



Faculty of Sciences

Department of Chemical and Forensic Sciences

**SYNTHESIS, CHARACTERISATION AND APPLICATION OF
RHENIUM(I)
COMPLEXES WITH NITROGEN-BASED HETEROCYCLIC LIGANDS**

by

MOREMI MODISE

Student ID number: 18100176

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Supervisor(s): DR. SEBUSI ODISITSE

Department of Chemistry

Faculty of Science, BIUST

E-mail Address

November, 2022

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ABSTRACT

This work aimed to develop, characterize, and investigate the cytotoxicity of five rhenium (I) based compounds. The compounds designated Re2-pico, Re3-pico, Re4-pico, Re(Phen), and Re2(Phen) were produced in yields of 35 to 60% using monodentate and bidentate pyridine derivatives as ligands. The complexes were synthesized using a similar protocol of refluxing in xylene for 4 hours using the appropriate stoichiometric ratios. Most of the complexes exhibited different physical properties and solubility. The ligands in the five complexes synthesized were found to all coordinate to the Rhenium metal centre through the lone pair of electrons found on the Nitrogen atom in the pyridine ring. The complexes' structures were determined using FT-IR, ESI-MS, ^1H -NMR, and Single Crystal-XRD. Three of the crystalline complexes were successfully elucidated using Single Crystal-XRD while the other two required more rigorous testing using other characterization methods to elucidate their structures. The data obtained from the SC-XRD was particularly helpful in characterization as it showed one of the complex's actual structures was different from the proposed one. The Single crystal XRD data gave us insight into the bond angles, bond length, geometry, and orientation of the atoms in space. The ^1H -NMR spectra showed characteristic aromatic peaks in the expected regions with a downfield chemical shift which could be attributed to bonding to the rhenium metal centre. MTT assay was used to determine the cytotoxicity of the compounds and the protocol was optimized to get the best results possible in an efficient manner. An absorbance plate reader determines the absorbance of samples in microplates by sending light of known intensity through each well vertically and detecting the intensity behind the sample. The absorbance values were measured at 450 nm using a microplate reader and IC_{50} values were calculated using the Graphpad Prism 8 plotting software. The experiment was repeated twice, with each complex and the control being measured in triplicate. The most potent complex based on the calculations of the IC_{50} values was Re2-pico, while the least potent was Re4-pico.

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DEDICATION

This work is dedicated to my grandmother Mrs. Segametsi Margret Maganu who passed away due to cancer. I wish she was here to celebrate this achievement with me and everything I am going to accomplish. And to my parents and siblings who have always nurtured, supported, and cared for me.

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LIST OF ABBREVIATIONS

ATP	Adenosine Triphosphate	UV	Ultraviolet
AR	Analytical Reagent	TMS	Tetramethylsilane
BMOV	Bis(maltolato)oxovanadium		
DILI	Drug-Induced Liver Injury		
DMSO	Dimethyl Sulfoxide		
DNA	Deoxyribonucleic Acid		
ER(+/-)	Oestrogen Receptor-Positive/Negative		
ESI-MS:	Electrospray Ionisation Mass Spectrometry		
FBS	Fetal Bovine Serum		
FDA	Food and Drug Administration		
FTIRS	Fourier Transform Infrared Spectroscopy		
GSH	Glutathione		
HIV	Human Immunodeficiency Virus		
KP1019	Indazole trans-[tetrachlorobis(1H-indazole)ruthenate(III)]		
LR	Laboratory Reagent		
MCF-7	Michigan Cancer Foundation-7		
MHz	Megahertz		
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide dye		
NADH	Nicotinamide adenine dinucleotide hydrogen		
NADPH	Nicotinamide adenine dinucleotide phosphate		
NAMI-A	Imidazolium-trans-tetrachloro(dimethylsulfoxide)imidazoleruthenium(III)		
NCI	National Cancer Institute		
NMR	Nuclear Magnetic Resonance		
PBS	Phosphate-buffered saline		
PDT	Photodynamic Therapy		
RAPTA	Ruthenium Arene 1,3,5-triaza-7-phosphaadamantane		
RNA	Ribonucleic Acid		
ROS	Reactive Oxygen Species		
SC-XRD	Single-Crystal X-Ray Diffraction		
SERM	Selective estrogen receptor modulator		

CHAPTER 1

1.0 INTRODUCTION

1.1 CANCER

One of the biggest health issues in the globe is cancer. It is second only to cardiovascular diseases as the leading cause of death worldwide. Making progress against cancer is one of the world's most serious issues because it is one of the top causes of mortality globally. Cancer is a condition when some bodily cells multiply out of control and spread to other parts of the body [1]. Some tumors are non-malignant, which means they don't represent a danger to life, while others are malignant (cancerous), which means they may prove fatal if left untreated. Human cells often divide (via a process known as cell division) to create new cells as the body requires them. Old or damaged cells die and are replaced by new cells when this occurs, figure 1 shows the difference between normal cells and cancerous cells [1].

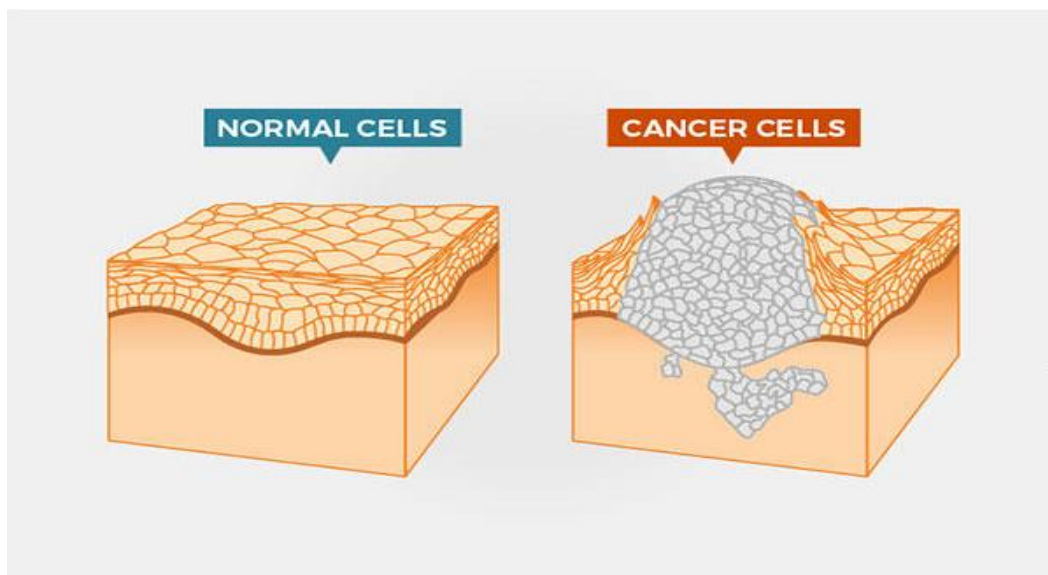


Figure 1: Comparison between normal cells and cancer cells [1].

In order to create new tumors, cancerous tumors in the body move to nearby and distant tissues (metastasis). While many malignancies develop solid tumors, blood-borne cancers like leukemias typically do not. No neighboring tissues are invaded by benign tumors [2]. Benign tumors typically don't come back after removal, although malignant tumors occasionally do. Depending on where they are, benign tumors can be quite large

and life-threatening, such as those in the brain as they can obstruct blood flow [2]. Tumors have characteristic cellular organelles as shown in figure 2.

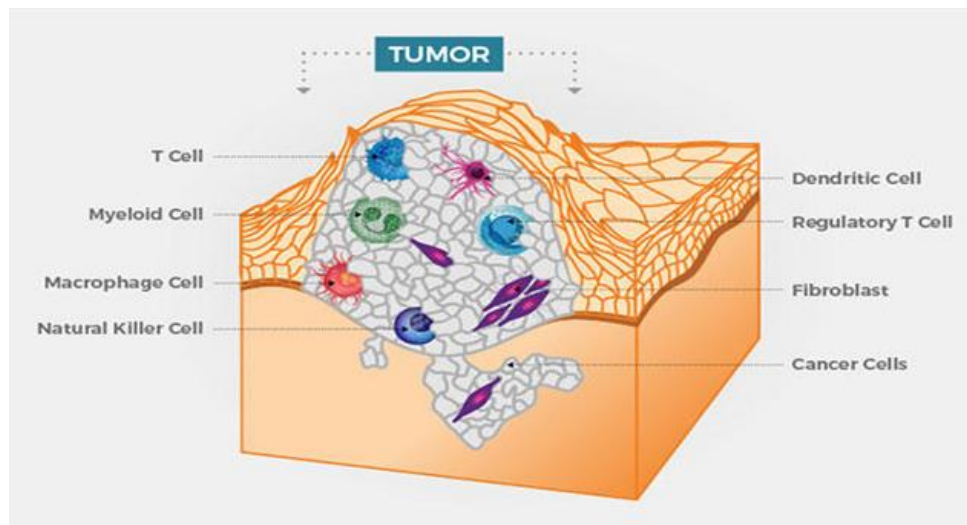


Figure 2: Anatomy of a tumor [1].

One of the biggest causes of death worldwide is cancer with nearly 10 million cancer-related deaths in 2020 [2]. In terms of new instances of cancer, the following were the most prevalent in 2020:

- breast (2.26 million cases).
- lung (2.21 million cases).
- rectum and colon (1.93 million cases).
- prostate (1.41 million cases).
- 1.20 million incidences of non-melanoma skin cancer
- stomach (1.09 million cases).

The most common cancer that results in mortality in Botswana is cervical cancer [3]. The mean age was 52 years, and between 2003 and 2011, cervical cancer represented 14% of all malignancies in Botswana and 26% of all cancers in women [3]. In Botswana, over 250,000 women or 25% of the entire female population are between the ages of 30 and 49, placing them at an elevated risk of getting cervical cancer [3].

1.1.1 Causes of cancer

Cancer has many causes ranging from preventable factors like a person's lifestyle to non-preventable factors like genetics [4]. For instance, research from 2014 showed that over 480,000 people in the United States pass away each year as a result of cigarette use [4]. The following categories apply to external risk factors:

- physical carcinogens like ionizing radiation and UV light.
- biological carcinogens, such as infections from specific viruses, bacteria, or parasites.
- chemical carcinogens, such as asbestos, substances in cigarette smoke, aflatoxin (a food contaminant), and arsenic (a drinking water pollutant).

Age is currently the most important inescapable risk factor. The American Cancer Society reports that 87 percent of cancer diagnoses in Americans aged 50 or older are identified by doctors. Normally, the body gets rid of DNA-damaged cells before they develop into cancer, but as we age, the body becomes less effective at doing this. This contributes to the increased chance of developing cancer later in life [4].

Cancer development may also be influenced by genetic factors [5]. When to divide and stop growing, a person's cells are given instructions by their genetic code. Cancer may develop as a result of changes in this genetic code that result in incorrect instructions. A large portion of the instructions for cellular growth and division are carried by proteins, which are produced by the cells in part by genes [5]. Some genes change the proteins that are typically in charge of repairing damaged cells, which can also cause cancer. A parent may convey these genes to their offspring if they possess them. Other genetic alterations take place after birth, and risk-raising behaviors like smoking and sun exposure exist. Other changes that can lead to cancer take place in the chemical signals that control how the body activates particular genes [5].

Finally, a person may inherit a propensity for a particular type of cancer [6]. This is referred to as a hereditary cancer syndrome by doctors. Numerous cancer instances are profoundly influenced by inherited genetic alterations. Each person's cancer has a

particular genetic mutational mix. More alterations will take place as the cancer progresses. Various cells in the same tumor may exhibit different genetic alterations [6].

1.2 TREATMENT

Every cancer kind requires a different treatment plan, thus an accurate cancer diagnosis is crucial for the right and expedient course of treatment. Surgery, chemotherapy, and/or radiotherapy are frequently used as treatments. Identifying the treatment's objectives is a significant initial step [7]. Generally speaking, the objective is to eradicate cancer or greatly extend life. A key objective is enhancing the patient's quality of life. Support for the patient's physical, psychological, and spiritual health as well as palliative care for those with terminal cancer can help achieve this [7].

When detected early and given the proper care, several of the most prevalent cancers, including breast cancer, cervical cancer, oral cancer, and colorectal cancer, have high cure rates [8]. Even when cancerous cells are present in other parts of the body, several cancer forms, like testicular and other types of leukemia and lymphoma in children, have good cure rates if adequate medical assistance is provided [8].

1.3. PROBLEM STATEMENT

Despite being used extensively to treat cancer, the platinum-based medication cisplatin and its derivatives have some significant drawbacks. For instance, these medications cause harmful side effects and can result in drug resistance. However, the prominence of platinum-based medications has stoked enthusiasm for creating new pharmacological candidates using alternative metal centers with fewer negative side effects and good potency [9]. These complexes exhibit high kinetic stability and reactivity due to the existence of a metal-carbon link and π -bound arenes. Researchers have concentrated increasingly on the creation of organometallic transition metal anticancer medicines [10]. As a result, using organometallic complexes opens up a lot of possibilities for rational drug design with greater cellular absorption, minimal side effects, and various mechanisms of action inside tumor cells [10]. Compared to cisplatin, these compounds are less toxic, exhibit alternative mechanisms of action, bind with biological target DNA, and go through ligand hydrolysis. Due to their great selectivity and low toxicity, rhenium-

based compounds have demonstrated remarkable usefulness in the fight against cancer [11].

1.4. AIMS AND OBJECTIVES

To synthesize, characterize and test cell proliferation ability of pyridyl derivative based rhenium (I) complexes.

1.4.1 OBJECTIVES

- a. To synthesize pyridyl-based rhenium(I) complexes.
- b. To characterize complexes using spectroscopic techniques such as FT-IR spectroscopy, ESI mass spectrometry, NMR and Single crystal X-Ray Diffraction.
- c. Cell proliferation screening through MTT assay.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 APPLICATIONS OF KEY METAL COMPLEXES IN MEDICINE

New therapeutic and diagnostic metal complexes are currently influencing medical practice in the fast-expanding field of inorganic chemistry's application to medicine [12]. The design of chemicals to minimize hazardous side effects and comprehend their methods of action must be improved, and this requires advancements in bio-coordination chemistry. The periodic table's d block elements, which includes groups 3 through 12, is represented by transition metals [12]. In any of their often-occurring oxidation states, they have partially filled d-shells. The use of transition metal complexes as medications to treat a variety of human ailments, such as cancers, lymphomas, infections, diabetes, and neurological problems, has advanced significantly in research [13]. Transitional metals can interact with a variety of negatively charged molecules and display various oxidation states [13]. Due to this potential, the creation of innovative metal-based therapies with fascinating pharmacological applications has begun [13].

The therapeutic application of metal complexes stimulates further investigation into the creation of novel metallodrugs, such as metal-mediated antibiotics, antivirals, antiparasitics, radio-sensitizing agents, and anticancer compound [14]. From the National Cancer Institute (NCI) tumor screening database, more than a thousand possible anticancer metal compounds were examined [15]. According to their affinity for binding to sulphahydryl groups, chelation, the creation of reactive oxygen species, and the production of lipophilic ions, they were divided into four major classes based on their methods of action [15]. Based on their ability to inhibit angiogenesis or promote apoptosis, numerous prospective anticancer drugs have been studied [15].

2.1.1 Platinum complexes

Rosenberg accidentally discovered that the platinum compound cisplatin (cis-[PtCl₂(NH₃)₂]) has anticancer properties in the late 1960s [16]. Since its clinical use was allowed in 1978, cisplatin has become one of the world's top-selling anticancer

medications and unquestionably the most well-known metal-based medication [16]. The enormous clinical success of cisplatin served as a powerful incentive to look for more new platinum complexes that may be employed as medications [16]. It is generally known that the primary mechanism underlying the cytotoxic effects of platinum-based anticancer medications is the association of the platinum core with DNA [17]. Platinum (II) center coordination to DNA results in structural alterations that ultimately trigger the development of apoptosis [17]. Cisplatin and its derivatives are used in the therapeutic treatment of a number of malignancies [17].

The cure rate for testicular cancer increased from 10% to 85% because to the use of the drug cisplatin [18]. Cisplatin is also employed in Auger therapy, a sort of radiation therapy for the treatment of cancer that uses a lot of low-energy electrons (emitted by the Auger effect) instead of high-energy radiation to kill cancer cell [18].

Cisplatin and carboplatin, two platinum analogues, are among the most frequently utilized chemotherapeutic drugs in oncology [19]. Both medications have a wide range of clinical action in a variety of malignancies, such as bladder cancer, head and neck cancer, thoracic cancers, germ cell tumors, and gynecological cancers [20]. Even though both drugs ultimately work through a similar method to cause tumor cell apoptosis, cisplatin has been found to be more efficient in treating particular tumor types [21]. Although carboplatin typically has more favorable toxicity profiles, this can commonly translate to a worse response in some cancers [21].

However, because platinum medications struggle to distinguish between cancerous and healthy cells, as well as because innate and acquired cancer resistance to platinum is common, the therapeutic usefulness of cisplatin and its analogues is constrained [22]. These significant drawbacks have prompted the search for substitutes, including platinum-based compounds and other transition metal derivatives [23]. Oxaliplatin and carboplatin, as well as two others nedaplatin in Japan and heptaplatin (figure 3) in Korea have received widespread approval [23].

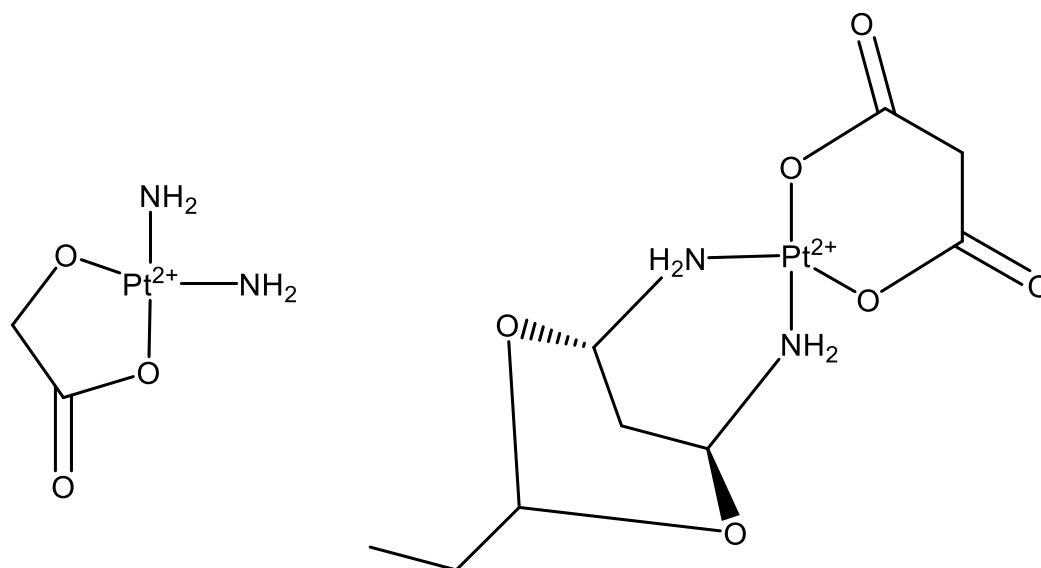


Figure 3: Cis-platin derivatives Nedaplatin and Heptaplatin [26].

