoithioyl)alaninato)silver(I) (9)



The procedure (3.4.1) was repeated using ligand **4** (0.193 g, 0.00065 mol), KOH (3.1 ml, 0.00065 mol) and AgNO₃ (0.0552 g, 0.000325 mol). Following several days of allowing the mixture to evaporate in a dark surrounding, a light grey precipitate of complex **9** (0.120 g, 43%) was obtained. FTIR ν_{max} 3279 (N-H), 1660 (C=O amide), 1532 (C=C aromatic), 848 (C=S), 1168 (C-N). Calcd. for (C₂₂H₃₈AgN₆O₁₉S₂); C: 30.53%, H: 4.96%, N: 9.71%, S: 7.41%, found: C: 30.57%, H: 3.91%, N: 9.60%, S: 10.08%. ¹H NMR (500 MHz; DMSO-d₆) δ 11.88 (2H, s, NH), 11.12 (2H, d, J = 10.6 Hz, NH), 8.28 (2H, d, J = 8.6 Hz, Ar-H), 8.12 (2H, d, J = 8.9 Hz, Ar-H), 4.33-4.27 (2H, m, CH), 1.37 (6H, d, J = 7.2 Hz, CH₃). ¹³C-NMR (125 MHz; DMSO-d₆) δ 174.2 (C=SNH), 167.6 (C=OO), 153.1 (C=ONH), 150.3 (Ar-C), 138.8 (Ar-C), 130.3 (Ar-C), 124.0 (Ar-C), 48.5 (CH), 18.5 (CH₃).

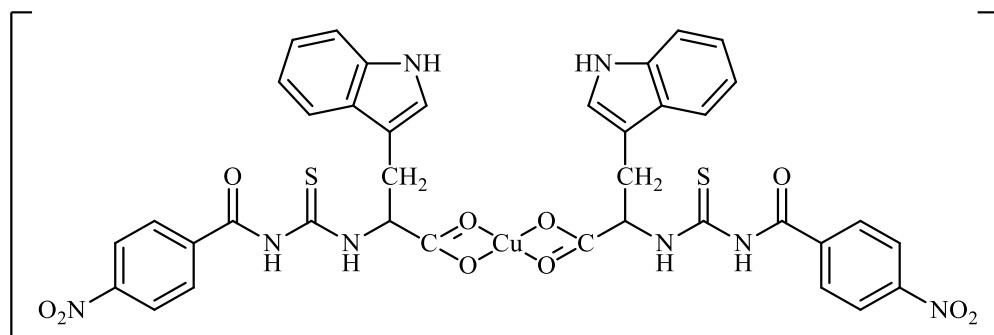
3.4.5 Synthesis of potassium di((4-nitrobenzoyl)carbamoithioyl)cysteinato)silver(I) (10)



The procedure (3.4.1) was repeated using Ligand **5** (0.181 g, 0.00055 mol), KOH (2.7 ml, 0.00055 mol) and AgNO₃ (0.0467 g, 0.000275 mol). Following several days of allowing the

mixture to evaporate in a dark surrounding, a dark brown precipitate of complex **10** (0.105 g, 43.6%) was obtained. FTIR $\nu_{\max}$ 3034 (N-H), 1680 (C=O amide), 1516 (C=C aromatic), 846 (C=S), 1161 (C-N). Calcd. for (C₂₂H₃₄AgN₆O₁₇S₄); C: 30.17%, H: 3.22%, N: 9.60%, S: 14.65%, found: C: 29.77%, H: 2.88%, N: 10.55%, S: 14.93%. ¹H NMR (500 MHz; DMSO-d₆) δ 11.93 (2H, s, NH), 11.23 (2H, d, J = 7.4 Hz, NH), 8.27 (2H, d, J = 5.7 Hz, Ar-H), 8.11 (2H, d, J = 22.1 Hz, Ar-H), 3.41 (2H, dd, J = 11.7, 3.7 Hz, CH), 2.49-2.43 (4H, m, CH₂). ¹³C-NMR (125 MHz; DMSO-d₆) δ 172.3 (C=SNH), 166.4 (C=OO), 149.9 (C=ONH), 142.3 (Ar-C), 136.8 (Ar-C), 130.6 (Ar-C), 124.3, (Ar-C), 57.4 (CH), 33.0 (CH₂).

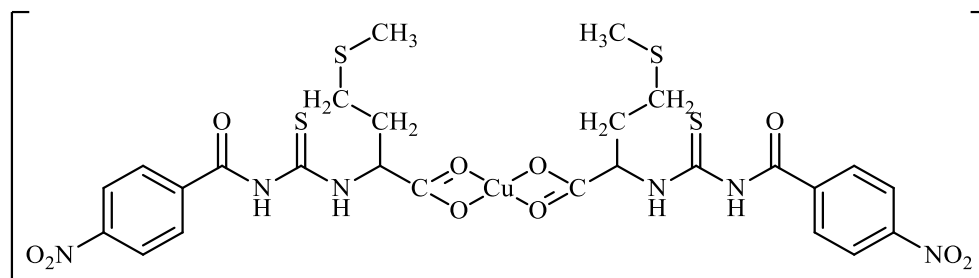
3.4.6 Synthesis of di((4-nitrobenzoyl)carbamothioyl)tryptophanatocopper(II) (**11**)



Ligand **1** (0.206 g, 0.5 mmol) was dissolved in 10 ml of ethanol followed by continuous stirring to make sure all ligand was dissolved. A (2.2 ml 0.5 mmol) of KOH was added into the solution and the mixture was stirred at room temperature for 30 minutes. A metal solution of CuCl₂·2H₂O (0.043 g, 0.25 mmol) in 2 ml of distilled water was later added into the solution containing ligand and KOH before the mixture was stirred for another 24 hours. Then, the mixture was filtered out and left to evaporate for few days to give out brownish precipitate of complex **11** (0.18 g, 68.70%). The complex was then subjected to purification with distilled water. FTIR $\nu_{\max}$ 3170 (N-H), 1681 (C=O amide), 1520 (C=C aromatic), 853 (C=S), 1171 (C-N). Calcd. for

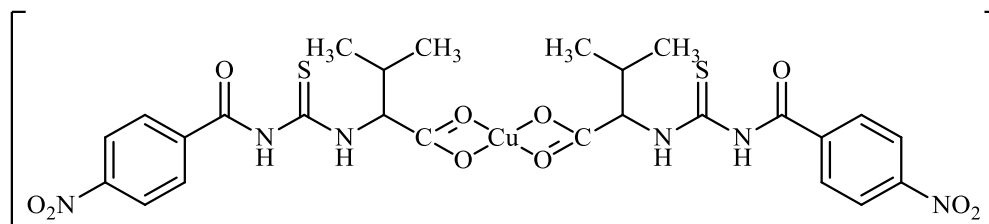
(C₃₈H₄₈CuN₈O₁₉S₂); C: 43.53%, H: 4.61%, N: 10.69%, S: 6.12%, found: C: 43.16%, H: 3.85%, N: 10.62%, S: 6.61%.

3.4.7 Synthesis of di((4-nitrobenzoyl)carbamothioyl)methionatocopper(II) (**12**)



The procedure (3.4.6) was repeated using ligand **2** (0.357 g, 1.0 mmol), KOH (4.7 ml 1.0 mmol) and CuCl₂·2H₂O (0.085 g, 0.5 mmol). After few days, green precipitate of complex **12** (0.239 g, 57.59 %) was obtained. FTIR $\nu_{\max}$ 2986 (N-H), 1682 (C=O amide), 1523 (C=C aromatic), 854 (C=S), 1169 (C-N). Calcd. for (C₂₆H₃₄CuN₆O₁₃S₄); C: 37.61%, H: 4.13%, N: 10.12%, S: 15.45%, found: C: 37.25%, H: 3.73%, N: 10.52%, S: 15.61%.

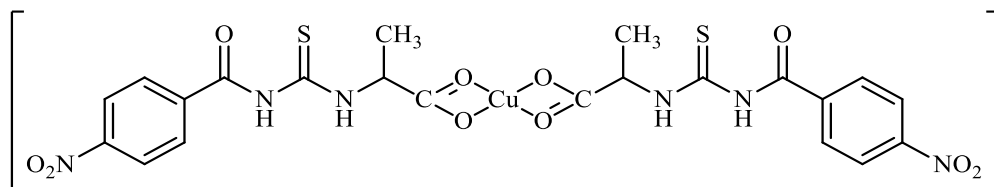
3.4.8 Synthesis of di((4-nitrobenzoyl)carbamothioyl)valinatocopper(II) (**13**)



The procedure (3.4.6) was repeated using ligand **3** (0.325 g, 1.0 mmol), KOH (4.7 ml 1.0 mmol) and CuCl₂·2H₂O (0.085 g, 0.5 mmol). After few days, brownish precipitate of complex **13** (0.261 g, 65.10%) was obtained. FTIR $\nu_{\max}$ 2964 (N-H), 1679 (C=O amide), 1519 (C=C aromatic), 853

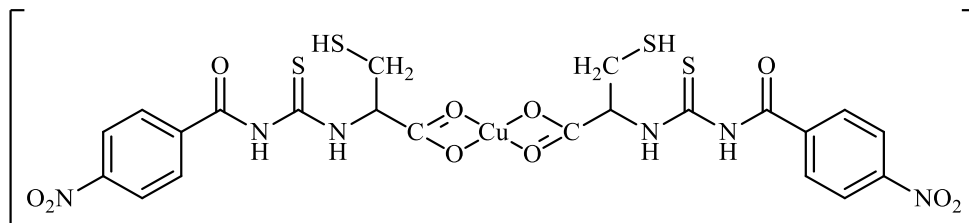
(C=S), 1166 (C-N). Calcd. for (C₂₆H₃₈CuN₆O₁₅S₂); C: 38.92%, H: 4.77%, N: 10.48%, S: 7.99%, found: C: 48.73%, H: 4.30%, N: 10.85%, S: 9.21%.

3.4.9 Synthesis of di((4-nitrobenzoyl)carbamothioyl)alaninatocopper(II) (**14**)



The procedure (3.4.6) was repeated using ligand **4** (0.193 g, 0.65 mmol), KOH (3.1 ml 0.65 mmol) and CuCl₂·2H₂O (0.0554 g, 0.325 mmol). After few days, brownish precipitate of complex **14** (0.137 g, 55.24%) was obtained. FTIR $\nu_{\max}$ 3116 (N-H), 1671 (C=O amide), 1519 (C=C aromatic), 854 (C=S), 1170 (C-N). Calcd. for (C₂₂H₃₂CuN₆O₁₆S₂); C: 34.58%, H: 4.22%, N: 11.00%, S: 8.39%, found: C: 34.30%, H: 3.90%, N: 11.04%, S: 9.89%.

3.4.10 Synthesis of di((4-nitrobenzoyl)carbamothioyl)tcysteinatocopper(II) (**15**)



The procedure (3.4.6) was repeated using ligand **5** (0.157 g, 0.48 mmol), KOH (2.3 ml, 0.48 mmol) and CuCl₂·2H₂O (0.041 g, 0.24 mmol). After few days, brownish precipitate of complex **15** (0.131 g, 76.15%) was obtained. FTIR $\nu_{\max}$ 3072 (N-H), 1703 (C=O carboxylic), 1651 (C=O amide), 1523 (C=C aromatic), 851 (C=S), 1168 (C-N). Calcd. for (C₂₂H₂₀CuN₆O₁₀S₄); C: 36.69%, H: 2.80%, N: 11.67%, S: 17.81%, found: C: 36.64%, H: 3.08%, N: 12.26%, S: 12.75%.

3.5 Antibacterial Test Using Agar-Disk Diffusion Method

Antibacterial properties of thiourea compounds were investigated against three Gram-positive bacteria namely *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes* and three Gram-negative bacteria namely *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*. The minimal inhibitory concentration (MIC) were estimated by agar-disc diffusion method (Nemati et al., 2011). Repeated sub-culturing of the bacteria were done using Mueller-Hinton broth while Mueller-Hinton agar was used for antibacterial assay. All bacteria were cultured in Mueller Hinton broth and incubated at 37 °C with stirring for 24 hours. To prepare the agar medium, 17.55 g of Mueller Hinton agar powder was dissolved in 450 ml of distilled water in a 450 ml Schott bottle. All apparatus involved such as tweezer and cotton swabs were placed in autoclave to sterilise the apparatus and avoid contamination. Laminar flow station was wiped with 70% ethanol to maintain aseptic technique. Mueller-Hinton agar solution was then poured into sterile new petri dishes and was allowed to gel and cooled in the laminar flow station. The bacterial suspension was adjusted to 0.5 McFarland standard and 20 µL of the inoculum was spread uniformly onto Mueller Hinton agar using a sterile cotton swab. The disc (20 microlitres of sample) with different concentrations (50 ppm, 100 ppm, 150 ppm, 200 ppm, 250 ppm, 500 ppm and 1000 ppm) of synthesised compounds dissolved in DMSO were placed onto the Mueller Hinton agar. The DMSO was used as negative control (Khan et al., 2008), while penicillin, vancomycin, ampicillin, tetracycline, ceftazidime and imipenem were used as the standard antibiotic (positive control) for *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* respectively. Subsequently, the plates were incubated at 37 °C for 24 hours. After the

incubation, the clear inhibition zone was observed, and the diameter of the inhibition zone was measured in millimetre (mm).

3.6 Molecular Docking

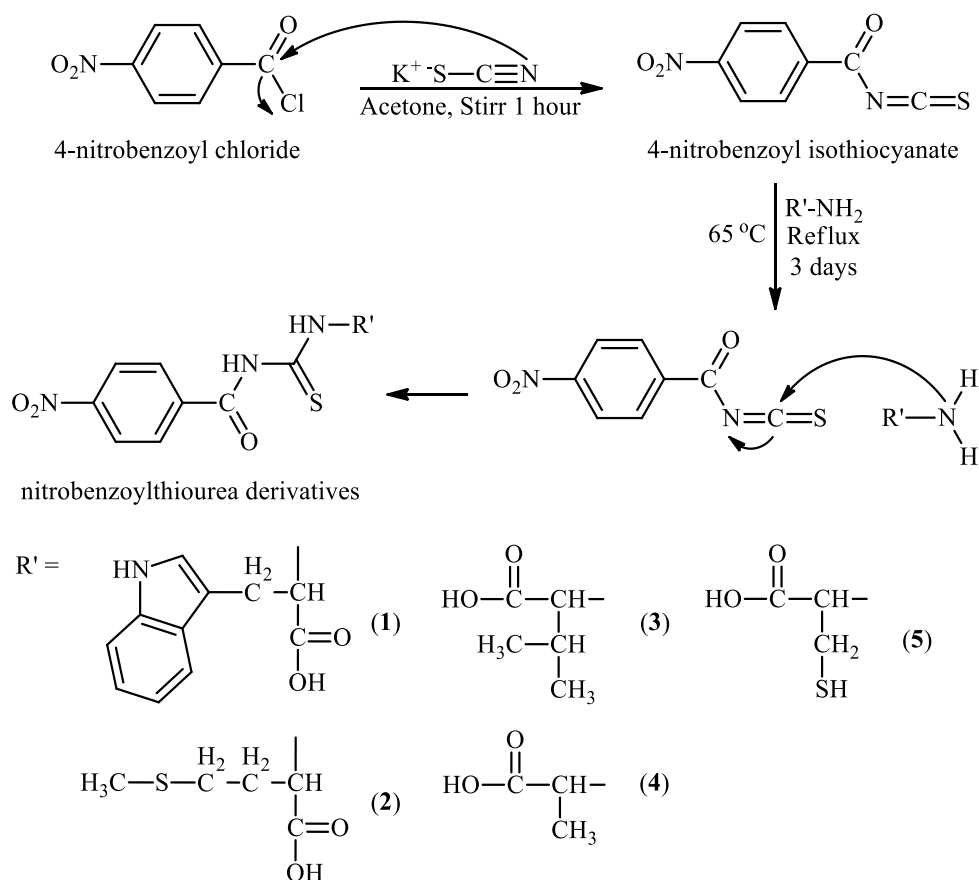
The crystal structure of the targeted bacteria's protein chain was retrieved in PDB format from the RSCB protein data bank. The protein was initially prepared using AutoDock Tools-1.5.7 by specifying the structure of the protein in terms of the presence of water hydrogen atoms and its polar characteristics. The resulting file was saved as a pdbqt file. The synthesised ligand structures were generated using Chemdraw software and were afterwards converted to pdbqt files using a variety of software programs, including Molview, Open Babel, and the AutoDock Tools program. Afterward, the AutoDock Tools were used to build docking put files (Khan et al., 2020) from both the protein and ligand files, in which a grid box size containing the centre of where the molecule may bond was allocated. Following that, molecular docking simulation was done using AutoDock Vina (Pingaew et al., 2018). The docked binding poses were visualised using PyMOL, an open source molecular visualisation application used to identify the hydrogen bonds inside the protein (Roche Allred et al., 2017) and a 3D ligand-protein interaction was displayed and discussed (Li et al., 2021). PyMOL is used to visualise the presence of intermolecular interaction (study focuses on hydrogen bonding) between the compounds and the amino acid residues in the binding protein. It is a commonly used open-source molecular visualisation application to find salt bridges and hydrogen bonds in molecular structures (Roche Allred et al., 2017). Few factors were investigated for a better understanding, including the conformation of the synthesised compounds in the protein's binding site, the assessment of binding affinity along with the hydrogen bond, and the number of interactions between the synthesised compounds with the protein residue.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Synthesis and Structural Analysis of Nitrobenzoylthiourea Ligand

Thiourea ligand (**1-5**) were synthesised by reacting the starting material of 4-nitrobenzoyl chloride with potassium thiocyanate (KSCN) in distilled acetone. The mixture was stirred for one hour under room temperature. The reaction resulted in milky-like yellow colour of solution that changed from yellow coloured solution together with the formation of potassium chloride (KCl) precipitate. The KCl precipitate was removed through filtration and the filtrate was further reacted with a series of amino acid namely tryptophan, methionine, valine, L-alanine and cysteine by heated to reflux for 3 days at 65 °C to give ligand (**1-5**) respectively. The synthesis pathway of monothiourea ligands were shown in Scheme 4.1.



Scheme 4.1: Synthesis pathway of thiourea ligands (1-5)

The solution obtained was filtered and the filtrate was left to evaporate at room temperature to give a yellow to orange solid precipitate of thiourea ligands. The precipitates were collected after few days and followed by recrystallisation using several organic solvents such as nitromethane, toluene, dimethyl sulfoxide, diethyl ether, hexane, acetone, chloroform, acetonitrile, methanol, ethanol, propanol and hexane. However, none of the single system solvents produced pure form of thiourea ligands. The absence of pure thiourea ligands despite recrystallization in various organic solvents can be attributed to the complex nature of the ligand-solvent interactions, wherein none of the individual solvents provided the ideal conditions for complete purification. Hence, all ligands were recrystallised using mixture of

organic solvents with variation of mixture ratios for each of the ligand under constant stirring and heat to give out pure compounds. The proposed structure of ligands (**1-5**) were then confirmed by CHNS elemental analysis, FTIR and ^1H and ^{13}C NMR spectroscopy.

Ligand **1** and **4** were obtained in pure form through recrystallisation in a mixture of ethanol:dichloromethane (2:1) ratio. For ligand **2**, pure compound was obtained through washing by distilled water for five times. Ligand **3** formed pure compound from recrystallisation in a mixture of propanol:dichloromethane (2:1) ratio while, pure ligand **5** was obtained from recrystallisation in a mixture of ethanol:hexane (1:2) ratio. All compounds were left to dry out at room temperature for one to two days before they were collected and analysed. Thiourea ligands (**1-5**) were successfully synthesised in good yields between 79.5% to 89.51%. The synthesised nitrobenzoylthiourea derivatives were characterised by FTIR, NMR and CHNS elemental analysis techniques. The disappearance of NCS peak in all compounds indicates the successful formation of thiourea compounds resulting from the conversion of -NCS to -NH (Nordin et al., 2017). The physical properties and analytical data of ligand (**1-5**) are shown in Table 4.1. Furthermore, for CHNS elemental analysis data, most ligand are in good agreement with the theoretical value. However, there are also compound (example for ligand **3**) that are not pure, with the experimental values surpassing the calculated ones. It is noteworthy that this discrepancy suggests the presence of impurities, which is also consistent with the observed antibacterial results later (Table 4.2).

Table 4.1: Physical properties and analytical data of thiourea ligand 1-5

Compounds	Molecular formula	Yield (%)	Colour
1	C ₁₉ H ₁₆ N ₄ O ₅ S	81.40	Yellow
2	C ₁₃ H ₁₅ N ₃ O ₅ S ₂	81.11	Yellow
3	C ₁₃ H ₁₅ N ₃ O ₅ S	89.51	Orange
4	C ₁₁ H ₁₁ N ₃ O ₅ S	79.5	Yellow
5	C ₁₁ H ₁₁ N ₃ O ₅ S ₂	82.15	Orange

Table 4.2: CHNS elemental analysis of thiourea ligand 1-5

Thiourea	Molecular Formula	Molecular Weight	Measured value (Theoretical Value)			
			% Carbon	% Hydrogen	%Nitrogen	%Sulphur
1	C ₁₉ H ₁₆ N ₄ O ₅ S	414.42	55.72(55.33)	4.18(3.91)	13.87(13.58)	6.51(7.77)
2	C ₁₃ H ₁₅ N ₃ O ₅ S ₂	357.41	43.71(43.69)	4.22(4.23)	11.82(11.76)	17.76(17.94)
3	C ₁₃ H ₁₅ N ₃ O ₅ S	325.34	48.16(47.99)	4.73(4.65)	12.97(12.92)	8.26(9.86)
4	C ₁₁ H ₁₁ N ₃ O ₅ S	297.29	44.84(44.44)	3.97(3.73)	14.53(14.13)	8.68(10.79)
5	C ₁₁ H ₁₁ N ₃ O ₅ S ₂	329.35	40.43(40.11)	3.57(3.37)	14.07(12.76)	16.49(19.47)

4.1.1 Characterisation of Nitrobenzoylthiourea Compounds using Fourier Transform Infrared (FTIR)

The room temperature attenuated total reflection Fourier Transform Infrared (FTIR-ATR) spectra of all synthesised ligands were recorded in the middle-IR region between 500 cm^{-1} and 4000 cm^{-1} . The FTIR spectrum of ligand **1** is shown in Figure 4.1 whereas FTIR spectra for ligands (**2-5**) are shown in Appendix A1-A4. The IR spectra reveal all the significant bands of important functional groups of thiourea ligands especially five distinctive bands of interest namely $\nu(\text{C=O})$ of carboxylic group, $\nu(\text{C=O})$ amide, $\nu(\text{C=S})$, $\nu(\text{C-N})$ and $\nu(\text{N-H})$. FT-IR spectra showed the successful formation of thiourea compounds through the absence of $\nu(\text{NCS})$ stretching band at 2000 cm^{-1} to 2400 cm^{-1} and the presence of expected absorption at 3100 cm^{-1} to 3400 cm^{-1} attributed to $\nu(\text{NH})$ band. This result from the conversion of (NCS) to (NH) which also indicates that all (NCS) has reacted with the amino acid. The strong indication of the formation of thiourea functionality can be indicated by the absence and presence of $\nu(\text{NCS})$ and $\nu(\text{NH})$ group respectively (Fakhar et al., 2018).

The $\nu(\text{N-H})$ stretching and bending vibration band can be identified at 3200 cm^{-1} to 3420 cm^{-1} which correspond to the presence of expected absorption of (N-H) group in the ligands. The stretching of $\nu(\text{C=O})$ of carboxylic group for ligands (**1-5**) were observed at 1717 cm^{-1} , 1722 cm^{-1} , 1716 cm^{-1} , 1709 cm^{-1} and 1716 cm^{-1} , respectively. Apart from that, distinctive peaks were also noticed at 1674 cm^{-1} , 1681 cm^{-1} , 1672 cm^{-1} , 1659 cm^{-1} and 1668 cm^{-1} that attributed to the presence of $\nu(\text{C=O})$ of amide group of ligands (**1-5**) respectively. The distinctive peak apparently were decreased in frequency compared to ordinary amides absorption range due to the formation of intramolecular hydrogen bond interaction (Khairul et al., 2016) between the

hydrogen atom of thio-amide group ($\text{H}-\text{N}-\text{C}=\text{S}$) and the oxygen atom of carbonyl group ($\text{C}=\text{O}$) (Fakhar et al., 2018), apart from low nucleophilic character of sulfur atom within $\text{C}=\text{S}$ bond. Moreover, the ($\text{C}=\text{O}$) group (benzoyl) occurred at 1681 to 1659 cm^{-1} which was lower than the usual carbonyl group at 1760-1685 cm^{-1} is due to the conjugation towards the benzene ring and the hydrogen bond formation with NH (Li et al., 2010).

In addition, the presence of $\nu(\text{C}=\text{C})$ of aromatic bond was also observed at peak between 1532 cm^{-1} to 1608 cm^{-1} for all ligands. The presence of thiourea also being supported by the presence of peak at 1167 cm^{-1} to 1156 cm^{-1} that were attributed to $\nu(\text{C}-\text{N})$ functional group. Additionally, $\nu(\text{C}=\text{S})$ stretching vibration frequency was observed at 854 cm^{-1} , 853 cm^{-1} , 854 cm^{-1} , 857 cm^{-1} and 858 cm^{-1} for ligand (**1-5**) respectively. The stretching band were observed at lower frequency as it has less double bond character, lower nucleophilic character of sulphur atom in $\text{C}=\text{S}$ moiety, existence of intramolecular hydrogen bonding with (N-H) and resonance effect with the phenyl rings in the molecule as reported by Hassan and co-workers (2011). Table 4.3 summaries all the FTIR stretching vibration values of nitrobenzoylthiourea ligand (**1-5**).

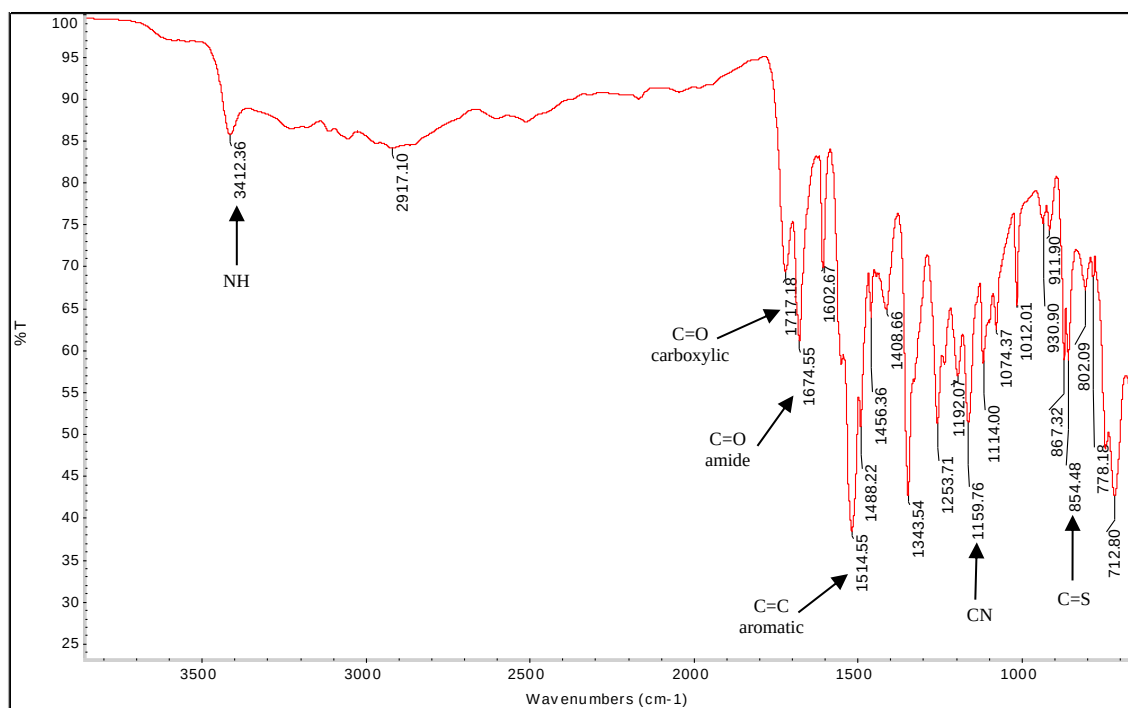


Figure 4.1: FTIR spectrum of ligand **1**

Table 4.3: Important bands observed in the FTIR spectra of nitrobenzoyl thiourea derivatives (**1-5**)

Thiourea ligand	$\nu(\text{NH})$	$\nu(\text{C=O})$ carboxylic	$\nu(\text{C=O})$ amide	$\nu(\text{C=C})$ aromatic	$\nu(\text{CN})$	$\nu(\text{C=S})$
1	3412	1717	1674	1514	1159	854
2	3218	1722	1681	1508	1156	853
3	3237	1716	1672	1521	1163	854
4	3281	1709	1659	1532	1167	847
5	3113	1716	1668	1519	1157	848

4.1.2 Characterisation of Nitrobenzoylthiourea Compounds using ^1H and ^{13}C -Nuclear Magnetic Resonance (NMR) Spectroscopy

The ^1H NMR and ^{13}C NMR spectroscopic analysis was performed for thiourea ligands (**1-5**) in DMSO- d_6 , in which some important resonance can be observed such as $\delta_{\text{H}}(\text{NH})$, δ_{H} (ArH), $\delta_{\text{H}}(\text{CH})$, $\delta_{\text{H}}(\text{CH}_2)$ and $\delta_{\text{H}}(\text{CH}_3)$. Figure 4.2 shows the ^1H NMR spectrum of ligand **1**, while ^1H NMR spectrum of ligand **2-5** are shown in Appendix (A5-A8).

For ^1H NMR, two (NH) peaks can be observed which represent resonance of (CONH) and (CSNH). The proton resonance of (CONH) in which the proton bonded to carbonyl group can be noticed at higher chemical shift compared to the resonance of (CSNH) in which the proton is bonded to thiocarbonyl group. This might be due to electron withdrawing effect of oxygen and sulphur, and the presence of deshielding aromatic ring near (CONH) group making it more deshielded and exist downfield at higher frequency compared to proton of (CSNH). The chemical shifts of the two (NH) signals were distinct, owing to the existence and influence of the electron withdrawing group and intramolecular hydrogen bond in the molecule (Saeed et al., 2010).

The presence of CONH of thiourea compound (**1-5**) was observed by single peak at δ_{H} 11.89, 11.88, 11.93, 11.88 and 11.90 ppm, respectively. Whereas the doublet peaks ranging from δ_{H} 11.04 ppm to 11.21 ppm was assigned for the resonance proton of CSNH. The presence of hydrogen attached to aromatic groups are observed as multiplet peaks in the range of δ_{H} 8.03 ppm to 8.28 ppm for ligand **1**, δ_{H} 8.0 ppm to 8.27 ppm for ligand **2**, δ_{H} 8.11 ppm to 8.28 ppm for ligand **3**, δ_{H} 8.09 ppm to 8.28 ppm for ligand **4** and δ_{H} 8.09 ppm to 8.27 ppm for ligand **5**. Distinctive feature of ligand **1** can also be observed from multiplet aromatic peaks presence at

δ_H 6.90 ppm to 7.49 ppm. (Ar-H₁) of all thiourea compounds exist downfield at low frequency compared to (Ar-H₂) due to powerful withdrawing group of NO₂ that is closer to (Ar-H₁). Apart from that, peaks at δ_H 2.47 ppm, 4.97 ppm, δ_H 4.77 ppm, 3.57 ppm were attributed to (CH) proton of ligand **1**, **2**, **4** and **5**, respectively. Multiplet peaks of (CH) resonance signal at δ_H 2.46 ppm to 2.33 ppm can be observed to characterise ligand **3**. Distinctive peaks at δ_H 2.47 ppm and 2.46 ppm owing to the presence of proton (CH₂) of ligand **1** and **5** respectively. Meanwhile, the unique characteristic of ligand **2** compared to other ligands is the presence of multiple (CH₂) resonance at δ_H 2.51 ppm and 2.46 ppm. The presence of (CH₃) resonance of ligand **2** and ligand **4** were observed as singlet peak at δ_H 2.03 ppm and doublet peak at δ_H 1.46 ppm respectively. The presence of an electron-donating group, sulphur, close to the (CH₃) group of ligand **2** has shifted the signal downfield for ligand **2** compared to ligand **4**. Moreover, distinctive feature of ligand **3** was also observed with a doublet peak between δ_H 0.91-0.98 ppm indicates the presence of two (CH₃) group split by CH proton into a 1:1.

