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Synthesis and characterization of novel [Pt(diimine)(acylthiourea)]+ complexes as potential anticancer agents and exploring the use of sulphobutyl-ether-B-cyclodextrin and surfactant micelles as a drug delivery system

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Synthesis and characterization of novel [Pt(diimine)(acylthiourea)]$^{+}$ complexes as potential anticancer agents and exploring the use of Sulphobutyl-ether-β-cyclodextrin and surfactant micelles as a drug delivery system

A Dissertation submitted to the Faculty of Science, University of the Witwatersrand, in fulfilment of the requirement for the degree Master of Science (MSc) in Chemistry

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Supervisor: Dr I.A Kotze

Declaration

I , the undersigned, hereby declare that the dissertation "Synthesis and characterization of novel [Pt(diimine) (acylthiourea)]$^{+}$ complexes as potential anticancer agents and exploring the use of Sulphobutyl-ether-β-cyclodextrin and surfactant micelles as a drug delivery system" is my original work and that I have not previously in its entirely or in part submitted it at any university for a qualification.

.....

Rachael Ntombikayise Magwaza

Abstract

A series of [Pt(diimine)(L^n-*O*,*S*)]Cl complexes, where L^n-*O*,*S* represents a series of *N*,*N*-dialkyl-*N'*-acylthiourea ligands and diimine represents (1,10-Phenanthroline; 5,6-dimethyl-1,10-phenanthroline or [3,2-d:2',3'-f]-quinoxaline were successfully synthesised and characterised. A new crystal structure was obtained for *N*,*N*-di(2-hydroxy)-*N*'-benzoylthiourea which revealed an interesting herringbone crystal packing arrangement.

The cytotoxicity of the series of complexes was evaluated on H1975 lung cancer cell lines at 50 µM and 5 µM. All the complexes were highly cytotoxic with cell death of 90-98% at 50 µM. However, at 5 µM there were much more variations on cell viability percentages. Although the structure–activity relationship can only be established when the IC_{50} (the concentration of an inhibitor where the response is reduced by half) values are determined, it is clear that the complexes containing the methyl substituents on the 5 and 6 positions of the phenanthroline moiety were the most cytotoxic with almost 98% cell death at 5 µM. The solubility of the complexes did improve by using *N*,*N*-dialkyl-*N'*-acylthiourea as ancillary ligands, however aqueous solubility remains a major problem.

Sulphobutyl-ether-β-cyclodextrin (captisol) and low-molecular-weight surfactant micelles as drug delivery systems were considered in attempt to improve the solubility. DOSY NMR Spectroscopy revealed that there was no inclusion complex formation between the complex and capstiol, although the chemical shift trend suggested that there is at least some interaction. The low-molecular-weight surfactant micelles were considered as an alternative, which showed some promise as a drug delivery system, since the aqueous solubility improved and a colloidal suspension was obtained.

And we know that God causes everything to work together for the good of those who love God and are called according to his purpose from them.

Romans 8 vs 28

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List of Abbreviations

WHO	World Health Organization
IARC	International Agency of Research on Cancer
DNA	Deoxyribose Nucleic Acid
RNA	Ribose Nucleic Acid
JM216	*cis, trans*-[$PtCl_2$(acetate)$_2$(NH_3)(cyclohexylamine)]
AMD 473	*cis*-[$PtCl_2$(NH_3)(2-methylpyridine)]
EIF3	eukaryotic initiation factor 3
HET	2-hydroxythanethiolato
terpy	2,2',2"-terpyridine
Uv-vis	ultra violet visible
dppz	dipyrido[3,2-a:2',3'-c]phenazine
S,S-dach	1*S*,2*S*-diaminocyclohexane
CpG	dinucleotide
FDA	food and drug administration
SBE	sulfobutyl ether
DMSO	dimethylsulfoxide
EPR	enhanced permeation and retention effect
CMC	critical micellar concentration
NMR	nuclear magnetic resonance
NOESY	nuclear overhauser effect spectroscopy
ROESY	rotating frame nuclear overhauser effect spectroscopy
DOSY	diffusion ordered spectroscopy
FT-IR	fourier transform infrared
m.p	melting point

MeOH	methanol
mol	moles
ppm	parts per million
FT-IR /IR	fourier transform infrared spectroscopy
dmp	5,6-dimethyl-1,10-phenanthroline
dpq	[3,2-d:2',3'-f] quinoxaline
phen	phenanthroline
CHNS	elemental analysis
CD_3CN	acetonitrile
MS	mass spectrometry
MTT	3-(4-5-dimethylthioazol-2-yl)-2,5-diphenyl tetrazolium bromide
EGFR	epidermal growth factor receptor
ATP	adenosine triphosphate
bpy	bipyridine
MEOH	methanol
PFG	pulsed field gradient

Chapter 1

Literature review: Platinum-based anticancer drugs

Chapter 1

Literature review: Platinum-based anticancer drugs

1.1 Background on cancer and the current platinum anticancer drugs used

Worldwide, an estimate of 14.1 million new cases of cancer and 8.2 million cancer deaths was reported in 2012 by the World Health Organisation (WHO).[1] It is estimated by The International Agency of Research on Cancer (IARC) that by 2030 the global burden will grow to 21.7 million new cancer cases and 13 million cancer deaths per annum, which will be attributed to the growth and ageing of the population. Cancer can be described as a group of diseases characterised by the uncontrolled growth and spread of abnormal cells.[2] Usually, certain genes make sure that cells grow and produce in an orderly and controlled way, but from time to time a mutation happens in the gene when a cell divides. This result in a gene being damaged lost or copied twice. These cells than typically do not respond to signals to induce cell death (apoptosis) , and invade nearby tissues and spread throughout the body.[3] In addition, cancer cells are very similar to normal cells and the immune system is not triggered to terminate them.