The success of platinum has also prompted substantial research on other metal complexes, including titanium, ruthenium, and gallium, that may have anticancer and other biological properties [24]. There are only a few non-platinum complexes that are now being utilized in clinical trials as anticancer medication [24]. Ruthenium complexes have been the subject of intense research, two substances NAMI-A ([imH]trans-[RuCl₄(dmsO-S)(im)]) and KP1019 ([indH]trans-[RuCl₄(ind)₂], ind = indazole), have entered clinical trials [24]. Additionally, half-sandwich organoruthenium(II) complexes, in which different ligands are coupled to the primary metal ion, have received a great deal of attention [24]. The interactions of metal ions and complexes with DNA are well recognized [25]. It is also well acknowledged that cisplatin's interaction with DNA is essential for its function. Due to this, a number of metal-based anticancer medications were developed and heavily examined for interactions with DNA [25]. Eventually, it became evident that many active metal-based medicines are more focused on other targets such as the mitochondria [25]. But there are still other uses for the interactions with DNA, such as bioanalysis. Thus, metal complexes have been used to investigate the structural and functional characteristics of nucleic acids [25].

2.1.2 Iron complexes

Recently, ferrocene, a compact metallocene with low toxicity, reversible redox behavior, and stability in non-oxidizing environments, has been crucial in bioorganometallics as an antiparasitic, antibacterial, and even anticancer agent [26]. Inadvertent preparation of ferrocene began in 1951. Based on its reactivity, R. B. Woodward and G. Wilkinson inferred a sandwich-like structure. For his work on metallocenes and other areas of organometallic chemistry, Wilkinson and E. O. Fischer later shared the Nobel Prize [26]. The Jaouen group successfully connected the ferrocene fragment to tamoxifen, an estrogen receptor modulator used to treat breast cancers. Several substituted ferrocenes have been discovered in recent years [26]. Tamoxifen was changed by the addition of a ferrocene fragment to produce ferrocifen, which is also extremely cytotoxic [27]. A new set of powerful antiproliferative compounds were created after a conventional McMurry cross-coupling process was used to prepare the compounds [27]. Similar methods of combining an organic substance with an organometallic substance have been applied to create more potent medications [27]. Embelin is a para-benzoquinone that occurs naturally and exhibits remarkable selectivity towards malignant cell lines as opposed to non-cancerous cell lines. One of the key ideas common to supramolecular chemistry is the concept of molecular self-assembly [28].

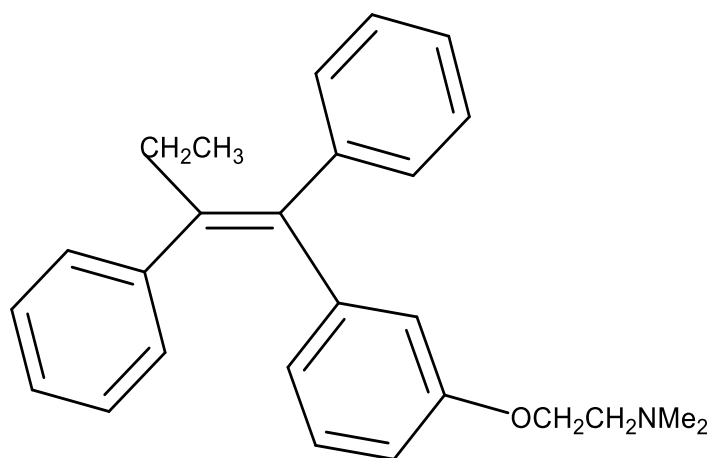


Figure 4: Tamoxifen, a selective estrogen receptor modulator (SERM), is used to treat both males and females with all stages of hormone receptor-positive breast cancer [33].

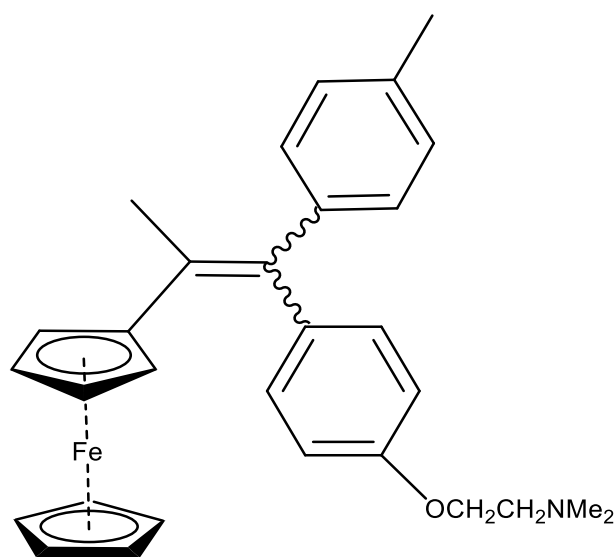


Figure 5: Ferrocifen [35].

There are several bioferrocene compounds with antitumoral potential [29]. They provide as an example of the depth of the activity in this area, the variety of structures used, and the wide range of potential attack angles [29]. Even if only a small portion of the several mechanisms involved have been reliably explained thus far, this is encouraging [29]. The initial step in studying the antiproliferative activity of these complexes was to determine their IC_{50} values on cancer cells (MDA-MB-231, MCF-7, and PC-3), as well as in certain cases, non-cancerous cells [30]. The cytotoxicity of these complexes is substantially stronger on cancer cells than on normal cells, which is their primary trait. Testing on NHEM, newborn human epidermal melanocytes, and astrocytes has demonstrated this [30].

Breast cancer is a common affliction among women; it affects about 1 in 8 women in the West [31]. Generally speaking, there are two categories of breast tumors that can be distinguished by the presence of either an estrogen receptor positive (ER(+)) or an estrogen receptor negative (ER(-)) [32]. The ER(+) type accounts for roughly two-thirds of cases and is sensitive to hormone therapy with selective estrogen receptor modulators (SERMs). Two receptor subtypes, $ER\alpha$ and $ER\beta$, are present in ER(+) cell lines [33]. Several ferrocenyl derivatives were created using the tamoxifen and hydroxytamoxifen structures as a guide [34]. The series of ferrocifens was biologically examined *in vitro* and *in vivo* [34]. To investigate how different hydroxy-substituted ferrocifens affect the cell

proliferation, two lines of breast cancer cells were used, one for tumors mediated by the ER receptor and the other for tumours mediated by ER [35].

Much emphasis was given to the creation of water-soluble ferrocenyl compounds that exhibit anticancer action [35]. Some findings indicated that these substances are strong anticancer agents, sometimes much more so than the water-insoluble molecules [35]. The differential in solubility between soluble and insoluble substances may be the cause of this phenomenon [36]. It is crucial to note that Koepf-Maier is a pioneer in this area in this context. In the 1980s, he created a number of ferrocenyl compounds with biological activity [36]. Ferrocenium tetrafluoroborate salt and ferricenium tri-iodide are two of the most significant ferrocenyl compounds with good anticancer activity [37].

Several scientists have investigated the ferrocene derivatives' mode of action in the treatment of cancer [37]. The findings demonstrate that the oxidation state of the iron in the ferrocene moiety affects the activity of ferrocenyl compounds. According to certain findings, Fe(II) ferrocenyl compounds are more effective than Fe(III) ones [38]. According to research on the ferrocifen's mechanism of action as one of the Fe(II) compounds, the ferrocifens affect receptor protein conformation [38]. Additionally, when ferrocifen binds to ER β , an 'oxidant/antioxidant' mechanism may occur [39]. Fe(II) complexes are known to be oxidized to Fe(III) by oxygen, producing extremely reactive OH $\cdot$ radicals, and the ferrocifen-ER complex is proposed to dimerize and attach itself to a specific area of DNA. [39]. The observed antiproliferative effect in relation to the ER β receptor could be caused by these radicals damaging the DNA strand adjacent to the binding site [39].

Some researchers have offered a different explanation for the mechanism, claiming that the reduction of ferrocenium ions in living organisms produces active oxygen radicals like hydroxyl that are responsible for the compound's anticancer activity by forming radical metabolites that cause biological damage to cancer cells [40]. In other words, the high activity of ferrocene compounds as an anticancer drug in the two processes is due to their strong redox characteristics and the production of OH $\cdot$ radicals [40].

2.1.3 Ruthenium complexes

Arene complexes of ruthenium (II) form an essential section of organometallics [41]. These compounds work on platinum-resistant cancer cells and are typically less harmful

than their platinum-based equivalents [41]. The synthesis of a wide variety of modified Ruthenium Arene 1,3,5-triaza-7-phosphaadamantane (RAPTA) compounds has contributed to the understanding of the anticancer activity's mechanism of action and the identification of strategies for optimizing therapeutic activity [42]. Although ruthenium compounds normally react with DNA less aggressively than platinum does, RAPTA complexes can nonetheless cause DNA damage [43]. Some ruthenium complexes were discovered to cause DNA damage in a pH-dependent manner [43]. But intriguingly, it was also discovered that while some of these complexes do not interfere with DNA at physiological pH or higher, which is characteristic of healthy cells, DNA damage is seen at lower pH (7) [44]. Since cancer cells have lower pH values than healthy cells due to hypoxia, it was hypothesized that the altered pH environment would enable targeted anticancer action [44].

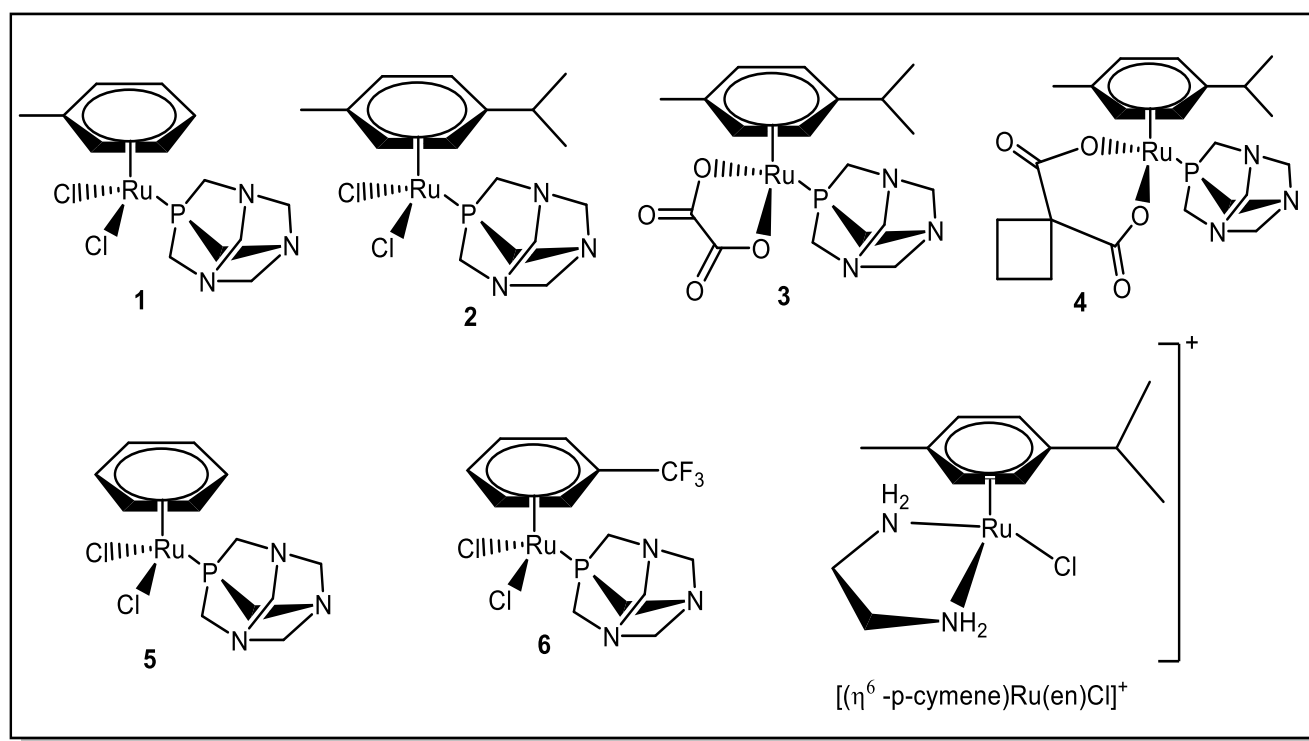


Figure 6: A core ruthenium (II) atom complexed to an arene group makes up the central component of RAPTA compounds, a class of investigational cancer medications [52].

As cisplatin resistance continues to be an issue in medicine, RAPTA medicines have an alternative protein-mediated method of anticancer activity that is of great interest as a

complementary therapy [45]. There has been substantial progress in our understanding of medication resistance mechanisms. Some of these techniques include lowered cellular absorption, improved drug efflux from cells, and deactivation through binding to cytosolic and nuclear proteins as well as glutathione [45]. The ability of cancer cells to repair DNA harm brought on by harmful drugs like cisplatin can also increase [45]. Additional structural alterations to Ru (II) complexes have been demonstrated to further inhibit some resistance mechanisms [46]. For instance, it has been demonstrated that P-glycoprotein inhibitors of the phenoxazine type are associated with drug resistance in some cancers, and that this leads to complexes that can overcome multidrug resistance by being connected to the Ru (II)-arene center via imidazole linkages [46].

Utilization of photoactive organometallic complexes or metal complexes coupled with an organometallic moiety has increased recently [47]. In the presence of light, photodynamic therapy (PDT) is a treatment that shows promise for treating cancer cells. Tetra(4-pyridyl)porphyrin (TPP) derivatives were synthesized and found to have potential as photosensitizing chemotherapeutic drugs [48]. On human melanoma tumor cells, these derivatives' biological effects were examined. When exposed to a 652 nm laser, the ruthenium compounds displayed outstanding phototoxicities toward melanoma cells [49]. Microscopy studies of cellular absorption and localisation of $[\text{Ru}_4(\eta^6\text{-C}_6\text{H}_5\text{CH}_3)_4(\text{TPP})\text{Cl}_8]$ and $[\text{Rh}_4(\eta^5\text{-C}_5\text{Me}_5)_4(\text{TPP})\text{Cl}_8]$ revealed that they collected in granular structures other than lysosomes in the cytoplasm of melanoma cells [50]. The metal component and the fluorescent porphyrin moiety were located in comparable cell structures. Because they combine chemotherapeutic activity with photodynamic therapy, the porphyrin areneruthenium (II) derivatives are a novel class of organometallic photosensitizers that are interesting for the treatment of cancer [41].

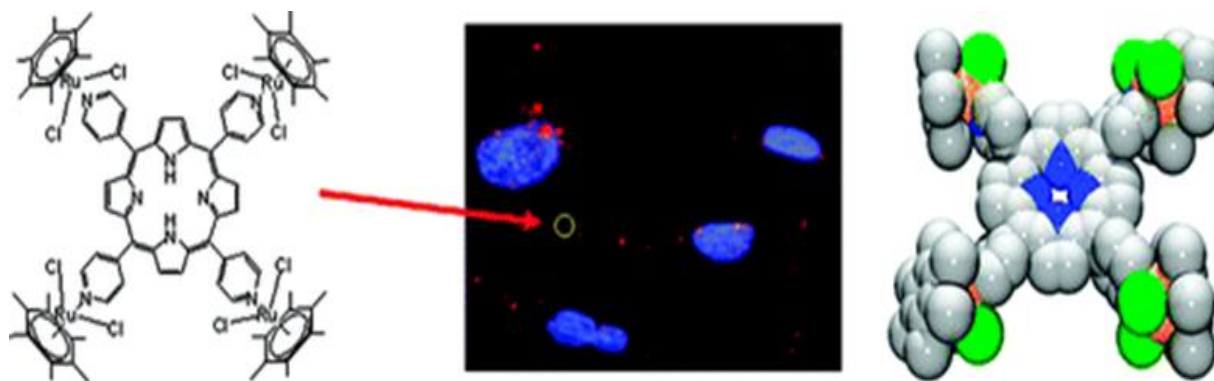


Figure 7: Photoactive Ruthenium complex $[\text{Ru}_4(\eta^6\text{-C}_6\text{H}_5\text{CH}_3)_4(\text{TPP})\text{Cl}_8]$.

2.2 OTHER COORDINATION COMPOUNDS IN MEDICINE

Arsenic trioxide appears to impact a wide range of intracellular signaling pathways and significantly change cellular function [51]. As a result, there are various routes by which arsenic trioxide causes cell death. There have been instances of apoptosis being induced, proliferation being suppressed, and even angiogenesis being blocked [52]. Arsenic trioxide raises the amount of hydrogen peroxide in cells and inhibits glutathione peroxidase, potentially via producing arsenic-GSH conjugates [52]. By increasing cellular H_2O_2 level, ascorbic acid increases arsenic trioxide-induced death in lymphoma cells but not in healthy bone marrow cells, which is in agreement with a broad role in redox processes [53]. Due to its combined actions, arsenic trioxide may be considered a potential mitochondriotoxic anticancer agent [53].

Galium nitrate, $\text{Ga}(\text{NO}_3)_3$, is most commonly used to treat hypercalcemia in cancer patients by lowering the increased Ca^{2+} levels in the blood. [54]. Rapid bone loss causes life-threatening calcium levels in the blood in this illness, which is frequently linked to bone malignancies [54]. By preventing osteoclasts from secreting acid onto the surface of bones, dissolving minerals and proteins, gallium slows down the pace of bone loss [55]. Gallium therefore has a well-established mechanism of action that involves inhibiting this "proton" pump. In order to transport the Ga^{3+} cation more efficiently or selectively, gallium compounds may be created. [55]. It has been recommended to combine gallium with bisphosphonates to increase its oral availability [60]. Well-defined Ga (III) complexes are produced by chelating agents such pyridoxal isonicotinoyl hydrazone and 8-hydroxyquinoline thiosemicarbazone [56].