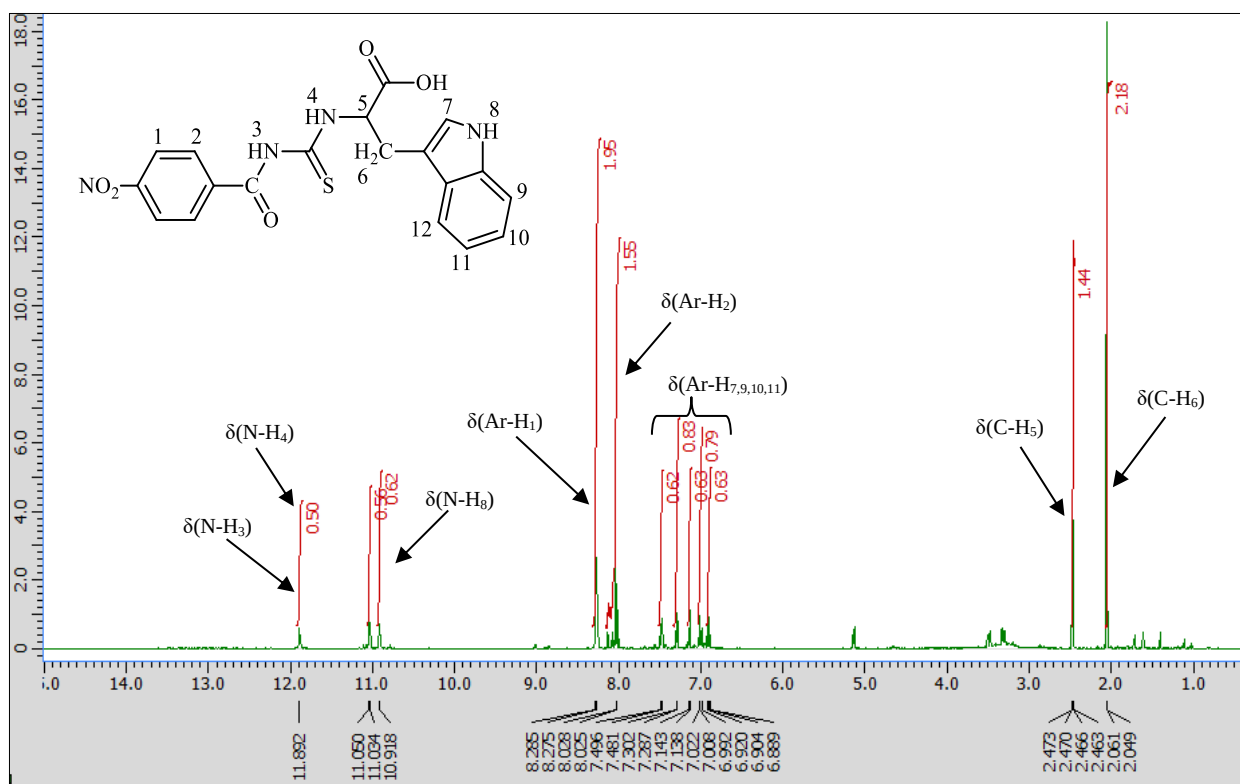


Figure 4.2: ^1H NMR spectrum of ligand **1**

The formation of thiourea ligand were further confirmed by the appearance of important carbon signals of $\delta\text{c}(\text{COOH})$, $\delta\text{c}(\text{CSNH})$, $\delta\text{c}(\text{CONH})$, $\delta\text{c}(\text{Ar-C})$, $\delta\text{c}(\text{C-H})$, $\delta\text{c}(\text{C-H}_2)$ and $\delta\text{c}(\text{C-H}_3)$. Figure 4.3 showed the ^{13}C NMR spectrum of ligand **1**, whereas ^{13}C NMR spectrum of ligand (**2-5**) are shown in Appendix (A9-A12).

The carbon peaks of (CONH) were more deshielded and present at the most downfield frequency followed by the carbon of (COOH) and (CSNH) due to the higher electronegativity value of oxygen compared to sulphur atom. The (N-H) groups in the ligands were shown with the presence of two distinctive absorption peaks of (CONH) and (CSNH). The presence of thiourea compound was also confirmed by the signal of carbon peaks (CSNH) observed at δc 149.9-167.6 ppm for ligand (**1-5**). Meanwhile, the spectra indicate the presence of carboxylic

group which were resonated at δ_c 166.3-172.3 ppm are observed for ligand (1-5). The carbon signals attributed to (CONH) were seen at δ_c 72.4-181.05 ppm for ligand (1-5) correspondingly. The signals were observed to be deshielded due to the deshielding effects by several reasons including the formation of intramolecular hydrogen bonding in the molecule, increasing electronegativity of sulphur and oxygen atoms as well as due to different environment (Hassan et al., 2020).

Apart from that, carbon of aromatic group can be observed at four different environments based on the structural formula of ligand (2-5). The peaks were observed between δ_c 123.8-150.4 ppm for ligand (2-5). Whereas multiplet peak signals between δ_c 130.6-150.3 ppm and δ_c 108.9-127.9 ppm of ligand **1** were shown from its unique presence of aromatic group in the compound's structure compared to other thiourea ligands. Furthermore, the presence of carbon (CH) was indicated by resonance signals at δ_c 53.7-58.9 ppm for ligand (1-5). The absorption peaks of (CH₂) were observed at δ_c 26.74 ppm for ligand **1**, δ_c 32.9 ppm for ligand **5** and two different environments appeared at δ_c 30.9 ppm and δ_c 29.7 ppm of ligand **2**. Distinctive peaks were also observed at δ_c 15.2 ppm, 19.2 ppm and 17.7 ppm attributed to (CH₃) of ligand **2**, **3** and **4** respectively. All detected ¹H and ¹³C NMR signals were consistent with the presence of the corresponding aliphatic or aromatic group in each compound, reflecting the conformation of the synthesised compound.

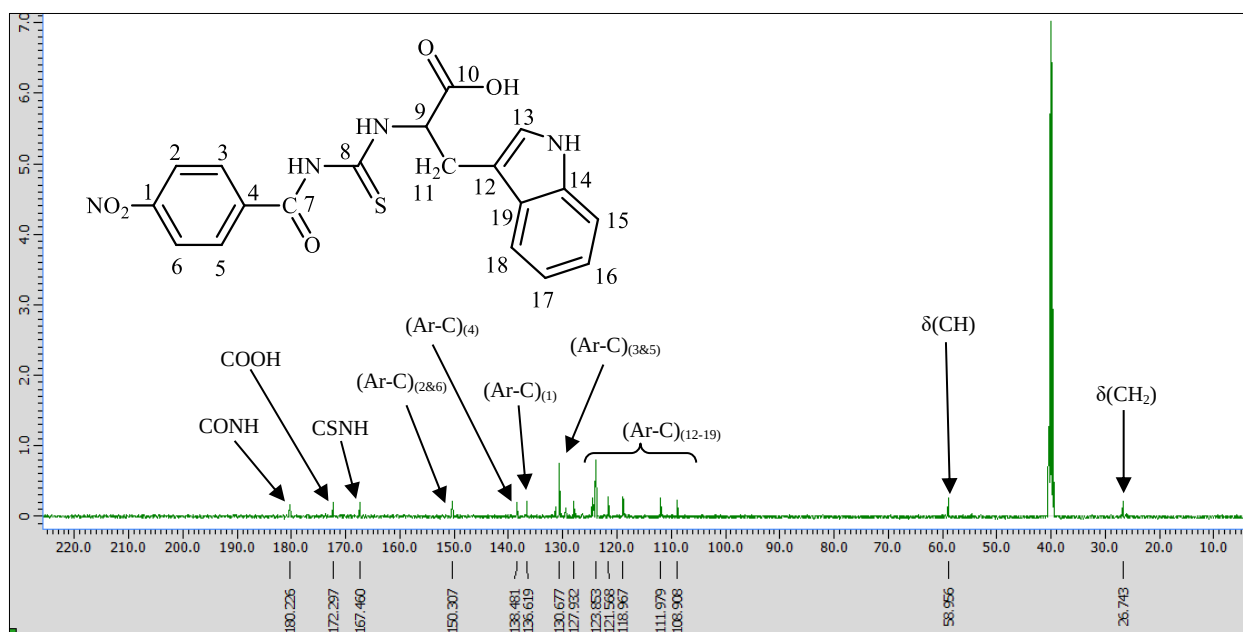
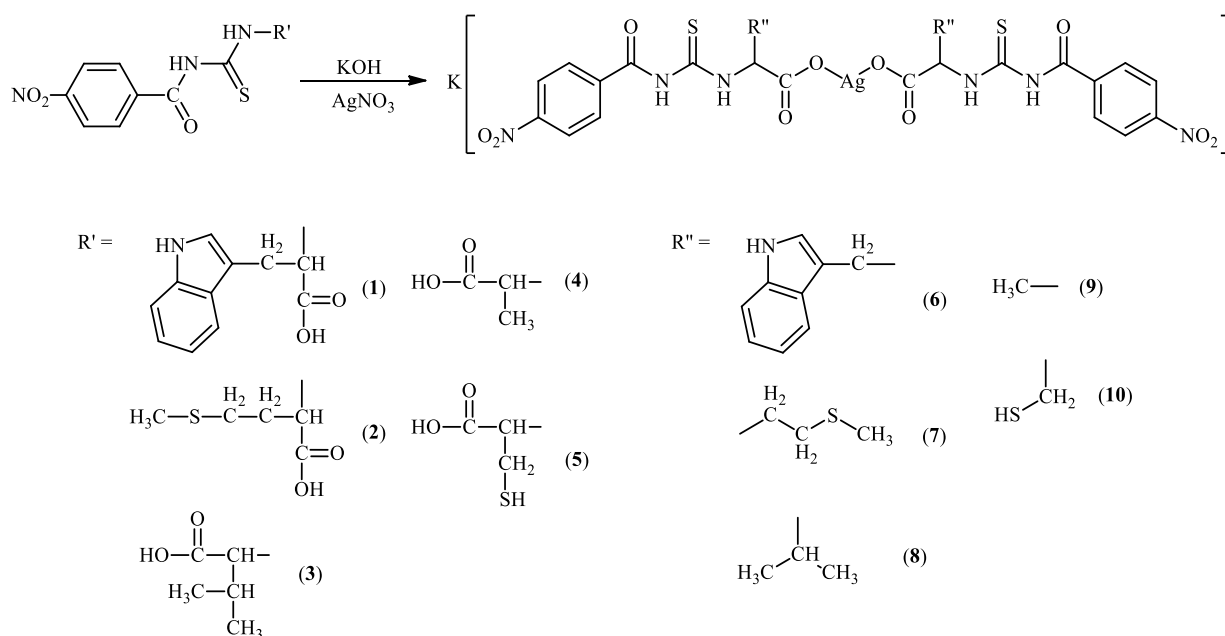


Figure 4.3: ^{13}C NMR spectrum of ligand **1**

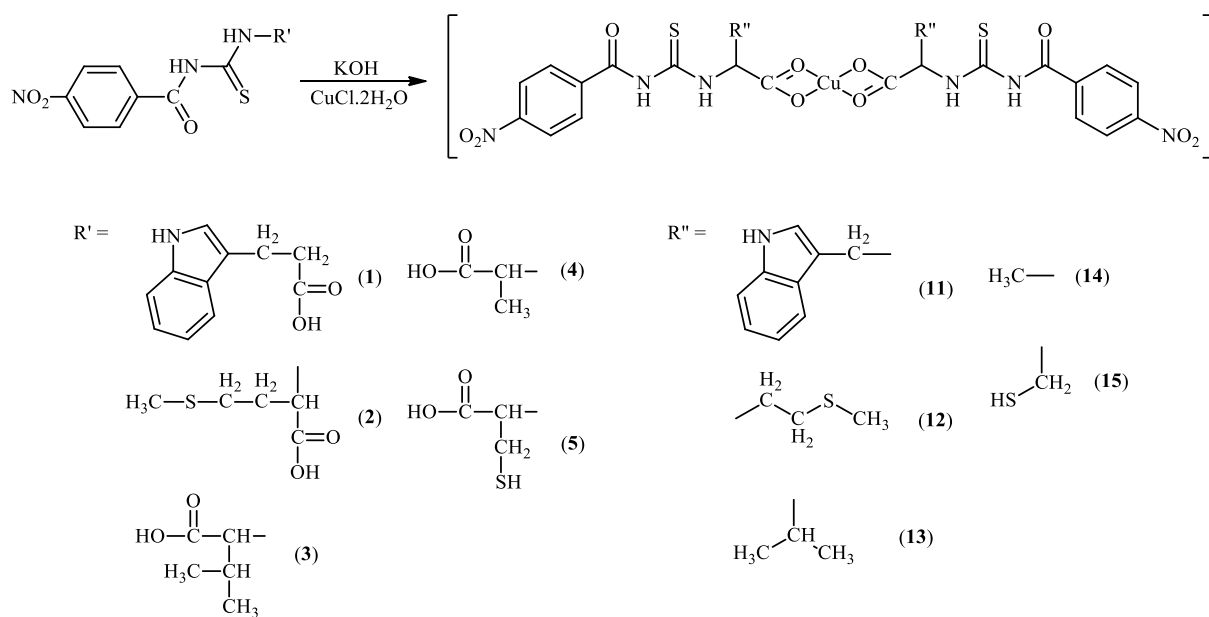
4.2 Synthesis of Ag(I) and Cu(II) Complexes of Thiourea

For the synthesis of silver(I) and copper(II) complexes, the ligands were first dissolved in 10 ml ethanol, then potassium hydroxide (KOH) solution was added with a ratio of 1:1 ligand to KOH. The mixture was stirred at room temperature for one hour before metal solution was added. The addition of KOH solution was done to deprotonate the hydrogen ion from the carboxylic acid functional group of the ligand. This will facilitate coordination of metal ions to the carboxylate group of the ligand, resulting in the formation of thiourea complexes. After one hour of stirring, AgNO_3 and $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ metal solutions were added to the ligands, producing complexes (**6-10**) after the addition of Ag(I) metal and complexes (**11-15**) after the addition of Cu(II) metal. The mixture was stirred for an additional 24 hours before the precipitates formed were filtered. The physical properties and analytical data of complex (**6-15**) are shown in Table 4.4. The filter paper holding the silver(I) complexes (**6-10**) was wrapped with

aluminium foil to minimize exposure to sunlight, which could reduce the silver(I) complexes into silver metal, and then dried in a fume hood. Additionally, filter paper containing complexes (**11-15**) was folded and allowed to dry in the fume hood. Scheme 4.2 and Scheme 4.3 illustrate the synthesis pathway of silver(I) complexes and copper(II) complexes. In addition, the CHNS elemental analysis of ligand (**6-10**) data are in good agreement with the theoretical values (Table 4.5).



Scheme 4.2: Synthesis pathway of thiourea silver(I) complexes (**6-10**)



Scheme 4.3: Synthesis pathway of thiourea copper(II) complexes (**11-15**)

Table 4.4: The physical properties and analytical data of thiourea complex **6-15**

Thiourea Complex	Molecular formula	Yield (%)	Colour
6	C ₃₈ H ₃₆ AgKN ₈ O ₁₃ S ₂	79.9	Dark brown colour
7	C ₂₆ H ₃₂ AgKN ₆ O ₁₂ S ₄	75.2	Grey colour
8	C ₂₆ H ₃₄ AgKN ₆ O ₁₃ S ₂	75.5	Dark brown colour
9	C ₂₂ H ₃₄ AgKN ₆ O ₁₇ S ₂	43.0	Dark grey colour
10	C ₂₂ H ₂₈ AgKN ₆ O ₁₄ S ₄	43.6	Dark grey colour
11	C ₃₈ H ₃₂ CuN ₈ O ₁₀ S ₂	68.7	Brownish colour
12	C ₂₆ H ₃₀ CuN ₆ O ₁₀ S ₄	57.6	Green colour
13	C ₂₆ H ₂₈ CuN ₆ O ₁₀ S ₂	65.1	Brownish colour
14	C ₂₂ H ₂₀ CuN ₆ O ₁₀ S ₂	55.2	Light orange colour
15	C ₂₂ H ₂₀ CuN ₆ O ₁₀ S ₄	76.2	Light orange colour

Table 4.5: CHNS elemental analysis of thiourea complex **6-15**

Thiourea Complex	Molecular Formula	Measured value (Theoretical Value)			
		%Carbon	%Hydrogen	%Nitrogen	%Sulphur
6	C ₃₈ H ₃₆ AgKN ₈ O ₁₃ S ₂	44.92(44.58)	3.54(3.54)	11.08(10.94)	6.72(6.26)
7	C ₂₆ H ₃₂ AgKN ₆ O ₁₂ S ₄	34.90(34.86)	3.68(3.60)	9.34(9.38)	15.08(14.32)
8	C ₂₆ H ₃₄ AgKN ₆ O ₁₃ S ₂	36.38(36.75)	3.54(4.03)	9.51(9.89)	8.48(7.55)
9	C ₂₂ H ₃₄ AgKN ₆ O ₁₇ S ₂	30.57(30.53)	3.91(3.96)	9.60(9.71)	10.08(7.41)
10	C ₂₂ H ₂₈ AgKN ₆ O ₁₄ S ₄	29.77(30.17)	2.88(3.22)	10.55(9.60)	14.93(14.65)
11	C ₃₈ H ₃₂ CuN ₈ O ₁₀ S ₂	43.16(43.53)	3.85(4.61)	10.62(10.69)	6.61(6.12)
12	C ₂₆ H ₃₀ CuN ₆ O ₁₀ S ₄	37.25(37.61)	3.73(4.13)	10.52(10.12)	15.61(15.45)
13	C ₂₆ H ₂₈ CuN ₆ O ₁₀ S ₂	48.73(38.92)	4.30(4.77)	10.85(10.48)	9.21(7.99)
14	C ₂₂ H ₂₀ CuN ₆ O ₁₀ S ₂	34.30(34.58)	3.90(4.22)	11.04(11.00)	9.89(8.39)
15	C ₂₂ H ₂₀ CuN ₆ O ₁₀ S ₄	36.64(36.69)	3.08(2.80)	12.26(11.67)	12.75(17.81)

4.2.1 Characterisation of Ag(I) Complexes of Nitrobenzoylthiourea Using Fourier Transform Infrared (FTIR)

FTIR analysis of thiourea complex (**6-10**) were carried out in the middle-IR region between 500 cm^{-1} and 4000 cm^{-1} . The FTIR spectrum of complex **6-10** are shown in Appendix A13-17. The FTIR analysis on Ag(I) complexes (**6-10**) were conducted to show the significant changes on the functional group stretching vibration upon complexation, as evidenced by shifts in peak positions or intensities in their spectra. These changes indicate alterations in chemical bonding and coordination environments, confirming the coordination sites of the ligands to the metal ions in the complex.

IR spectra reveal all the significant bands of important functional groups. The most significant changes that can be observed is the presence of relatively broad peak of (N-H) functional group between 2968 cm^{-1} to 3279 cm^{-1} . In addition, a disappearance and shifting of $\nu(\text{C=O})$ carboxylic towards a lower region in the IR spectra was observed, which may indicate that the complexation of Ag ion at the carboxylate site of the ligand occurs. The disappearance of the (C=O) carboxylic peaks and their shift to lower wavenumbers compared to the free ligand, coupled with their overlap with the C=O amide peaks which resulted in broadening of the C=O amide peak, suggests that ligands coordinate to the Ag(I) metal through the carboxylate group (Saeed et al., 2010). The disappearance was due to the deprotonation that induced the delocation of the electron and conforming the coordination through oxygen (Mishra et al., 2012). The carbonyl of $\nu(\text{C=O})$ of amide group showed slight chemical shifted from 1659 cm^{-1} to 1681 cm^{-1} for the free ligand to 1660 cm^{-1} to 1682 cm^{-1} for the metal complexes could be attributed to changes in the electronic environment around the carbonyl group upon coordination to the metal

ion. Absorption band observed around 1516 cm^{-1} to 1532 cm^{-1} were attributed for $\nu(\text{C}=\text{C})$ aromatic group of Ag(I) complexes that shifted from 1508 cm^{-1} to 1532 cm^{-1} of the free ligand. Furthermore, there was very little to no significant shift of $\nu(\text{C}=\text{S})$ and $\nu(\text{C}-\text{N})$ in the complex compared to the free ligands. This observation suggests that the $(\text{C}=\text{S})$ group does not form any coordinate covalent bond with the metal ions. This interpretation is consistent with the unchanged spectral features between the free ligands and the complex, indicating that the chemical environment around these functional groups remains largely unaffected by metal coordination. The FTIR stretching vibration values of silver(I) complexes (**6-10**) were shown in Figure 4.4.

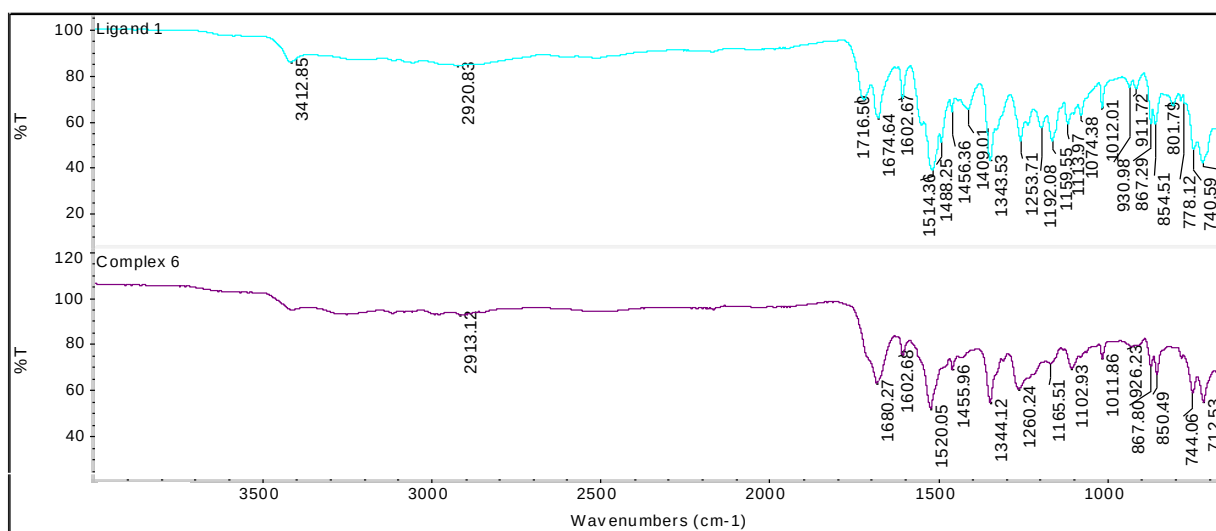


Figure 4.4: FTIR spectrum of ligand **1** and complex **6**

Table 4.6: Comparison of FTIR stretching vibration (cm⁻¹) of thiourea ligand **1-5** with silver(I) complex **6-10**

Stretching vibration (cm ⁻¹)	Compounds									
	(1)	(6)	(2)	(7)	(3)	(8)	(4)	(9)	(5)	(10)
$\nu(\text{N-H})$	3412	2913	3218	2978	3237	2968	3281	3279	3113	3034
$\nu(\text{C=O})$ carboxylic	1716	-	1722	-	1716	-	1709	1714	1716	-
$\nu(\text{C=S})$	854	850	853	852	854	849	847	848	848	846
$\nu(\text{C-N})$	1159	1165	1156	1166	1163	1172	1167	1168	1157	1161
$\nu(\text{C=C})$ aromatic	1514	1520	1508	1521	1521	1518	1532	1532	1519	1516
$\nu(\text{C=O})$ amide	1674	1680	1681	1682	1672	1679	1659	1660	1668	1680

4.2.2 Characterisation of Cu(II) Complexes of Nitrobenzoylthiourea Using Fourier Transform Infrared (FTIR)

The room temperature attenuated total reflection Fourier transform infrared (FT-IR ATR) spectra of thiourea complexes **11**, **12**, **13**, **14** and **15** were recorded. The FTIR analysis on Cu(II) complexes (**11-15**) were conducted to show the significant changes on the functional group stretching vibration analysis upon complexation as evidenced by shifts in peak positions or intensities in their spectra. These changes indicate alterations in chemical bonding and coordination environments, confirming the coordination sites of the ligands to the metal ions in the complex. The FTIR spectrum of complex (**11-15**) are shown in Appendix A18-A22. IR spectra reveal all the significant bands of important functional groups such as $\nu(\text{N-H})$, $\nu(\text{C=O})$ amide, $\nu(\text{C=C})$ aromatic, $\nu(\text{C-S})$ and $\nu(\text{C-N})$ functional group.

The most significant changes can be observed with the presence of relatively broad peak of $\nu(\text{N-H})$ functional group between 2964 cm^{-1} to 3170 cm^{-1} . In addition, a disappearance of peak at 1709 cm^{-1} to 1722 cm^{-1} and shifting of $\nu(\text{C=O})$ carboxylic towards lower region in the IR spectra of thiourea complexes (**11-15**) was observed, which might indicate that carboxylate group have involved in the coordination to the Cu(II) metal centre to form the thiourea complexes. The disappearance was due to the deprotonation that induced the delocation of the electron and conforming the coordination through oxygen (Mishra et al., 2012). Whereas, the decrease in shifting frequency observed in complex **15**, compared to its free ligand, suggests a reduction in the bond order of the C=O bond upon coordination to copper (Welch et al., 2022). The (C=O) carboxylic peaks were observed shifted to lower wavenumber from its free ligand and overlapped with the C=O amide peaks result in the broadening of the C=O amide peak. The

carbonyl of $\nu(\text{C}=\text{O})$ of amide group showed slight chemical shift from 1659 cm^{-1} to 1681 cm^{-1} in the free ligand to 1651 cm^{-1} to 1682 cm^{-1} of metal complexes. Absorption band observed ranging from 1519 cm^{-1} to 1523 cm^{-1} were attributed for $\nu(\text{C}=\text{C})$ aromatic group of thiourea complexes that shifted from 1508 cm^{-1} to 1532 cm^{-1} of the free ligands. Furthermore, there was very little to no significant shift of $\nu(\text{C}=\text{S})$ in the complex compared to the free ligands. It might indicate that $\nu(\text{C}=\text{S})$ group does not form any coordinate covalent bond with the metal ions. This observation suggests that the $(\text{C}=\text{S})$ group does not form any coordinate covalent bond with the metal ions. This interpretation is consistent with the unchanged spectral features between the free ligands and the complex, indicating that the chemical environment around these functional groups remains largely unaffected by metal coordination. The vibrations of the $\nu(\text{C}-\text{N})$ bond in the ligands can be observed had shifted from $1156\text{--}1167\text{ cm}^{-1}$ in the free ligands to $1157\text{--}1171\text{ cm}^{-1}$ in the complexes. Summarization on the FTIR stretching vibration values of complex (**11-15**) are shown in Table 4.2.3, while the FTIR stretching vibration values of copper(II) complexes (**11-15**) were shown in Figure 4.5.

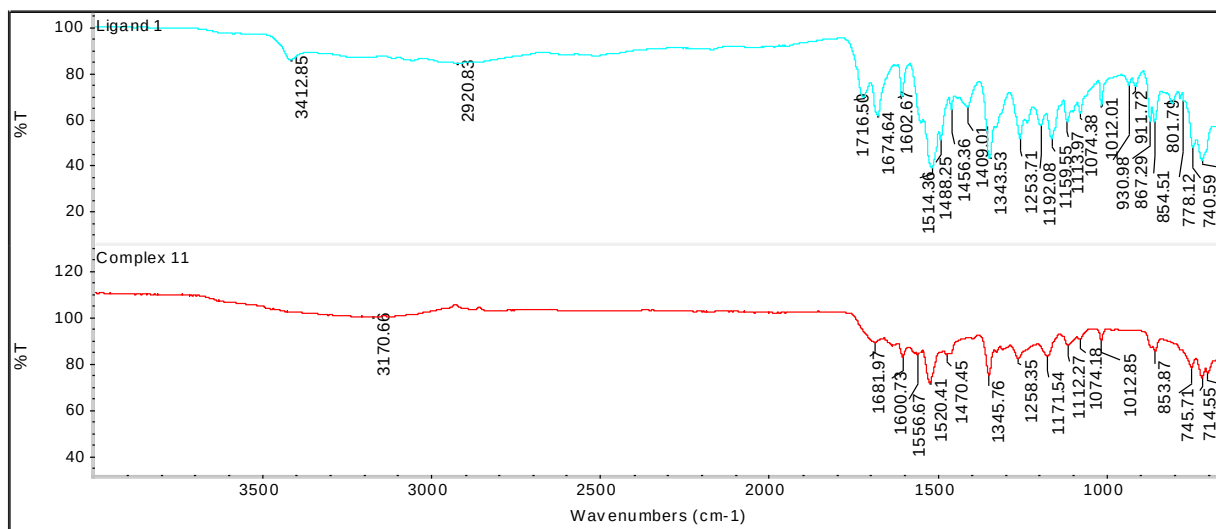


Figure 4.5: FTIR spectrum of ligand **1** and its complex **11**

Table 4.7: Comparison of FTIR stretching vibration (cm^{-1}) of thiourea ligand **1-5** with copper(II) complex **11-15**

Stretching vibration (cm^{-1})	Compounds									
	(1)	(11)	(2)	(12)	(3)	(13)	(4)	(14)	(5)	(15)
$\nu(\text{N-H})$	3412	3170	3218	2986	3237	2964	3281	3116	3113	3072
$\nu(\text{C=O})$ carboxylic	1716	-	1722	-	1716	-	1709	-	1716	1703
$\nu(\text{C=S})$	854	853	853	854	854	853	847	854	848	851
$\nu(\text{C-N})$	1159	1171	1156	1169	1163	1166	1167	1170	1157	1168
$\nu(\text{C=C})$ aromatic	1514	1520	1508	1523	1521	1519	1532	1519	1519	1523
$\nu(\text{C=O})$ amide	1674	1681	1681	1682	1672	1679	1659	1671	1668	1651

4.2.3 Characterisation of Monothiourea Complexes (6-10) Using ^1H And ^{13}C -Nuclear Magnetic Resonance (NMR) Spectroscopy

The ^1H and ^{13}C NMR spectroscopic analysis was performed on thiourea Ag(I) complexes. Figure 4.6 shows the ^1H NMR spectrum of complex **6** in DMSO- d_6 , while the ^1H NMR spectrum of complex **7-10** are shown in Appendix A23-A26. Significant chemical shift of $\delta_{\text{H}}(\text{NH})$, $\delta_{\text{H}}(\text{ArH})$, $\delta_{\text{H}}(\text{CH})$, $\delta_{\text{H}}(\text{CH}_2)$ and $\delta_{\text{H}}(\text{CH}_3)$ were observed.

For ^1H NMR, there were two (N-H) peaks observed which represent resonance of (CONH) and (CSNH). The chemical shifts of the two (N-H) signals were distinct, owing to the existence and influence of the electron withdrawing group and intramolecular hydrogen bond in the molecule (Saeed et al., 2010). Singlet peak attributed to (CSNH) for complex (**6-10**) were observed in the range from δ_{H} 11.88-11.94 ppm. Whereas the doublet peaks of (CONH) bonds for complex (**6-10**) were observed from δ_{H} 11.09-11.23 ppm. The presence of hydrogen attached to aromatic groups are observed as multiplet peaks in the range of δ_{H} 8.0-8.30 ppm for complex (**6-10**). It was observed that only slight shifts occurred in aromatic peaks of thiourea complexes especially at Ar- H_2 in which chemical shift appeared slight downfield compared to their free ligands. The overlapping of proton signals in the aromatic ring might contributed to these characteristics along with suggestion that complexation have also taken place. Distinctive feature of complex **6** can also be observed from multiplet aromatic peaks presence at δ_{H} 6.89-7.49 ppm. Ar- H_1 of all thiourea compounds exist downfield at lower frequency compared to Ar- H_2 due to the strong withdrawing group of NO_2 that is closer to Ar- H_1 .

Apart from that, resonance proton (CH) of thiourea complex **6**, **7**, **9** and **10** were observed ranging between signals δ_{H} 3.22-4.96 ppm. Multiplet peaks of (CH) resonance signal at δ_{H} 2.16-2.46 ppm can be observed to characterise the structure of complex **8** that shifted from the free

ligand, δ_H 2.33-2.46 ppm. Most (CH) resonance shown slight chemical shift shielded to low frequency at δ_H 4.97 ppm of free ligand to δ_H 4.96 ppm of complex **7**, δ_H 4.77 ppm of free ligand to δ_H 4.33 ppm of complex **9** and δ_H 3.57 ppm of free ligand to δ_H 3.41 ppm of complex **10**, while complex **6** appeared slight deshielded towards high frequency shifting to δ_H 3.22 ppm from its free ligand of δ_H 2.47 ppm. Furthermore, distinctive peaks at δ_H 2.45 ppm and 2.49-2.43 ppm owing to the presence of proton (CH₂) of complex **6** and **10** respectively. The unique characteristic of complex **7** compared to other complexes is the presence of two (CH₂) resonance at δ_H 2.40-2.53 ppm. Meanwhile, the presence of (CH₃) resonance of complex **7** and complex **9** were observed from δ_H 2.05-1.37 ppm. The presence of electronegative group (sulphur) near (CH₃) have shifted the signal downfield for complex **7** compared to complex **9**. Moreover, distinctive feature of complex **8** is shown with the presence of peak at δ_H 0.96-0.88 ppm that are attributed to two (CH₃) presence in the compound. The values of chemical shift in ¹H NMR spectroscopy for the complexes observed a shift from the ¹H NMR spectroscopy of thiourea ligands corresponding to carboxyl moieties that are bound to silver ion (Uznanski et al., 2017).

In addition, paramagnetic nature of thiourea Cu(II) complexes will result in broader signals, hence, NMR spectroscopic analysis was not carried out for these complexes. The selection of Cu(II) complexes in our antibacterial study was grounded in the rationale of exploring the antimicrobial potential of transition metal complexes, particularly copper, which has been recognized for its diverse biological activities including antibacterial properties. While NMR analysis of paramagnetic complexes such as those involving Cu(II) presents challenges, alternative spectroscopic characterization techniques such as FTIR spectroscopy, can offer valuable insights into their molecular structures and coordination.

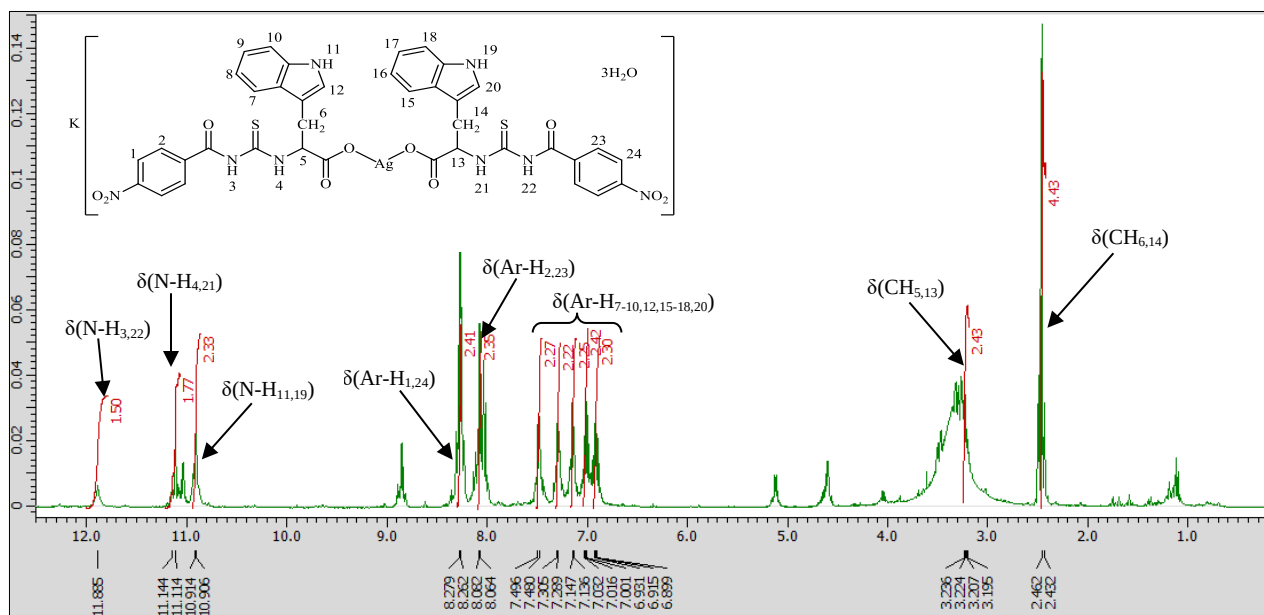


Figure 4.6: ^1H NMR spectrum of complex **6**

The chemical structures of thiourea complex **6**, **7**, **8**, **9** and **10** were further established by the appearance of important carbon signals of $\delta_{\text{C}}(\text{COO})$, $\delta_{\text{C}}(\text{CSNH})$, $\delta_{\text{C}}(\text{CONH})$, $\delta_{\text{C}}(\text{ArC})$, $\delta_{\text{C}}(\text{CH})$, $\delta_{\text{C}}(\text{CH}_2)$ and $\delta_{\text{C}}(\text{CH}_3)$. Figure 4.7 shows the ^{13}C NMR spectrum of complex **6**, whereas ^{13}C NMR spectrum of complex (**7-10**) are shown in Appendix A27-A30.

The carbon peaks of (CONH) were more deshielded and exist at the most downfield of high frequency followed by carbon of (COO) and (CSNH) due to the higher electronegativity value of C=O compared to sulphur atom. The (N-H) groups were shown with the presence of two distinctive absorption peaks of (CONH) and (CSNH). The signals were observed to be deshielded due deshielding effects by several reasons which are formation of intramolecular hydrogen bonding in the molecule, increasing electronegativity of sulphur and oxygen atoms as well as due to different environment (Hassan et al., 2011). The presence of the complex was also confirmed by the signal of carbon peaks (CSNH) observed from δ_{C} 149.9-167.4 ppm for

complex (**6-10**). Furthermore, the carbon signals attributed to (CONH) for complex (**6-10**) were resonated at δ_C 172.3-180.6 ppm. Slight chemical shift toward lower frequency were observed in complex **6**, **8** and **9** compared to their free ligands while, no obvious shift detected for signals in complex **7** and **10**. Meanwhile, larger chemical shift was seen on all carboxylate group of complexes compared to their ligand. The peaks attributed to the presence of carbon of carboxylate group is observed shifting from δ_C 172.3 ppm of free ligands to δ_C 167.4 ppm of complex **6**, δ_C 172.3 ppm of free ligands to δ_C 172.3 ppm of complex **7**, δ_C 171.8 ppm of free ligands to δ_C 167.8 ppm of complex **8**, δ_C 173.4 ppm of free ligands to δ_C 167.6 ppm of complex **9** and δ_C 166.3 ppm of free ligands to δ_C 166.4 ppm of complex **10**. The significant shift has suggested that silver bonded to carboxylate to form complex (Uznanski et al., 2017).

Apart from that, carbon of aromatic group can be observed by multiple peaks in the range of δ_C 123.8-150.5 ppm for complex **7-10**, showing slight shifting from their free ligand of δ_C 123.7-150.4 ppm. Whereas multiplet peak signals between δ_C 124.0-150.3 ppm and δ_C 109.3-121.5 ppm of complex **6** were shown for its unique presence of aromatic group in the compound's structure compared to other thiourea complexes. Slight chemical shifts were observed in carbon of aromatic group compared to their free ligands. Furthermore, the presence of carbon (CH) for complex (**6-10**) was indicated by resonance signals from δ_C 48.5-58.04 ppm shifted from their free ligand of δ_C 53.7-58.9. The absorption peaks of (CH₂) were observed at δ_C 27.7 ppm for complex **6** shifting from ligand **1** of δ_C 32.9 ppm, followed by 3 δ_C 3.1 ppm for complex **10** shifting from ligand **5** of δ_C 32.9 ppm and two different environments appeared at δ_C 29.7 ppm and δ_C 30.9 ppm of complex **7** which also showed chemical shift from ligand **2** of δ_C 30.9 ppm and δ_C 29.7 ppm. Distinctive peaks were also observed at δ_C 15.2 ppm, 19.6-18.0 ppm and 18.5 ppm attributed to (CH₃) of complex **7**, **8** and **9** respectively. The (CH₃) aliphatic

groups of complex **7**, **8** and **9** show significant chemical shift from their free ligands **2** (δ_C 15.1 ppm), **3** (δ_C 19.17 ppm) and **4** (δ_C 17.70 ppm) correspondingly. All detected signals were consistent with the presence of the corresponding aliphatic or aromatic group in each complex, reflecting the conformation of the synthesised complex.

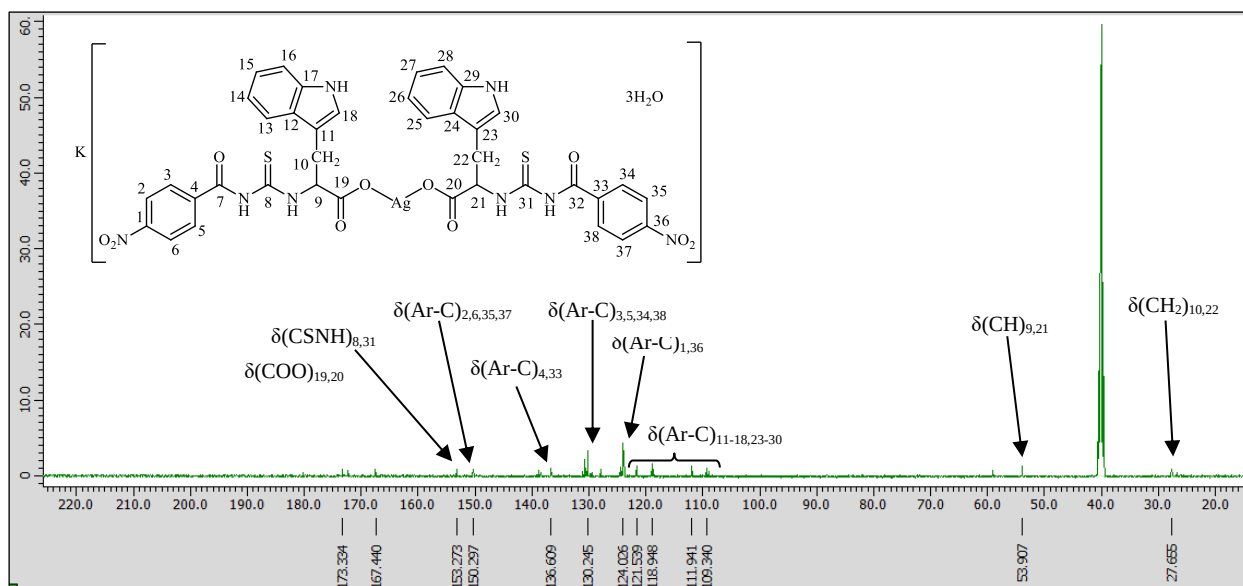


Figure 4.7: ^{13}C NMR spectrum of complex **6**

4.3 Antibacterial Activity

The antibacterial activity of all the synthesised compounds in various concentrations (50 ppm, 100 ppm, 150 ppm, 200 ppm, 250 ppm, 500 ppm and 1000 ppm) were determined using the disc diffusion method using various concentrations. The bacteria such as *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* were all tested against compounds (1–15). Each bacteria's standard antibiotic was employed as positive control. In contrast, DMSO was used as the negative control (Ngaini & Mortadza, 2019). The outcomes were categorised based on the observed zone of inhibition, with measurements falling within the range of 6-13 mm designated as 'mild', and those ranging from 14-16 mm categorised as 'moderate' (Abd Halim & Ngaini, 2017).

4.3.1 Antibacterial Activity of Thiourea Ligands (1-5)

The result obtained from antibacterial study of ligand (1-5) is summarised and comprehensive analysis of the data was present in Table 4.8 and Table 4.9. Notably, when tested against gram positive bacteria, all ligands exhibited negligible bactericidal action at concentrations less than 500 ppm. The negligible bacterial action could be attributed to the steric hindrance effect caused by the slight modulation involving the bulky group of tryptophan and nitrobenzoyl group found in the structure of ligand **1** for instance, thus increasing the compound's steric hindrance. The reduced antibacterial activity can further be attributed to the low lipophilicity character of the ligands and complexes, due to the presence of bulky groups in their structure and the resulting steric hindrance (Ngaini et al., 2012). The occurrence of steric hindrance generated by these bulky groups has created a force that obstructs the interaction between active sites in the compound and the receptor site of the bacteria (Fakhar et al., 2018).

This hindrance decreases the lipophilicity character of the compound, making it more challenging to penetrate the bacterial cell membrane and ultimately reducing its antibacterial activity.

Only ligands **1** and **2** exhibit mild antibacterial action at concentrations of 500 ppm and above. Ligand **1** inhibits *B. cereus* with a mild inhibition of 8 and 9 mm at 500 and 1000 ppm concentrations, respectively. Similarly, ligand **2** inhibits *S. aureus* and *L. monocytogenes* with inhibition zones of 10 and 7.5 mm at 500 ppm and 10 and 9 mm at 1000 ppm, respectively (Appendix A31-A33). The antibacterial activity shown by ligand **2** could be possibly due to the increase in the substituted aliphatic chain length of thiourea compound containing methionine side chain, thus increases the biological activity (Liang et al., 2018). As the alkyl chain gets longer, the inclusion of a functional group such as carboxylic acid and an increase in the amino acid side chain may increase the hydrophobicity of the carbon chain and enhancing the compound's lipophilicity. The increased carbon chain length enhances lipophilicity and might be the reason for the improved cytotoxicity (Fatima et al., 2017). However, the presence of an inhibition zone around the DMSO disc, despite its role as a solvent in the study (negative control), may indicate a potential issue with the experimental setup, such as contamination or unintended effects, necessitating further investigation and corrective measures.

Table 4.8: Zone of inhibition (mm) of synthesised compound (1-5) on gram positive bacteria

Table 4.6: Zone of inhibition (mm) of synthesised compound (1-5) on gram positive bacteria																					
	Zone of Inhibition (mm)																				
	<i>S. aureus</i>							<i>B. cereus</i>							<i>L. monocytogenes</i>						
Concentration (ppm)	50	100	150	200	250	500	1000	50	100	150	200	250	500	1000	50	100	150	200	250	500	1000
Compound																					
1	-	-	-	-	-	-	-	-	-	-	-	-	8	9	-	-	-	-	-	-	-
2	-	-	-	-	-	10	10	-	-	-	-	-	-	-	-	-	-	-	-	7.5	9
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DMSO (-): 6.5 mm Standard antibiotic (+): Penicillin = 17 mm Vancomycin =15 mm Ampicillin = 19 mm																					
*Note: (-) = No activity																					

Table 4.9: Zone of inhibition (mm) of synthesised compound (1-5) on gram negative bacteria

Concentration (ppm) Compound	Zone of Inhibition (mm)																				
	<i>E. coli</i>							<i>K. pneumoniae</i>							<i>P. aeruginosa</i>						
	50	100	150	200	250	500	1000	50	100	150	200	250	500	1000	50	100	150	200	250	500	1000
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	7	-	-	-	-	-	8	8	-	-	-	-	-	9	12
3	-	-	-	-	-	-	-	-	-	-	-	-	7	7	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	7	8	12	13	13	13	14	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	10	11	-	-	-	-	-	-	-
DMSO (-): 6.5 mm Standard antibiotic (+): Tetracycline = 19 mm Ceftazidime =17 mm Imipenem = 17 mm																					
*Note: (-) = No activity																					

On the other hand, when tested against gram negative bacteria, it was observed that ligand **4** shows the most pronounced antibacterial activity against *K. pneumoniae* beginning from 50 ppm, with the inhibition zones continuously increasing from 7 ppm until 14 ppm at 1000 ppm (Appendix A35-A36). Meanwhile, ligands **2**, **3**, and **5** have only shown mild antibacterial activity against gram negative bacteria at concentrations of 500 and 100 ppm (Appendix A34, A37-A39), similar to the results found for gram positive bacteria. The cell wall penetration ability of any antibacterial agent is expected to be greater in the case of gram-positive bacteria compared to gram-negative bacteria agents (Mukherjee et al., 2017). The difference in cell wall penetration ability between gram-positive and gram-negative bacteria can be attributed to their structural differences. Gram-positive bacteria have a relatively simple cell wall structure (lack of outer membrane) composed mainly of a thick layer of peptidoglycan, which is loosely packed and porous, allowing easier penetration by antibacterial agents (Silhavy et al., 2010; Soares et al., 2012). In contrast, gram-negative bacteria have an additional outer membrane that surrounds the peptidoglycan layer which acts as a barrier, preventing the entry of foreign molecules including host defence molecules and antibiotics (Saxena et al., 2023), thereby making it more difficult for antimicrobial agents to penetrate the cell wall and reach the inner cytoplasmic membrane. These ligands might also contain an unfavourable side chain that hindered the antibacterial action of the thiourea molecule. This could be due to the surface membrane of the bacteria, which is constructed by membrane layers of anionic sites such as phosphate and carboxyl group that offer potential sites for electrostatic binding (Wan Zullkiplee et al., 2016). Compounds with unfavourable properties may prevent the binding process with the cellular component of the bacteria, resulting in the absence of antibacterial activity. This shown that the thiourea bearing amino side chain also played important roles in activity as it

might hindering the active site of thiourea and have no effect on the compound's selectivity for targeted cell and the character of the compound might be unfavourable for binding process to those specific sites of bacteria cell. The graphs shown in (Appendix A87-A88) were employed to visually illustrate the pattern and represent the inhibition zone of synthesised ligands against gram-positive bacteria and gram-negative.

4.3.2 Antibacterial Activity of Thiourea Complexes (6-15)

The result obtained from antibacterial activity of complexes (6-15) were shown in Table 4.10 and Table 4.11. When tested against gram positive bacteria, all complexes also showed negligible bactericidal action at concentration less than 500 ppm. Apart from that, incorporation of silver(I) and copper(II) into thiourea ligands were seen to enhanced the antibacterial activity compared to their free ligand. It was observed that only complex **15** demonstrated mild antibacterial activity against *S. aureus* with an inhibition zone of 9 mm at 50 ppm and continuously increasing until 10 mm at 1000 ppm (Appendix A46- A47). It can be observed that complex **13** showed the highest activity against *S. aureus* followed by complex **14, 8, 11, 15, 12** and **9**, respectively. Whereas against *B. cereus*, the incorporation of both copper and silver metal in complex **9, 10, 14, 15** shows significant increase in antibacterial activity, in contrast with their free ligand **4** and **5** that failed to show inhibition. The enhancement in the antibacterial activity of silver(I) and copper(II) metal chelates possibly because of the effect of the incorporation of metal ion on the normal cell process. Chelation enables the ligand to act as more powerful and efficacious bactericidal agents by inhibiting the growth of bacteria, in which the zone of inhibition of metal complexes was found to be larger than that of their free ligands (Nagesh et al., 2014). Chelation possibly enhance the lipophilic nature of the central metal atom,

which then favours its penetration through the lipid layers of cell membrane as chelation decreases the polarity of the metal from the partial sharing of its positive charge with donor groups and probable electron delocalisation over the whole chelate ring (Ramesh et al., 2016).

Furthermore, in the experiment against gram negative bacteria, only few complexes have demonstrated antibacterial activity at concentration less than 500 ppm. For instance, complex **14** shown inhibition against *E. coli* bacteria at 100 ppm concentration with 7 mm inhibition zone and increasing to 9 mm at 1000 ppm (Appendix A58-A59). Meanwhile, complex **7** shown mild antibacterial activity against *K. pneumoniae* beginning from 100 ppm, with the inhibition zones continuously increasing from 8 mm until 9.5 mm at 1000 ppm (Appendix A63-A64). This is followed by complex **9** exhibited mild antibacterial action with 7 mm zone of inhibition and increasing to 8 mm at 1000 ppm (Appendix A66-A67). In addition, only complex **7** exhibited mild antibacterial action against *P. aeruginosa* at concentrations of 100 ppm and above (Appendix A73-A74). On the other hand, most of the complex (**6-15**) demonstrate mild to moderate antibacterial action against gram negative bacteria at concentrations of 500 and 100 ppm. The difficulty to show their inhibitory action might be attributed to the occurrence of steric hindrance force that obstructs the interaction between active sites in the compound with receptor site of the bacteria (Fakhar et al., 2018). Moreover, the presence of electron-withdrawing (nitro-group) might also affect the antibacterial activity. This is because electron-withdrawing groups do not favour the hydrogen bonding formation (Zheng et al., 2011), thus the more electron-withdrawing groups there are, the weaker the interaction that will be formed. Incorporation of silver(I) and copper(II), which exhibit more inhibition than their respective ligand precursors, demonstrates the positive effect of the metal ions in the complexes. The desired biological feature formed from the complexation might also play a role in chemical qualities of ligand-

metal bond that helps to boost the selectivity toward targeted cell. The reason behind this could be because the ligands utilised in metal complexes operates as carrier for the silver(I) ions and copper(II) ions into the biological system, and the magnitude of complex's antibacterial capabilities may be connected to their ease participation in ligand exchange process (Azócar et al., 2014), implying that the ease with which ions released from the complex was crucial in the process. The synergistic effect of the increased lipophilicity of the central metal atom also played a role, which allows the complex to penetrate the membrane through the lipid layer of the cell membrane and inactivate the active enzyme site of the microorganism (Liang et al., 2018; Arslan et al., 2009), thus inactivate the bacteria.

It can be summarised that complexes (**6–15**) had significantly higher antibacterial activity than their free ligands (**1–5**). Copper(II) complex **15** was observed to have the highest inhibition against gram-positive bacteria, whereas copper(II) complex **14** showed the highest inhibition against gram-negative bacteria. It was also demonstrated that gram-positive bacteria were more vulnerable to thiourea complexes than ligands since each of the thiourea complexes successfully inhibited certain gram-positive bacteria. The graphs represent the inhibition zone of synthesised silver(I) complex (**6–10**) against gram-positive bacteria and gram-negative are shown in (Appendix A89-A90). Whereas graph represent the inhibition zone of synthesised copper(II) complex (**11–15**) against gram-positive bacteria and gram-negative are shown in (Appendix A91-A92).

Table 4.10: Zone of inhibition (mm) of synthesised compound (6-15) on gram positive bacteria

Concentration (ppm) Compound	Zone of Inhibition (mm)																				
	<i>S. aureus</i>							<i>B. cereus</i>							<i>L. monocytogenes</i>						
	50	100	150	200	250	500	1000	50	100	150	200	250	500	1000	50	100	150	200	250	500	1000
6	-	-	-	-	-	-	-	-	-	-	-	-	-	7.5	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8
8	-	-	-	-	-	10	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	8	8	-	-	-	-	-	-	8	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-	7.5	8	-	-	-	-	-	-	-
11	-	-	-	-	-	7	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	7	10	-	-	-	-	-	-	-	-	-	7.5	7.5	7.5	7.5	7.5
13	-	-	-	-	-	13	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	9	15	-	-	-	-	-	9.5	10	-	-	-	-	-	-	-
15	9	9	9.5	9.5	9.5	9.5	10	-	-	-	-	-	8	9.5	-	-	-	-	-	-	-
DMSO (-): 6.5 mm Standard antibiotic (+): Penicillin = 17 mm Vancomycin =15 mm Ampicillin = 19 mm																					
																					*Note: (-) = No activity

Table 4.11: Zone of inhibition (mm) of synthesised compound (6-15) on gram negative bacteria

Concentration (ppm) Compound	Zone of Inhibition (mm)																				
	<i>E. coli</i>							<i>K. pneumoniae</i>							<i>P. aeruginosa</i>						
	50	100	150	200	250	500	1000	50	100	150	200	250	500	1000	50	100	150	200	250	500	1000
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	11	11
7	-	-	-	-	-	-	-	-	8	8	8	8	9	9.5	-	7	7.5	7.5	7	7.5	8
8	-	-	-	-	-	-	9.5	-	-	-	-	-	9	13	-	-	-	-	-	-	8.5
9	-	-	-	-	-	-	8	-	7	7	7	7.5	8	8	-	-	-	-	-	-	-
10	-	-	-	-	-	8	8	-	-	-	-	-	-	7	-	-	-	-	-	-	8
11	-	-	-	-	-	8	9.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	7	7	7	7	9	-	-	-	-	-	8	8	-	-	-	-	-	-	7.5
13	-	-	8	8	8	8	8	-	-	-	-	-	12	16	-	-	-	-	-	-	-
14	-	7	7	7.5	7.5	8.5	9	-	-	7	7.5	7.5	8.5	9	-	-	-	8	8	8	8
15	-	-	-	-	-	7.5	7.5	-	-	-	-	-	-	7	-	-	-	-	-	8	9
DMSO (-): 6.5 mm Standard antibiotic (+): Tetracycline = 19 mm Cefazidime = 17 mm Imipenem = 17 mm																					
																					*Note: (-) = No activity

In conclusion, the bacterial growth inhibitory properties of the thiourea ligands and complexes were evaluated based on the zone of inhibition. A clear zone of inhibition was observed in some of the compounds which indicates a moderate bacterial growth inhibitory effect. The antibacterial activity was categorised into mild and moderate activity in which all ligands showed mild activity in both gram-positive and gram-negative bacteria except for ligand **4** that showed moderate activity against *K. pneumoniae*. Furthermore, metal complexes (**6-12** and **15**) displayed mild activity against gram-positive bacteria compared to complex **13** and **14** which showed a moderate activity against *S. aureus*. Inhibition against gram-negative bacteria revealed mild antibacterial activity by all complexes except for complex **13** that showed moderate activity against *K. pneumoniae*. The antibacterial properties were later tested for docking via Autodock vina to better understand their interaction with the protein of the bacteria. From the inhibition zone result, it can be concluded that antibacterial activities of thiourea complexes are higher compared to their precursor ligand which supported the hypothesis of this research. (Ikokoh et al., 2015). Given the importance of lipids in cell and mitochondrial membranes, the presence of silver(I) and copper(II) in the molecular structure of the compound could possibly increase the lipophilicity and improve the bioavailability of silver(I) and copper(II) that may cause cytotoxicity by suppressing cellular respiration. Metal complexes exhibit much greater antibacterial activity than their corresponding ligands, which may be attributed to the chelation process, that decreases the polarity of metal ions by coordinating with ligands (Sumrra et al., 2014). In complexes, ligand delocalizes its π electrons with metal ion and metal ion partially shares its positive charge with the ligand, which results in the decrease of the polarity of the complexes (Khan et al., 2020). This decrease in polarity contributes to an increase

in the lipophilicity of the metal complexes. Since lipophilic molecules have an affinity for lipid environments, an increase in lipophilicity enhances the ability of the metal complexes to interact with lipid-rich environments, such as cell membranes. The decrease in the polarity of the metal thiourea complexes has consequently increase the lipophilicity of metal complex which helped the molecules to interact or penetrate more easily through the cell membrane of microbes and thus deactivating them (Ikokoh et al., 2015). The coordination of the metal ions with thiourea ligand can also lead to the formation of bulky molecular structures with hydrophobic regions and these hydrophobic regions contribute to the overall lipophilicity of the metal complex, further enhancing its ability to interact with lipid membranes.

Gram-negative bacteria were more susceptible to all tested thiourea ligand and complexes (**1-15**) than gram-positive bacteria. This could be possibly due to the thickness of the peptidoglycan layer of gram-positive bacteria, which prevents the silver ions from passing through the bacterial cell wall (Woo et al., 2008). The composition of peptidoglycan, which includes components such as lipopolysaccharide, lipoprotein, and phospholipids, can influence the thickness and diffusion properties of the cell wall in gram-positive bacteria (Mohamed et al., 2013; Ngaini et al., 2020). However, against gram-negative bacteria, metal complexes (**6-15**) showed more antibacterial activity than their free ligands; although in certain instances, the thiourea ligand demonstrated a larger inhibitory zone than their silver complexes. Although the mechanism by which ligand-metal complex compounds exert antibacterial activity is not completely understood, it has been suggested that the activity of metal-based complexes is strongly connected to their stability, lipophilicity, redox ability, and rate of metal ion release, with the coordinating ligand acting as a carrier for the metal ion (Claudel et al., 2020). Thus,

these factors may play a critical role in the inactivation of bacteria's cell functioning, and their absence may render the compounds bacterial activity.

4.4 Molecular Docking Study of Thiourea Ligands

The docking studies were conducted on compounds exhibiting an inhibition zone greater than 10 mm. This threshold was chosen to prioritise the investigation of the most promising antibiotic candidates, streamlining the analysis and focusing on compounds with the highest inhibitory potency while excluding compounds with lower inhibitory potency. Molecular docking was performed after synthesis to elucidate the potential binding modes and interactions of the synthesized compounds with target proteins, providing insights into how these compounds interact with bacterial targets, and aids in validating the observed antibacterial activities. The compounds with such activity were ligand **2** for inhibiting *S. aureus* and *P. aeruginosa*, and ligand **4** for inhibiting *K. pneumoniae*. The crystallographic structures of targeted protein of *S. aureus* (PDB entry: 4xwa), *K. pneumoniae* (PDB entry: 5xun) and *P. aeruginosa* (PDB entry: 7ci4) were obtained from the Protein Data Bank. These proteins were identified as a possible target enzyme for compounds **2** and **4** based on their greatest affinity scores showed in the Table 4.12. The increase in the affinity score, reflecting the enhanced intermolecular force between the protein receptor and ligand, varies depending on the specific compounds and protein targets under study. Previous research, such as that by Meng et al. (2012), has shown that prediction of the ligand within the active site of the protein leads to increased affinity scores, which can be quantified through scoring functions or binding affinity assessments. The interactions between the synthesised ligand **2** and **4** with the targeted protein receptors were studied, and the best docking poses were analysed. The scoring function

generates a score for each pose, and then resulting values are used to rank the different poses and compounds. The more fitting the compounds into the targeted protein, the lower the binding energy will be (Siqueira et al., 2021), which might suggest that the scoring is required for a quantitative estimation of a better predicted pose quality. Prior to determining the optimal conformation for each compound, it may be necessary to investigate docking poses for the presence of significant intermolecular forces. These docking poses correspond to a better binding mode of the ligand in term of abundance of hydrogen bond interaction with the protein residue. The inhibition of bacteria is formed via hydrogen bonding interaction of the compound with the active site of the enzyme (Ngaini & Mortadza, 2019). For comparative purposes, the antibiotic penicillin, imipenem and ceftazidime were used as positive control for *S. aureus*, *P. aeruginosa* and *K. pneumoniae* bacteria respectively (Refat et al., 2022). The results revealed that the potential binding energy of all the ligands were slightly higher than antibiotics in the respected bacteria receptors.

Table 4.12: The molecular docking results for thiourea ligand **2**, **4** and its respected antibiotic with *S. aureus* (PDB entry: 4xwa), *K. pneumoniae* (PDB entry: 5xun) and *P. aeruginosa* (PDB entry: 7ci4).

Ligand	Chemical formula	Binding Affinity (kcal/mol)		
		PDB entry: 4xwa	PDB entry: 7ci4	PDB entry: 5xun
2	C ₁₃ H ₁₅ N ₃ O ₅ S ₂	-7.7	-6.7	-
4	C ₁₁ H ₁₁ N ₃ O ₅ S	-	-	-6.9
Penicillin	C ₁₆ H ₁₈ N ₂ O ₄ S	-7.4	-	-
Imipenem	C ₁₂ H ₁₇ N ₃ O ₄ S	-	-5.6	-
Ceftazidime	C ₂₂ H ₂₂ N ₆ O ₇ S ₂	-	-	-6.9

Among the compounds, ligand **2** showed the highest docking energy with *S. aureus* (PDB entry: 4xwa) which suggest a stronger interaction compared to *P. aeruginosa* (PDB entry: 7ci4). Docking energy reflects the stability of the ligand-protein complex in which higher docking energy suggests stronger binding affinity between the ligand and protein, indicating a more favourable interaction. The selected binding protein was identified as a possible target enzyme for compounds **2** and **4** based on their greatest affinity scores of (-7.7 kcal/mol) for *S. aureus*, (-6.7 kcal/mol) for *P. aeruginosa*, and (-6.9 kcal/mol) for *K. pneumoniae*. From the docking log analysis, these poses were chosen due to their excellent value and interaction with the amino acid residue of protein target. Additionally, molecular docking was performed on methionine and alanine as the amino acid side chains of compounds **2** and **4**, respectively, to evaluate the role of amino acid in the compound's antibacterial action. However, docking revealed a low binding affinity for both amino acids that are involved in compounds **2** and **4**. From the docking, methionine had a binding affinity of (-4.4 kcal/mol) against *S. aureus* and (-3.8 kcal/mol) against *P. aeruginosa*, respectively. Additionally, the binding affinity of alanine was found to be (-3.9 kcal/mol) against *K. pneumoniae*. The result obtained shows that the binding energy of thiourea ligand at the binding site of receptor protein is greater compared to free amino acid (Table 4.13).

Table 4.13: The molecular docking results for thiourea ligand **2**, **4** and its respected antibiotic with *S. aureus* (PDB entry: 4xwa), *K. pneumoniae* (PDB entry: 5xun) and *P. aeruginosa* (PDB entry: 7ci4) including the binding affinity and important amino acid residues of proteins that interact with the compounds.

Ligand	PDB entry	Binding score value (kcal/mol)	Important Residue
2	4xwa	-7.7	Gly12, Ser13, Gly14, Lys15 and Thr16
2	7ci4	-6.7	Glu55 and Arg272
4	5xun	-6.9	Thr64, Thr93 and Tyr86
Methionine	4xwa	-4.4	Thr16, Gly14, Ser13 and Cys15
Methionine	7ci4	-3.8	Asn251 and Asp89
Alanine	5xun	-3.9	Arg96 and Ser66

Considered their respective roles in the binding energy at the binding site of the receptor protein, thiourea ligand observed higher binding energy compared to the free amino acid suggests a stronger interaction between the thiourea ligand and the receptor protein. This indicates that the reactive functional group of thiourea, which is involved in binding to the receptor protein, may have greatly contributed to the bacteriostatic activity of the molecule. On the other hand, the amino acid thiourea side chain, while also present in the ligand structure, may only make a minimum contribution to the ligand's antibacterial activity, as reflected by its lower binding energy. Therefore, by comparing the binding energies of the thiourea ligand and the free amino acid provides insight into the roles of their respective functional groups in the ligand's antibacterial activity. This is because the synthesised compounds interact with the amino acid residues of the protein to occupy the active site, and eventually inhibit the activity of the protein enzyme (Tao et al., 2020).

The binding affinity of these reactions was later visualised with the presence of polar hydrogen bond interaction. In molecular docking, hydrogen bond interactions are crucial in biochemistry and biological recognition processes as they enhance interactions with cell membrane (Ngaini et al., 2020). It can also be observed later that presence of thiourea active group such as NH, C=O and C=S formed the most interaction with the amino acid residue of the bacteria. An illustration of the molecular docking for the interactions of the ligands and receptor is depicted in Figure 4.8 to Figure 4.10.

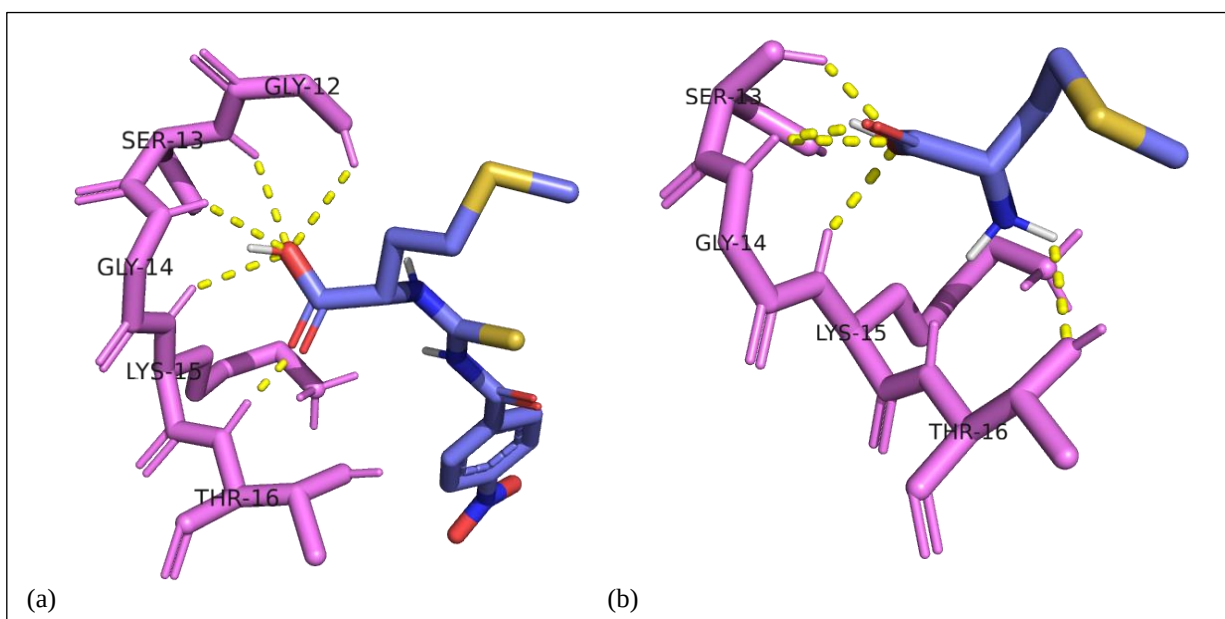


Figure 4.8: The 3D representation of the interactions for *S. aureus* (PDB ID: 4xwa) docked with (a) ligand 2 and (b) methionine.