There is increasing attention in the role of vanadium in biological systems in general [57]. Studies exploring using vanadium compounds as insulin analogues in medicine was sparked by the discovery that vanadate ions have characteristics similar to insulin [57]. Diabetes mellitus results from inadequate glucose metabolism, which can be caused by either an absence of endogenous insulin or insulin resistance. Insulin, a tiny protein, is not orally active, hence the promise for insulin-mimetic chemicals as medications resides in their oral delivery [58]. When taken orally, the vanadate (V) ion is effective. In the transition state structure of protein tyrosine phosphatases, vanadate is thought to replace phosphate as a vanadium mechanism (PTP) [58]. Both V^{4+} and V^{5+} coordination compounds have been investigated as insulin analogues [58]. Important considerations for drug design in terms of delivering the physiologically active vanadate include ligand displacement and lability control [59]. Up to now, clinical trials have mostly explored sodium orthovanadate and the bis(maltolato)oxovanadium (IV) compound (BMOV) [59].

It is generally known that vanadium complexes have medicinal uses [60]. Recent research has shown the effectiveness of oxovanadium (V_2O_3) compounds of thiourea and vanadium substituted polyoxotungstates against HIV-infected immortalized T-cells [61]. However, it had repeatedly been seen that vanadium (IV) was unstable under physiological conditions [62]. A Porphyrin ring was then introduced to stabilize the VO^{2+} ion [62]. In solutions with GSH, the oxovanadium porphyrin rings remain stable. They were tested for their capacity to prevent HIV-1 (BaL) multiplication in Hut/CCR5 cells [61]. The water-soluble analog with aminosulfonyl functional groups displayed the maximum effectiveness at the 5 μ M level with over 97 percent inhibition, though all the complexes exhibited anti-viral activity in comparison to the vehicle control [61].

Antibacterial agents made of silver and mercury salts have been used for a very long time [63]. For the treatment of serious burns, silver sulfadiazene is used; the polymeric substance releases the antimicrobial Ag^+ ion over time [63]. Many nations still employ silver nitrate to protect newborn children's eyes from ophthalmic illness. The reason for the specificity of bacteriocidal effect is provided by the mechanism of action of Ag and Hg, which involves the gradual release of the active metal ion and suppression of thiol function in bacterial cell walls [63].

2.3 Rhenium (Re) as an anti-cancer agent

Rhenium is a heavy transition metal with a wide range of oxidation states (-1 to +7) providing it with unique properties [64]. Re deserves special consideration because of its large range of oxidation states, not only because it makes it possible to create complexes with a wide range of structural variety, but also because of its capacity to influence the redox status of cancer cells [65]. Pt, in contrast, only possesses the common oxidation states of +2 and +4. Only Mn has the same wide range of oxidation states as Re. Although being an essential metal, Mn carries a significantly higher risk of toxicity than Re, especially when it comes to the brain [65]. To circumvent the therapeutic limits of platinum medicines, many rhenium (Re) compounds with potential anticancer characteristics have been created recently [66]. The most typical Re compounds are Re(I) tricarbonyl complexes, however other Re compounds with higher oxidation states, hetero-metallic complexes, and Re-loaded self-assembling devices have also been studied. All of these chemicals are still in the preclinical study stage, however many of them show potential cytotoxic and phototoxic characteristics against malignant cells [66]. The rhenium compounds have several potential modes of action, such as phototoxicity, DNA binding, mitochondrial effects, oxidative stress modulation, or enzyme inhibition.

It has been demonstrated that rhenium can produce DNA adducts with one or two guanine bases, similar to those produced by cisplatin, but with an octahedral structure as opposed to a square-planar one using the drug Re-diselenoether [67]. Malignant cells have also been shown to take up Re after being exposed to the Re-diselenoether complex [67]. One rhenium atom and two selenium atoms are combined to form the water-soluble metal complex rhenium (I)-diselenoether (Re-diselenoether). The rhenium(I)-diselenoether, which has a core Re atom coupled with two Se atoms, was created with the intention of combining the antiproliferative capabilities of Re with the distinctive apoptotic modulator properties of Selenium (Figure 8.) [67].

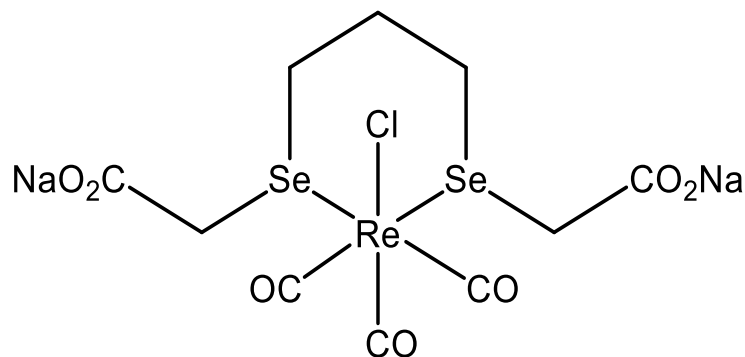


Figure 8: A medication called Rhenium(I)-diselenoether (Re-diSe) was created to treat metastatic triple-negative breast cancer[67].

Only lately has rhenium's potential anticancer uses been investigated [65]. These investigations have shown a particular interest in rhenium(I) tricarbonyl compounds. These substances, which are mostly used as CO₂ reduction catalysts, have several properties that make them suitable for use as anticancer agents [65]. For instance, they can attach to DNA nucleobases covalently, just like platinum-based medications [68]. Additionally, the ligand substitution kinetics for rhenium(I) tricarbonyl complexes are on par with those for platinum-based medications in terms of magnitude [68]. The rich spectroscopic features of these compounds, which may be used for imaging, provide a major advantage over conventional platinum anticancer medicines [69]. These rhenium(I) tricarbonyl complexes have been effectively exploited in cellular fluorescence microscopy imaging applications according to their triplet-based luminous emission, and vibrational microscopy imaging is possible thanks to their unique C≡O stretching frequency [69].

2.5 Heterocyclic ligands

Heterocycles are crucial structural components of many of the anti-cancer drugs currently on the market [70]. In actuality, heterocyclic rings were present in approximately two-thirds of the novel molecular anti-cancer therapies that the Food and Drug Administration (FDA) approved between 2010 and 2015 [70].

Because they are so prevalent in nature and have developed interactions with a wide variety of cellular processes and systems, they are frequently used in the design of anti-cancer drugs [71]. Because of their versatility, heterocycle-based medicines have the

potential to affect a variety of cellular processes and metabolic pathways involved in cancer pathogenesis [71].

Heterocycles, which are classified as cyclic compounds with at least one other element (such as nitrogen, oxygen, or sulfur) and ring member atoms of carbon, are prevalent in biology and can be found in a variety of forms, including amino acids, proteins, and co-factors for enzymes [72]. They are used at almost all phases of the vast biochemical processes required to support life and play a crucial part in the metabolism of all living things [72].

Heterocycles can interact with other molecules in a wide range of ways, including hydrophobic forces, pi-stacking interactions, metal coordination bonds, hydrogen bond donor/acceptor capabilities [73]. Heterocycles also occur in a variety of forms and sizes due to their large range of ring diameters and structural permutations, which enables them to fit the equally diverse structural range of enzyme binding pockets [73].

2.5.1 Sulfur-based heterocycles

In addition to being a crucial component of many vitamin cofactors, sugars, and nucleic acids, sulfur also plays a significant role in controlling translation by sulfurating transfer RNA [73]. Considering the significance of sulfur in biological systems, the creation of anti-cancer medications has given great attention to heterocycles containing sulfur, just as their counterparts based on oxygen and nitrogen [74]. In a recent screening investigation, for instance, thiophene derivatives were tested for their ability to suppress the proliferation of human breast cancer cells. Several of the compounds showed encouraging inhibitory effects. According to the researchers, their findings could serve as a foundation for the development of tyrosine kinase inhibitors in the future that have fewer adverse effects [74].

2.5.2 Nitrogen-based heterocycles

Close to 75% of the heterocyclic anticancer treatments permitted by the FDA between 2010 and 2015 contained nitrogen-based heterocycles, demonstrating the significance of these molecules in the creation of anti-cancer drugs [75]. Indoles are among the most significant nitrogen heterocycles, and studies have shown that they can cause cell death in a few cancer cell types [75].

It has been established in recent years that indole and its derivatives can alter several cellular routes linked to the development of cancer [76]. These comprise the capacity to induce cellular oxidative stress and cell death, as well as the inhibition of cell signaling, typical cell cycle progression, tumor vascularization, and DNA repair [77]. Vincristine and vinblastine are two of the most significant early indole-based anticancer drugs. Both have clinical relevance today and have been known to impede tubulin polymerization since the early to mid-1960s [78].

Finding new and more potent analogs of compounds with already-known activity is a crucial component of pharmaceutical research [79]. Transition metal ions coupled to nitrogen-containing ligands, such as 1,10-phenanthroline and/or 2,2'-bipyridine, have been discovered to have significant applicability in chemotherapy [80]. With numerous first-row transition metals, the ligands (1,10-phenanthroline and 2,2'-bipyridine) form highly stable chelates [80]. The nitrogen atoms of 1,10-phenanthroline (phen), a stiff, planar, hydrophobic, heteroaromatic molecule, are well positioned to work together in cation binding [81].

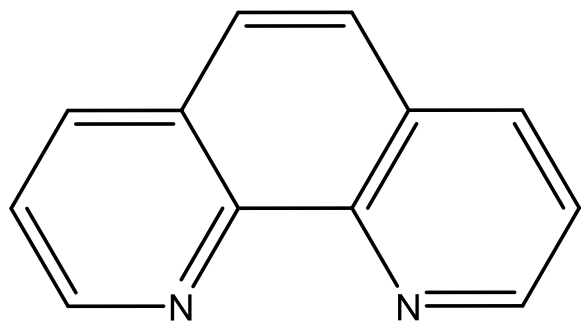


Figure 9: 1,10 phenanthroline [81].

There are diazaphenanthrene analogs called phenanthrolines (phens) which are found in sterols, sex hormones, cardiac glycosides, bile acids, and morphine alkaloids [82]. Phens and their derivatives represent an important class of organic molecules among nitrogen heterocycles that have drawn significant interest from both synthetic and medicinal chemists attributed to the presence of the phen ring system as a structural pattern in quite a few naturally occurring biologically significant products, particularly alkaloids [82].

Phen derivatives exhibit a diverse spectrum of pharmacological and physiological actions; as a result, the synthesis of novel molecules containing this core is currently a fascinating research topic [83]. In addition to the growing interest in the synthesis of new phen derivatives to produce biologically active molecules, 1,10 phenanthroline (o-phen) deserves special consideration, especially as a heterocyclic moiety, because the arrangement of the atoms in its skeleton is ideal for chelating a variety of metal cations, including lanthanide ions [83].

Owing to their diverse structural and chemical properties, including as rigidity, planarity, aromaticity, basicity, and chelating capacity, o-phen has drawn particular interest from researchers [84]. They are also useful as an electron transfer reagent and in the synthesis of photoactive compounds [84]. O-Phens have been utilized as significant heterocyclic ligands for numerous metal complexes that are crucial to numerous vital technological and therapeutic applications [85]. For instance, because of their capacity to attach to or interact with the DNA biomacromolecule, chemical nucleases and therapeutic agents [86]. Research into the interactions between metal complexes made from o-phen as well as derivatives of modified o-phen moieties and DNA is of great interest due to the strong selectivity that o-phen compounds have demonstrated at the intercalation sites of the DNA molecule [86].

For the treatment of cancer patients, non-platinum organometallic compounds have not yet received approval, but Re compounds may offer interesting clinical options. Numerous Re-drugs have been used to demonstrate the inhibitory effects on cancer cells, however few of these chemicals have been examined in tumor-bearing animals in experimental models. Further testing is required to ascertain their mode of action under different conditions. Ligand design is an essential part of the research and development of these metal complexes as the ligand of choice has significant implications on the efficacy, lipophilicity, toxicity and the mode of action of the metal complex.

CHAPTER 3

3.0 METHODOLOGY AND EXPERIMENTAL SECTION

3.1 CHEMICALS

The chemicals used were sourced from different companies and were of high purity without need for further purification, with xylene being used as the solvent for all reactions. A similar synthesis protocol adapted from Haddad et al was used to synthesize all the complexes with variations in temperature and mole ratio to obtain the desired compound.

Table 1: List of chemicals used for the experiments.

Chemical Name	Purity	Supplier
Methanol	98%	Skylabs
Ethanol	98%	Skylabs
Xylene	98%	Skylabs
Dichloromethane	95%	Skylabs
Toluene	98%	Skylabs
Acetonitrile	95%	Skylabs
1,10 phenanthroline	98%	Rochelle chemicals
Pentacarbonylchlororhenium (I) 98%	98%	Sigma-Aldrich
2-(Chloromethyl)pyridine hydrochloride 98%	98%	Sigma-Aldrich
3-(Chloromethyl)pyridine hydrochloride 98%	98%	Sigma-Aldrich
4-(Chloromethyl)pyridine hydrochloride 98%	98%	Sigma-Aldrich
Dimethylsulfoxide	98%	Skylabs
Dimethyl formamide	98%	Skylabs

3.2 INSTRUMENTS

3.2.1 NMR Spectroscopy

A physicochemical method called nuclear magnetic resonance (NMR) spectroscopy is used to identify the structural characteristics of molecules. It is based on how atomic nuclei react to externally applied radiofrequency radiation [87]. There is a net energy exchange during this interaction, and the nuclear spin of the atomic nuclei changes as a

result [87]. The nuclear spin is defined by the quantum value (I), which varies depending on the isotope under consideration. Atomic nuclei with $I \neq 0$ can only be found by NMR spectroscopy (NMR-active nuclei, such as ^1H , ^2H , ^{13}C and ^{15}N) [88]. These NMR-active magnetic nuclei, which behave like microscopic magnets, can synchronize with the magnetic fields around them (a process called magnetization) [88]. The constant that characterizes the force of the tiny magnets is called the magnetogyric ratio (γ), and its value varies depending on the isotope [89].

Chemical shift refers to a nuclear spin's Larmor frequency with respect to its chemical surroundings. The chemical shift of substances is typically measured using the standard compound tetramethylsilane [TMS; $(\text{CH}_3)_4\text{Si}$]: $\delta_{\text{TMS}} = 0 \text{ ppm}$ [90]. In other terms, the ^1H or resonance of TMS is used to detect the frequencies of molecules in a sample's ^1H nucleus [90]. Understanding the chemical shift trend is crucial for interpreting NMR data. The closeness of unsaturated groups ($\text{C}=\text{C}$, $\text{C}=\text{O}$, aromatic) and electronegative atoms (O , N , halogen) has an effect on the proton NMR chemical shift [90].

NMR spectra were recorded at the NMR Laboratory Centre at the University of Limpopo, South Africa. The ^1H NMR spectra were run on Bruker AC 400 MHz Spectrophotometer. TMS was used as the internal standard. CDCl_3 , acetone- d_6 or $\text{DMSO}-\text{d}_6$ were used as solvents, and chemical shifts (δ) were reported in parts per million (ppm) downfield from TMS with the solvent resonance as the internal standard.

3.2.2 ESI-MS

After analyte molecules are converted to ions, mass spectrometry is a method of analysis that can offer both qualitative (information about structure) and quantitative (information about molecular mass or concentration) data [91]. The mass spectrometer's ionization source receives the target molecules first, where they are subsequently ionized to acquire positive or negative charges [91]. The ions then travel through the mass analyser and arrive at different parts of the detector according to their mass/charge (m/z) ratio [91]. A computer system generates and stores useful signals after the ions contacts the detector. The signals are graphically represented by a mass spectrum on the computer, which shows their relative abundance in relation to their m/z ratio [91].

In order to help ions move from the solution into the gaseous phase and then undergo mass spectrometric measurement, ESI employs electrical energy [91]. Thus, ESI-MS can analyze ionic species in solution with higher sensitivity [92]. By protonation or cationization, such as metal cationization, neutral substances can also be changed from their non-ionic state to an ionic state in solution or in gaseous phase [92]. ESI/MS is one of the most effective analytical techniques, although its use for the detection of some low polar rhenium complexes is restricted [93]. Ionization of these molecules is challenging due to the absence of acidic or basic groups in the structure. By adding groups that can undergo protonation or deprotonation, the analyte can be chemically changed to boost the effectiveness of ionization [93].

The acquisition parameters for the data was acquired using Sciex Qtrap 6500+ mass spectrometer, using electron spray ionisation mode. Some compounds were analysed using positive ESI others Negative ESI. The instrument was at the National Veterinary Laboratory in Sebele, Gaborone.

3.2.3 Single Crystal X-Ray Diffraction

Atomic-scale X-ray Diffraction is a non-destructive analytical technique that provides exact information on the intrinsic crystal lattice, including unit cell dimensions, bond lengths, bond angles, and site ordering data [94]. When X-rays interact with the crystal, they diffract [94]. To create a 3D representation of the electron density, which reflects the average positions of the atoms in the crystal, the angles and intensities of these diffracted X-rays will be measured [94]. Single-crystal refinement, in which the data produced by the X-ray examination is interpreted and refined to determine the crystal structure, is directly related. The samples can include proteins, minerals, metals, vitamins, and medicines [95].

A Bruker APEX II CCD diffractometer was used to analyse suitable crystals. The instrument was at the University of Botswana in the Chemistry department. The crystal was kept at 294.0 K during data collection. Using Olex2 the structure was solved with the SHELXT structure solution program using Intrinsic Phasing and refined with the SHELXL refinement package using Least Squares minimisation.

3.2.4 FT-IR Vibrational spectroscopy

The electromagnetic spectrum's IR region is where IR spectroscopy measures the amount of light that a substance absorbs [96]. A molecule must contain a bond inside its structure that can display a "dipole moment," which indicates that electrons inside a bond are not equally distributed [96]. Because electrons in a specific bond, such as the O-H bond, are not uniformly distributed, the oxygen is more strongly attracted to the electrons than the hydrogen [96]. The O atom in the bond has a tiny negative charge due to the fact that electrons have a negative charge, while the H atom has a slight positive charge [97]. This bond's ability to absorb IR light due to the charge difference creates a peak in the IR spectrum [97]. In functional groups having a dipole moment, certain bonds will absorb in the infrared spectrum at varying wavenumbers [97].