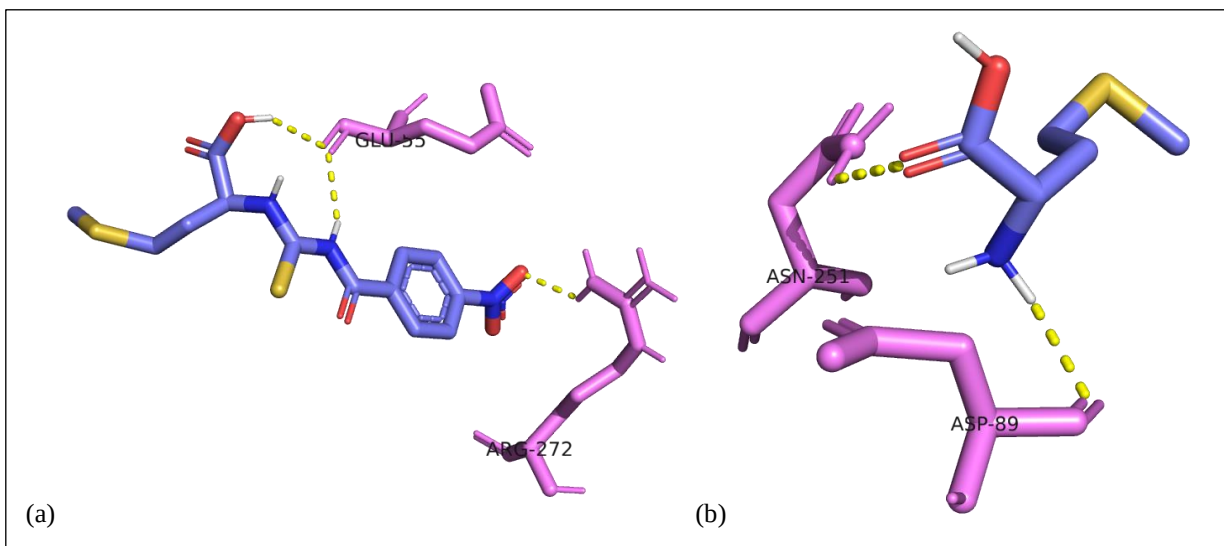


Figure 4.9: The 3D representation of the interactions for *P. aeruginosa* (PDB entry: 7ci4) docked with (a) ligand 2 and (b) methionine

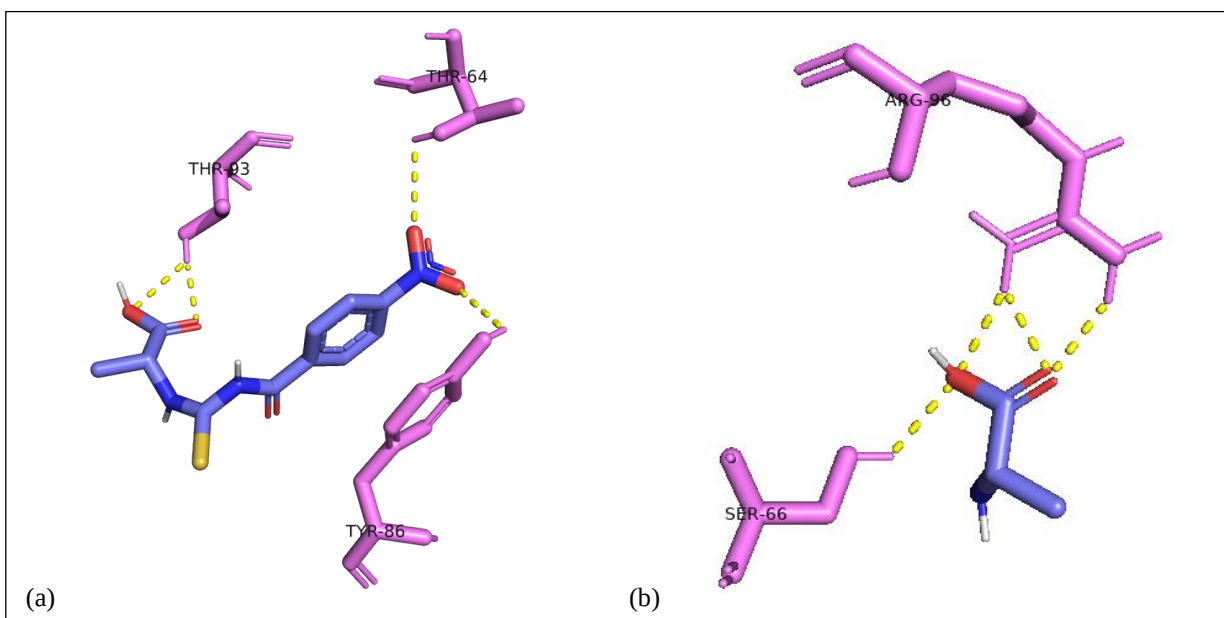


Figure 4.10: The 3D representation of the interactions for *K. pneumoniae* (PDB entry: 5xun) docked with (a) ligand 4 and (b) alanine.

From the PyMOL visualisation, there are numerous protein residues that are in proximity or interact with the ligand molecules. The amino acid residues of proteins interact via hydrogen bonds and are classified as polar interactions, as opposed to non-interacting amino acids, which are classified as non-polar interactions. The presence of H-bond interactions is essential to biochemistry and biological recognition processes because they facilitate interactions with cell membranes (Ngaini et al., 2020). Five hydrogen bonds and five residues were involved in the interaction in ligand **2**. It exhibited excellent molecular docking in the *S. aureus* binding site, with a binding affinity of (-7.7 kcal/mol), as well as hydrogen bond interaction between the oxygen of -NO₂ with hydrogen (NH) of GLY-12, SER-13, GLY-14, and LYS-15 residue. Oxygen of C=O showed hydrogen bonding interaction with hydrogen (-NH) of THR-16 residue. This could indicate that the considerable electrostatic contact occurred during the binding phase (Halim & Ngaini, 2016). Additionally, docking ligand **2** against the *P. aeruginosa* protein residues revealed that two residues produced three hydrogen bonds with a binding affinity of (-6.7 kcal/mol). Polar hydrogen bond interactions between the hydrogen of -NH and hydrogen of (-OH) with oxygen (C=O) of GLU-55 residue were observed, whereas the oxygen of -NO₂ interacts with the hydrogen (-NH₂) of ARG-272 residue. Although it might not show much of hydrogen bonds interaction against *P. aeruginosa*, but the docking score is high indicating the importance of electrostatic and hydrophobic interactions shown by the compound with the active site of the protein (Mathews et al., 2020). Similarly, a strong interaction between ligand **4** and *K. pneumoniae* was observed, with a binding affinity of (-6.9 kcal/mol). Four hydrogen bonds involving three residues were found in the polar interaction between oxygen of -NO₂ with hydrogen (-OH) of THR-64 and hydrogen (-OH) of TYR-86. Other than that, there were also interaction between oxygen of C=O and oxygen of -OH with hydrogen (-OH) of THR-93. A lot

of previous published study reported that ligands tend to dock in the binding cavity of receptor protein and directly interact with the residues of the catalytic site through hydrogen bonds (Drzewiecka-Antonik et al., 2020). Figure 4.11 depicts the conformation of ligand **2** and **4** in the binding site of bacterial structure.

Furthermore, molecular docking studies were also conducted on the amino acids that are involved in the side chains of ligands **2** and **4** against bacteria molecules. In the case of methionine against *S. aureus*, five hydrogen bonds were established with four residues through polar interaction between the oxygen -OH of carboxylic group with hydrogen (-NH) of SER-13 and LYS- 15. Oxygen (C=O) of the carboxylic group interacted with hydrogen (-NH) of GLY-14, and finally the hydrogen of -NH₂ interacted with oxygen (-OH) of THR-16. Similarly, docking between methionine against *P. aeruginosa* binding site revealed two hydrogen bonds with two amino acid residues. The hydrogen bond interactions were shown between oxygen (C=O) of carboxylic group with hydrogen (-NH₂) of ARG-96 and hydrogen of -NH₂ with oxygen (C=OOH) of ASP-89. Alanine, a side chain of ligand **4** generated four hydrogen bonds formed by two residues between the oxygen of the carboxylic group with hydrogen (NH₂) and hydrogen (C=NH₂) of ARG-96, whereas oxygen of -OH interact with hydrogen (C=NH₂) of ARG-96. Other hydrogen bond interaction was observed between oxygen of -OH and hydrogen (-OH) of SER-66 residue. Figure 4.12 depicts the binding pocket created by the interaction between methionine and alanine against the bacteria's molecule.

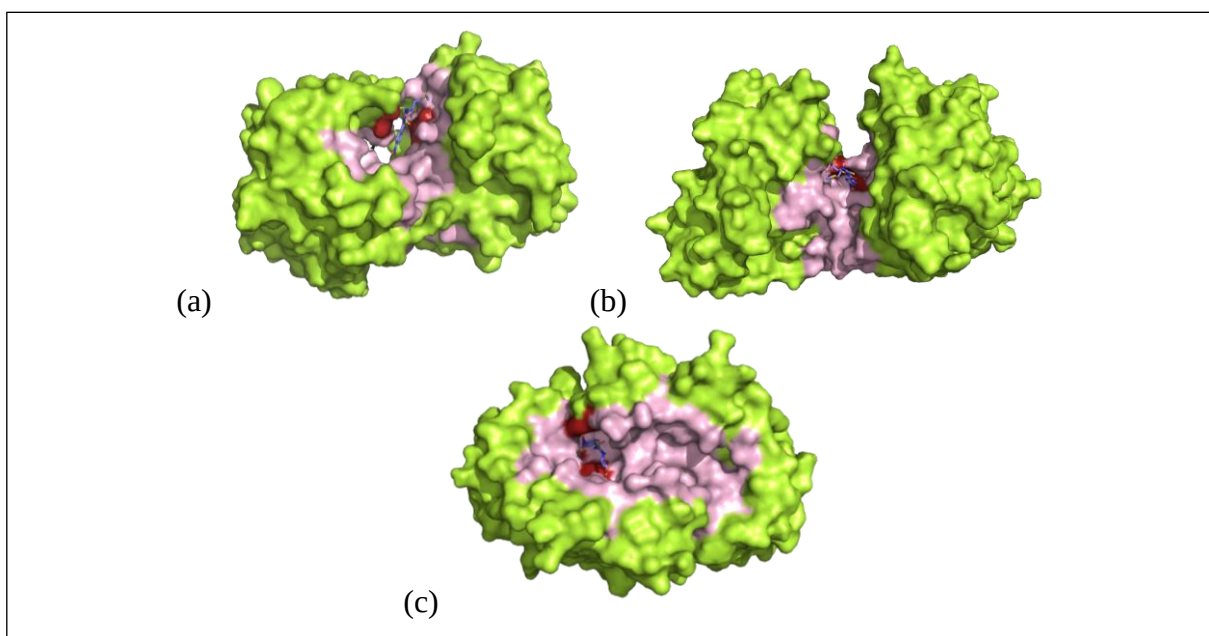


Figure 4.11: The molecular binding pocket of (a) ligand 2 docked with *S. aureus*, (b) ligand 2 docked with *P. aeruginosa* and (c) ligand 4 docked with *K. pneumoniae*.

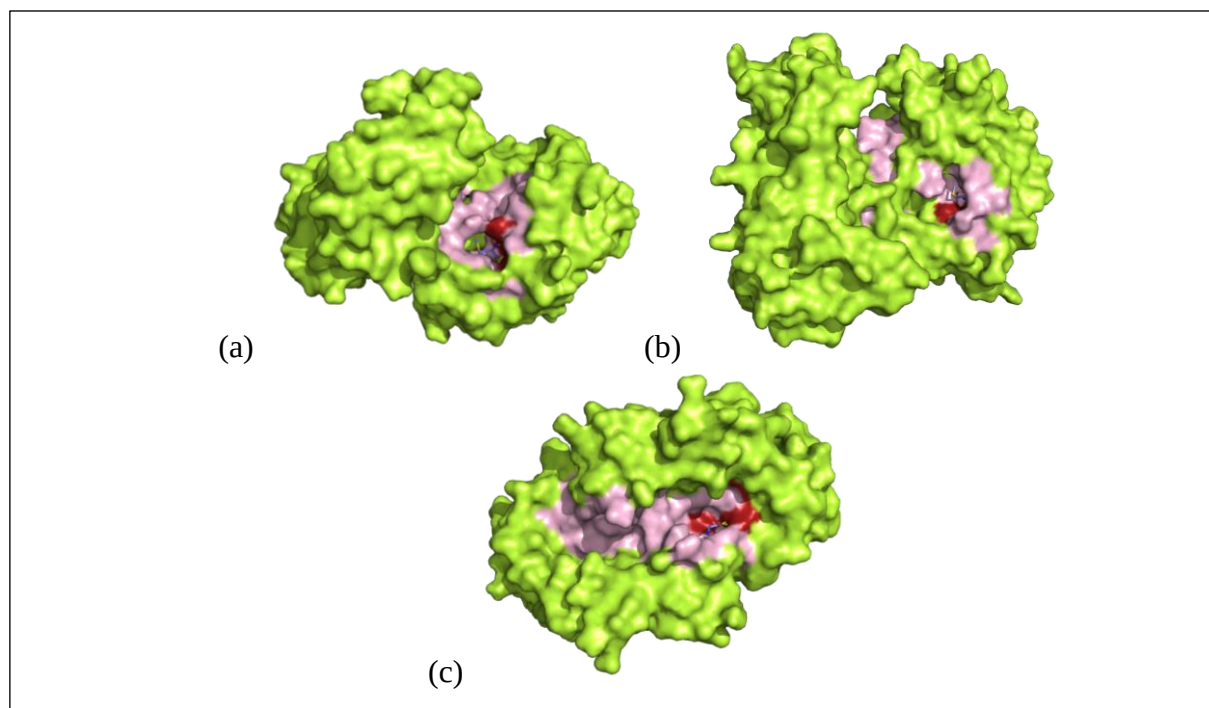


Figure 4.12: The molecular binding pocket of (a) methionine with *S. aureus*, (b) methionine docked with *P. aeruginosa* and (c) alanine docked with *K. pneumoniae*.

Figure 4.11 and Figure 4.12 depicted a binding site for interacting compounds to the amino acid residue of the bacteria. Protein-ligand interactions are critical for deciphering the molecular mechanisms underlying cell activity (Pérez de la Lastra et al., 2021). The green conformation represented the bacteria's chain involved in the docking, whereas the red conformation represented the polar interaction, and the pink conformation represented the non-polar interaction between the compounds and the residues. The binding sites might be accepted as the compound's potential poses because they perform well under the various binding scores acquired from docking. Thus, the computational approach of molecular docking interactions with amino acid residues with high binding affinity provided additional support for the inhibitory activity of ligand **2** and **4**.

4.5 Molecular Docking Study of Thiourea Complexes

Moreover, antibacterial activity of thiourea complexes showed a reasonably high inhibition as compared to thiourea ligand. For this consideration, docking studies was purposely carried out to understand the various possible complex-protein interactions. In this study, silver and copper metal were involved in complexation to thiourea ligand. Copper metal complex had a stronger inhibitory activity from inhibition zone of disc-diffusion method compared to silver metal complex. Due to limitations in the Autodock Vina program, the molecular docking analysis was restricted to the copper(II) complex. The software encountered difficulties when handling metal ions (silver metal) during docking procedures in which it was recognised as a valid AutoDock type, leading to constraints in the analysis. The analysis was only performed for copper(II) complexes that shown inhibition zone greater than 10 mm which were complex **11**, **12**, **13**, **14**, **15** against *S. aureus*, complex **14** against *B. cereus* and complex

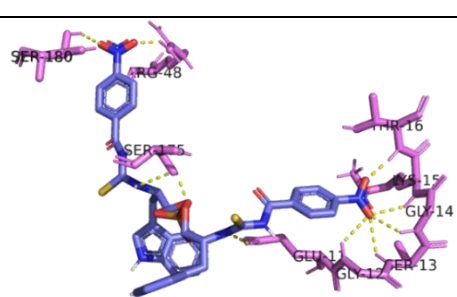
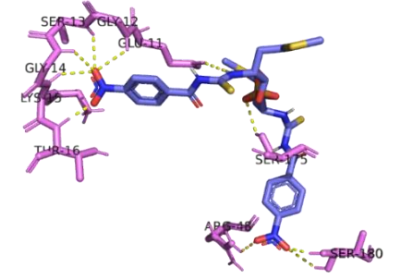
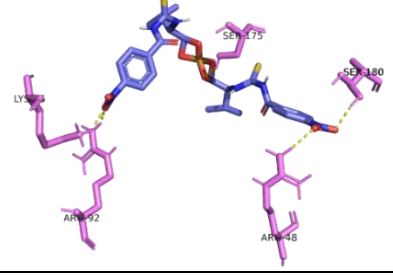
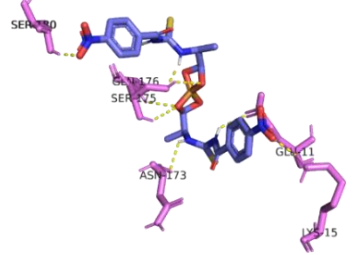
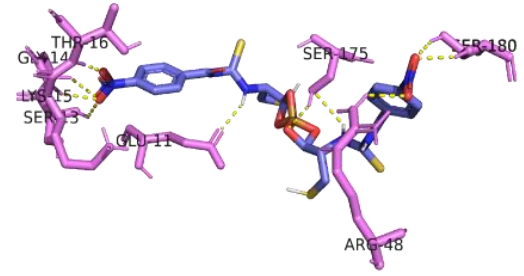
13 against *K. pneumoniae*. The crystallographic structures of targeted protein of *S. aureus* (PDB entry: 4xwa), *B. cereus* (PDB entry: 5bca) and *K. pneumoniae* (PDB entry: 5xun) were obtained from the Protein Data Bank. The selected binding proteins were identified as a possible target enzyme for these complexes due to their greatest affinity scores and are presented in Table 14. From the result, docking poses correspond with better polar hydrogen bond interactions were determined. Whereas the interaction energies were quite favourable due to negative free energy (Gordon et al., 2020).

Table 4.14: The molecular docking results for thiourea complex **11**, **12**, **13**, **14** and **15** with *S. aureus* (PDB entry: 4xwa), *B. cereus* (PDB entry: 5bca) and *K. pneumoniae* (PDB entry: 5xun) including the binding affinity score and important amino acid residues of proteins that interact with the compounds.

Complex	PDB entry	Binding score value (kcal/mol)	Important Residue
11	4xwa	-10.4	Ser180, Arg48, Ser 175, Glu11, Thr16, Lys15, Gly14, Ser13 and Gly12
12	4xwa	-9.0	Thr16, Lys15, Ser13, Gly12, Glu11 and Ser175
13	4xwa	-9.4	Ser180, Arg48, Ser175, Arg92 and Lys15
14	4xwa	-9.8	Ser180, Asn173, Glu11, Lys15, Ser175 and Gly176
15	4xwa	-9.3	Ser180, Arg48, Ser 175, Glu11, Thr16, Lys15, Gly14 and Ser13
14	5bca	-8.3	Tyr343, Lys379, Arg380 and Ala444
13	5xun	-9.2	Cys69, Asn88, Ser66 and Arg96

Based on the antibacterial study, the majority of complexes that inhibit gram positive bacteria were found to be effective against *S. aureus* bacteria. From the docking against *S. aureus* (PDB entry: 4xwa), complex **11** showed the most favourable negative free energy of (-10.4 kcal/mol) followed by complex **14** (-9.8 kcal/mol), complex **13** (-9.4 kcal/mol), complex **15** (-9.3 kcal/mol) and complex **12** (-9.0 kcal/mol). 2020). It was revealed before that the highest inhibition against gram-positive bacteria were shown by complex **14**. However, docking observed that complex **11** is the most active compound, thus the lower number of interactions shown by complex **11** as compared to complex **14** were unexpected. This unexpected result could be due to the different ways of these complexes bind to their target. The same finding in the result published by Maalik and coworkers (2019) were observed, in which they explained that the higher bonding energies and good docking score calculated for this compound (complex **11** in this case) may support the overall stability of the possible predicted complex and hence its activity. The 3D visualisations of the docked compound were displayed in Table 4.15.

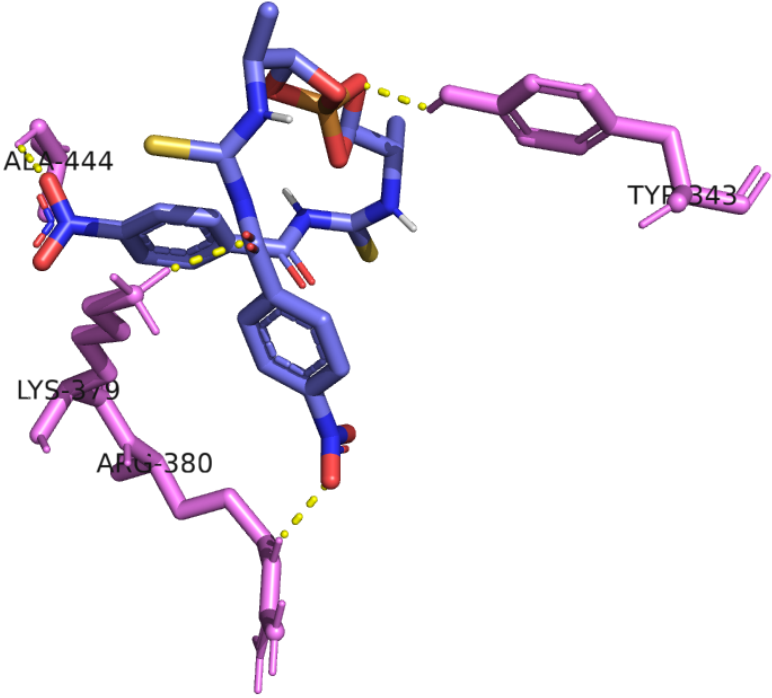
Table 4.15: 3D docking illustration between complex **11**, **12**, **13**, **14** and **15** against *S. aureus* (PDB entry: 4xwa)

Complex	Binding poses (kcal/mol)	PyMol Modelling
11	-10.4	
12	-9.0	
13	-9.4	
14	-9.8	
15	-9.3	

From the PyMOL visualization, there are numerous protein residues that are in proximity or interact with the ligand molecules. The amino acid residues of proteins interact via hydrogen bonds and are classified as polar interactions, as opposed to non-interacting amino acids, which are classified as non-polar interactions. In complex **11** against *S. aureus*, ten hydrogen bonds and nine amino acid residues were involved in the interaction. They displayed excellent molecular docking with a binding affinity of (-10.4 kcal mol). The oxygen of -NO₂ forms hydrogen bonds with the hydrogen (-OH) of SER-180 and the hydrogen (-NH) of ARG-48 residues. Oxygen of -NO₂ interacted with hydrogen (-NH) of THR-16, LYS-15, GLY-14, SER-13, and GLY-12. In addition, hydrogen interactions were discovered between the oxygen bonded to the metal centre and the hydrogen of the -NH residue in SER-175. This highest binding activity of complex **11** probably due to the stronger interaction involves between the complex and protein residue (resulted in higher biological activity). This is because the resulting binding energy of docked complexes defines the degree of stability of binding between the protein receptor and the target compound (Gordon et al., 2020). A higher docking activity (lowest binding energy) reflects greater degree of complementarity and tighter binding between the ligand and the protein receptor, leading to a more stable complex formation and better predicted affinity profile (Siquera et al., 2021). Furthermore, docking of complex **12** with *S. aureus* protein residues revealed the presence of ten hydrogen bonds and nine residues involved in the hydrogen bond interaction. They displayed remarkable molecular docking with a binding affinity of (-9.0 kcal/mol). Hydrogen bond interaction take place between oxygen of -NO₂ with hydrogen (-NH₂) of ARG-48 and hydrogen (-OH) of SER-180 residues. Other interactions were between oxygen of -NO₂ and hydrogen (NH) from THR-16, LYS-15, GLY-14, SER-13, and GLY-12. In addition, hydrogen interactions were found between the oxygen bonded to the metal

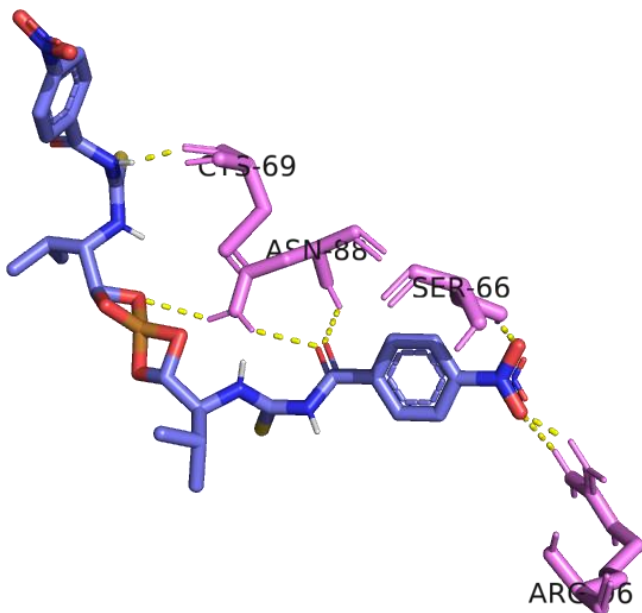
centre with hydrogen (-OH) of SER-175 and the hydrogen of -NH with oxygen (C=O) of GLU-11. These abundance of hydrogen bonds may justify the high antibacterial activity of copper complex against the bacteria (Souza et al., 2021). Moreover, five hydrogen bonds and five residues are involved in the interaction of complex **13** with *S. aureus*. They displayed outstanding molecular docking with a binding affinity of (-9.4 kcal/mol). Oxygen of -NO₂ interacted with hydrogen (-NH₃) from LYS-15 and hydrogen (NH₂) from ARG-92. Also disclosed were hydrogen interactions between the oxygen bonded to the metal centre and the SER-175 residue. Other hydrogen interactions were observed between the oxygen of -NO₂ and the hydrogen (-OH) of SER-180 and hydrogen (-NH₂) of ARG-48. As for complex **14**, eight hydrogen bonds were formed with six residues through the interaction of oxygen bonded to the metal centre with the hydrogen (-NH) and (-OH) of SER-175 and the hydrogen (-NH) of GLY-176. Other hydrogen bond interactions were between hydrogen of -NH with oxygen (-C=O) of ASN-173 and GLU-11, and between the hydrogen of -NH with oxygen (-OH) of SER-175. Oxygen of -NO₂ was observed to form more hydrogen bonds with hydrogen (-NH₃) of LYS-15 and hydrogen (-OH) of SER-180. They displayed superior molecular docking with a binding affinity of (-9.8 kcal/mol). Being the second active compound in molecular docking study, complex **14** inhibit bacteria effectively. This is due to the nucleophilic, light and polar substituents like NO₂, NH and C=O in the synthesised compound showed better interaction modes with target protein and therefore have good inhibitory activities (Maalik et al., 2019). Lastly, interaction of complex **15** against *S. aureus* revealed ten hydrogen bonds interact with six amino acid residues. They displayed superior molecular docking with a binding affinity of (-9.8 kcal/mol). The oxygen of -NO₂ forms hydrogen bonds with the hydrogen (-OH) of SER-180 and the hydrogen (-NH₂) of ARG-48. Other hydrogen interactions involved oxygen from -

NO₂ and hydrogen (-NH) from THR-16, LYS-15, GLY-14, and SER-13. Also discovered were hydrogen interactions between the oxygen bonded to the metal centre and the hydrogen of -NH with SER-175 residue. The last interaction was between the hydrogen of -NH and the oxygen (-C=O) of GLU-11. The 3D visualisations of the docked compound were displayed in Table 4.16.

Table 4.16: 3D docking illustration between complex 14 against <i>B. cereus</i> (PDB entry: 5bca)		
Complex	Binding poses (kcal/mol)	PyMol Modelling
14	-8.3	

Apart from inhibition against *S. aureus*, other complexes such as complex **14** and **13** showed high inhibition against *B. cereus* and *K. pneumoniae* respectively. These complexes were docked and later visualized via PyMOL. In the case of complex **14** against *B. cereus*, four hydrogen bonds were established with four amino acid residues. They exhibited excellent molecular docking with binding affinity of (-8.3 kcal/mol). Oxygen of -NO₂ was observed to

interact with hydrogen (NH) of ARG-380 and hydrogen (NH) of ALA-444. Also observed was a hydrogen bond at the oxygen bonded to the metal centre with hydrogen (-OH) of TYR-343 and oxygen (C=O) of complex **14** to hydrogen (NH₃) of LYS-379. Table 4.17 depicts a visual representation of these interactions.

Table 4.17: 3D docking illustration between complex 13 against <i>K. pneumoniae</i> (PDB entry: 5xun)		
Complex	Binding poses (kcal/mol)	Pymol Modelling
13	-9.2	

In addition, Table 4.17 showed a docking of complex **13** against *K. pneumoniae* which involved seven hydrogen bonds and four residues that were seen through hydrogen bond interaction between hydrogen of -NH with oxygen (C=O) of CYS-69. Other hydrogen bond interactions were between oxygen of -NO₂ with hydrogen (-OH) of SER-66, hydrogen (C=NH₂) of ARG-96 and hydrogen (NH₂) of ARG-96. Oxygen of (C=O) was also observed to interact with hydrogen (NH₂) and hydrogen (NH) of ASN-88. Lastly, the oxygen bonded to the metal

center formed a hydrogen bond interaction with the hydrogen (NH₂) of ASN-88. Excellent molecular docking was observed, with a binding affinity of (-9.2 kcal/mol). Interestingly, it can be observed that none of the complexes shown interaction at the aromatic ring. As mentioned by other study, there was no interaction between the group located in the aromatic ring of the inhibitor against the bacteria (Haldys et al., 2021).

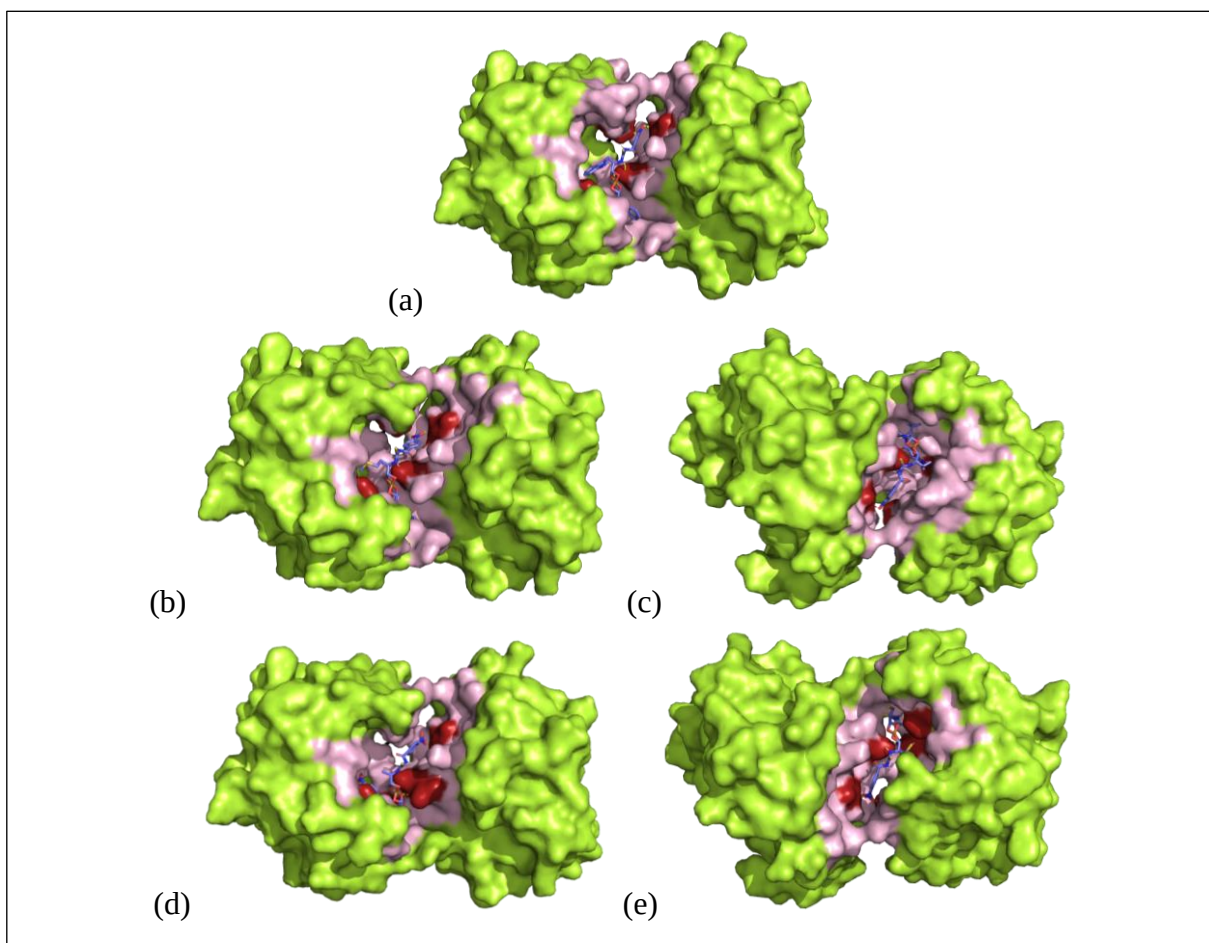


Figure 4.13: The molecular binding pocket of *S. aureus* docked with (a) complex 11, (b) complex 12, (c) complex 13, (d) complex 14 and (e) complex 15.

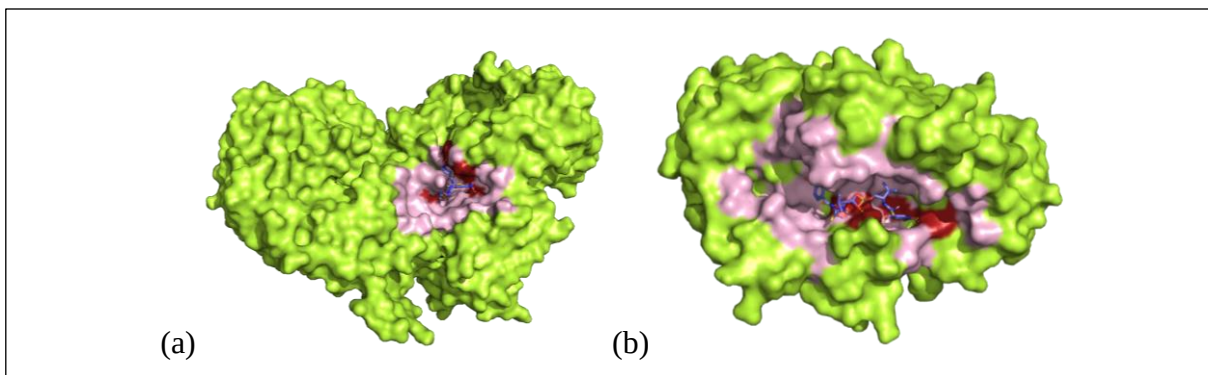


Figure 4.14: The molecular binding pocket of (a) complex **14** docked with *B. cereus*, (b) complex **13** docked with *K. pneumoniae*.

Figure 4.13 and Figure 4.14 showed a binding pocket of synthesised thiourea complexes in their active site of respected protein molecule. The green area represented the bacteria's chain involved in the docking, whereas the red area represented the polar interaction, and the light pink area represented the non-polar interaction between the compounds and the amino acid residues. It can be observed that in every docking simulation, the negative binding energy is greater, suggesting that the interacting complex is more stable and greater will be the propensity of the compound to associate with the protein. As higher energy (kcal/mol), hydrogen bonding and van der Waals interaction are proposed as the major forces stabilising the protein residue and metal complex (Qureshi & Javed, 2022). All complexes were docked against *S. aureus*, as these compounds exhibited satisfactory antibacterial activity. From the docking against the *S. aureus* active site, complex **11** resulted in the highest negative value of binding energy compared to complex **12**, which has the lowest negative value of binding energy. This could be the presence of many hydrogen donor and acceptor atoms in complex 11, resulting in the formation of more hydrogen bonds. However, these results do not necessarily imply that a higher negative binding energy always results in a more polar interaction, as other factors, such as the number

of hydrogen donor/acceptor atoms in the molecule, must be taken into account. Although more presence of hydrogen bonds may not determine whether or not a molecule is a good binder, they do determine the binding affinities for the targeting side, which explaining the fitting of molecule in the binding pocket, as in the case of complex **13** having a slightly lower negative value of binding energy than complex **12**, despite having fewer hydrogen bond interactions than complex **12**. Amino acid chains in the structure of complexes **11-15** also could contribute to the favourable orientation and crucial interactions between the complex and protein residue.

In conclusion, docking of synthesised thiourea complexes revealed more negative value of the affinity binding compared to the selected synthesised thiourea ligand. Simulating the binding mode between synthesised compound and protein residue allow deeper understanding of the action take place within the active site. It is also observed that selected synthesised thiourea complexes form much more hydrogen bonds interaction compared to selected synthesised thiourea ligand. Other studies have linked the biological activity of copper complexes to the interaction formed between the metal ion and nucleic acids, which causes DNA damage (Souza et al., 2021). Interaction between protein residue and electron substituent of copper complexes were also seen in all docking visualization. Thus, it supports the experimental result although further research might need to be conducted to verify the outcome.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

In this study, thiourea derivatives derived from 4-nitrobenzoyl chloride and their Ag(I) and Cu(II) complexes were synthesised and characterised. The antibacterial activity of these compounds against six different bacterial strains was evaluated, and molecular docking was employed to elucidate the molecular interactions at the binding site of proteins.

FTIR spectroscopy supported the formation of thiourea ligands (**1-5**) and thiourea complexes (**6-15**). The formation of the ligands was also supported by the disappearance of the NCS peak at 2000 cm^{-1} . The disappearance of the $\nu(\text{COOH})$ peak in the spectra, indicating that the ligands were coordinated to the metal through the carboxylate group, confirmed the coordination of the ligands to the silver(I) and copper(II) metals. The complexes can be identified based on their distinctive NMR chemical shift.

The antibacterial study revealed that thiourea complexes with silver(I) and copper(II) exhibited a broader spectrum of antibacterial activity than thiourea ligand alone. Complex **8** of silver metal and complex **13** of copper metal exhibited the greatest inhibition zone, measuring 13 mm and 16 mm, respectively. Although the presence of amino acid side chain does not directly contribute to the antibacterial property, but it did influence the lipophilicity and availability of the thiourea compound, particularly when combined with silver(I) and copper(II) metals within the active site of bacteria. Therefore, chirality has a significant effect on the enhancement of the compound's biological activity. By reacting with the carboxyl groups of the

bacteria, the C=S, C=O, and NH groups induce antibacterial activity by forming hydrogen bond interactions.

From antibacterial activity results, the most active compounds were visualised to determine its interactions in the catalytic site of the protein. Simulating the binding mode by docking the thiourea complex with a protein residue revealed that the oxygen incorporated in the metal centre played a huge role in inducing antibacterial activity by reacting with the protein residue. Thus, metal ions can penetrate the bacterial cell wall, enhancing the antibacterial efficacy of complexes. Simulation of the binding mode between the compounds and protein residues provided a deeper understanding of the interactions within the active site, with the complexes forming significantly more hydrogen bond interactions than the free ligands.

While the results of this study contribute to our understanding on the antibacterial properties and molecular interactions of thiourea complexes, further research may be needed to verify these outcomes and explore additional biological activities and toxicity profiles. Integrating experimental and computational approaches, such as molecular docking, can provide valuable insights into the design and optimization of targeted compounds for future studies in this field. Although it does not completely replace actual experimentation, it is important to note that molecular docking can be used as a tool for verifying the results of experiments or as preliminary screening tools prior the synthesis of the targeted compounds.

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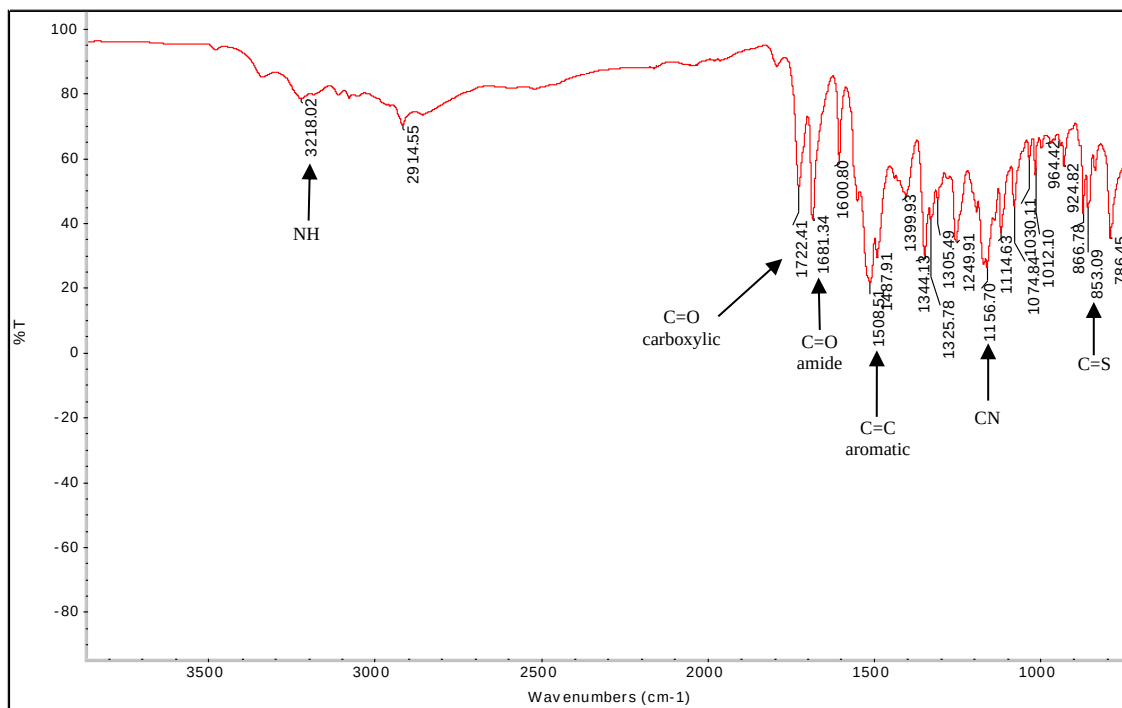
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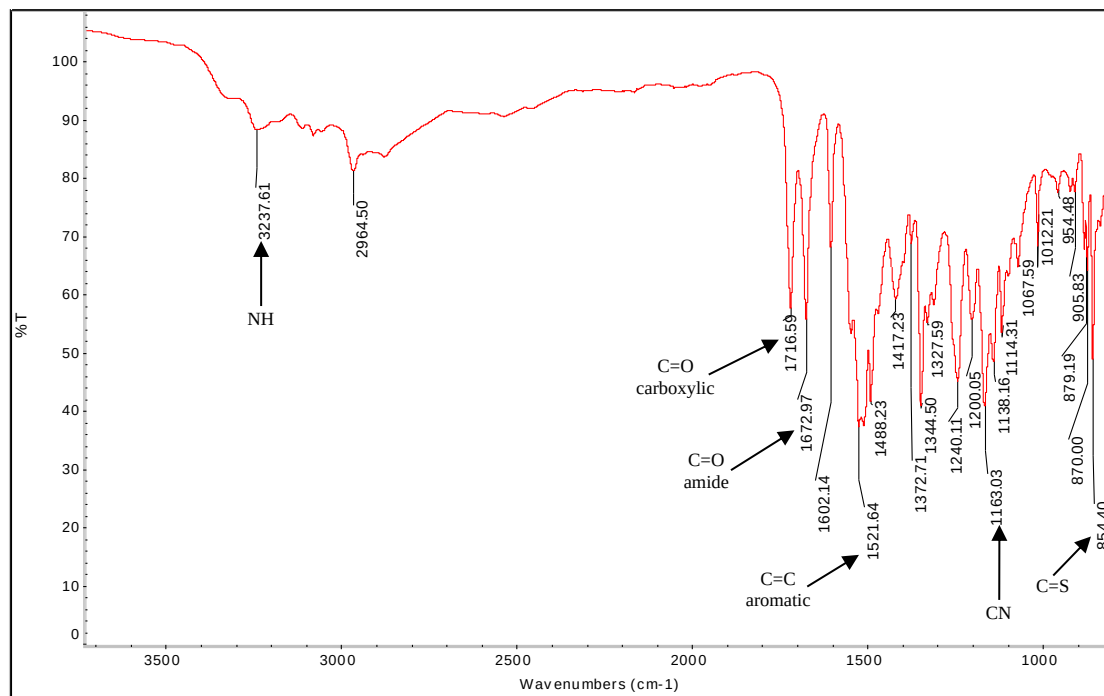
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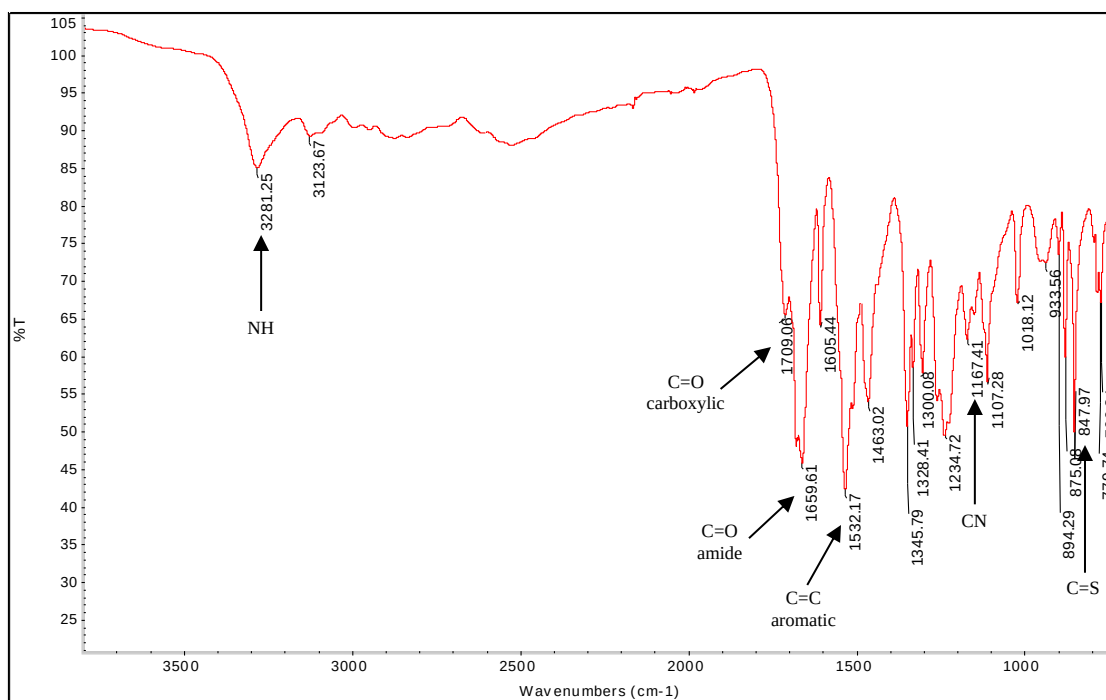
APPENDICES



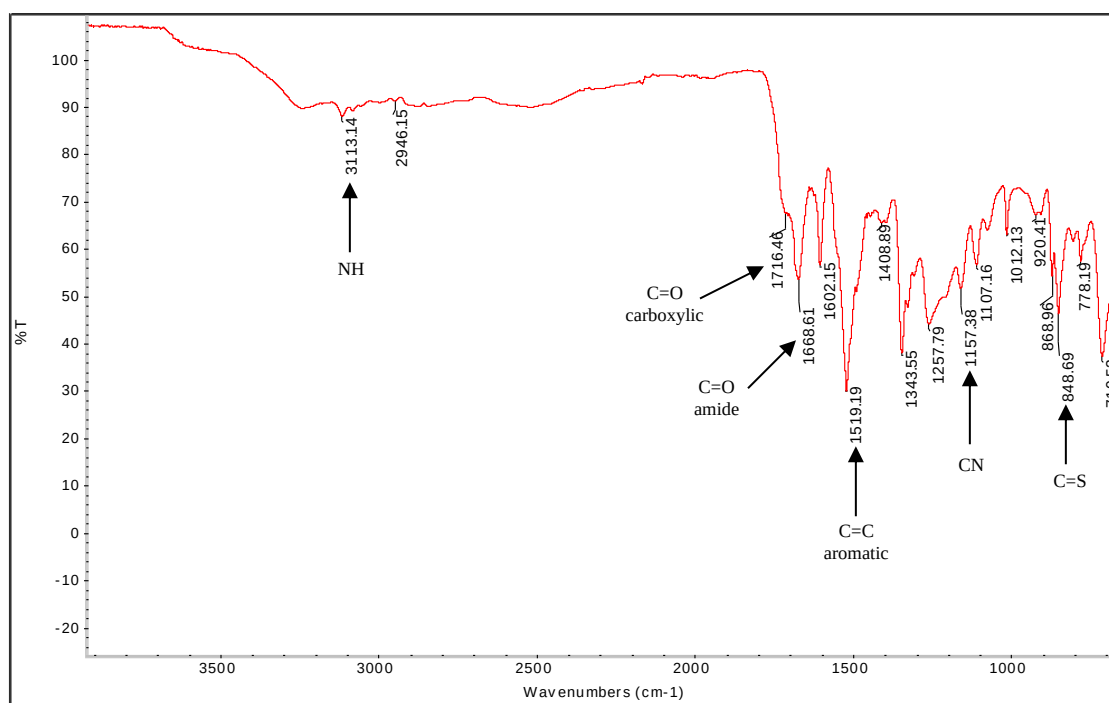
A1: FTIR spectrum of ligand 2



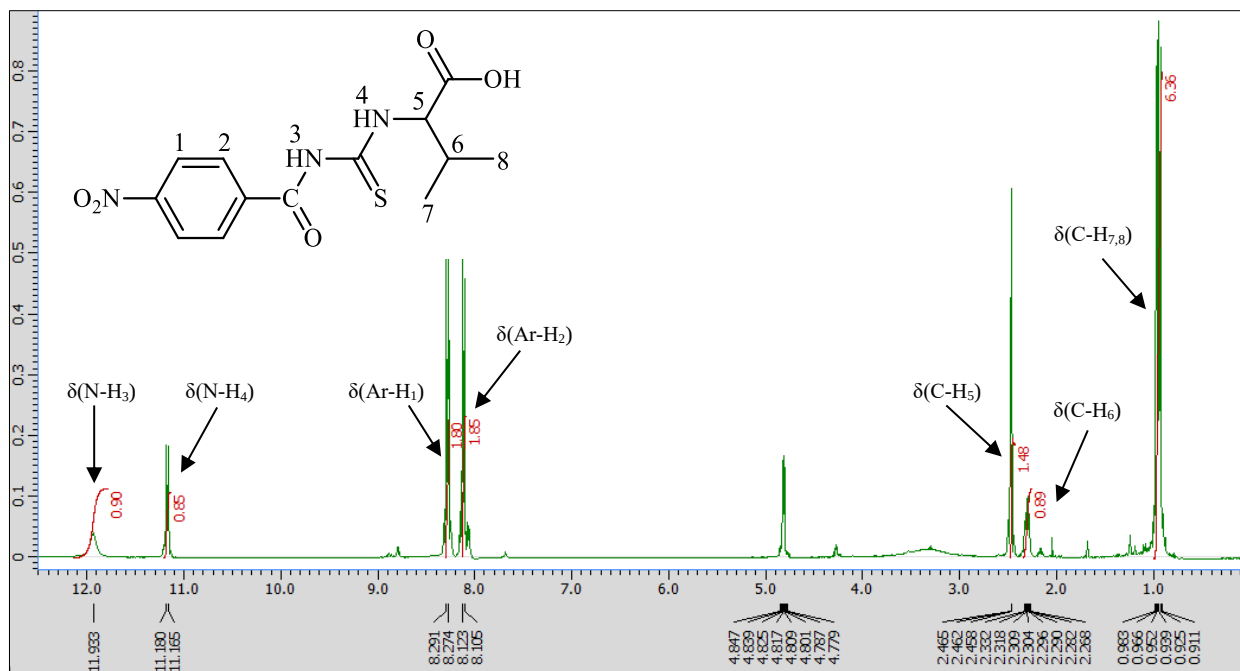
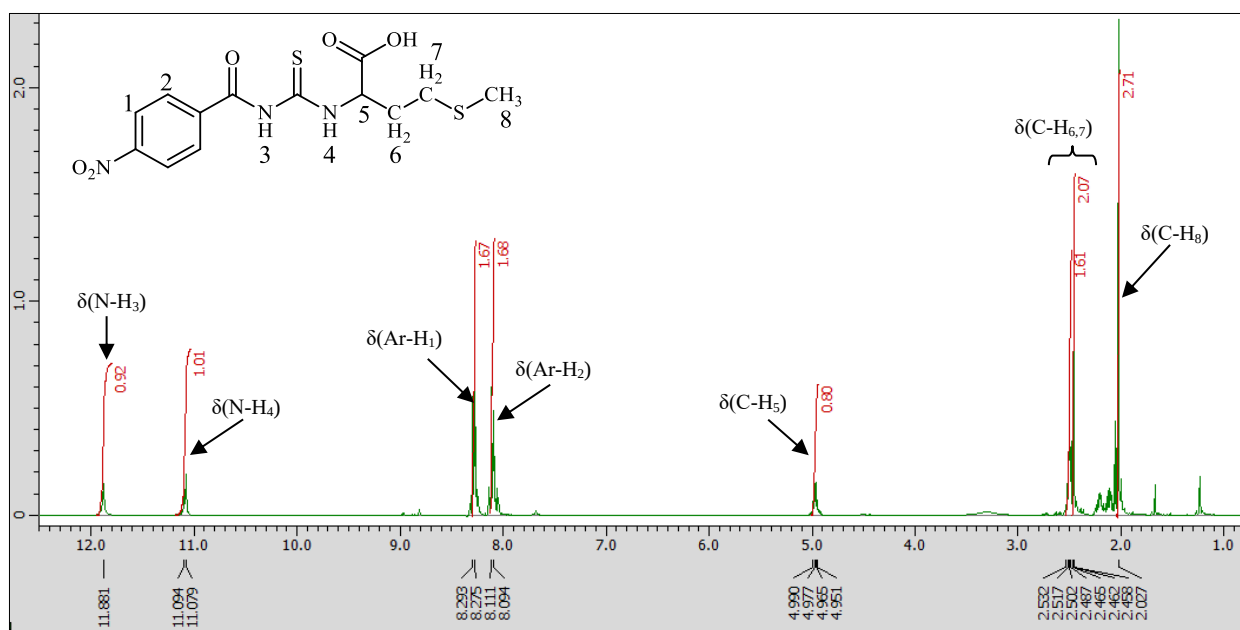
A2: FTIR spectrum of ligand 3