A lamp or heated rod that emits light in the infrared spectrum is the basic component of an IR spectrometer [97]. Then, a detector gathers all IR radiation wavelengths that have passed through the sample and transforms them into wavenumbers [98]. Each chemically diverse molecule will exhibit a unique absorption pattern depending on the quantity and variety of bonds it contains as well as the presence of various functional groups [98].

A Nicolet iS50 FT-IR spectrometer was used for identification of functional groups of interest in the 4000-500 cm^{-1} region. The FT-IR spectrometer used was at the Botswana Institute for Technology Research and Innovation in Gaborone.

3.2.5 Microplate reader

A Thermo Scientific Multiskan FC microplate reader was used for measuring absorbance when conducting the MTT assay. The absorbance was measured between 450 nm and 620 nm. It was located at the University of Botswana in the School of Medicine building.

3.2.6 Microplate Incubator/Shaker

An Awareness Technology Inc. Stat Fax-2200 microplate incubator/shaker was used to shake and incubate the microplate. The microplate was incubated while shaking for 15 minutes at a temperature of 37.5° C. It was located at the University of Botswana in the School of Medicine building.

3.3 SYNTHESIS OF COMPLEXES

All complexes were synthesized using an amended reaction procedure from Haddad et al. [99]. The complexes synthesized exhibited different physical appearances. From their morphology and color to the solvents they dissolved in. Re(phen) and Re₂(phen) displayed similarities in their appearance and solubility. While Re₂-pico, Re₃-pico and Re₄-pico exhibited vastly different physical appearances despite having similar ligands. This implies that the positioning of the methyl chloride (-CH₂Cl-) group on the pyridine ring plays a part in the morphology of the complexes formed. All the complexes were insoluble in water and were all soluble in DMSO. The crystalline complexes were insoluble in dichloromethane, while the powdered complexes were soluble.

Table 2: List of complexes, their physical appearance and solubility in different solvents

Complex	Appearance	Solubility			
		Acetone	DMSO	Dichloromethane	Water
Re(phen)	Fine, yellow powder	Soluble	Soluble	Soluble	Insoluble
Re ₂ -pico	Thick, dark brown oil	Soluble	Soluble	Soluble	Insoluble
Re ₃ -pico	Translucent, brown shard-like crystals	Partially soluble	Soluble	Insoluble	Insoluble
Re ₄ -pico	Translucent, brown cube-like crystals	Insoluble	Soluble	Insoluble	Insoluble
Re ₂ (phen)	Fine, yellow powder	Soluble	Soluble	Soluble	Insoluble

3.3.1 Synthesis of tricarbonylchloro (1,10 phenanthroline) rhenium(I)

Tricarbonylchloro (1,10 phenanthroline) rhenium(I) was synthesized according to the synthetic route shown in Figure 10. Pentacarbonylchlororhenium (I) (0.28mmol) was reacted with 1,10 phenanthroline (0.28 mmol) in a 1:1 ratio. The reaction was carried out in a 50 mL round bottom flask and refluxed for 6 hours at 110°C while stirring in 30 mL of xylene. The mixture was then allowed to cool at room temperature before filtration. The resultant yellow powder was allowed to dry at room temperature before being recrystallized using hot dichloromethane. The excess dichloromethane was evaporated off to allow formation of crystals. Resultant crystals were yellow flaky and very brittle, giving a yield of 60%. The crystals were characterized using FT-IR, SC-XRD, NMR and ESI-MS.

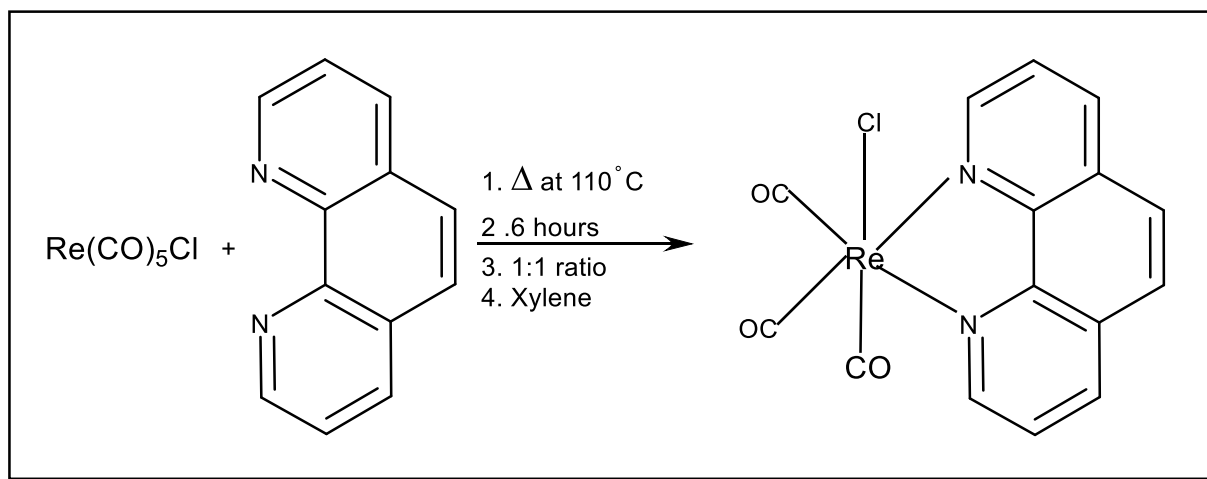


Figure 10: Schematic representation of synthesis of Tricarbonylchloro (1,10 phenanthroline) rhenium(I), $\text{Re}(\text{Phen})$.

3.3.2 Synthesis of hexacarbonyl di-4-chloromethyl pyridine dirhenium (I) dichloride

Hexacarbonyl di-4-chloromethyl pyridine dirhenium (I) dichloride was synthesized according to the synthetic route showed in Figure 11. A 1:2 reaction between pentacarbonylchlororhenium (I) (0.28 mmol) and 4-(Chloromethyl)pyridine hydrochloride (0.55 mmol) took place. In a 50 mL round bottom flask, the reaction was conducted and refluxed for 6 hours at 80°C while being stirred in 30 mL of xylene. After that, the mixture was left to cool at ambient temperature. The reaction solvent was decanted into a 200 mL beaker and put aside after producing an oily brown material. A portion of the solvent had evaporated after a few days, and clear crystals that resembled cubes were produced and the yield was found to be 38%. The crystals were characterized using FT-IR and SC-XRD.

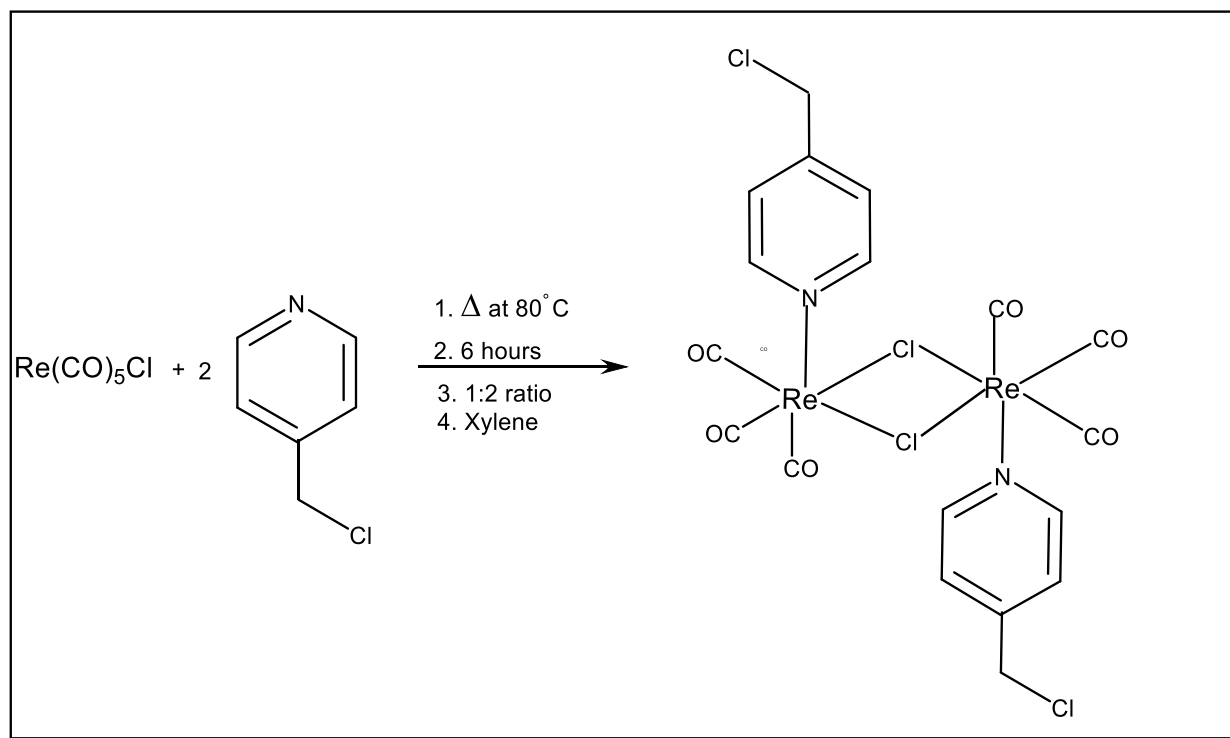


Figure 11: Schematic representation of synthesis of hexacarbonyl di-4-chloromethyl pyridine dirhenium (I) dichloride, Re₂-pico.

3.3.3 Synthesis of tricarbonyl di-2-chloromethyl pyridine rhenium (I) chloride

Tricarbonyl di-2-chloromethyl pyridine rhenium (I) chloride was synthesized according to the reaction scheme in Figure 12. Figure 12 illustrates the reaction between pentacarbonylchlororhenium (I) (0.28 mmol) and 2-(Chloromethyl)pyridine hydrochloride (0.55 mmol) in a 1:2 ratio. A 50 mL round bottom flask was used for the reaction, which was refluxed at 90°C for 6 hours while being stirred in 30 mL of xylene. After that, the mixture was allowed to cool at room temperature. The reaction solvent was decanted into a 200 mL beaker and put aside after producing a brown oily liquid. The percentage yield was calculated and found to be 51%. The brown oily liquid was characterized using ESI-MS.

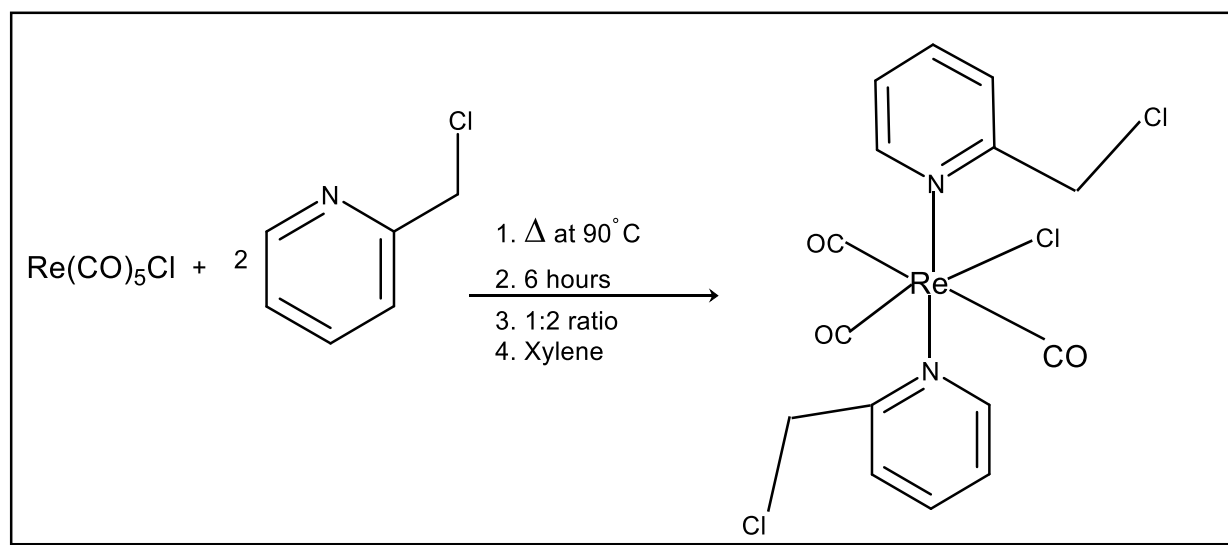


Figure 12: Schematic representation of synthesis of tricarbonyl di-2-chloromethyl pyridine rhenium (I) chloride, Re₂-pico.

3.3.4 Synthesis of tricarbonyl di-3-chloromethyl pyridine rhenium (I) chloride

Tricarbonyl di-3-chloromethyl pyridine rhenium (I) chloride was synthesized according to the reaction scheme in Figure 13. Figure 13 exhibits the reaction between pentacarbonylchlororhenium (I) (0.28 mmol) and 3-(Chloromethyl)pyridine hydrochloride (0.55 mmol) in a 1:2 ratio. A 50 mL round bottom flask was used for the reaction, which was refluxed at 110°C for 6 hours while being stirred in 30 mL of xylene. After that, the mixture was left to cool at room temperature. The reaction solvent was decanted into a 200 mL beaker and left aside after producing a dark green oily material. Greenish translucent shard-like crystals formed in the reaction vessel after a few days and the yield was 48%. The crystals were characterized using SC-XRD and FT-IR.

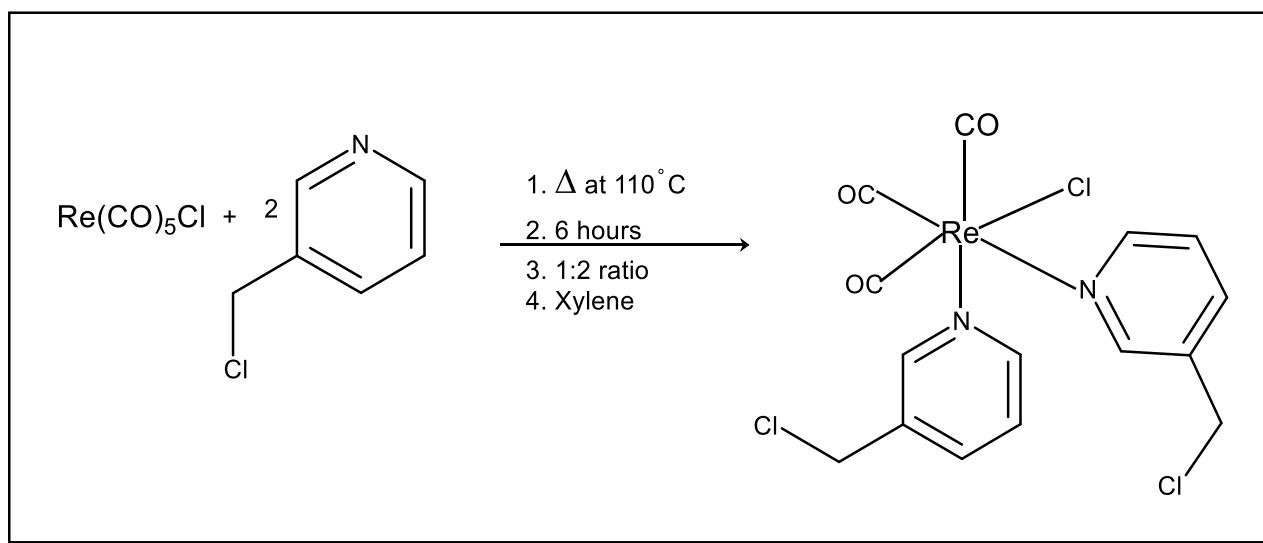


Figure 13: Schematic representation of synthesis of tricarbonyl di-3-chloromethyl pyridine rhenium (I) chloride, $\text{Re}_3\text{-pico}$.

3.3.5 Synthesis of carbonyl-bis(1,10 phenanthroline) rhenium (I) chloride

Carbonyl-bis 1,10 phenanthroline rhenium(I) was synthesized according to the reaction scheme in Figure 14. The scheme demonstrates the reaction between 1,10 phenanthroline (0.55 mmol) and pentacarbonylchlororhenium (I) (0.28 mmol) in a 1:2 ratio. A 50 mL round bottom flask was used for the reaction, which was refluxed at 110°C for 6 hours while being stirred in 30 mL of xylene. After the mixture had cooled to room temperature, filtration was performed. After drying at room temperature, the resulting yellow powder was recrystallized using hot dichloromethane. To enable crystal formation, surplus dichloromethane was evaporated out. Crystals that formed were exceedingly fragile, too small to be analyzed using SC-XRD and yellow in color. It was found to have a yield of 35%. The compound was analyzed using NMR and ESI-MS.

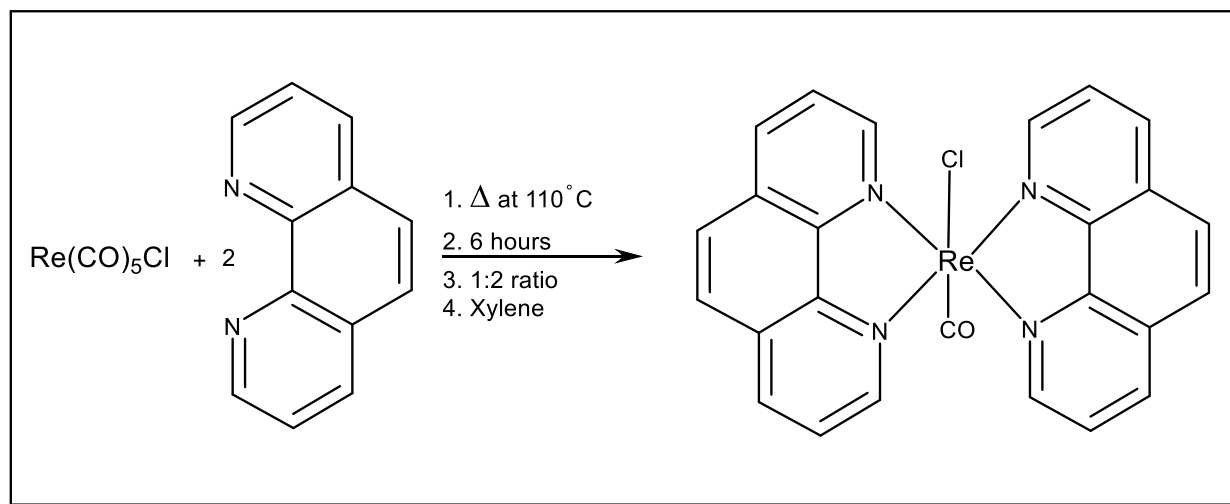


Figure 14: Schematic representation of synthesis of carbonyl-bis 1,10 phenanthroline rhenium (I) chloride, $\text{Re}_2(\text{phen})$.