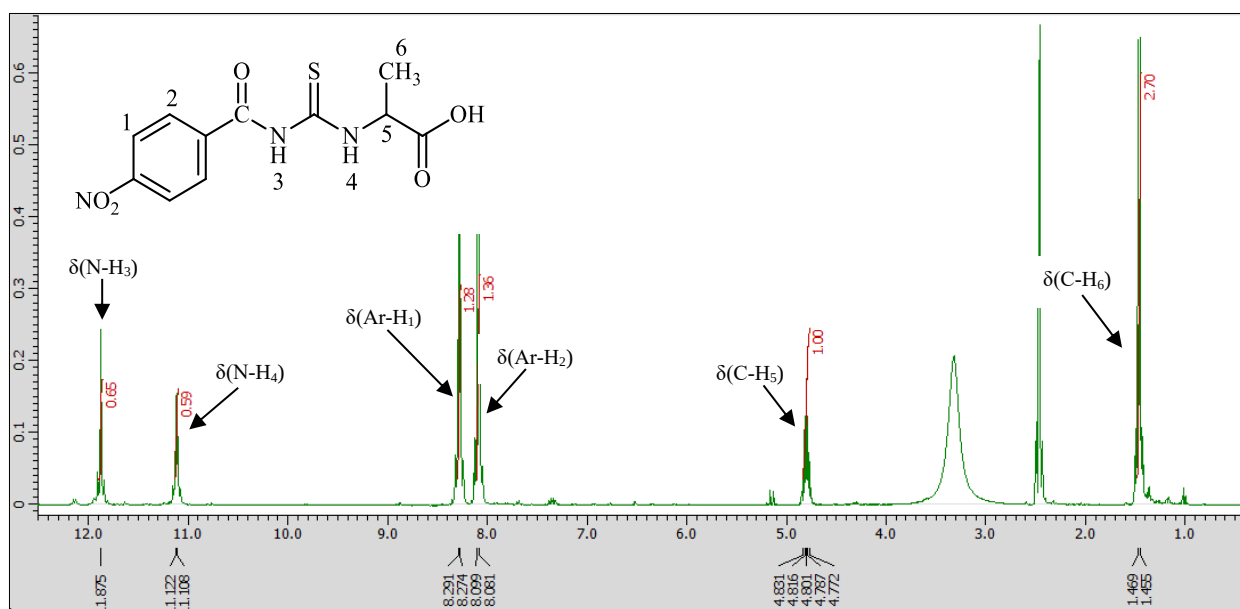


A3: FTIR spectrum of ligand 4

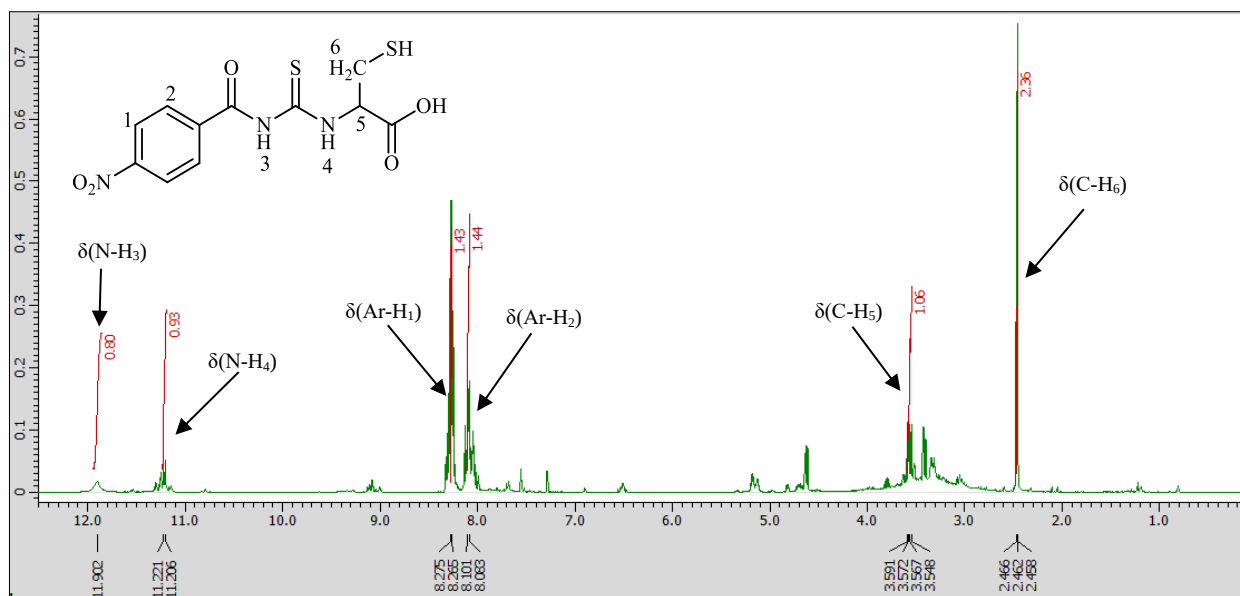


A4: FTIR spectrum of ligand 5

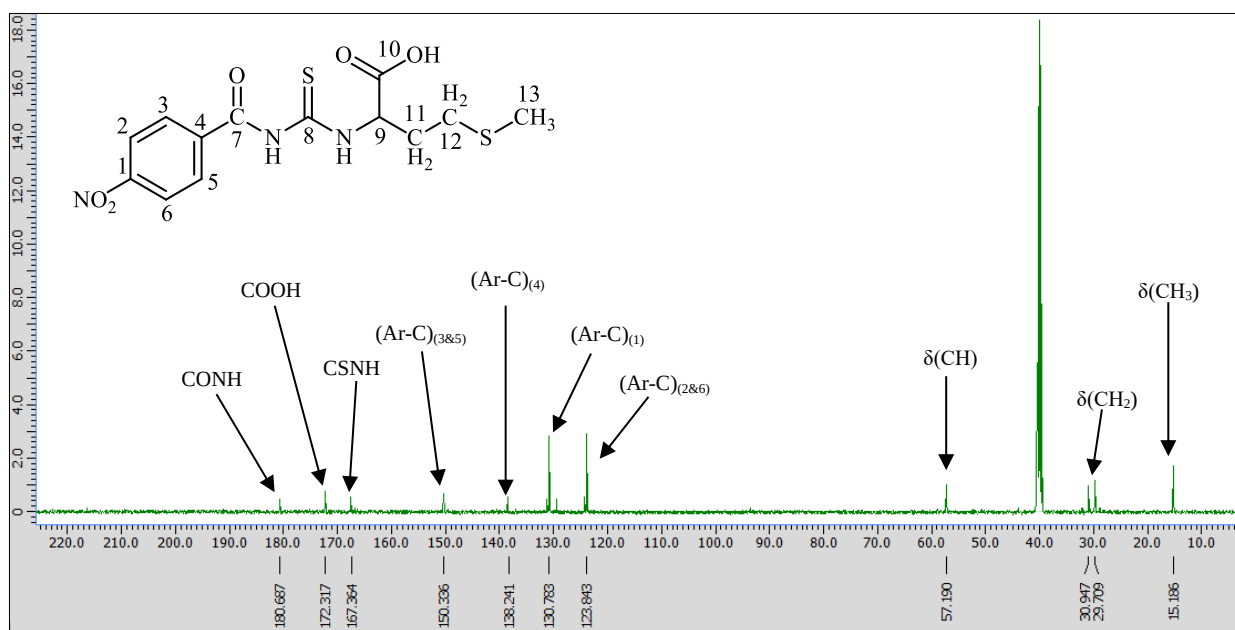




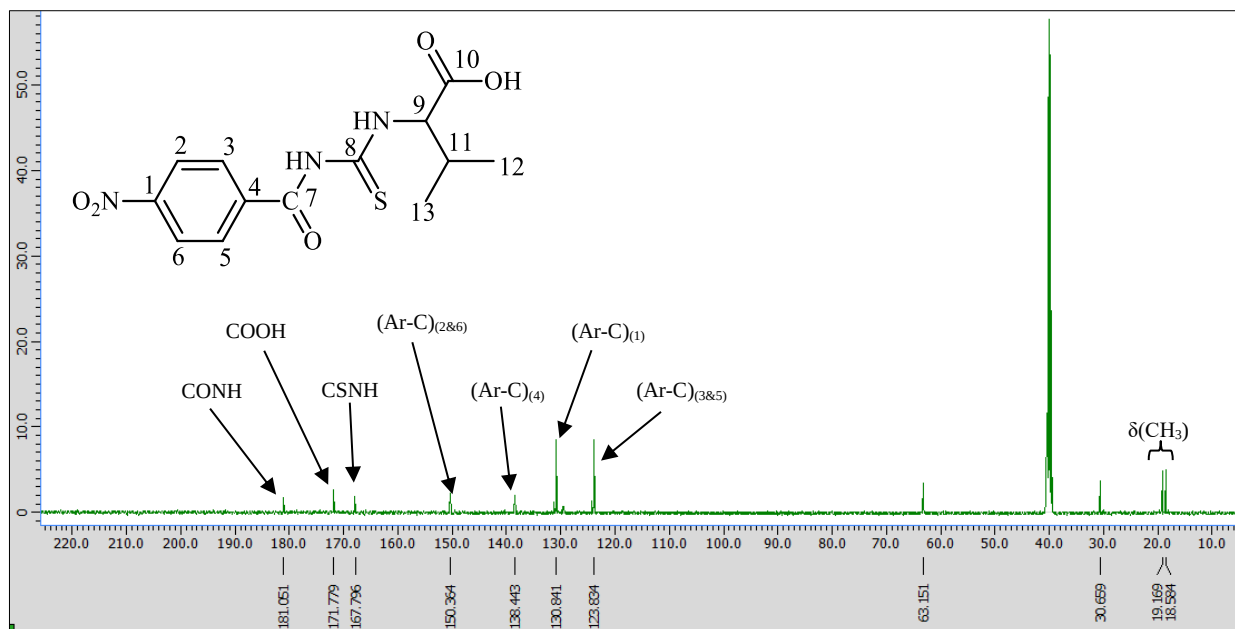
A7: ¹H NMR spectrum of ligand 4



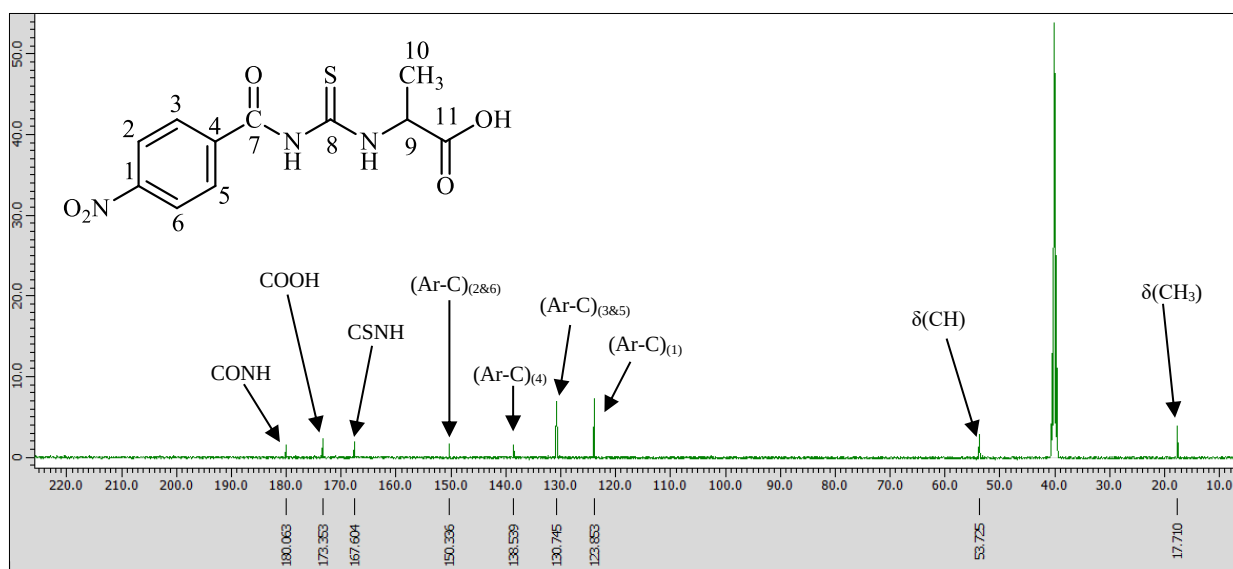
A8: ¹H NMR spectrum of ligand 5



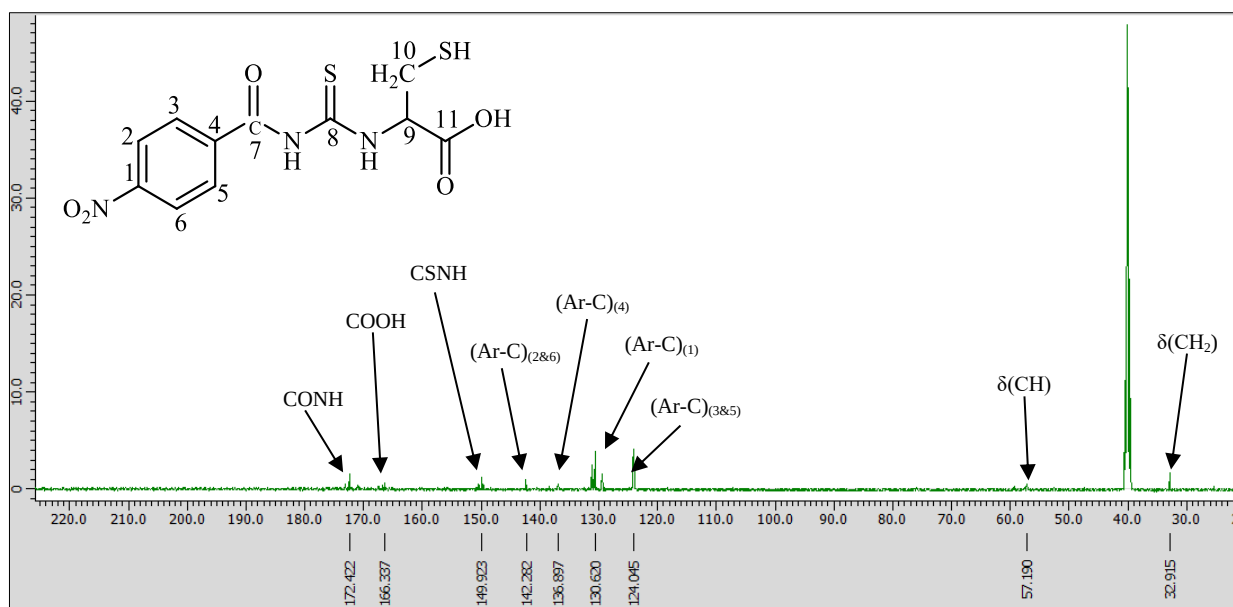
A9: ¹³C NMR spectrum of ligand 2



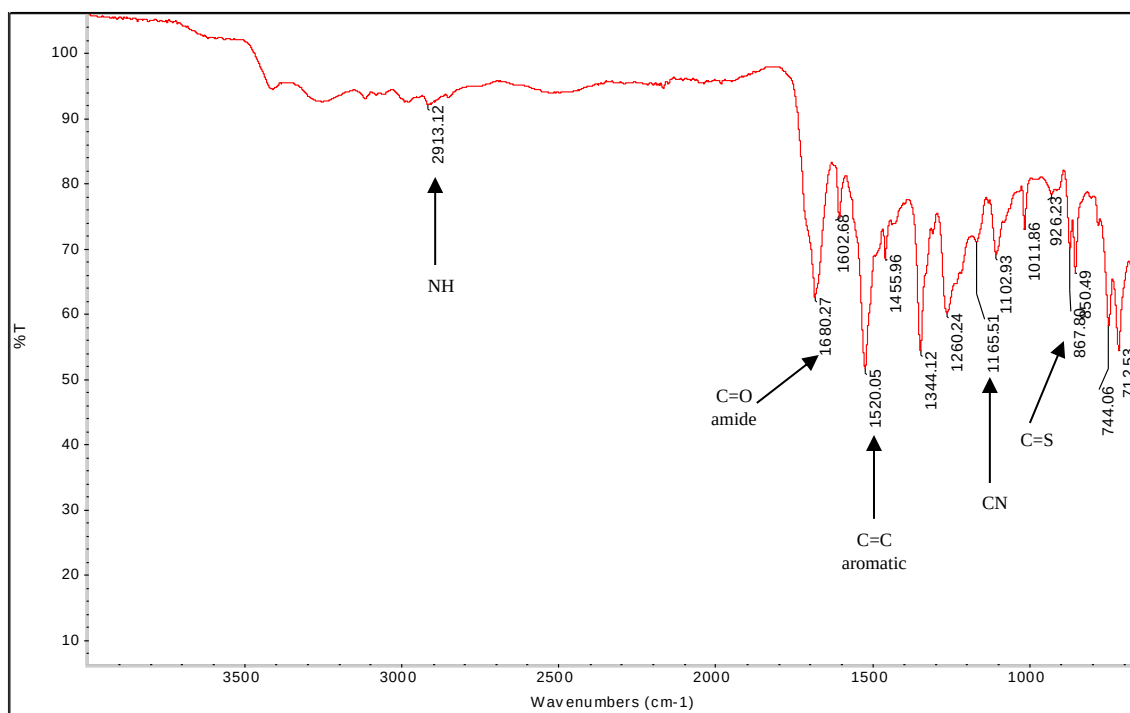
A10: ¹³C NMR spectrum of ligand 3



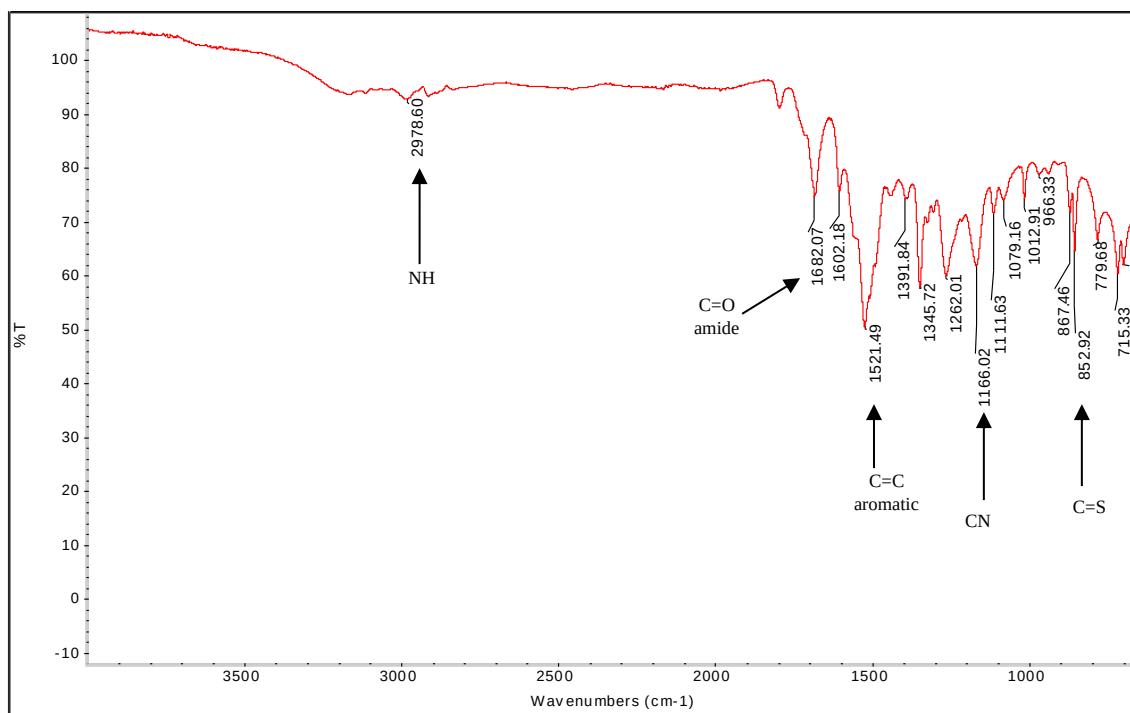
A11: ¹³C NMR spectrum of ligand 4



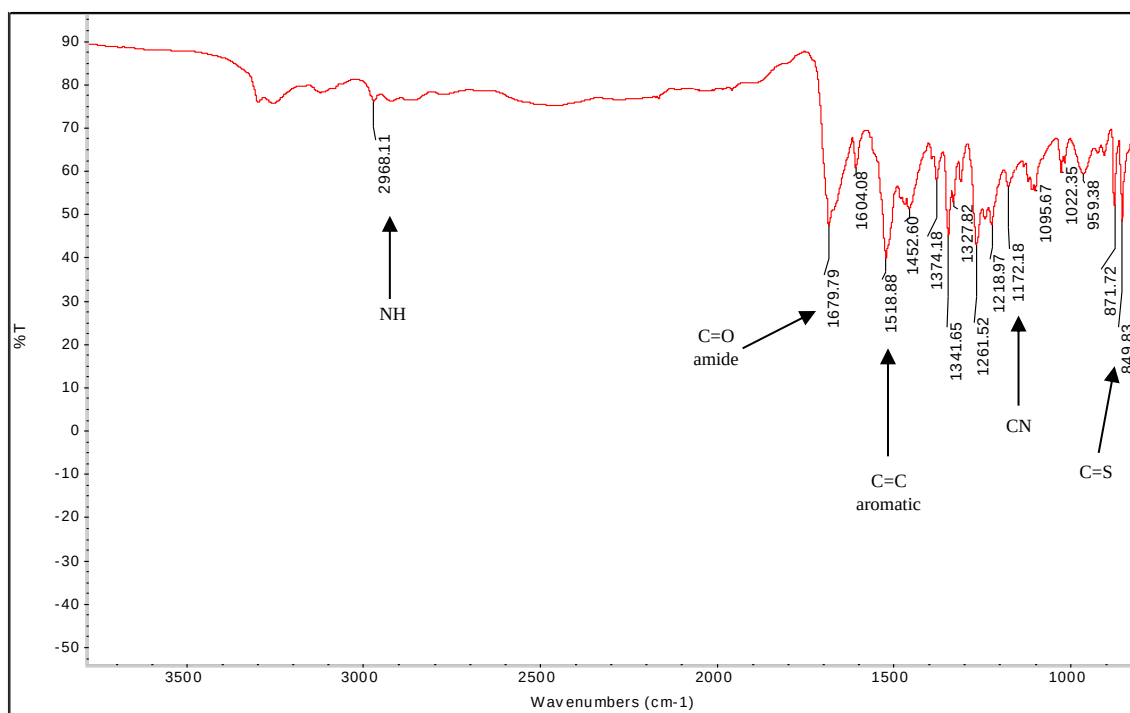
A12: ¹³C NMR spectrum of ligand 5



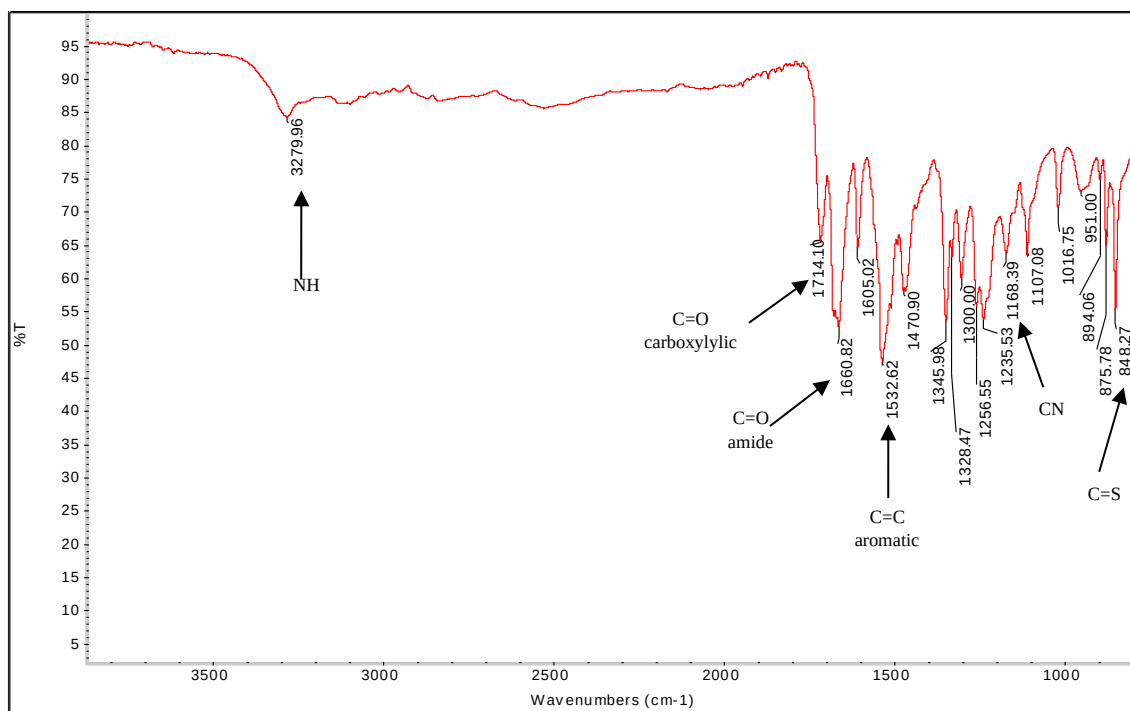
A13: FTIR spectrum of complex 6



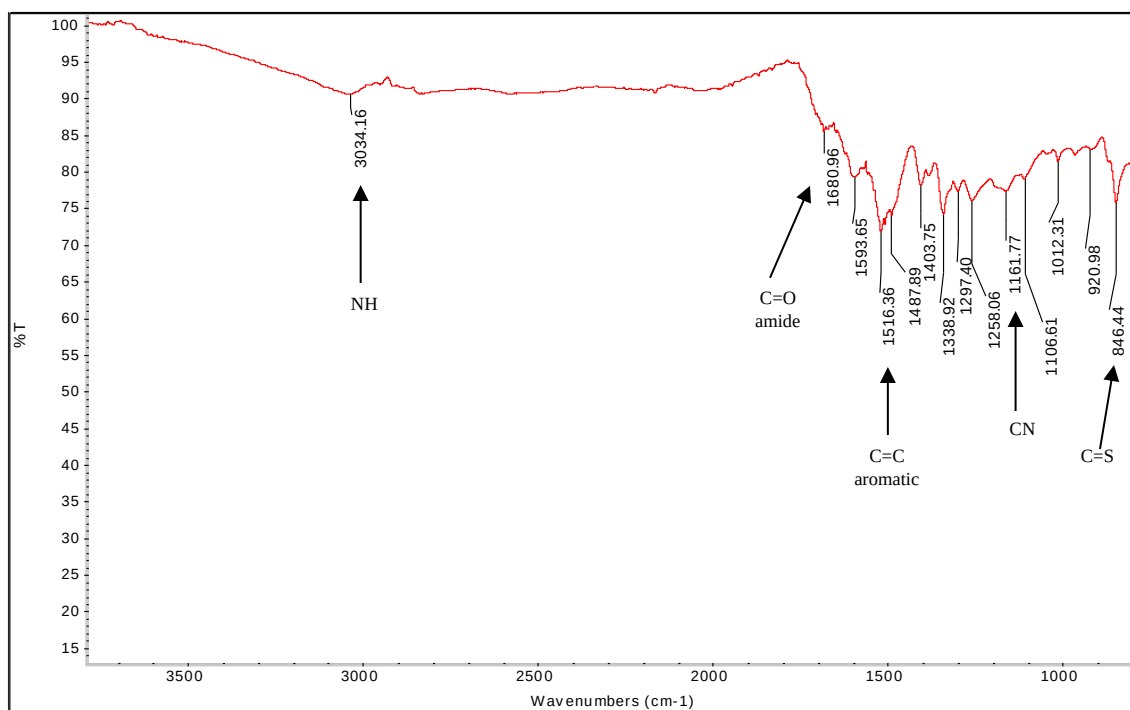
A14: FTIR spectrum of complex 7



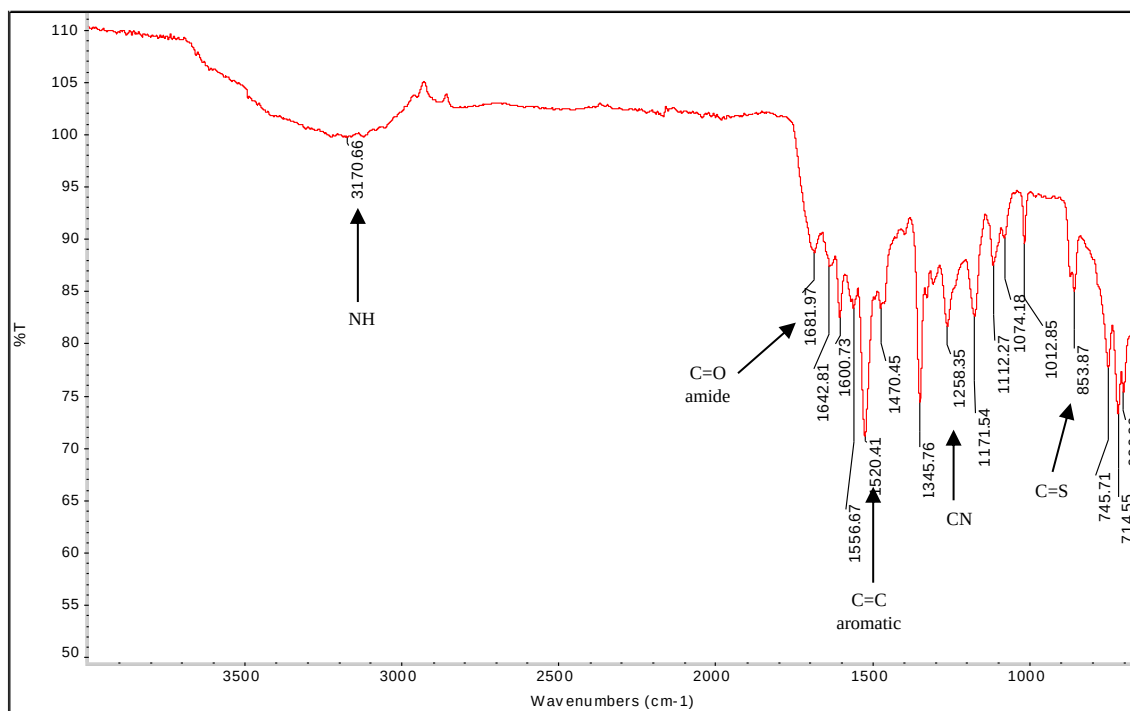
A15: FTIR spectrum of complex 8



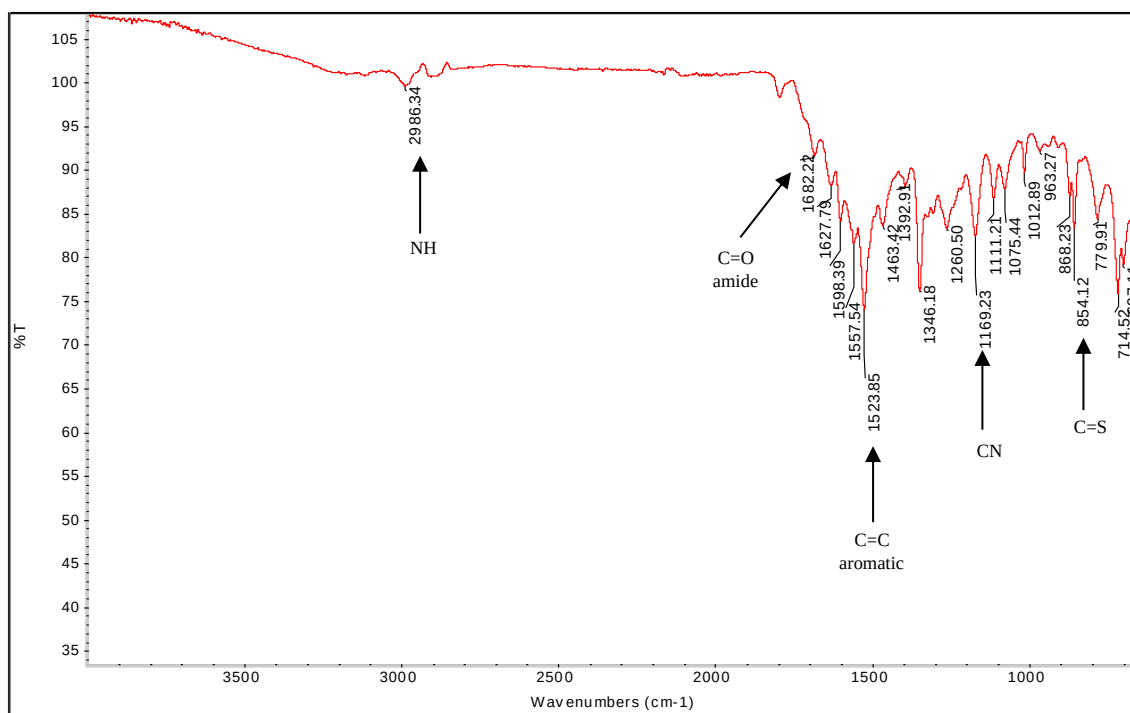
A16: FTIR spectrum of complex 9



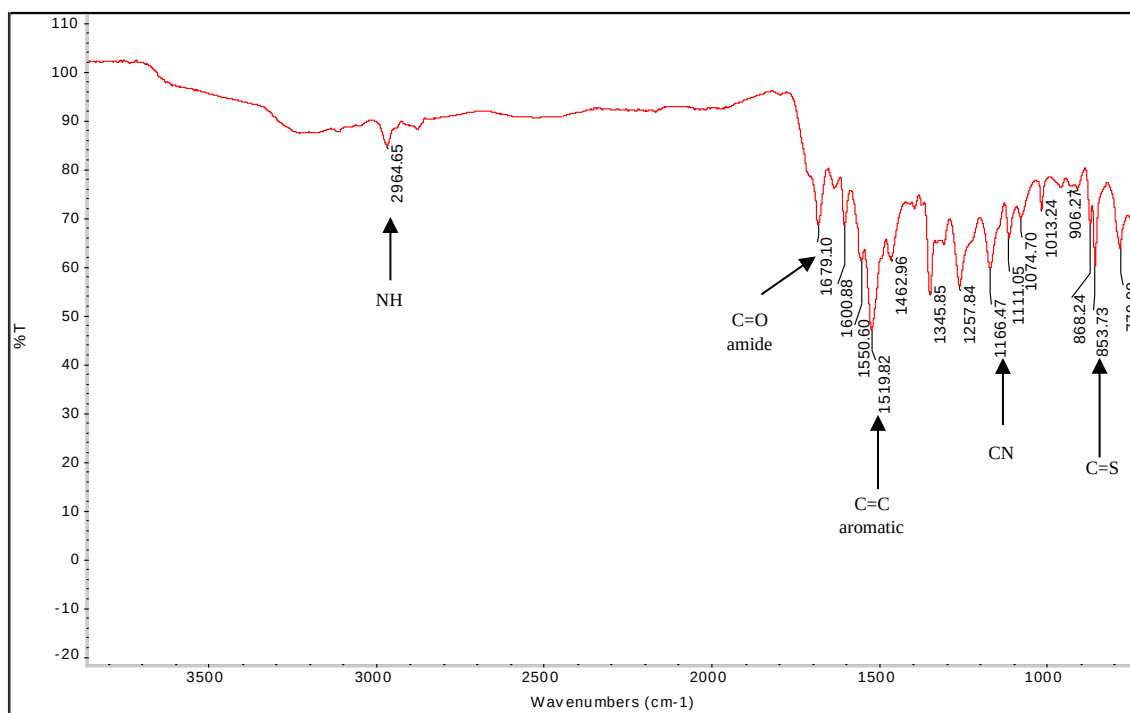
A17: FTIR spectrum of complex 10



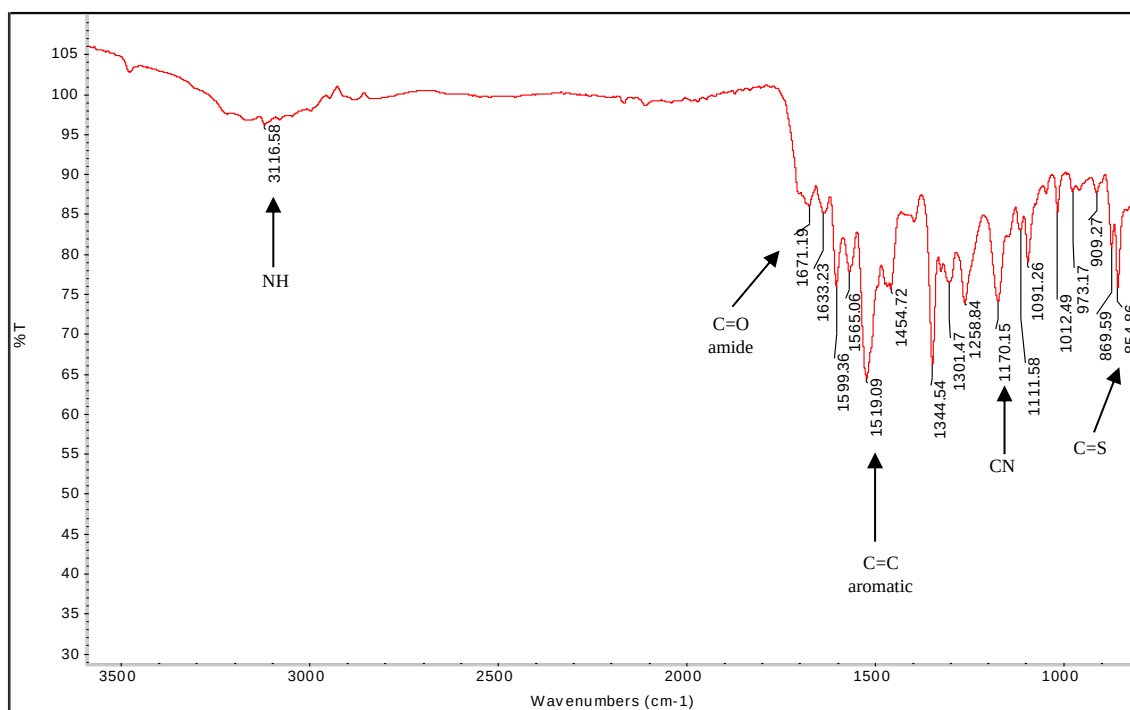
A18: FTIR spectrum of complex 11



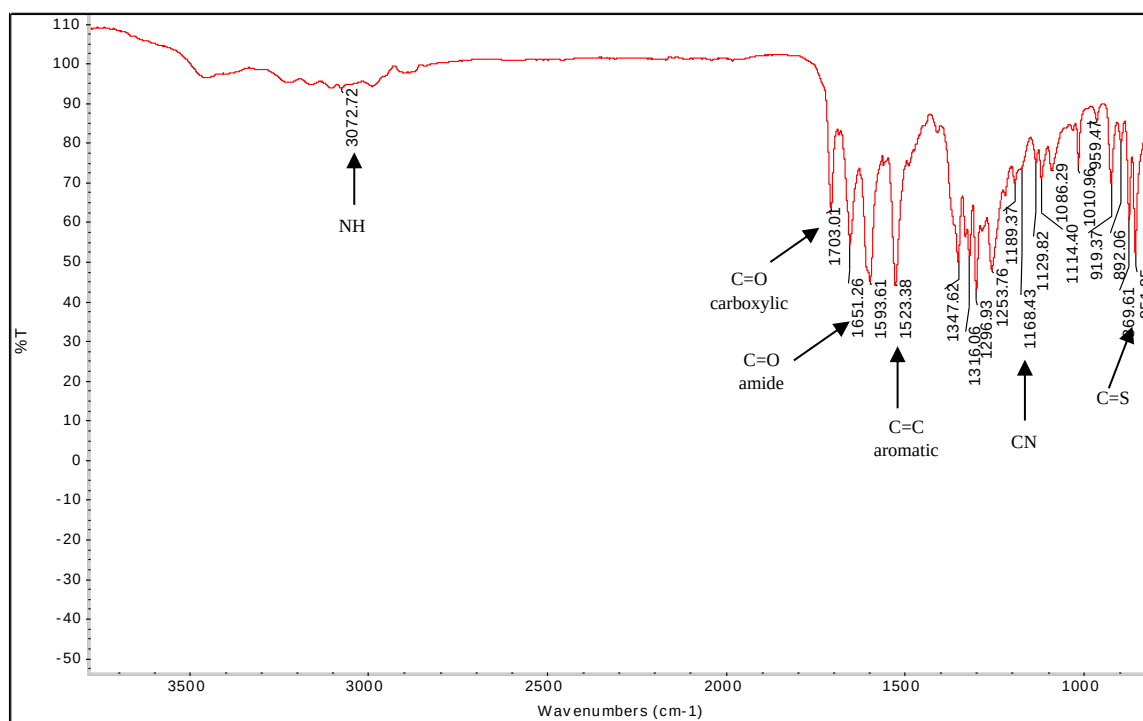
A19: FTIR spectrum of complex 12



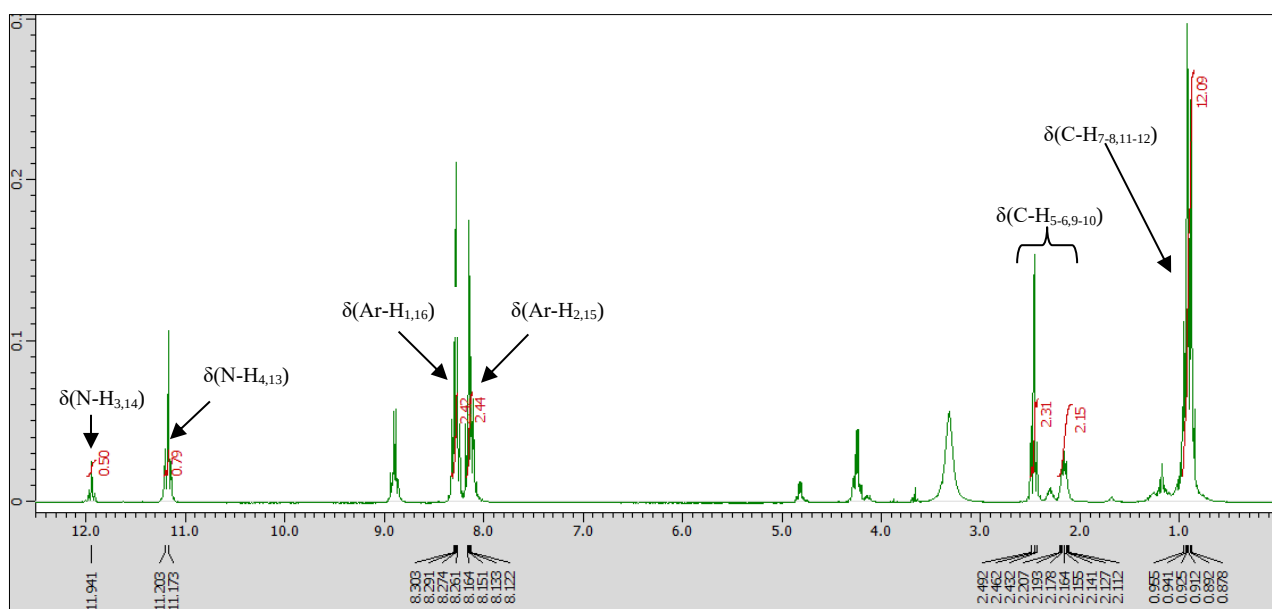
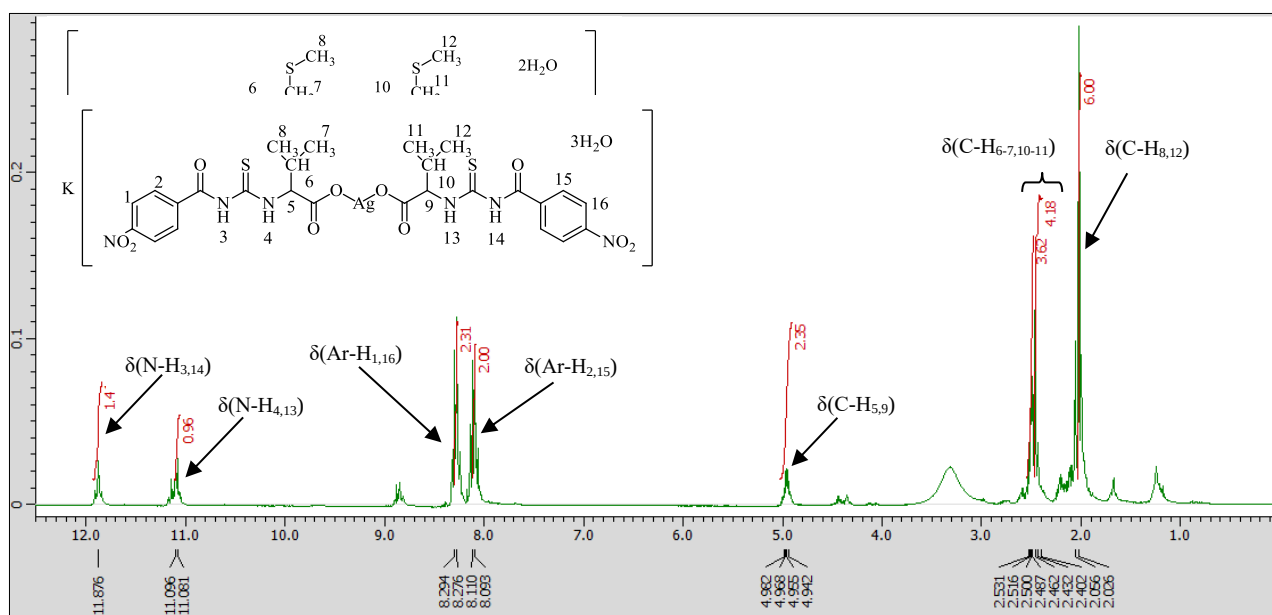
A20: FTIR spectrum of complex 13

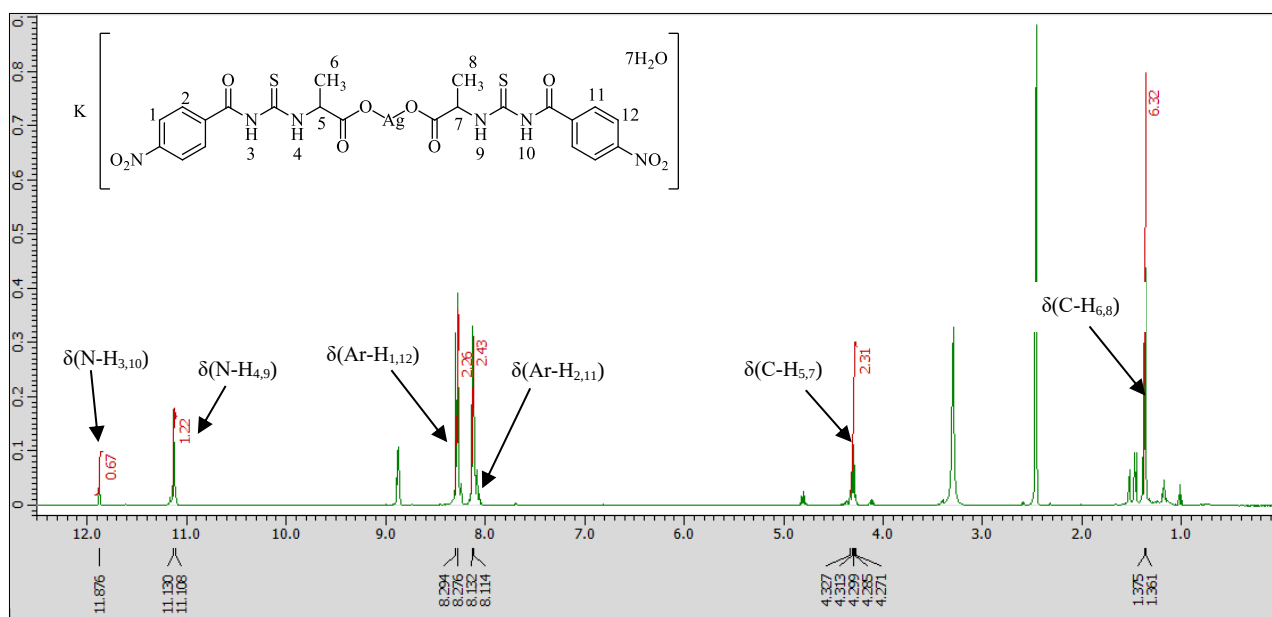


A21: FTIR spectrum of complex 14

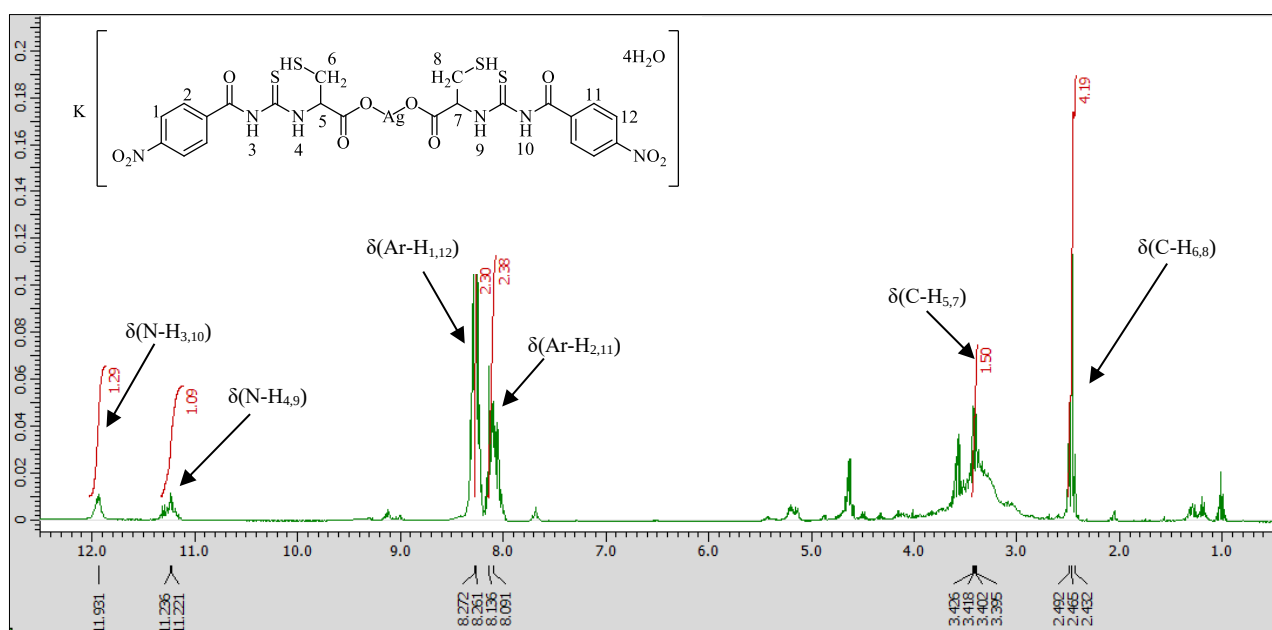


A22: FTIR spectrum of complex 15

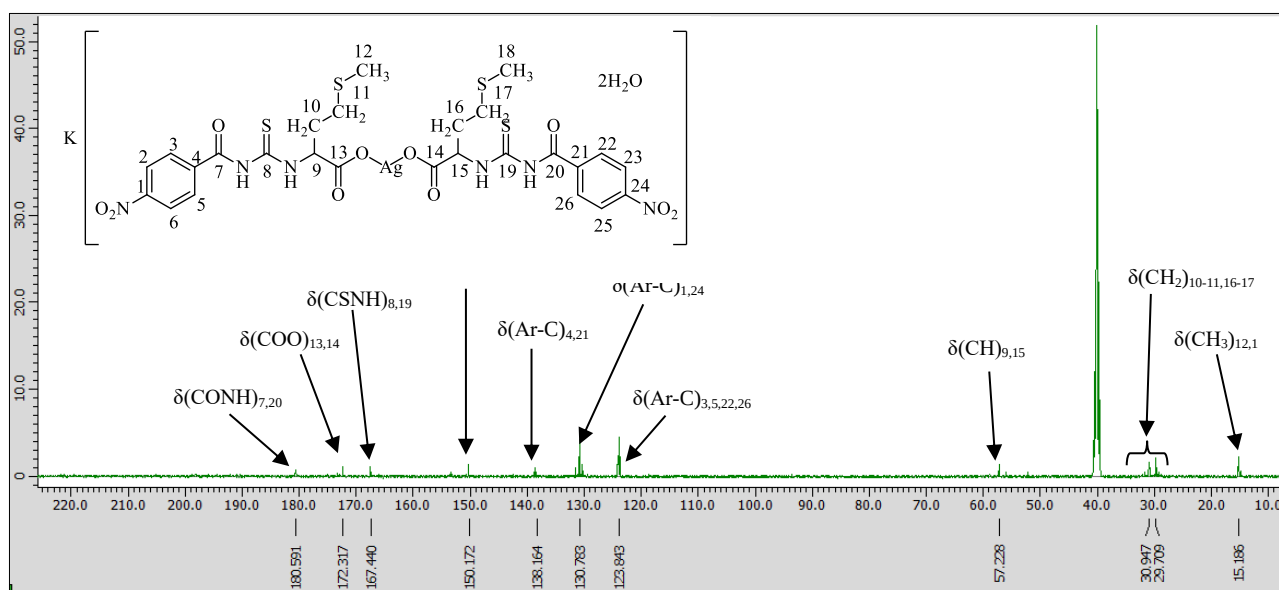




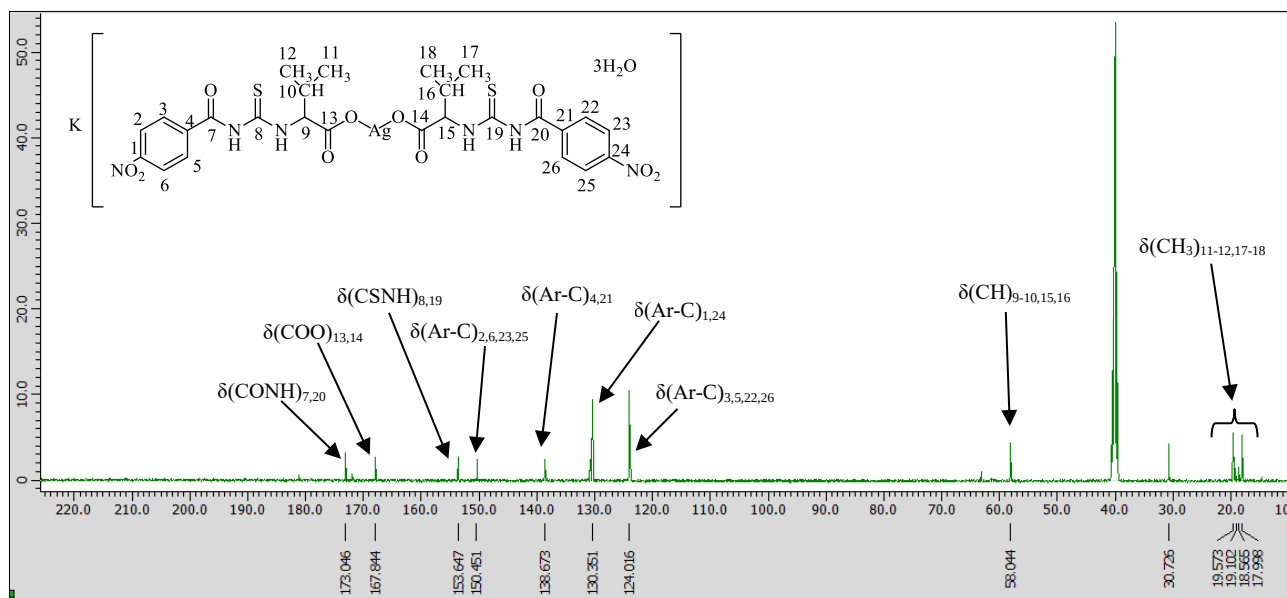
A25: ¹H NMR spectroscopy of complex 9



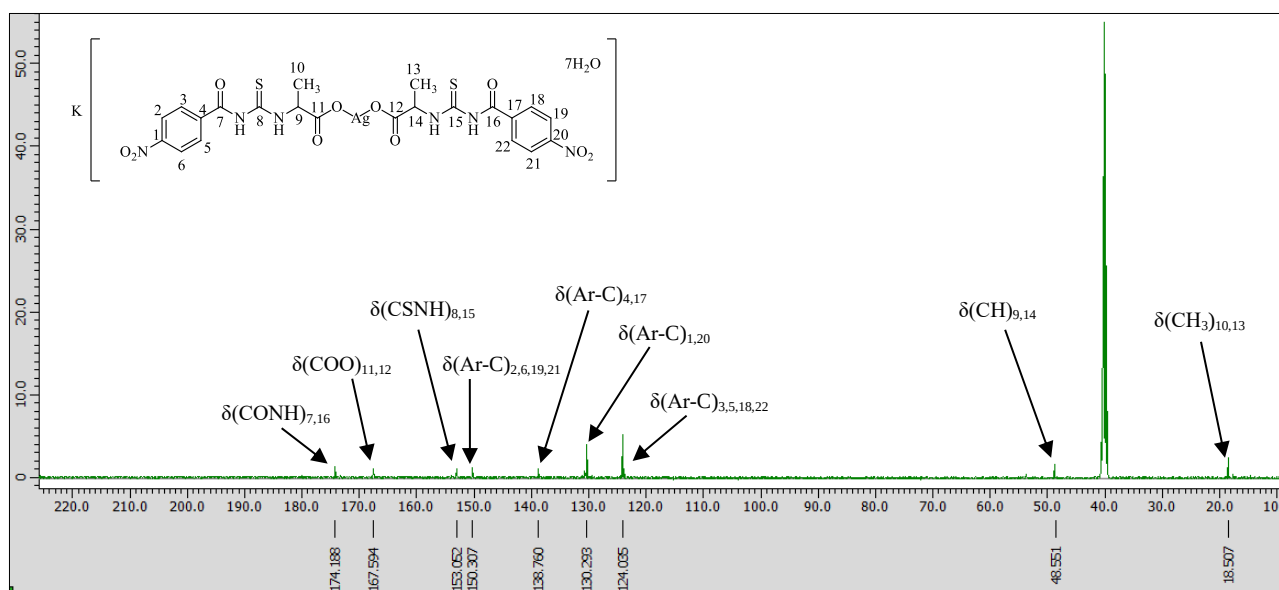
A26: ¹H NMR spectrum of complex 10



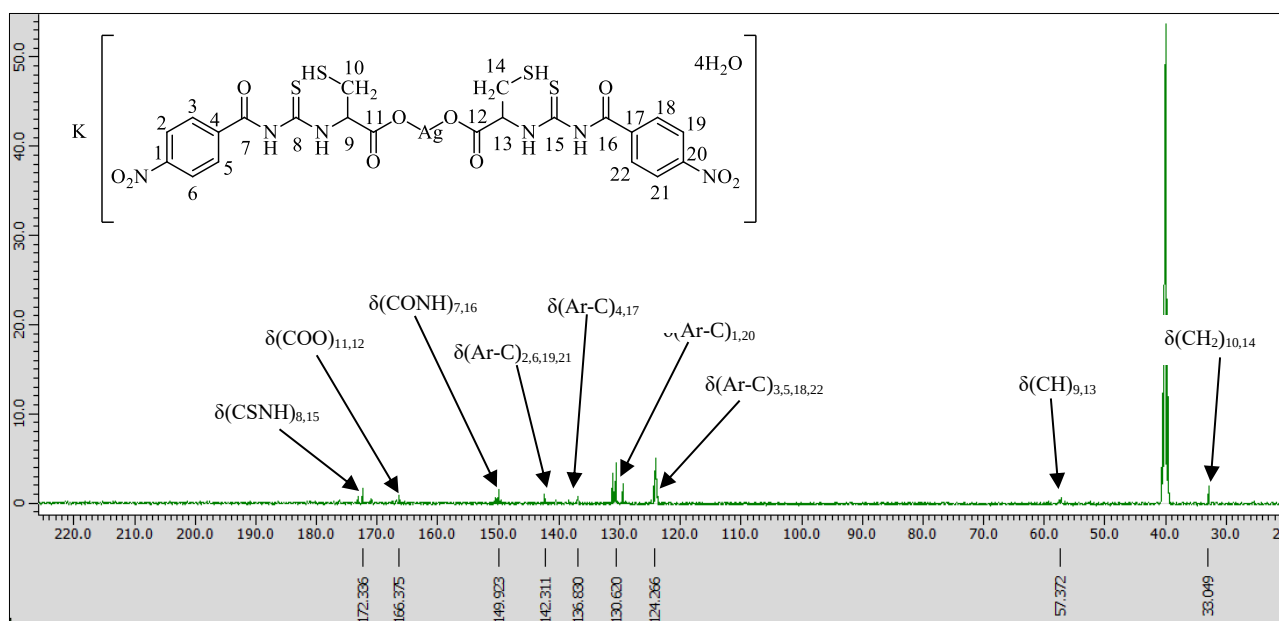
A27: ^{13}C NMR spectrum of complex 7



A28: ^{13}C NMR spectrum of complex 8

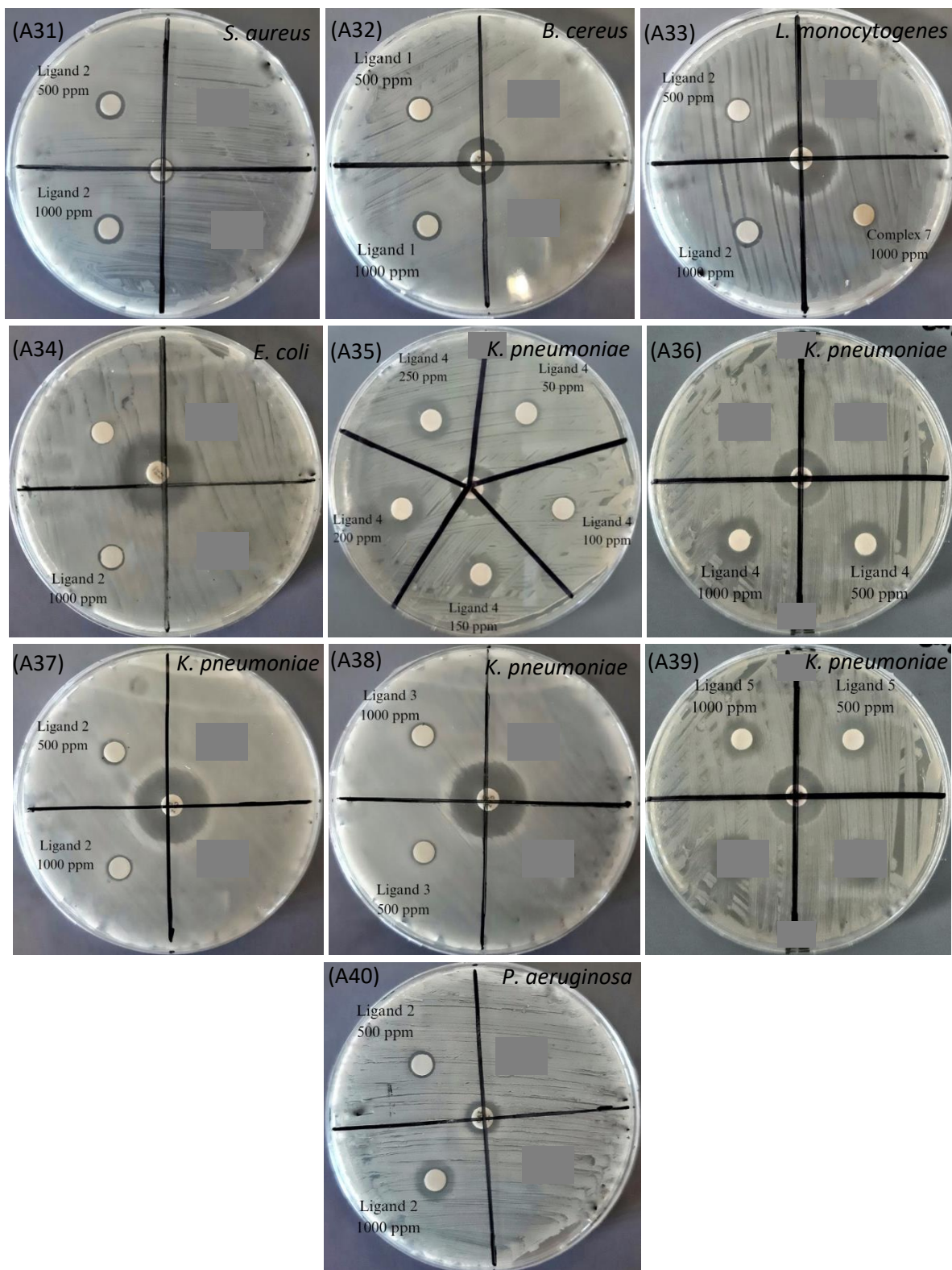


A29: ^{13}C NMR spectrum of complex **9**

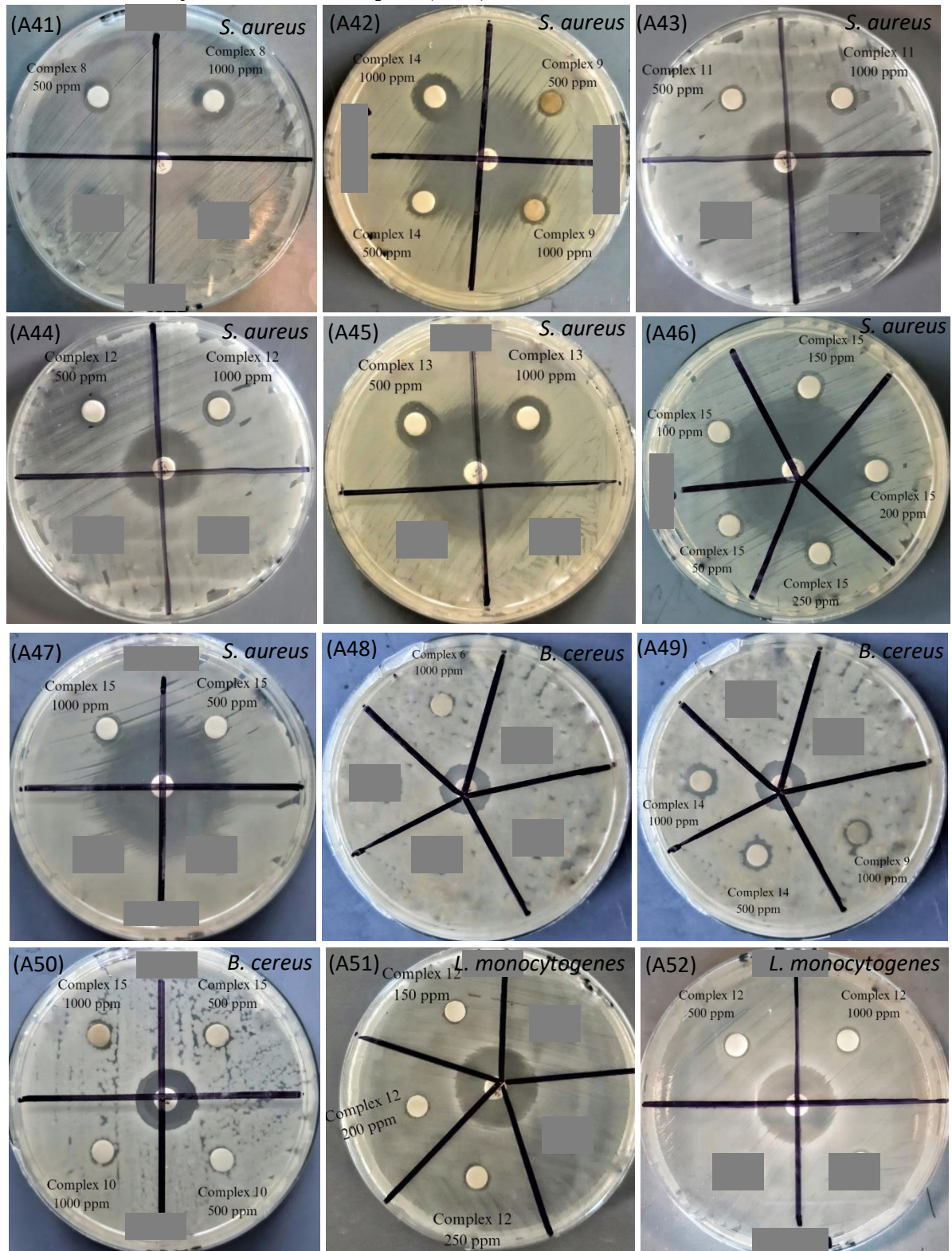


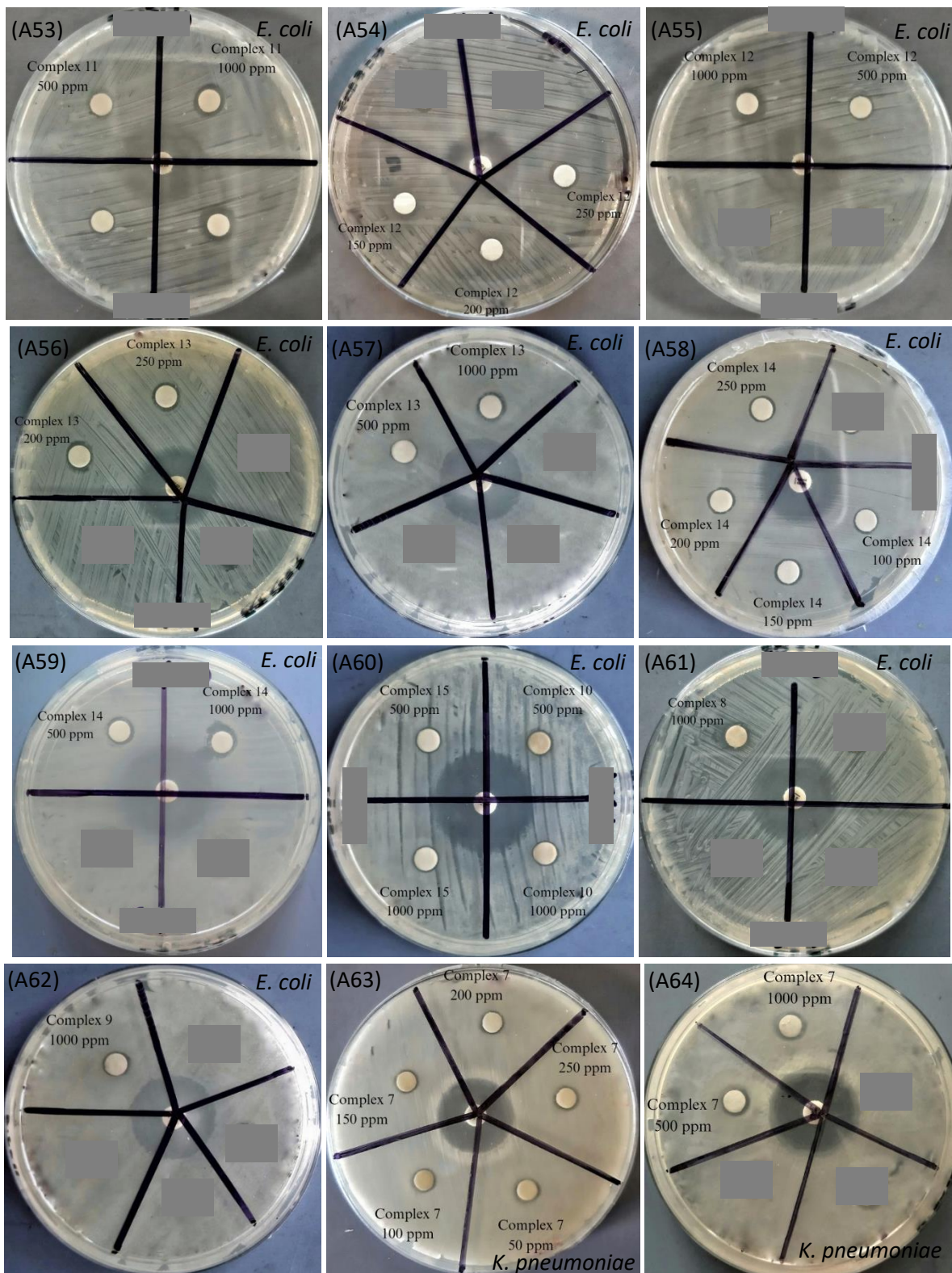
A30: ^{13}C NMR spectrum of complex **10**

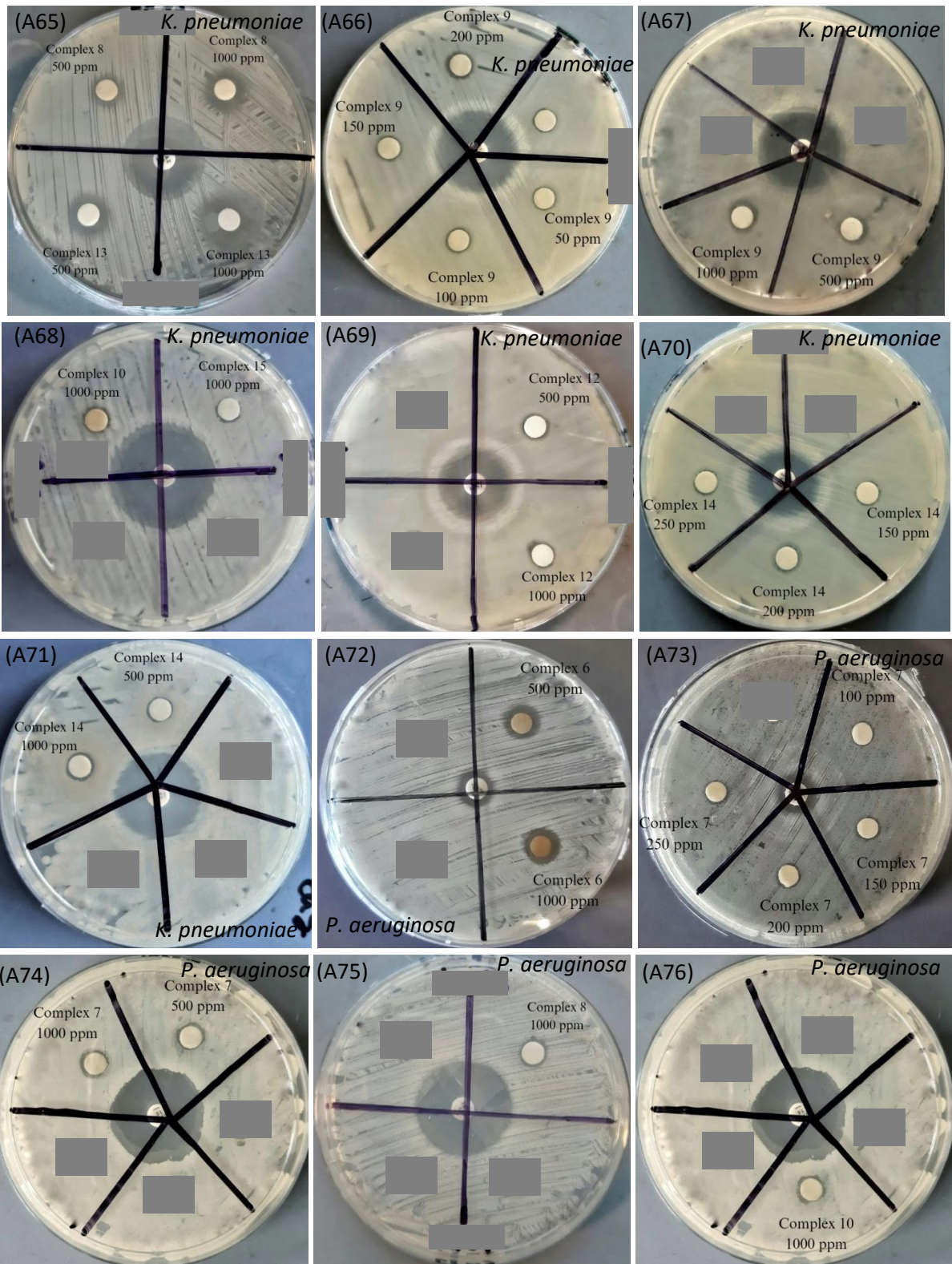
Antibacterial Study of Thiourea Ligand (1-5)

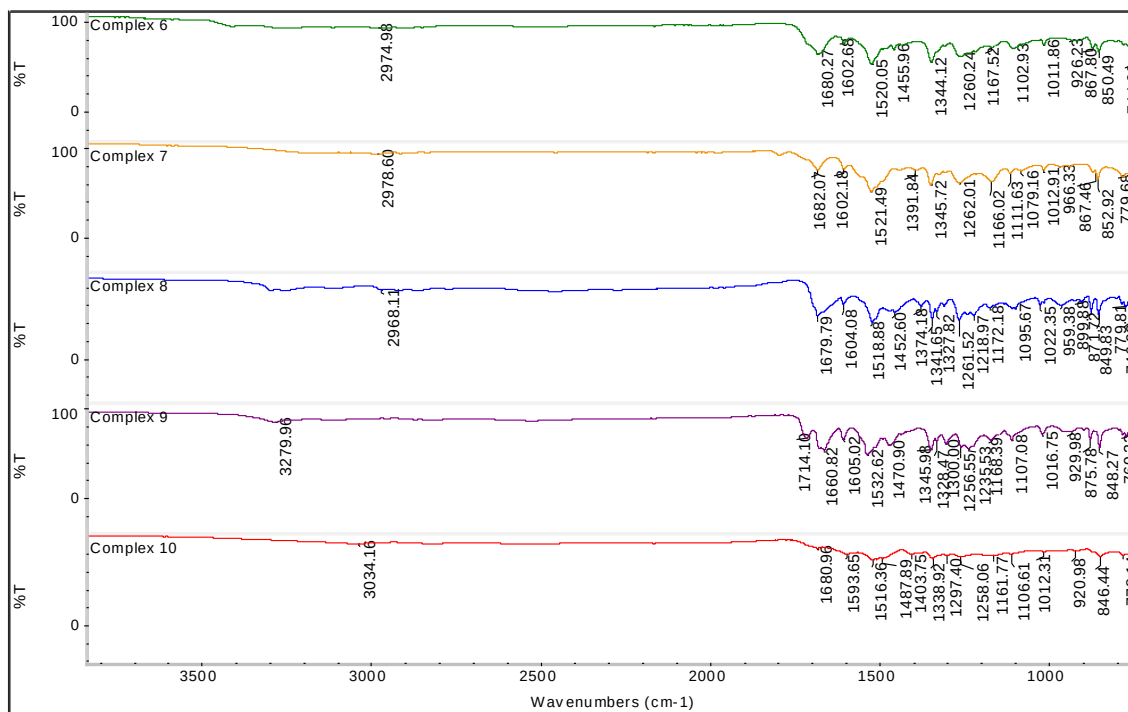
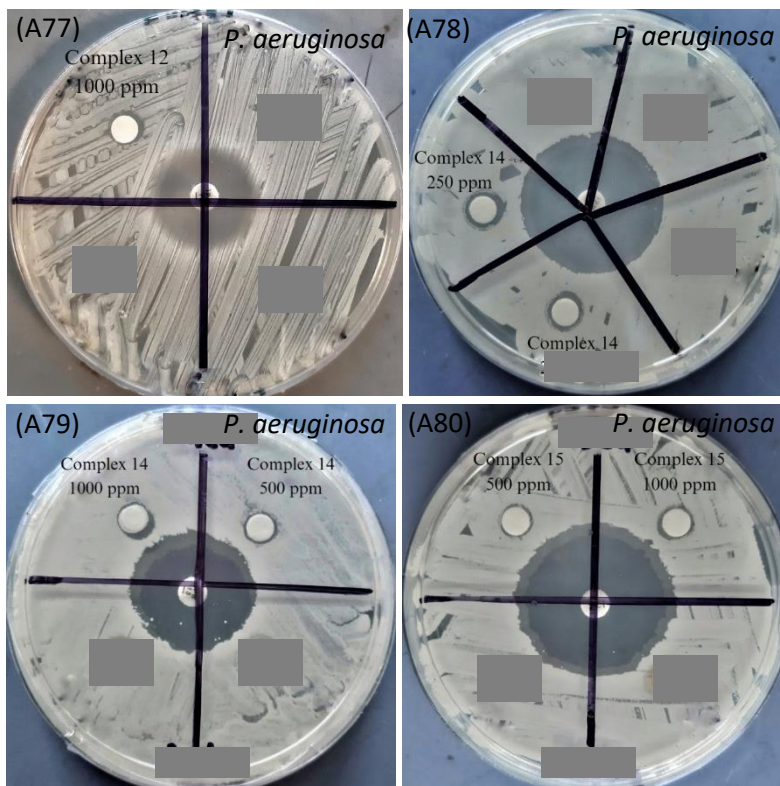