3.4 BIOLOGICAL STUDIES

3.4.1 MTT Assay

Numerous in vitro tests of a cell population's reaction to outside influences are based on measurements of cell viability and proliferation. Tetrazolium salt reduction is now recognized as a trustworthy method for assessing cell growth. Dehydrogenase enzymes help metabolically active cells reduce the yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) to produce reducing equivalents like NADH and NADPH. By using spectrophotometric techniques, the resulting intracellular purple formazan can be solubilized and measured. The MTT Cell Proliferation Assay calculates the rate of cell division. To speed up sample processing, as few assay steps as possible have been used. In the absence of cells, the MTT Reagent produces low background absorbance values. The linear link between cell number and signal production is established for each kind of cell, enabling precise measurement of changes in the rate of cell proliferation.

3.4.1.1 MTT Assay Methodology

Stock solutions of 2000 μM of the five complexes were prepared in a solvent mixture of acetone and DMSO mixed at a ratio of 6:4. Appropriate concentrations of the complexes were prepared by doing serial dilutions in the growth media. Solvent controls were also prepared in the same manner as the preparation of the compounds.

For MTT assay, cells were cultured in a clear, flat-bottom 96-well plate at a density of 5×10^3 cells per well in 100 μL of growth medium with 10% FBS. A set of wells with media only and no cells served as background controls. The cells were allowed to attach for 24 hours before the media was changed and cells were incubated for a further 24 hours. Cells were treated with serially diluted compounds with concentrations ranging from 6.25 to 200 μM and incubated at 37.5°C for 48 hours. Control cells were treated with solvent (DMSO: acetone) at concentrations matching those of the ligands and the complexes. All the controls were also incubated at 37.5°C for 48 hours. After incubating the compounds and all the controls, the old media was replaced with fresh media. To determine the cytotoxicity of the compounds and all the controls, the MTT Assay was used as per manufacturer instructions (BOSTER-AR1156). Using a multi-channel micropipette, 10

μL of MTT labelling reagent was added to each well and incubated at 37°C for 4 hours in a humidified 5% CO₂ incubator. Following this 4-hour incubation period, yellowish formazan crystals were formed. To dissolve these formazan crystals, 50 μL of DMSO was added to each well and incubated with shaking in a plate shaker for 15 minutes at 37.5°C. Optical density was then measured at 450 nm using a 96-well Thermo Scientific Multiskan FC microplate reader. Percentage cell viability was calculated using the following equation.

$$\begin{aligned} & \% \text{ cell viability} \\ &= \frac{\text{Absorbance of treated cells} - \text{Absorbance of background controls}}{\text{Absorbance of matched solvents} - \text{Absorbance of background controls}} \times 100 \end{aligned}$$

Inhibitory concentration (IC₅₀) which is the concentration of a compound that can inhibits 50% of the cell growth was calculated using Prism GraphPad software. Each complex and control were assayed in triplicates and the experiment was repeated twice.

CHAPTER 4

4.0 RESULTS AND DISCUSSION

4.1 CHARACTERISATION

4.1.1 Nuclear Magnetic Resonance

4.1.1.1 *Re(Phen)*

The protons on the aromatic ring are pushed downfield in aromatic molecules. As 1,10 phenanthroline is an aromatic compound its chemical shifts are in a similar region between 7 and 9 ppm as shown in figure 15. Also, due to the symmetrical nature of the compound 4 major peaks from the 8 structurally equivalent hydrogens are observed. Four doublets were observed for each pair of coupled hydrogens and were assigned their peaks as follows: 2, 11 δ = 8.66 ppm, 4, 9 δ = 7.71 ppm, 6, 7 δ = 7.10 ppm and 3, 10 δ = 6.74 ppm. De-shielding effects were observed after the ligand had been coordinated to the rhenium metal center and were found to cause a slight shift to the downfield region.

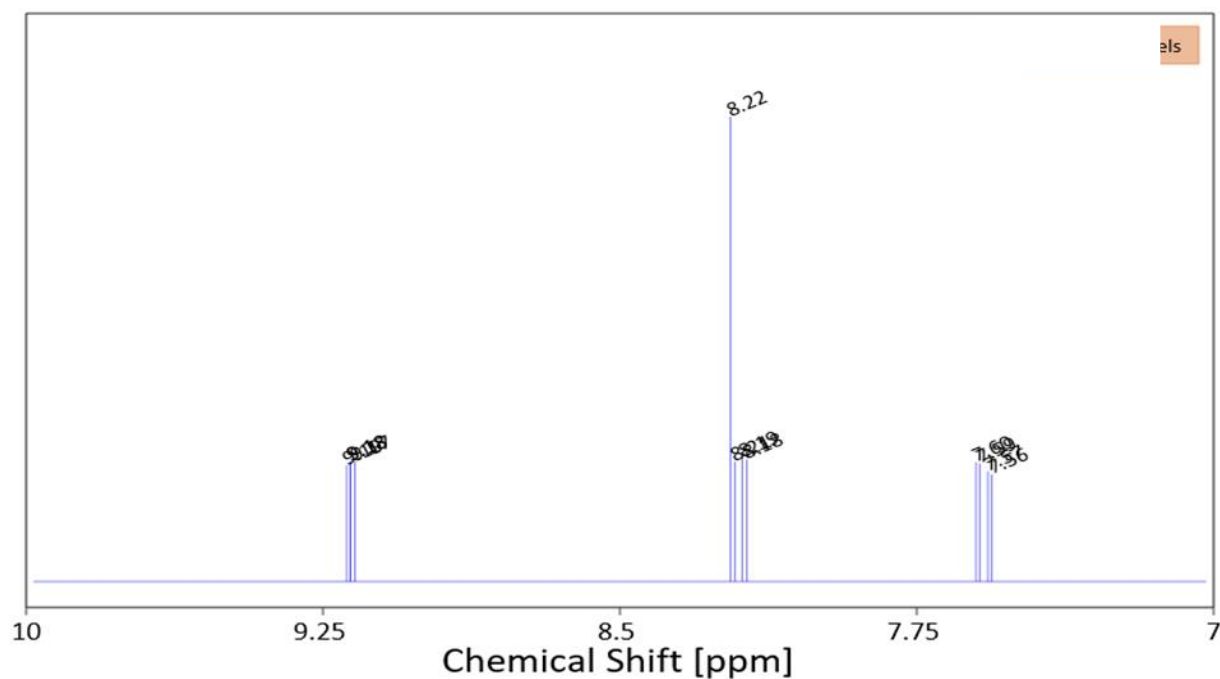


Figure 15: NMR spectrum of 1,10 phenanthroline

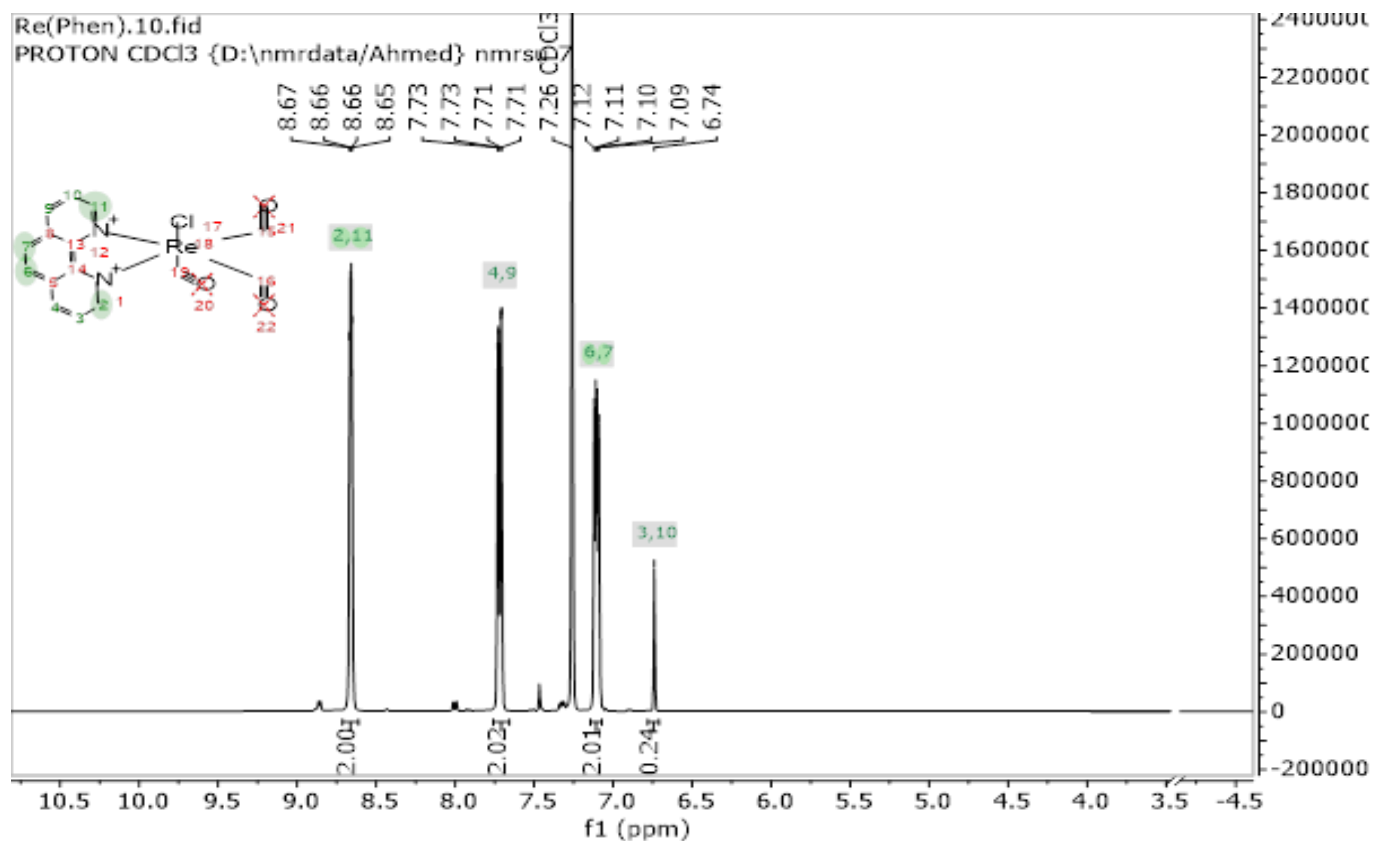


Figure 16: NMR spectra of the complex Re(Phen)

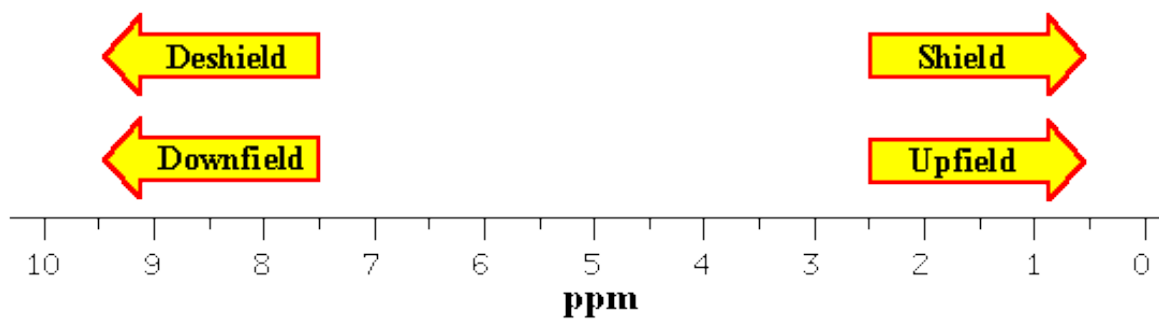


Figure 17: Illustration showing correlation between shielding and position of the signal

4.1.1.2 *Re2(Phen)*

In nuclear magnetic resonance (NMR) spectroscopy, the heavy atoms have a significant impact on the nearby light atoms' NMR shielding constants [100]. Molecules with electronegative atoms tend to attract the electron density to themselves and de-shield the nucleus [100]. Due to the nucleus's deshielding, a decrease in electron density and an increase in chemical shift value follow from the surrounding groups' increased electronegativity [100]. A considerable shift to the downfield region between 13.5 ppm and 11.5 ppm is observed when compared to the region where the ligand 1,10 phenanthroline is expected. This suggests increased deshielding after coordination. One of the reasons could be because the electronegative nature of the nitrogen atom on the ligand is increased as it is directly coordinated to the rhenium metal center. It coordinates to the metal center by donating its lone pair of electrons and it could be that it draws more electrons from the aromatic system away from the hydrogens and more towards itself. Another possible reason for the significant shift downfield is the anisotropic effect of the two bulky aromatic ligands. The property of a molecule known as anisotropy is when one part of the molecule opposes the applied field while the other section strengthens the applied field [101]. Chemical shifts hinge on the configuration of nearby bonds, the π bonds [101]. Aromatics, alkenes, and alkynes are examples of nuclei that exhibit chemical alterations brought on by π bonding. Due the symmetry of the complex all hydrogens were in an identical chemical environment with at least one other hydrogen, so no singlet peaks were observed. Four peaks that are doublets of doublets were expected and observed and upon integration, all 16 hydrogen atoms were successfully accounted for. The peaks detected were labelled and assigned were as follows: (13.25 ppm = 2,11,16,25) , (12.45 ppm = 4,9,18,23) , (11.91 = 6,7,20,21) and (11.77 ppm = 3,10,17,24).

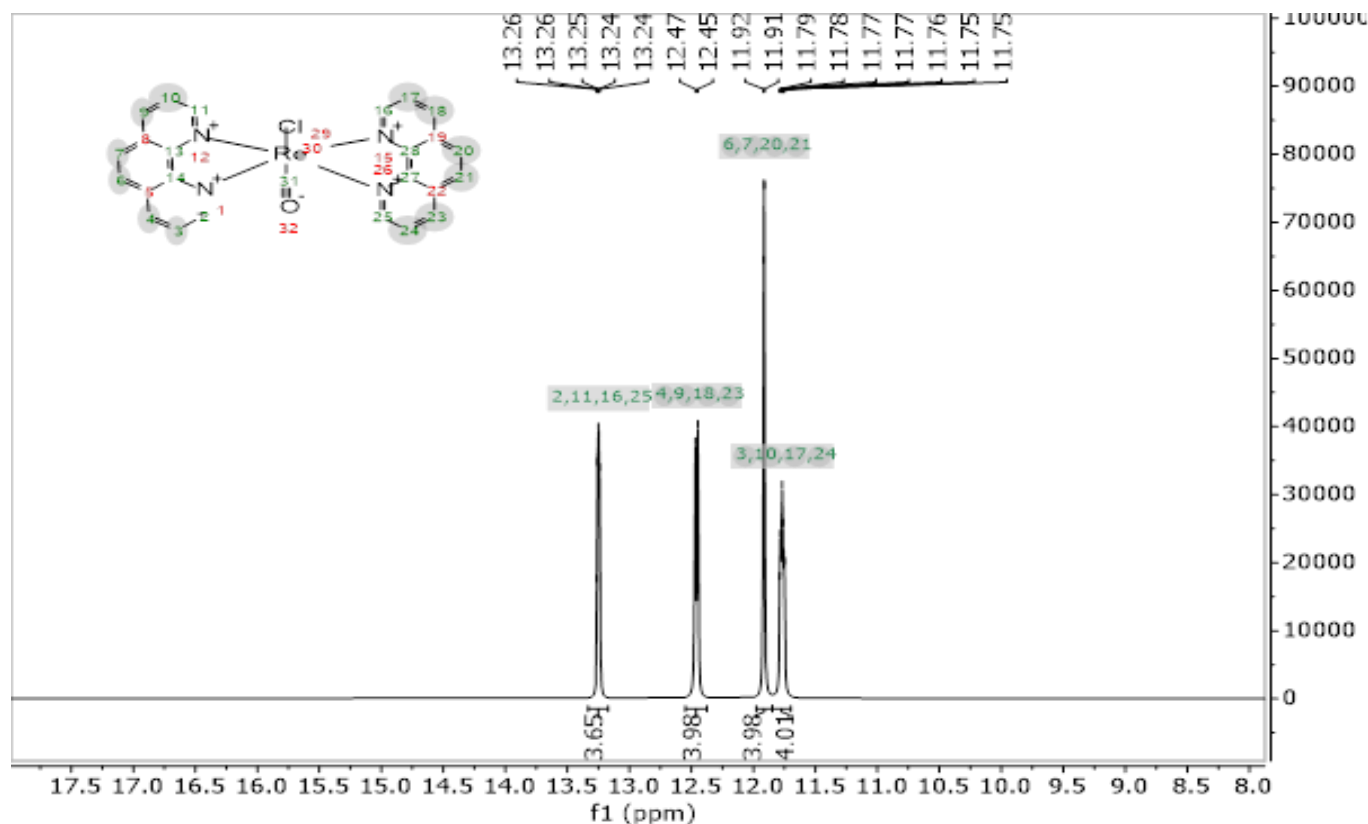


Figure 18: NMR spectra of the complex Re 2(Phen)

4.1.2 SC XRD

4.1.2.1 Re3-pico

Figure 18 shows the proposed structure and the elucidated SC-XRD structure of the synthesized complex Re3-pico. The crystal structure refinement is summarized in Table 3. The Figure 18 shows that two 3-(Chloromethyl) pyridine ligands coordinated to the rhenium metal center via the nitrogen atoms on the pyridine ring donating their lone pair of electrons. This is in line with the proposed structure and gives enough confidence that the complex was successfully synthesized. However, it is also observed that one of the ligands is at the axial position while the other is at the equatorial position, this configuration theoretically may not be the most stable due to steric hindrances between the ligands. In order to attain the optimal inter and intramolecular energy balance, molecular geometry in a crystal structure may be changed by intermolecular interactions due to the flexibility of intramolecular degrees of freedom [102]. Diverse conformers may result in highly different crystal packing configurations because conformation controls the

overall molecule shape and position of functional groups [102]. Molecules can crystallize in conformers other than their lowest energy state; in some circumstances, a higher energy conformer offers the best compromise between inter and intramolecular interactions [103]. This means the conformer obtained was the most suitable under the synthesis conditions, hence it was formed. The geometrical positions of the hydrogen atoms were calculated and placed accordingly. From the crystal structure it was determined that the compound crystallized in monoclinic crystal system. The structure also has a space group $P2_1/c$ with Z equals to 4. Where, Z means there were 4 molecules in the unit cell.