Antibacterial Study of Thiourea Complex (6-15)

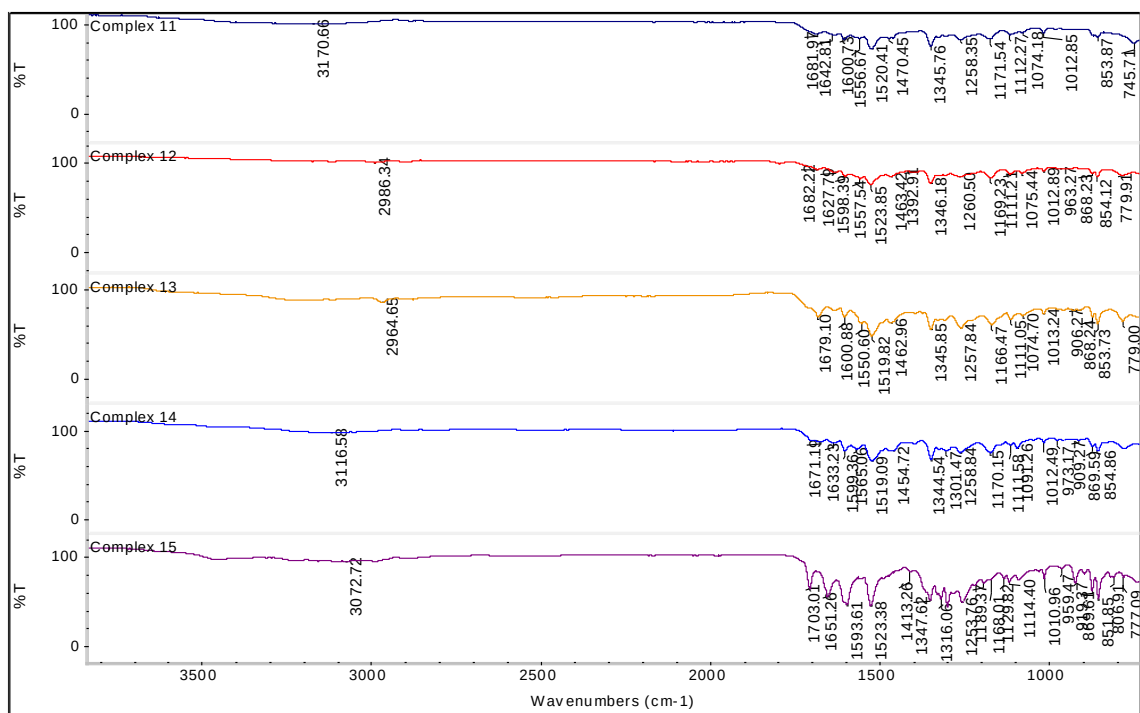




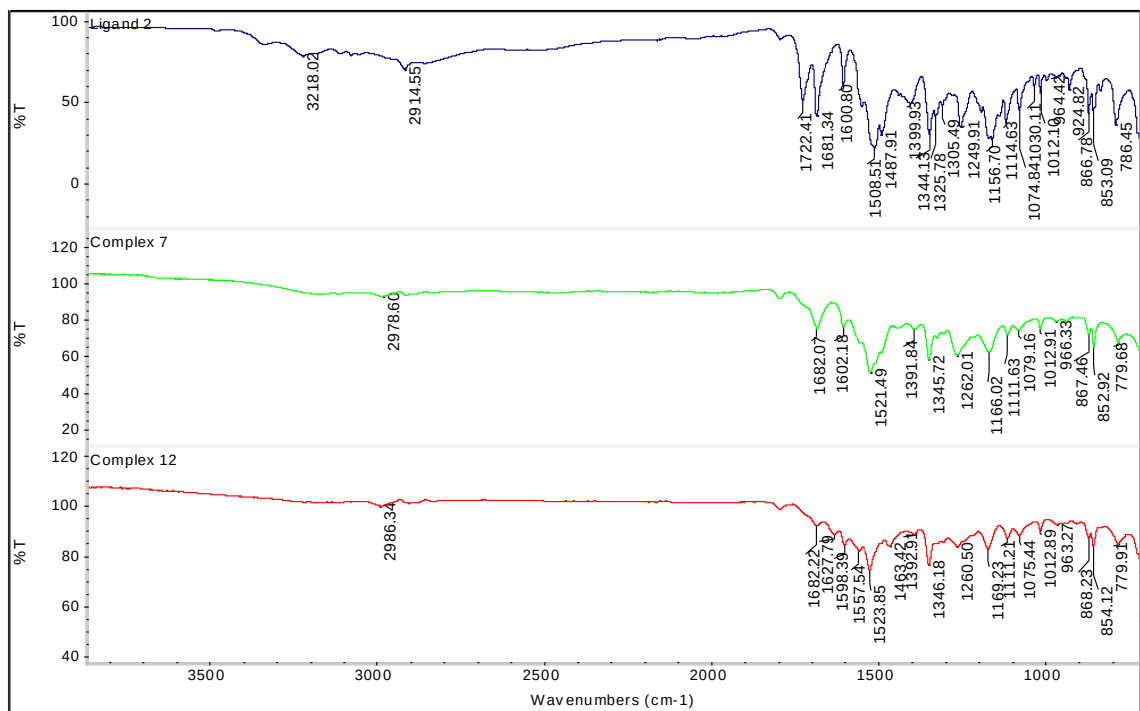




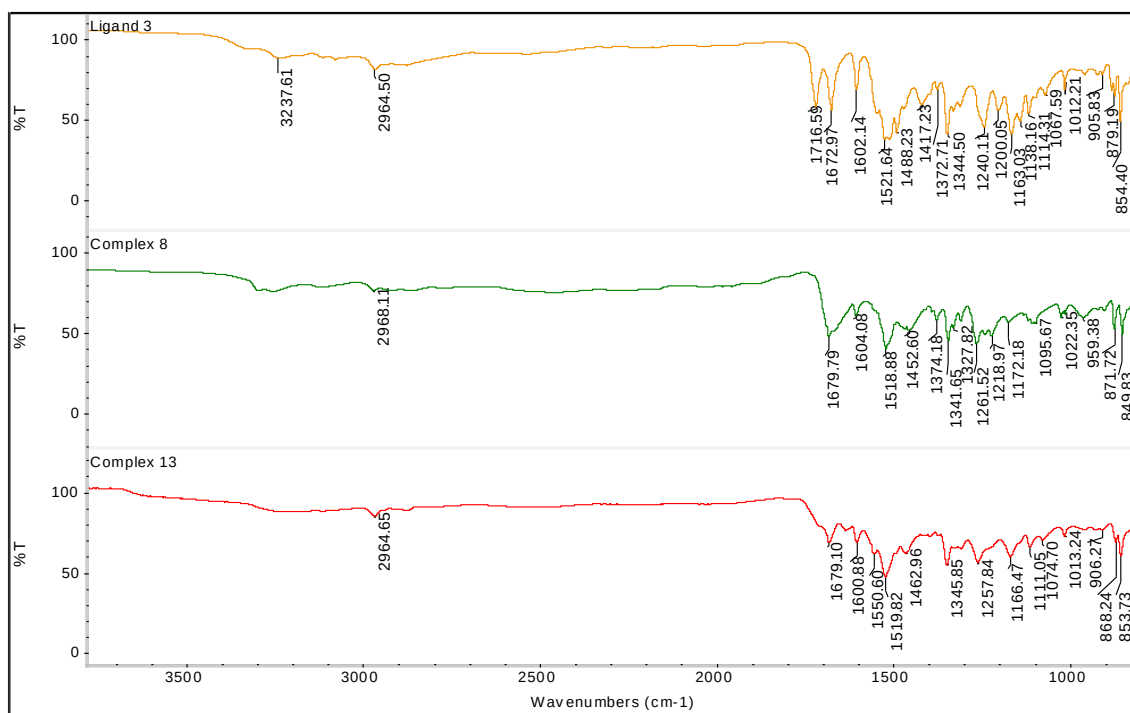
A81: Difference in FTIR spectrum of each complex (6-10)



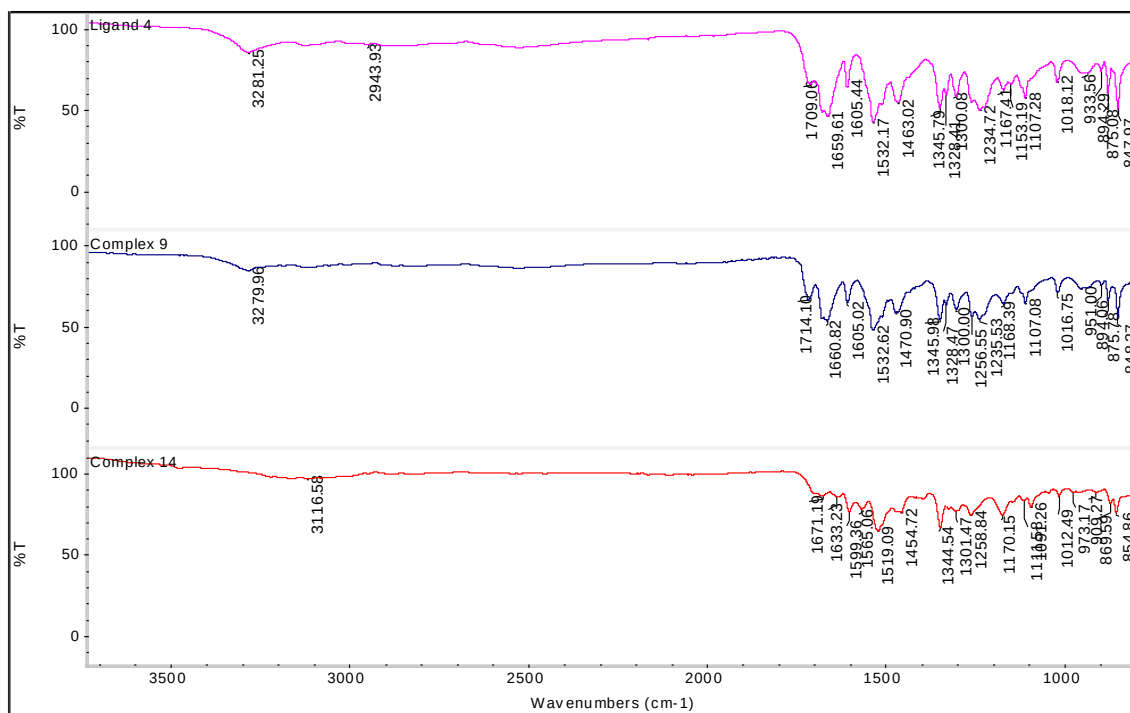
A82: Difference in FTIR spectrum of each complex (11-15)



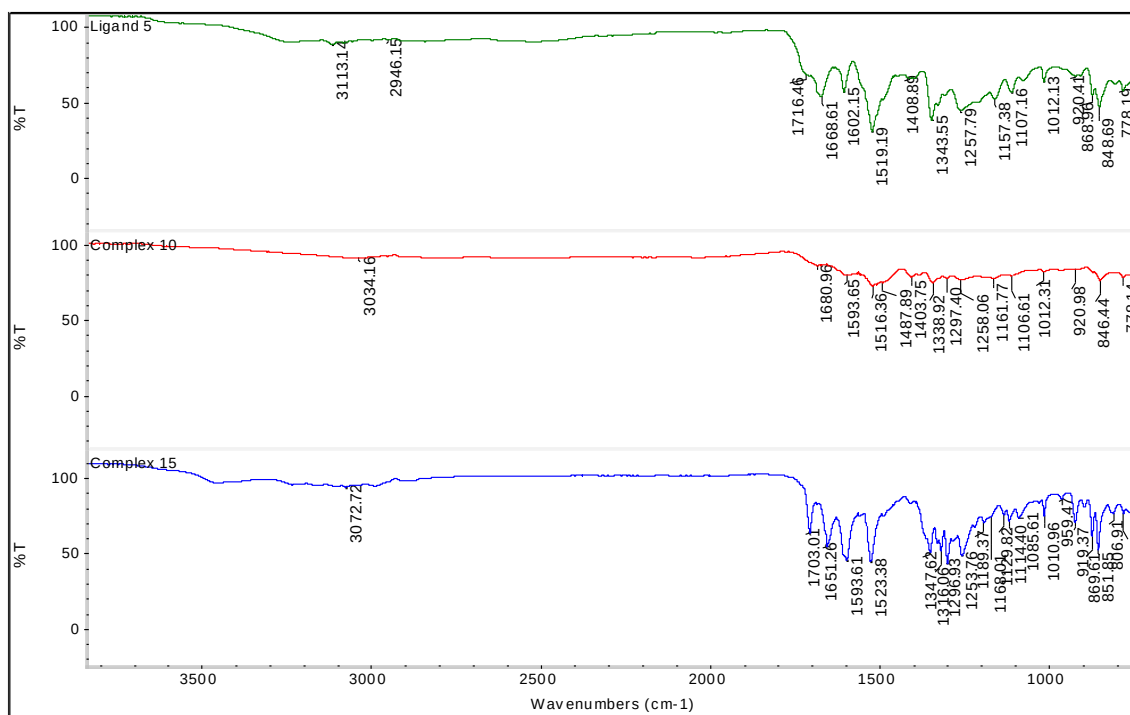
A83: FTIR spectrum of ligand 2 and it complexes



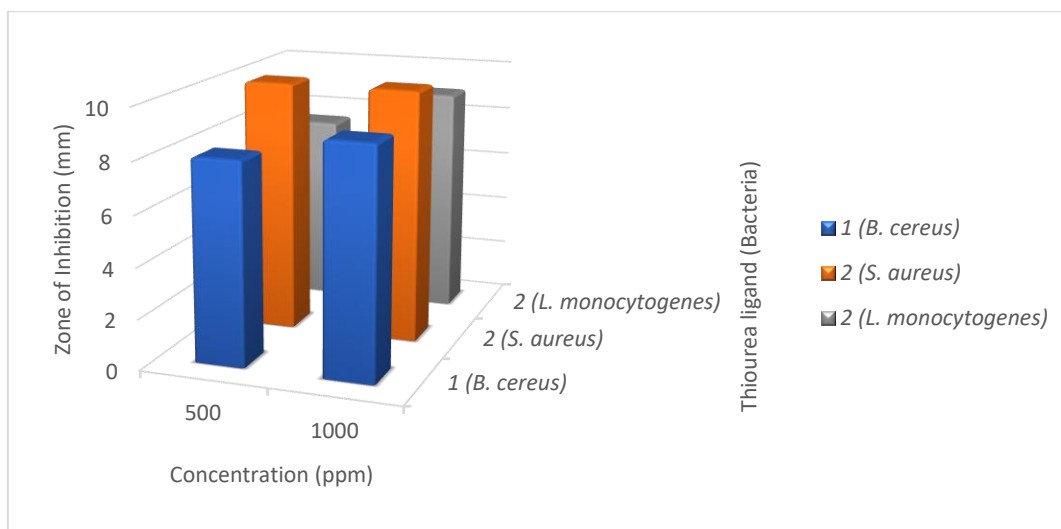
A84: FTIR spectrum of ligand **3** and it complexes



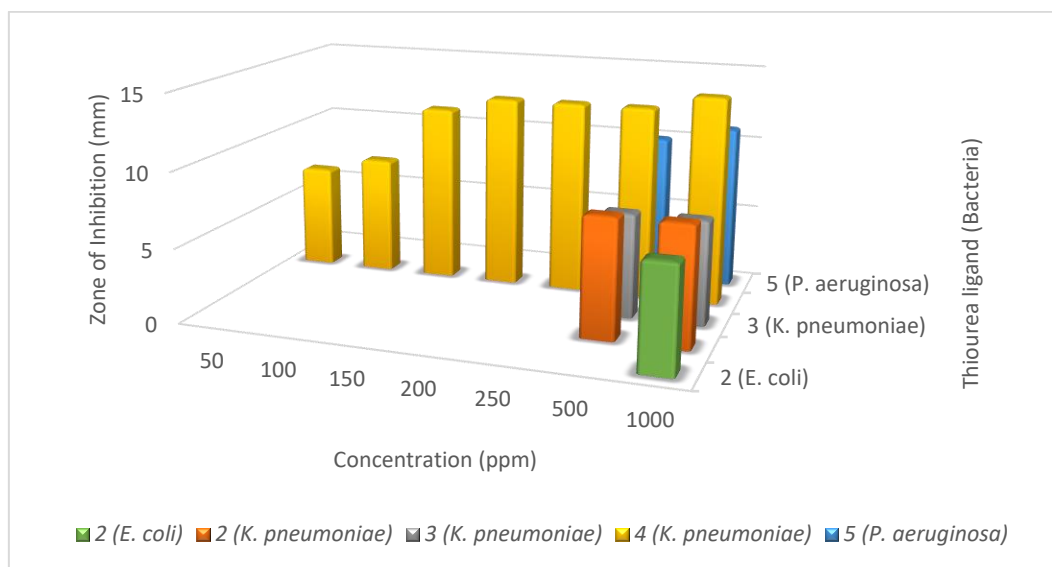
A85: FTIR spectrum of ligand **4** and it complexes



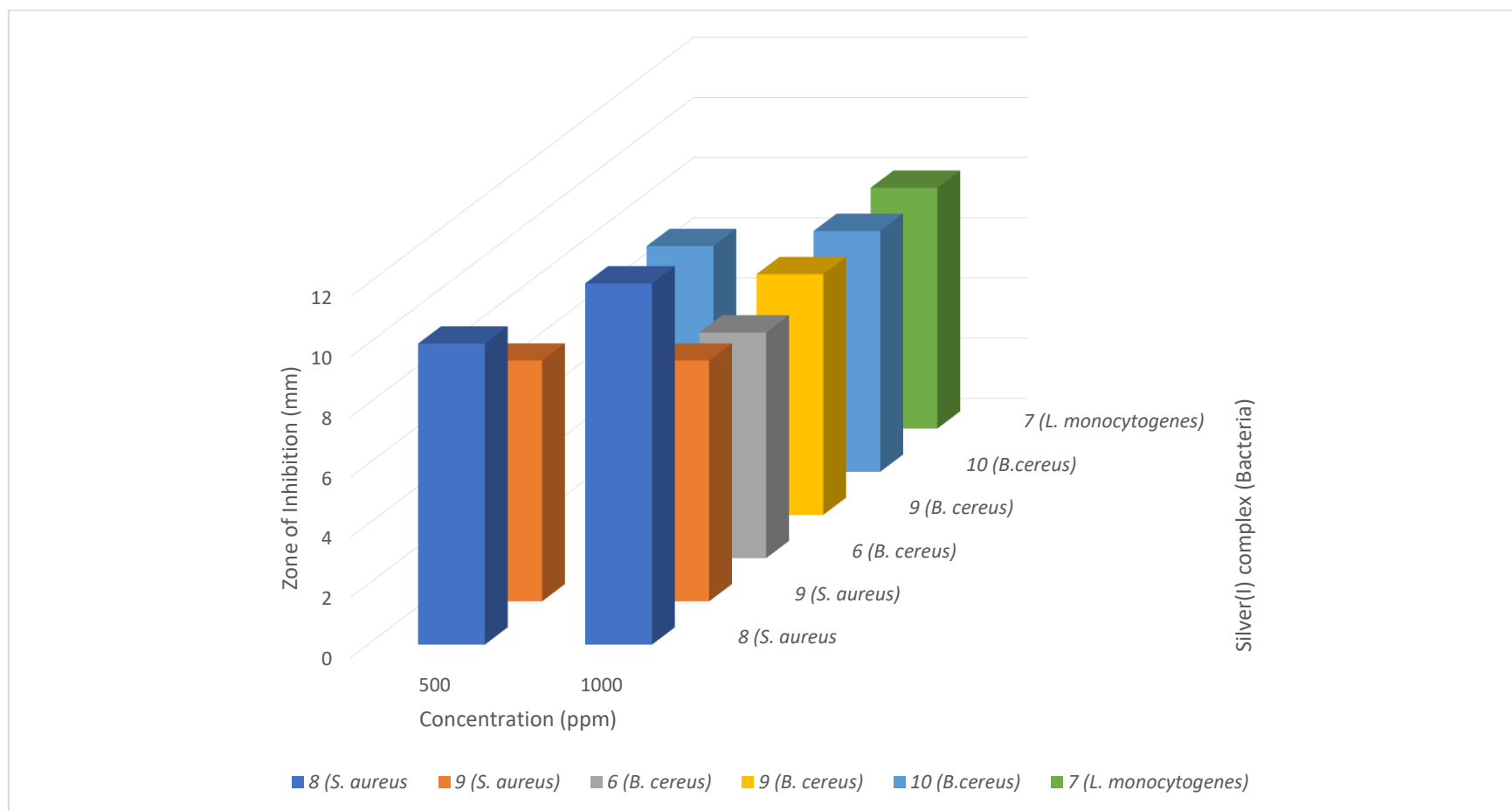
A86: FTIR spectrum of ligand **5** and it complexes



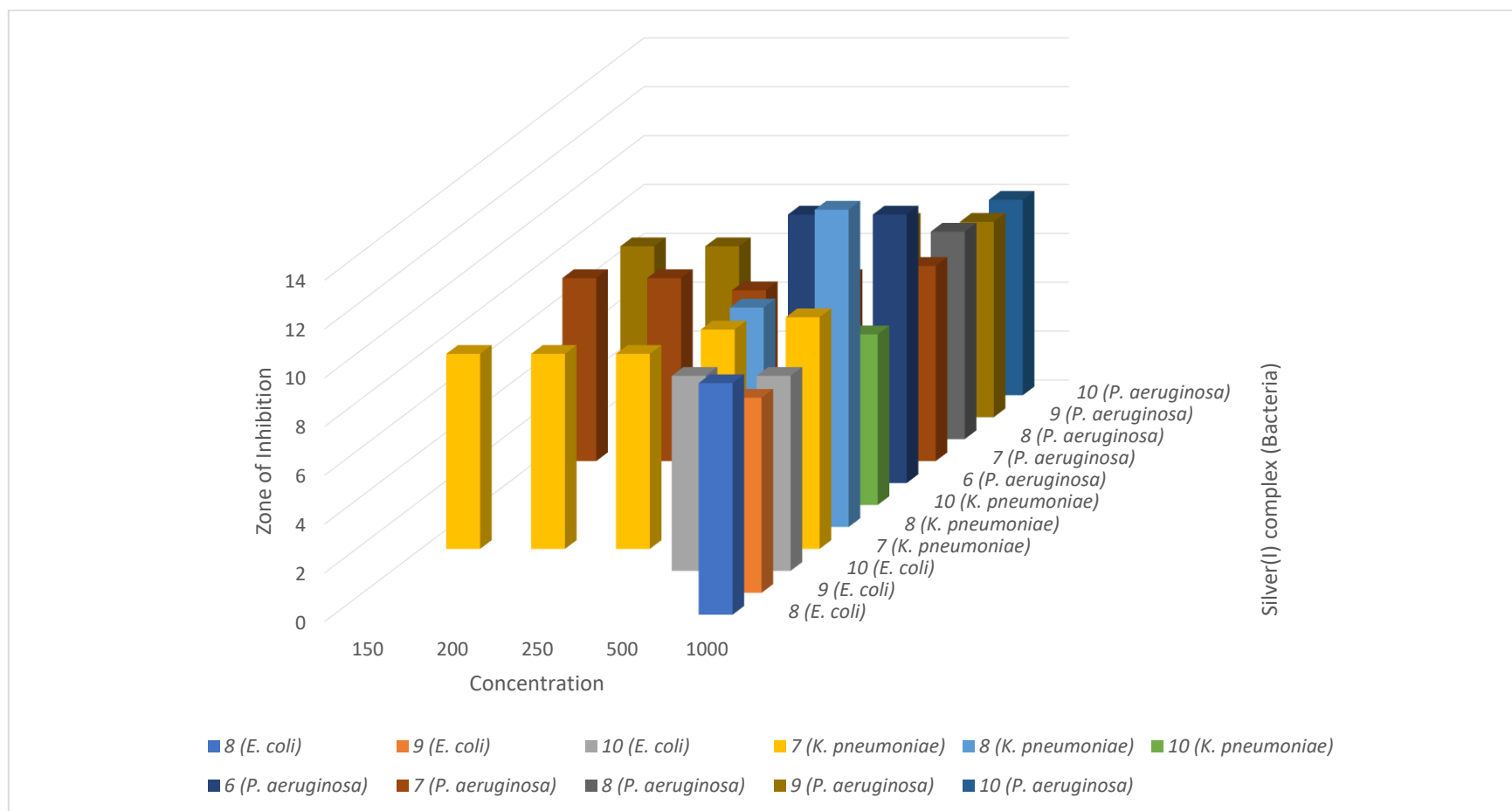
A87: Inhibition activity by thiourea ligand (**1-5**) on gram-positive bacteria



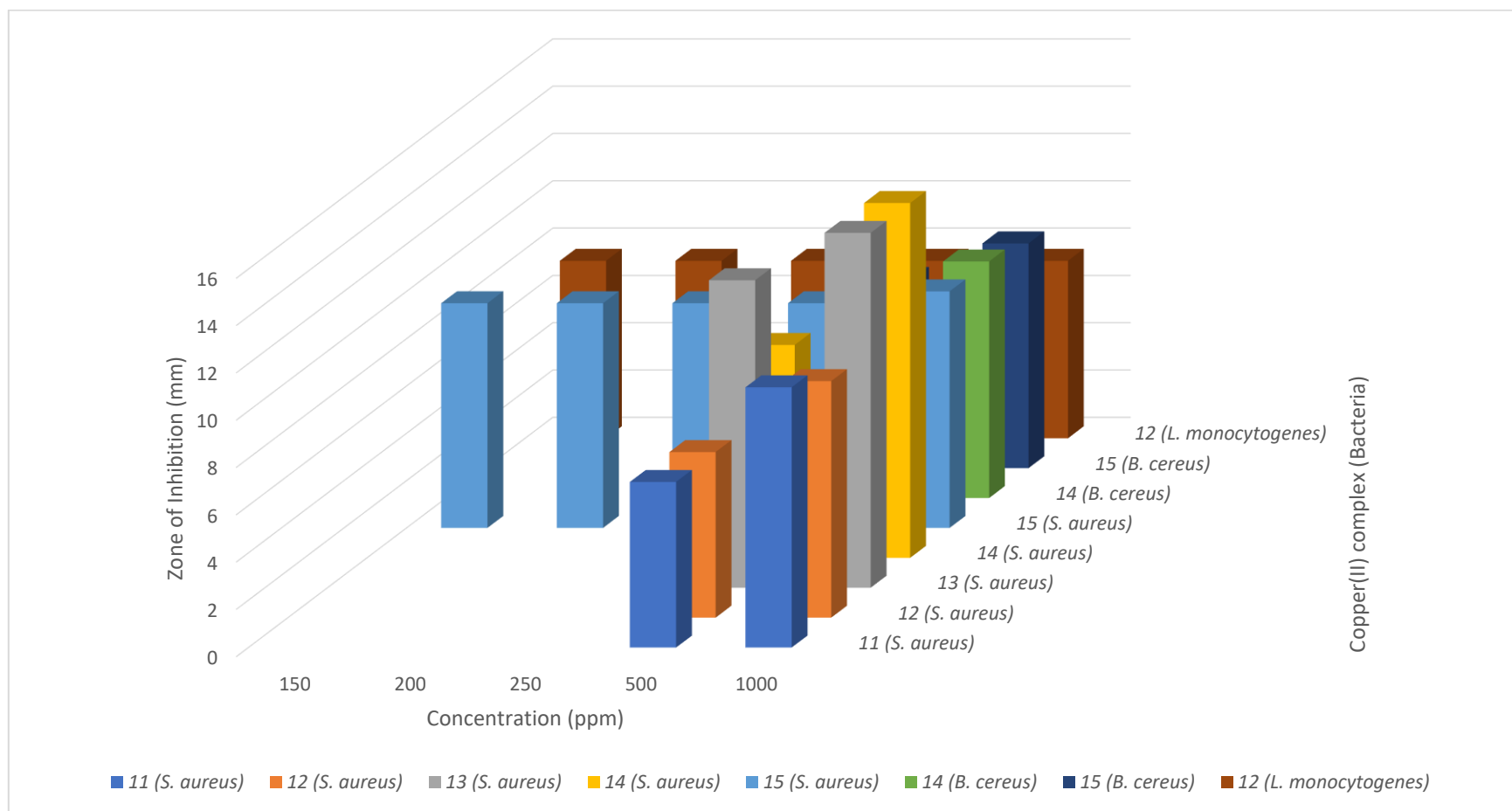
A88: Inhibition activity by thiourea ligand (1-5) on gram-negative bacteria



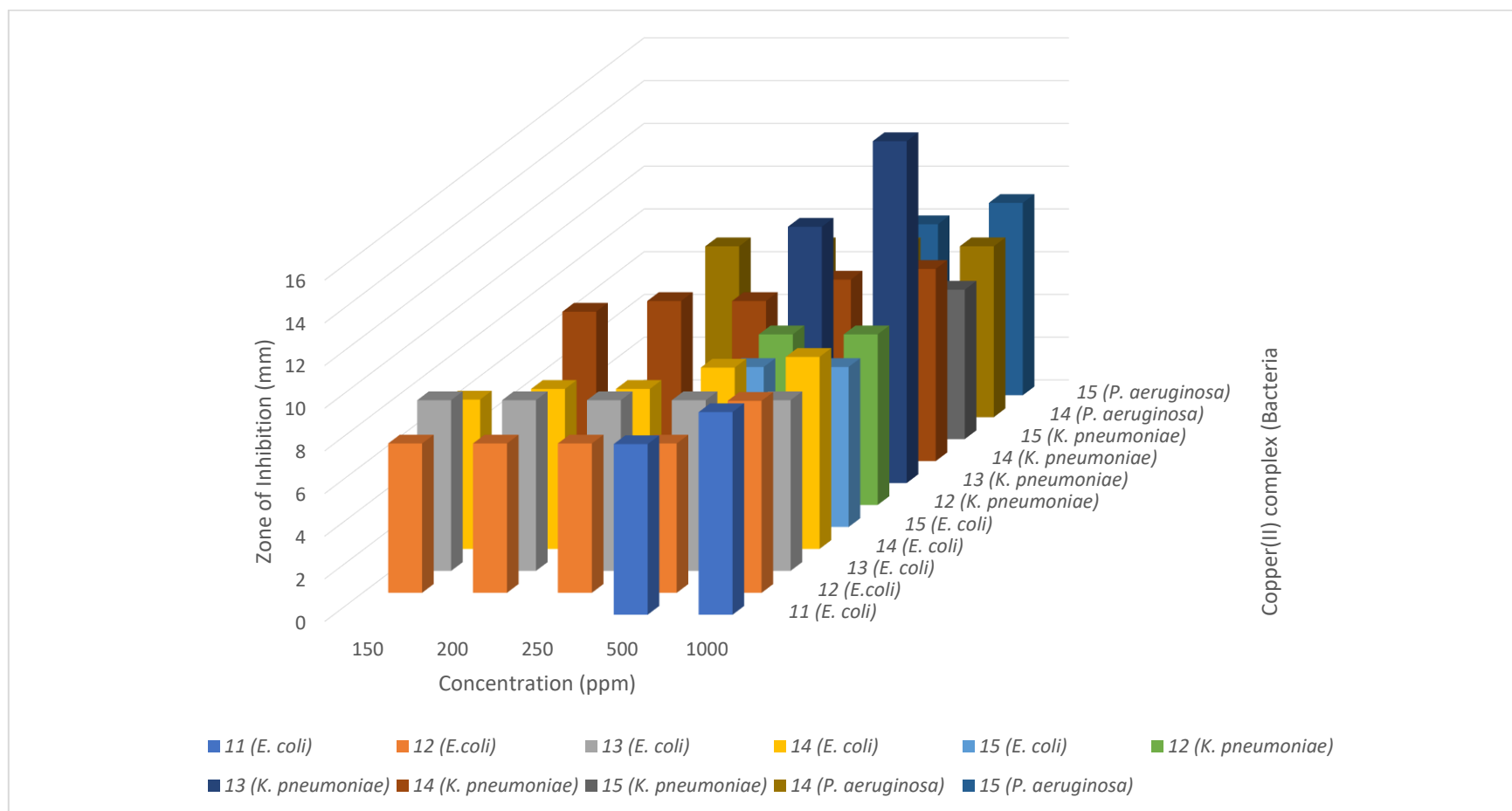
A89: Inhibition activity by thiourea complex (6-10) on gram-positive bacteria



A90: Inhibition activity by thiourea complex (6-10) on gram-negative bacteria



A91: Inhibition activity by thiourea complex (11-15) on gram-positive bacteria



A92: The number of inhibitions take place by thiourea complex (6-15) on gram-negative bacteria