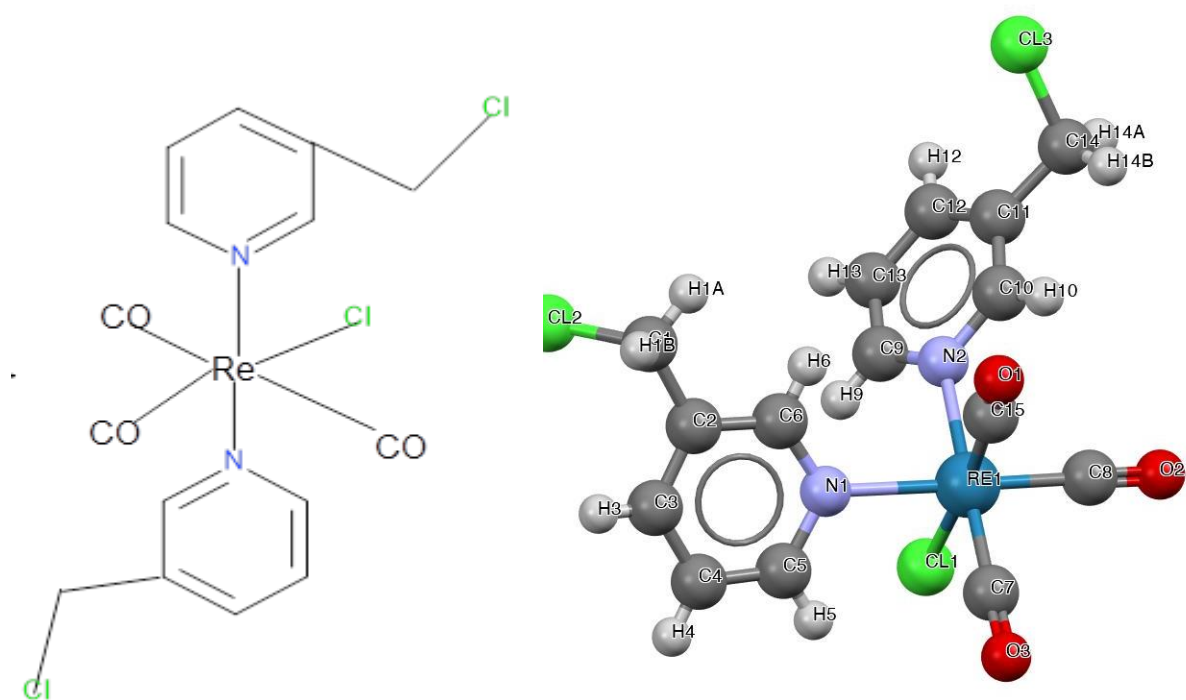


Figure 19: Illustration of the proposed structure of the complex, Tricarbonyl di-3-chloromethyl pyridine rhenium (I) chloride, Re3-pico and the elucidated SC-XRD crystal structure.

Table 3: Crystal structure data of Re3-pico

Empirical formula $C_{15}H_{12}Cl_3N_2O_3Re$
Formula weight 560.82
Temperature/K 297.0

Crystal system monoclinic

Space group $P2_1/c$

a/Å 12.2819(10)

b/Å 11.2612(8)

c/Å 12.9822(11)

α /° 90

β /° 95.198(3)

γ /° 90

Volume/Å³ 1788.2(2)

Z 4

$\rho_{\text{calc}}/\text{cm}^3$ 2.083

μ/mm^{-1} 7.259

F(000) 1064.0

Crystal size/mm³ 0.4 × 0.4 × 0.03

Radiation MoK α (λ = 0.71073)

2 θ range for data collection/° 5.676 to 54.262

Index ranges $-15 \leq h \leq 15$, $-14 \leq k \leq 14$, $-16 \leq l \leq 16$

Reflections collected 29557

Independent reflections 3954 [R_{int} = 0.1278, R_{sigma} = 0.0657]

Data/restraints/parameters 3954/0/212

Goodness-of-fit on F^2 1.090

Final R indexes [$|I| \geq 2\sigma(I)$] R_1 = 0.0669, wR_2 = 0.1793

Final R indexes [all data] R_1 = 0.0844, wR_2 = 0.1948

Largest diff. peak/hole / e Å⁻³ 2.76/-3.72

Table 4: Bond angle data of Re3-pico

Bond	Angle	Bond	Angle
Re1-N1-C6-C2	-172.7(10)	C1-C2-C3-C4	-178.8(14)
Re1-N1-C5-C4	172.2(10)	C6-C2-C1-Cl2	125.9(12)

Re1-N2-C10-C11	171.6(9)	C10-C11-C14-Cl3	-117.8(12)
Re1-N2-C9-C13	-172.5(10)	C14-C11-C12-C13	179.1(14)
N1-C6-C2-C1	177.2(13)		
N2-C10-C11-C14	-179.0(11)		

Table 5: Bond length data of Re3-pico

Bond	Length, Å	Bond	Length, Å
Re1 N1	2.213(10)	O2 C8	1.124(15)
Re1 Cl1	2.468(4)	O3 C7	1.152(17)
Re1 N2	2.221(9)	C6 C2	1.385(16)
Re1 C8	1.912(10)	C2 C1	1.505(17)
Re1 C7	1.919(14)	C2 C3	1.370(17)
Re1 C15	1.975(15)	C3 C4	1.385(19)
N1 C6	1.340(15)	C4 C5	1.367(18)
N1 C5	1.361(14)	C10 C11	1.396(15)
Cl2 C1	1.754(16)	C11 C14	1.503(18)
N2 C10	1.354(14)	C11 C12	1.352(19)
N2 C9	1.353(15)	C12 C13	1.39(2)
Cl3 C14	1.767(13)	C13 C9	1.375(18)
O1 C15	0.956(18)		

4.1.2.2 Re4-pico

Crystals and space groups are separated into many crystal systems according to their point groups [103]. The information in table 6 depicts the complex Re4-pico as being in an orthorhombic crystal structure, which has three axes that all meet at an acute angle. However, the lengths of each axis vary [104]. The complex has a space group *Pbca* with *Z* equals to 4. Figure 19 shows the elucidated crystal structure of the complex. It was revealed that contrary to the proposed structure the crystal is actually dinuclear. A

bridging ligand in coordination chemistry is a ligand that joins two or more atoms, typically metal ions. A polyatomic or atomic ligand could be used [105]. In this case the bridging ligands are the chlorine atoms and connect the two Rhenium metal centers. Due to their π -basicity, the halide ligands are weak field ligands. Chlorine bridges are usually bent and this is supported by the bond angle data obtained and represented in table 7. The bond angle between Re-Cl-Re was found to be 99.03° .

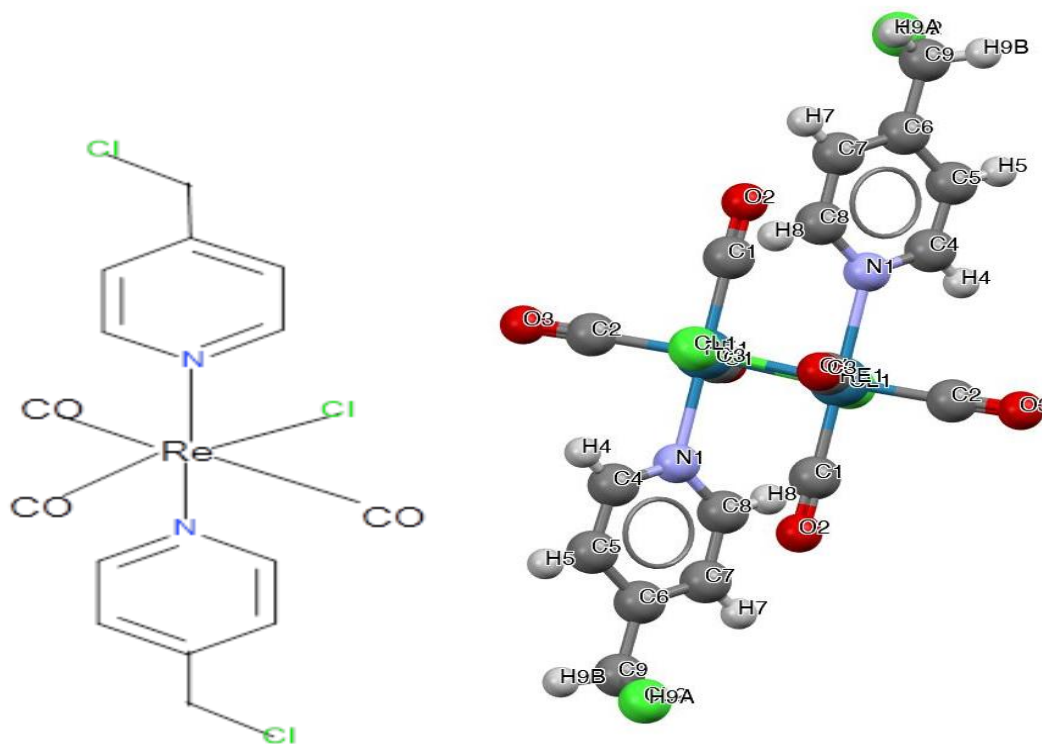


Figure 20: Illustration of the proposed structure of the complex, Tricarbonyl di-4-chloromethyl pyridine rhenium (I) chloride, Re4-pico and the elucidated SC-XRD crystal structure.

Table 6: Crystal structure data of Re4-pico

Empirical formula	$C_{18}H_{12}Cl_4N_2O_6Re_2$
Formula weight	866.50
Temperature/K	294.0
Crystal system	orthorhombic

Space group Pbca

a/Å 10.3715(9)

b/Å 11.2609(9)

c/Å 20.5756(19)

α /° 90

β /° 90

γ /° 90

Volume/Å³ 2403.1(4)

Z 4

$\rho_{\text{calc}}/\text{cm}^3$ 2.395

μ/mm^{-1} 10.547

F(000) 1600.0

Crystal size/mm³ 0.4 × 0.4 × 0.3

Radiation MoK α (λ = 0.71073)

2 θ range for data collection/° 5.696 to 55.112

Index ranges -13 ≤ h ≤ 13, -14 ≤ k ≤ 14, -26 ≤ l ≤ 26

Reflections collected 29386

Independent reflections 2765 [R_{int} = 0.0958, R_{sigma} = 0.0429]

Data/restraints/parameters 2765/0/146

Goodness-of-fit on F^2 1.179

Final R indexes [$|I| \geq 2\sigma(I)$] R = 0.0477, wR2 = 0.1113

Final R indexes [all data] R1 = 0.0586, wR2 = 0.1178

Largest diff. peak/hole / e Å⁻³ 2.37/-1.46

Table 7: Bond angle data of Re4-pico

Bond	Angle	Bond	Angle
Cl1 ¹ Re1 Cl1	80.97(8)	C6 C9 Cl2	111.0(10)
N1 Re1 Cl1 ¹	84.8(2)	Re1 ¹ Cl1 Re1	99.03(8)
N1 Re1 Cl1	85.1(2) C8	N1 Re1	121.9(7)
C3 Re1 Cl1	96.1(3) C8	N1 C4	116.3(9)

C3 Re1 Cl1	176.7(3)	C4 N1 Re1	121.8(7)
C3 Re1 N1	93.6(4)	O1 C3 Re1	177.7(9)
C3 Re1 C1	88.9(5)	O3 C2 Re1	176.1(10)
C2 Re1 Cl1	173.7(3)	N1 C8 C7	122.3(11)
C2 Re1 Cl1 ¹	93.9(3)	C7 C6 C9	122.6(13)
C2 Re1 N1	90.9(4)	C7 C6 C5	116.9(11)
C2 Re1 C3	89.0(4)	C5 C6 C9	120.5(12)
C2 Re1 C1	89.6(4)	O2 C1 Re1	176.9(12)
C1 Re1 Cl1 ¹	92.6(4)	C6 C7 C8	120.5(11)
C1 Re1 Cl1	94.2(4)	N1 C4 C5	123.4(11)
C1 Re1 N1	177.4(4)	C4 C5 C6	120.5(11)

Table 8: Bond length data of Re4-pico

Bond	Length, Å	Bond	Length, Å
Re1 Cl1 ¹	2.503(2)	O1 C3	1.133(12)
Re1 Cl1	2.503(2)	O2 C1	1.122(14)
Re1 N1	2.207(8)	N1 C8	1.344(13)
Re1 C3	1.904(9)	N1 C4	1.347(13)
Re1 C2	1.889(10)	C8 C7	1.399(16)
Re1 C1	1.938(11)	C6 C7	1.369(18)
C9 Cl2	1.747(15)	C6 C5	1.372(18)
C9 C6	1.483(16)	C4 C5	1.370(17)
O3 C2	1.132(13)		

4.1.2.3 *Re(Phen)*

The crystal structure elucidated was in line with the proposed structure and corroborated the that the ligand, 1,10 phenanthroline has coordinated with the metal center via donation of the lone pair of electrons on the nitrogen atoms. The complex possesses a monoclinic crystal system, which is a three-axis crystal system with two of them at right angles to one another and one inclined. Each of the three axes is varied in length [104]. It has a space group C2/m and Z equals to 4 which implies there are 4 molecules in the unit cell. Data collection and refinement details are in table 9. The number of bound electrons determines the bond's length (the bond order). With increasing bond order, the pull between the two atoms becomes stronger and the bond length decreases [106]. In most cases, the length of the connection between two atoms is about equal to the total of their covalent radii [106]. On average, the shortest bond length of all the atoms sharing a bond with the rhenium metal center was between the rhenium metal and the carbon from the carbonyl group and thus the strongest bond. When the t_{2g} orbitals of a metal and π^* of a ligand overlap, a ligand like carbon monoxide can provide the metal its sigma (nonbonding) electrons while also absorbing electron density from the metal [107]. Backbonding is the term used to describe the process wherein the metal receives σ -electron density from the ligand while the ligand receives π -electron density from the metal [107]. As a result, the ligand serves as both a σ -donor and a π -acceptor. In "backbonding," the metal gives π electrons to the ligand's π^* orbital, increasing electron density of the antibonding molecular orbital [107].

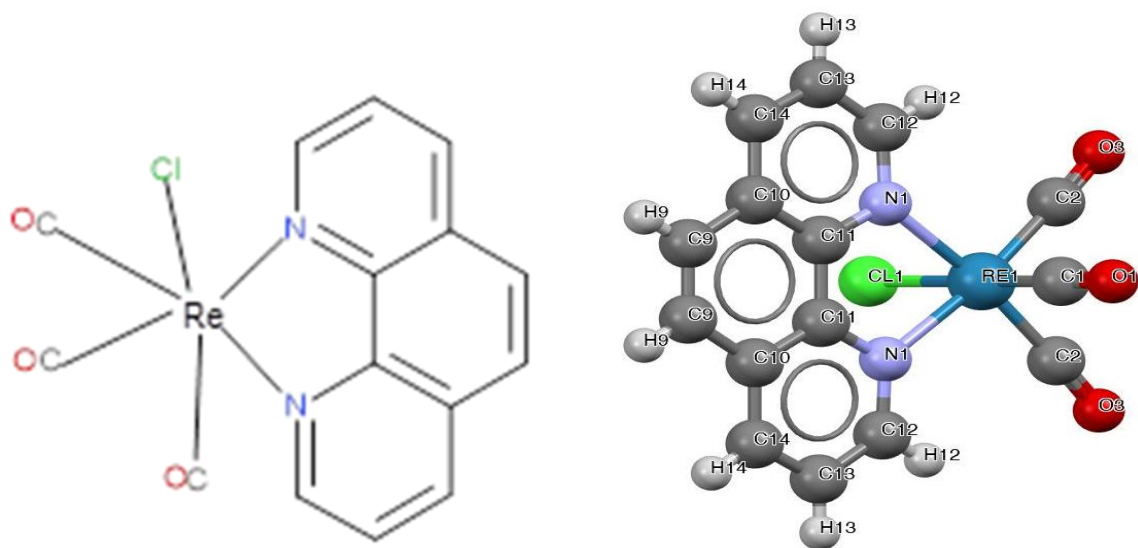


Figure 21: Illustration of the proposed structure of the complex, Tricarbonyl-1,10 phenanthroline rhenium (I) chloride and the elucidated SC-XRD crystal structure.

Table 9: Crystal structure data of Re(Phen)

Empirical formula	C ₁₅ H ₈ ClN ₂ O ₃ Re
Formula weight	485.88
Temperature/K	294.0
Crystal system	monoclinic
Space group	C2/m
a/Å	15.859(4)
b/Å	11.834(3)
c/Å	7.952(2)
α/°	90
β/°	107.042(8)
γ/°	90
Volume/Å³	1426.7(6)
Z	4
ρ_{calc}/cm³	2.262
μ/mm⁻¹	8.717
F(000)	912.0

Crystal size/mm³ 0.3 × 0.2 × 0.1

Radiation MoK α (λ = 0.71073)

2 θ range for data collection/° 6.274 to 41.696

Index ranges -15 ≤ h ≤ 15, -11 ≤ k ≤ 11, -7 ≤ l ≤ 7

Reflections collected 5788

Independent reflections 795 [R_{int} = 0.1482, R_{sigma} = 0.0668]

Data/restraints/parameters 795/0/106

Goodness-of-fit on F^2 1.176

Final R indexes [$|I| \geq 2\sigma(I)$] R_1 = 0.0496, wR_2 = 0.1164

Final R indexes [all data] R_1 = 0.0575, wR_2 = 0.1232

Largest diff. peak/hole / e $\text{\AA}^{-3}$ 2.30/-0.90

Table 10: Bond angle data of Re(Phen)

Bonds	Angles	Bonds	Bonds
C2 Re1 C21	91.5(9)	O3 C2 Re1	176.4(13)
C21 Re1 Cl1	94.2(4)	C11 N1 Re1	113.8(9)
C2 Re1 Cl1	94.2(4)	C12 N1 Re1	127.7(9)
C2 Re1 N11	96.2(5)	C12 N1 C11	118.5(12)
C2 Re1 N1	172.3(5)	O1 C1 Re1	175(2)
C21 Re1 N1	172.3(5)	N1 C11 C111	118.0(8)
N11 Re1 Cl1	96.2(5)	N1 C11 C10	122.4(13)
N1 Re1 Cl1	85.3(3)	C10 C11 C111	119.6(8)
N11 Re1 N1	85.3(3)	N1 C12 C13	122.9(14)
C1 Re1 C2	76.2(6)	C11 C10 C9	118.9(13)
C1 Re1 C21	88.4(6)	C14 C10 C11	117.0(13)
C1 Re1 Cl1	88.4(6)	C14 C10 C9	124.0(14)
C1 Re1 N1	176.3(7)	C14 C13 C12	120.0(15)
C1 Re1 N11	91.8(6)	C91 C9 C10	121.5(9)
		C13 C14 C10	119.1(14)

Table 11: Bond length data of Re(Phen)

Bond	Length, Å	Bond	Length, Å
Re1 C2	1.911(17)	N1 C12	1.308(18)
Re1 C2 ¹	1.911(17)	C11 C11 ¹	1.42(3)
Re1 Cl1	2.477(6)	C11 C10	1.41(2)
Re1 N1 ¹	2.175(11)	C12 C13	1.38(2)
Re1 N1	2.175(11)	C10 C9	1.44(2)
Re1 C1	1.89(3)	C10 C14	1.40(2)
C2 O3	1.135(18)	C13 C14	1.36(2)
O1 C1	1.10(3)	C9 C9 ¹	1.30(3)
N1 C11	1.350(18)		

4.1.3 Electrospray Ionisation Mass Spectrometry

4.1.3.1 Re(Phen)

The signal detected at 485.8 m/z in the spectrum (figure 22) corresponds to the mass of the complex 485.98, calculated based on the proposed structure. This gives good indication that the complex is present and stable in solution. At 450 m/z the signal is in line with the loss of a Chlorine atom which is a labile group $[M - 35]^+$. The adjacent peak of 448 m/z could be attributed to the ^{185}Re isotope. The large peak observed at 478 m/z can be assigned to the loss of a chlorine atom and four carbonyl groups coordinated to the metal center instead of three, $[M - 35 + 28]^+$.

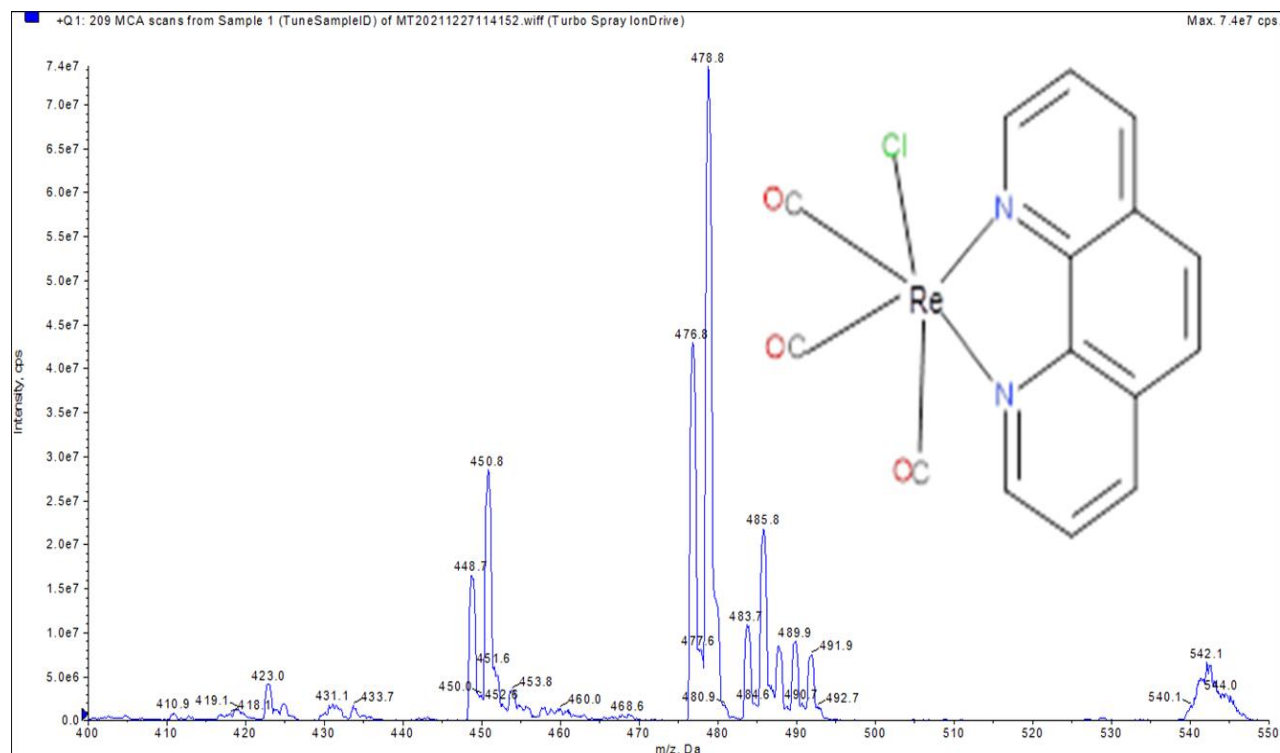


Figure 22: ESI-MS spectrum of Re(Phen)

4.1.3.2 Re₂(phen)

Based on the calculations from the proposed structure the atomic mass of the complex is 610.06 g/mol. Upon running the sample through ESI-MS the data was used to assign the complexes peak at as the peak at 610.8 m/z as shown in the spectrum, figure 23 . The data suggests the loss of a carbonyl group $[M - 28]^+$, to give the peak observed at 582 m/z. The adjacent peak 580 m/z could be attributed to the Rhenium isotope ^{185}Re which is the second most abundant Rhenium isotope, $[M - 28 - 2]^+$.

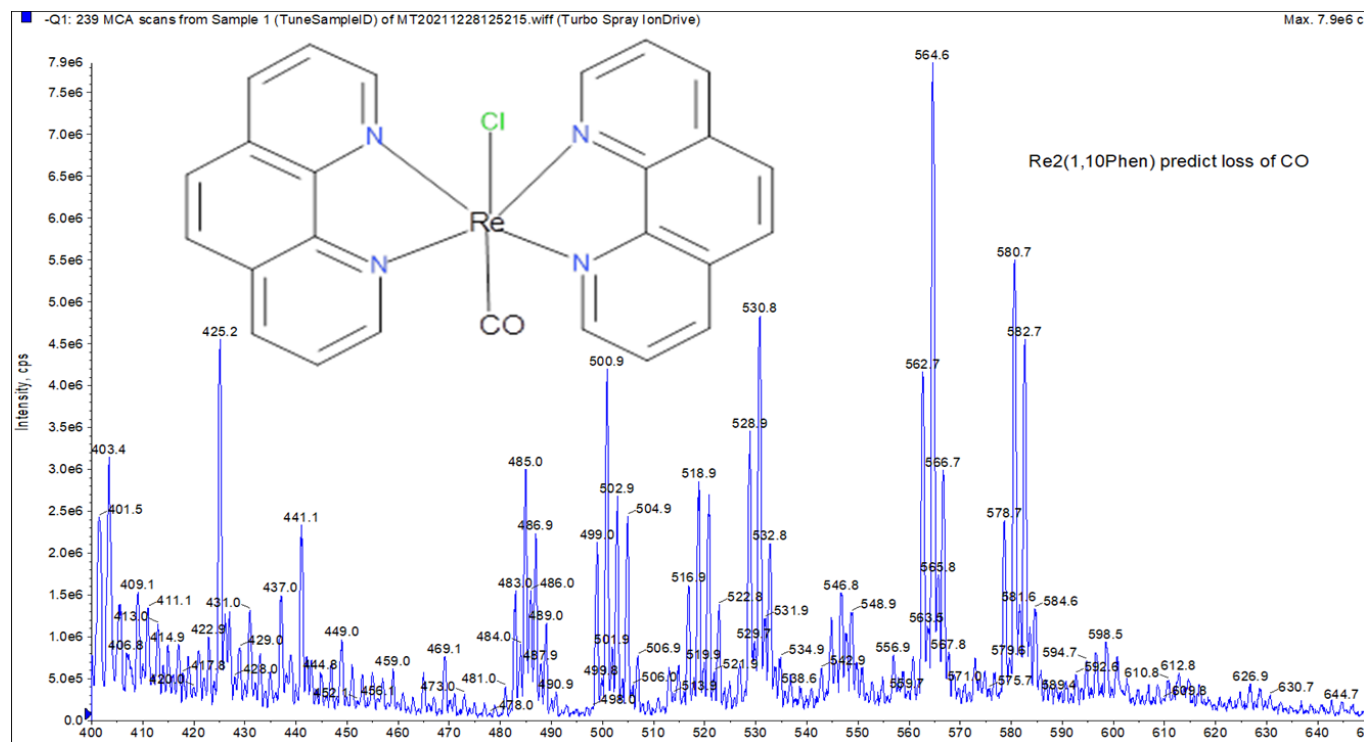


Figure 23: ESI-MS spectrum of $\text{Re}_2(\text{Phen})$

4.1.3.3 $\text{Re}_2\text{-pico}$

The electrospray ionization (ESI) mass spectrum of the complex shows a signal at 534.6 m/z from figure 24 assigned to the molecular ion $[\text{M}]^+$, verifying the stability of the complex in solution. Adducts are generally produced by both the positive and negative modes of electrospray ionization [108]. Protonated molecules are frequently seen, although sodium, potassium, and ammonium adducts are also frequently seen [109]. These species are actualized by the possibility of different cationic species carrying the surface excess charge of ESI nanodroplets [109]. Protons are by far the most prevalent of them, but sodium, ammonium, and potassium cations must also be taken into consideration [110]. These ions are produced by the additions in the mobile phase, solvent impurities, glassware, etc [110]. The peak at 556.6 can be attributed to the sodium adduct $[\text{M} + \text{Na}]^+$.

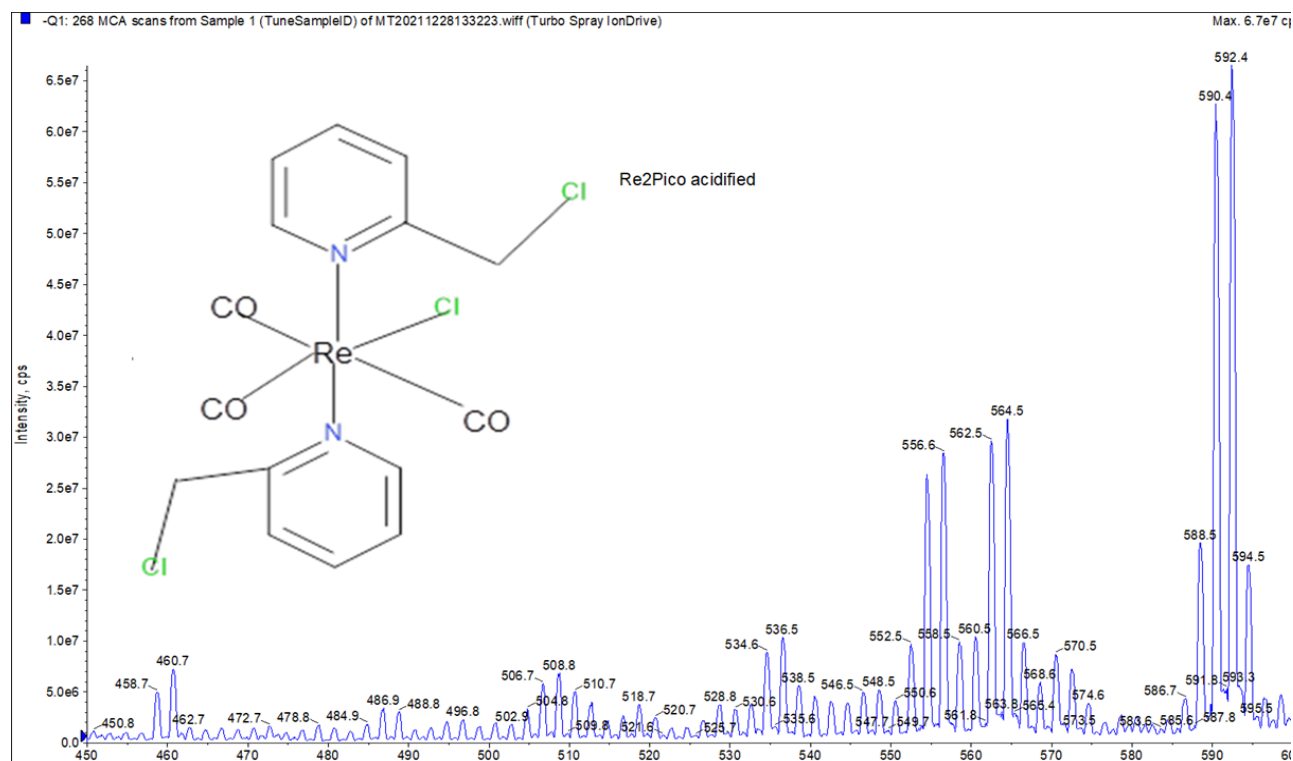


Figure 24: ESI-MS spectrum of Re2-pico

4.1.4 FT-IR Vibrational Spectroscopy

4.1.4.1 Re(Phen)

The IR spectrum, figure 25, of Re(Phen) showed the peak of $\nu(\text{C}=\text{N})$ aromatic stretch was found at 1424.59 cm^{-1} as shown in table 12. This is line with several literature sources that reported the peak to be in a similar region. The other prominent functional group observed was the $\nu(\text{C}=\text{O})$ stretch that characterizes the carbonyl group bound to the rhenium metal center. Because there is a significant change in the dipole in the mode where the $\text{C}=\text{O}$ bond is stretched, the absorption bands associated with that mode are typically very strong [111]. Furthermore, the aromatic $\nu(\text{-C}=\text{C-})$ was observed at 1538.64 cm^{-1} . These vibrational bands accounted for the full molecular structure.

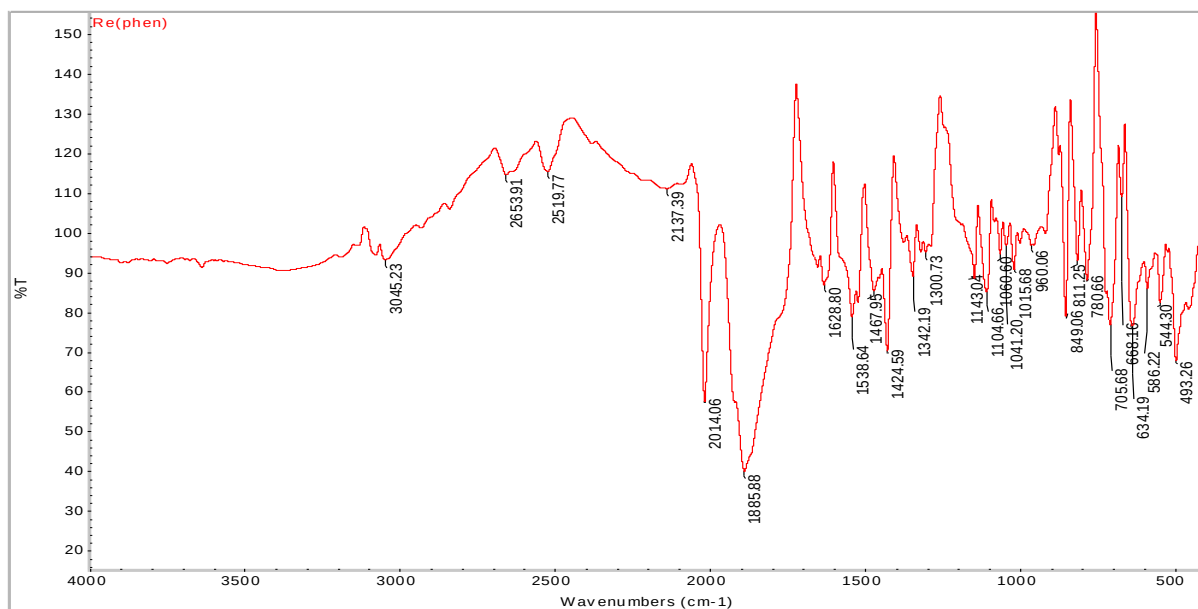


Figure 25: IR spectrum of Re(Phen)

Table 12: IR frequencies of functional groups of interest for Re(Phen)

Functional group	Frequency, ν (cm ⁻¹)
Aromatic C=N	1424.59
Aromatic C-H	1628.80
Aromatic C=C	1538.64
Carbonyl (C=O)	1885.88

4.1.4.2 Re3-pico and Re4-pico

The complexes Re3-pico and Re4-pico used very similar ligands and as such the same functional groups were sought after for both. Characteristic C-H stretching bands were observed at 1632.23 cm⁻¹ and 1632.16 cm⁻¹ for Re3-pico and Re4-pico respectively. The carbonyl bands for the complexes exhibited a strong peak at 1886.97 cm⁻¹ for Re3-pico and 1874.80 cm⁻¹ for Re4-pico. The CO's bonding mode (terminal or bridging) and the metal's electron density are the key determinants of the frequency shift of the carbonyl peaks in the infrared spectrum [112]. The following variables affect the carbonyl bands' strength and number of peaks: The metal ligand complex's shape and number of CO ligands. Ligands trans to a carbonyl can have a major impact on the success of the CO ligand to backbond to the metal [112]. The free metal to CO π -backbonding can

strengthen the M-CO bond if the trans ligand is a π -donor ligand. It is common knowledge that pyridine and amines are weak π -donors. They are worse than π -backbonding ligands, though [112]. As a result, there is no competition for the CO and it is simple to donate. As a result, it naturally lowers the CO IR stretching frequencies for the ligand donation effect in metal carbonyl complexes [112]. The methylene peaks observed at 1468.61 cm^{-1} and 1470.80 cm^{-1} can be attributed to the H-C-H out of plane wagging for Re3-pico and Re4-pico respectively.

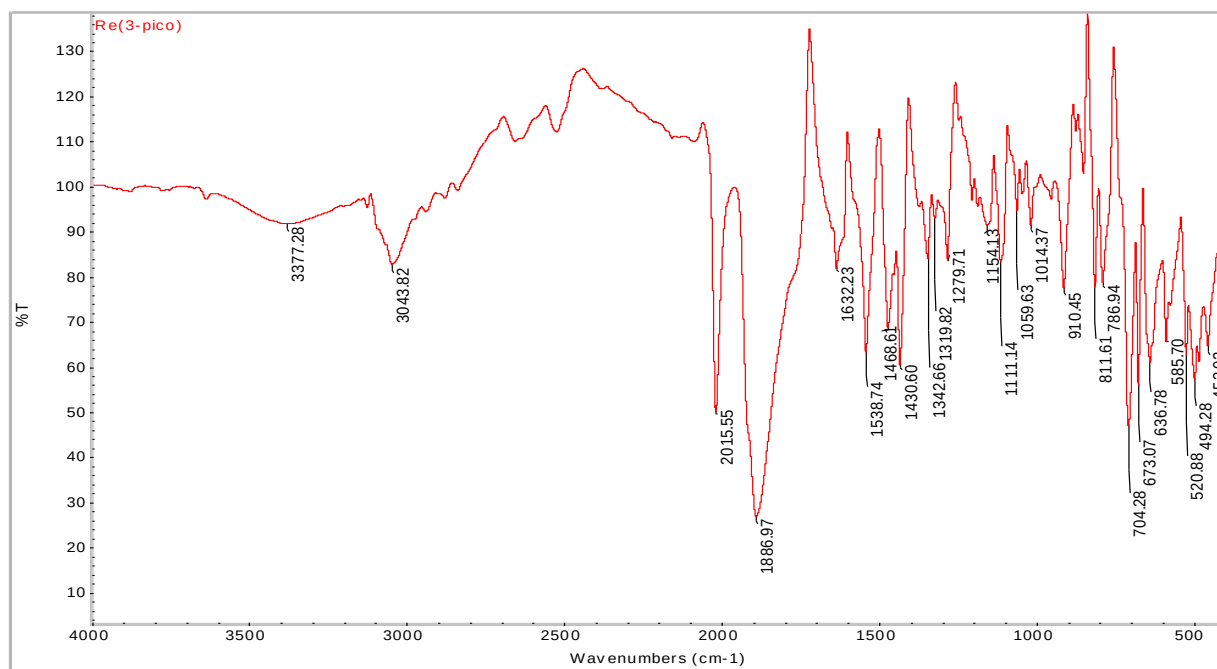


Figure 26: IR spectrum of Re3-pico

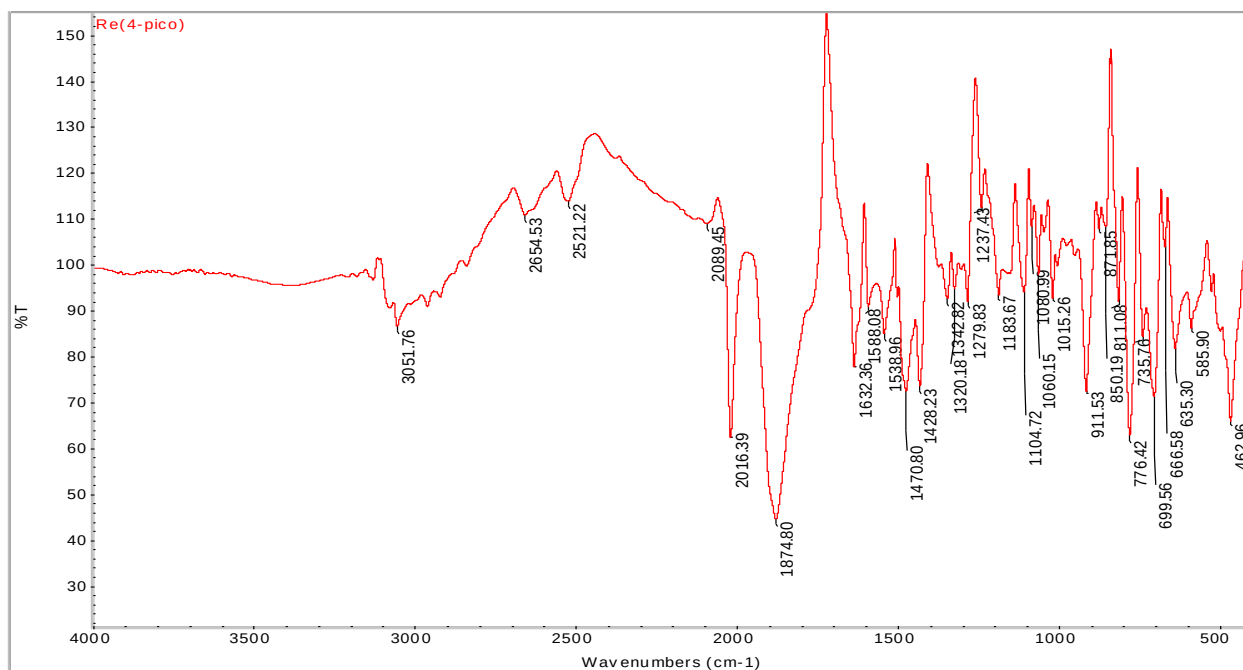


Figure 27: IR spectrum of Re4-pico

Table 13: IR frequencies of functional groups of interest for Re3-pico and Re4-pico

	Re3-pico	Re4-pico
Functional group	Frequency, ν (cm ⁻¹)	Frequency, ν (cm ⁻¹)
Aromatic C=N	1430.60	1428.23
Aromatic C-H	1632.23	1632.16
Aromatic C=C	1538.78	1538.96
Carbonyl (C=O)	1886.97	1874.80
Methylene (-CH ₂ -)	1468.61	1470.80

4.2 CYTOTOXICITY STUDIES

4.2.1 Cytotoxicity results

The graphs shown in Figure 26 show the correlation between the complexes at a given concentration and the subsequent cell viability. Graphs were plotted having already considered the effects of the solvent on cell viability as the solvent matrix used to dissolve the complexes also killed cells. From the data obtained IC_{50} values for each complex were calculated and were tabulated in Table 14. The order starting with the most potent is as follows: **Re2-pico<Re(Phen)<Re3-pico<Re2(Phen)<Re4-pico**. One of the most promising rhenium anti-cancer agents, Re(I)-diselenoether, has previously demonstrated significant cytotoxicity against the MCF-7 breast cancer cell line *in vitro*, with an IC_{50} value of 4.75 μM [113]. It exhibits significantly better cytotoxicity than the compounds synthesised, this is because it has selenium atoms coordinated to the rhenium metal centre. Selenium is a crucial trace element with growth-modulating qualities [114]. Recent studies have revealed selenium compounds to have extraordinary tumour-specific cytotoxicity, suggesting that selenium may have a role in the treatment of cancer [115]. The redox-active selenium compounds with the ability to kill tumour cells are of particular interest [116]. Re-diselenoether is a powerful anticancer drug due to the synergistic antiproliferative activities of Re and the distinct apoptotic modulator capabilities of Se [117].

Table 14: In vitro cytotoxicity assays (IC_{50} values) of the complexes determined using MTT assay after 24-hour treatment of MCF-7 cells.

Complex	IC_{50} (μM)
Re(Phen)	19.51
Re2(Phen)	38.22
Re2-pico	13.10
Re3-pico	32.58
Re4-pico	53.73

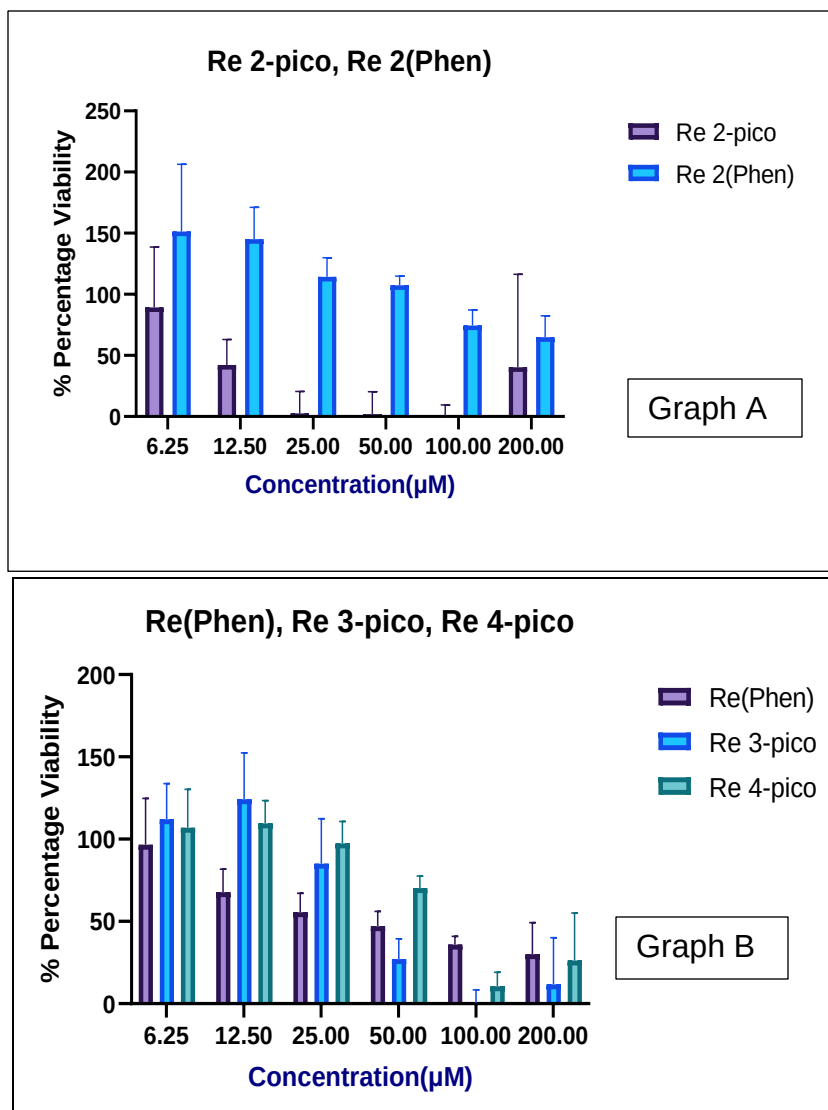


Figure 28: Comparison percentage viability bar graph of the complexes that show the difference in cytotoxicity amongst them at different concentrations.

One of the biggest challenges encountered in terms of getting precise results was that of faulty multi-channel micropipettes and deformed pipette tips due to autoclaving. This made it difficult to get a consistent exact volume of media, complex solution and even seeding cells across all the wells in the 96 well plate. It is possibly for this reason that in Graph A, an increase in cell viability is observed at 200 μM for Re2-pico despite the cell viability being 0 or close to 0 at concentrations of 25, 50 and 100 μM. However, this error was mitigated by using as many pre-sterilized micropipette tips as possible as they did not need to be autoclaved, therefore were not deformed. Re2(Phen) showed a consistent

correlation of decreasing cell viability and increasing concentration. Even though it was less potent than Re2-pico. In Graph B, Re3-pico showed the greatest efficacy of the other two complexes at concentrations higher than 25 μ M, having a cell viability of less than 50%. While Re(Phen) showed consistent correlation between an increase in concentration and decrease in cell viability.

4.2.2 Analysis of Cytotoxic results

Of the numerous rhenium (Re) complexes with potential anticancer characteristics that have been produced recently, Re(I) tricarbonyl complexes are the most prevalent [118]. By attaching to a range of biologically pertinent targeting molecules, Re-based anticancer medications can also be utilized in targeted therapy [118]. Numerous ligands have been identified that affect the pharmacological and toxicological profile, cellular uptake, biodistribution, cytotoxicity, and lipophilicity [118].

4.2.2.1 Number of CO groups

Specific little CO doses cause apoptosis, which kills cancer cells. Metal carbonyl complexes have been used as carriers to transport CO to cells [119]. The ability of the majority of transition-metal carbonyl compounds to emit carbon dioxide (CO) via a wide range of methods involving light, enzymes, or chemicals is a property they share [119]. Of all the complexes synthesized Re2(Phen) had the fewest number of carbonyl groups coordinated to the metal center and showed to be one of the least cytotoxic. Its counterpart Re(Phen) that had 3 carbonyl groups showed much better efficacy. This could be attributed to Re(Phen) being able to release a higher concentration of the carbonyl group into the cells to induce apoptosis [120]. Other complexes, except Re4-pico, containing more than one carbonyl group also showed better efficacy than Re2(Phen).

4.2.2.2 Re DNA Binding (Ligand lability)

It is well known that Re(I) compounds bind to DNA bases and create single- or double-strand adducts. Re may bind to adenine via the sites N1, N6, or to guanine via the location N7 [121]. In contrast to cisplatin, which forms irreversible square-planar Pt adducts, reversible binding of Re agents to one or two bases results in the production of less stable octahedral-Re adducts, which may be crucial to their anticancer properties [121]. Re(CO)₃ complexes can substitute their labile ligand to bind covalently to guanine nucleobases

[122]. This could be the reason the complex with the poorest efficacy is Re4-pico, which is dinuclear with the labile group chlorine acting as a bridging ligand. The configuration may not allow for an easy substitution of the labile group and formation of DNA adducts at the binding site hence hindering the cytotoxic mode of action of the complex.

4.2.2.3 Localization and Lipophilicity

The absorption and metabolism of the target medication are determined by lipophilicity, which is an important consideration in drug development [123]. This is necessary for medication molecules to get through the lipid layer that covers the majority of cellular membranes. It follows that in order to have effective absorption, medication molecules must be lipophilic [123]. However, very high lipophilicity can have negative effects in terms of drug uptake and effectiveness [124]. Very high lipophilicity has a number of drawbacks, including increasing permeability through a variety of biological membranes, including those that aren't the intended target [125]. Due to its exceptionally high lipophilicity, a chemical may adhere to the cell membrane excessively strongly and prevent access to the aqueous portion of the cell [126]. Adding halogen substituents (Cl, Br or I) increases the lipophilicity of molecules: the element becomes larger, more polarized and the London dispersion forces are increased accordingly [127]. As these forces are essential for lipophilic substances to interact within themselves and with others [127]. The poor efficacy of the complex Re 4-pico can be attributed to it having the highest number of chlorine atoms of all the complexes synthesized. The 4 chlorine atoms possibly make it too lipophilic to transition into the aqueous domain of the cells and perform optimally. This makes such compounds potential high Drug-Induced Liver Injury (DILI) risk factors [128]. It is therefore important in drug development and research to create compounds that aren't too lipophilic as this can be counterproductive and harmful. The rest of the complexes that had fewer chlorine atoms than Re 4-pico showed better efficacy. This suggests there was a good balance between their hydrophilic and lipophilic affinities.

It has been demonstrated that the subcellular localization of Re molecules and their chemical structure are related. $\text{Re}(\text{CO})_3$ complexes, especially those with lipophilic ligands, can localize to the mitochondria of cancer cells. Higher lipophilicity Re complexes selectively accumulated in mitochondria and triggered caspase-independent apoptosis in

cancer cells, whereas lower lipophilicity Re complexes localized in lysosomes and triggered caspase-dependent apoptosis. The mode of action of Re complexes can be extrapolated from this information based on their level of lipophilicity, which determines where they are localized.

For the picolyl based complexes, results show a correlation between the position of the methylene chloride ($-\text{CH}_2\text{Cl}$) substituent with respect to the nitrogen on the pyridine ring. The most potent complex is Re 2-pico, where the methylene chloride group is closest to the nitrogen at the ortho position, followed by Re 3-pico at the meta position. Finally, Re 4-pico where the substituent is at the para position. A possible reason for this apparent correlation could be based on that the anticancer efficacy of many metal-based drugs results from their direct contact with DNA. The position of the methylene chloride substituent at the ortho position may provide a more favorable pharmacokinetic configuration for the metal center to bind with the DNA. Thus, the further away the substituent moves from the nitrogen the weaker the DNA binding ability of the metal complex center.

CHAPTER 5

5.0 CONCLUSION

The aim of this work was to synthesize and characterize rhenium (I) complexes with pyridyl derivatives as ligands and test their antiproliferative properties against cancerous cell lines. The complexes were successfully synthesized using commercially available pyridyl derivatives 1,10 phenanthroline, 2-chloromethyl pyridine, 3-chloromethyl pyridine and 4-chloromethyl pyridine. The complexes were synthesized using the same starting material pentacarbonyl rhenium (I) chloride and they are:

- Tricarbonylchloro (1,10 phenanthroline) rhenium(I) (Re(phen))
- Carbonyl-bis1,10 phenanthroline rhenium (I) chloride (Re2(phen))
- Tricarbonyl di-2-chloromethyl pyridine rhenium (I) chloride (Re2-pico)
- Tricarbonyl di-3-chloromethyl pyridine rhenium (I) chloride (Re3-pico)
- Hexacarbonyl di-4-chloromethyl pyridine dirhenium (I) dichloride (Re4-pico)

The complexes were characterized using FT-IR, ¹H-NMR, ESI-MS and Single Crystal XRD. All these techniques confirmed the successful synthesis of the complexes. Single Crystal XRD was particularly useful in determining the structure of Re(phen), Re3-pico and Re4-pico and gave insight into the conformations and orientation of the substituents. It showed certain compounds differed from their expected structures which was key in explaining their efficacy in the cytotoxicity studies.

In vitro cytotoxicity studies were conducted against the MCF 7 breast cancer cell line. The complex Re2-pico showed to be the most potent with an IC₅₀ value of 13.10 while the least potent complex was Re4-pico with an IC₅₀ value of 53.73. The ligands coordinated were crucial in dictating the complex's efficacy and solubility.

5.1 FUTURE STUDIES

Future studies will include further characterization of the complexes especially those that were in non-crystalline form and conducting computational chemistry studies. Studying the cytotoxicity of the free ligands against the MCF 7 breast cancer cell line and the cytotoxicity of the complexes against healthy cells. DNA binding studies to determine the mechanism of action of the complexes. And to test the complexes against cisplatin-resistant cancerous cell lines.

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