1.1.1 Types of Cancer Treatment

Even though cancer does not have a permanent cure, it can be treated by common therapies like surgery, chemotherapy and also radiotherapy.[4] Surgery involves an operation to physically remove cancer cells while radiotherapy is the use of X-rays to kill the cancer cells. Chemotherapy involves the use of drugs to be taken orally or intravenously to kill the cancer cells.[4] There are a wide range of chemotherapeutic drugs that are currently being used, which are grouped according to their mechanism of action, chemical structures and relationship to

other drugs. The types of the chemotherapeutic drugs include alkylating agents, topoisomerases inhibitors, mitotic inhibitors, corticosteroids and antimetabolites. Alkylating agents inhibit reproduction by damaging DNA by forming covalent bonds at two nucleophilic sites on different DNA bases to induce *inter*strand and/ or *intra*strand cross-linking.[5,6] The examples of alkylating agent include cisplatin, oxaliplatin and carboplatin. These drugs work in all phases of the cell cycle and are used to treat many different cancers, including cancers of the lung, breast and ovary. Topoisomerase inhibitors, on the other hand, are drugs that interfere with enzymes that are called topoisomerases which are responsible for separating the DNA strands so that they can be copied.[7] There are also mitotic inhibitors which are derived from natural products which work by blocking cell division and corticosteroids which are natural hormones or hormones-like drugs that are useful in treating many cancer types by reducing inflammation and the immune response.[8]

The majority of the current anticancer drugs target DNA or proteins overexpressed in tumour cells. It is worth mentioning that recently there has been a new advance in the anticancer drugs target, where they are looking at a new set of potential targets which include RNA intermediaries between DNA and proteins. A new way was discovered in which the human cells control cancer genes expression, at the step where the genes are translated into proteins. They potentially target messenger RNA where these tags binds with the eukaryotic initiation factor 3 (EIF3).[9] A typical example of these drugs includes antimetabolites. Antimetabolites interfere with DNA and RNA growth by substituting for the normal building block of RNA and DNA.[5] While the chromosomes are being copied the antimetabolites damages the cells.

1.1.2 Current used platinum-based anticancer drugs

Even though the vast majority of pharmaceutical used for chemotherapy are based on organic molecules, a number of metal based pharmaceutical have also been discovered since the discovery of the platinum metallodrug, cisplatin. Cisplatin [$Pt(NH_3)_2Cl_2$] was discovered by Barnet Rosenburg in the 1960's in an attempt to understand whether electrical current played a role in cellular division. It was found that the phenomenon was not due to electric current but rather platinum hydrolysis products formed the inert platinum electrode.[10] Later in the discovery, it was envisioned that cisplatin may be interesting to test for anticancer activity. When cisplatin was tested against 180 tumours in Swiss white mice, it demonstrated potent activity, shrinking large solid tumours and the mice survived and were healthy. After 6 months, the cured mice did not show any signs of cancer.[10] From the results obtained, cisplatin entered the clinical trial phase and has been used as an anticancer drug ever since and falls under the alkylating group of anticancer therapies.[10] Cisplatin is able to cure over 90% of testicular cancer and it plays a fundamental role in the treatment of cancers such as ovarian, head, neck, bladder, cervical, melanoma, lymphomas and many other types of cancers.[10] However, despite its widely uses to treat cancer, its utility is limited by high toxicity, tumour resistance and relevant low solubility hence the drug cannot be administered orally.[11] Due to these limitations of cisplatin, new anticancer drugs have been developed. Out of the many anticancer drugs that have been synthesized, only a few have been on the clinical trials and these include drugs such as Carboplatin, Oxaliplatin, JM216 ({*cis*, *trans*-$[PtCl_2(acetate)_2(NH_3)(cyclohexylamine)]$}) and AMD473 (*cis*-$[PtCl_2(NH_3)$(2-methylpyridine)]) .[10] These drugs have gained clinical approval because they possess at least one distinct clinical advantage over cisplatin. These advantages include activity against cancer with intrinsic or acquired resistance to cisplatin treatment, reduced toxic side effects and the ability to be administered orally.[10]

1.1.3 Modes of reversible binding to DNA

The DNA-targeted drugs interact with DNA in different ways namely intercalation, non-covalent groove binding, covalent binding or cross-linking, DNA cleavage or nucleoside-analog incorporation.[12] These interactions are governed by 3 principal modes. The first mode includes controlling the transcription factors and polymerases, in which drugs interact with a protein that binds to DNA. The second mode is through RNA binding either to the DNA double helix to form nucleic acid triple helix structure or rather by exposing DNA single strand forming DNA-RNA hybrids that may interfere with transcriptional activity. The last mode includes the binding of small aromatic ligands molecules to DNA double helical structures.[12] The binding of small aromatic ligands usually takes place through noncovalent interactions. There are five noncovalent interaction sites which include interaction with the DNA major groove, the interaction with the DNA minor groove, the intercalation between base pairs *via* the DNA minor groove, a threading intercalation mode and the electrostatic attraction with the anionic sugar-phosphate backbone of DNA.[13] The groove binding is typically based on polyamines which are protonated under physiological conditions that strongly binds to the DNA. These molecules normally are crescent-shaped which bind to the major or minor groove of DNA based on the particular polyamine in addition to its overall charge.[14] The minor groove is the part of DNA where backbones are close together in the double helix while major grooves are where the DNA backbones are far apart (Figure 1.1) .

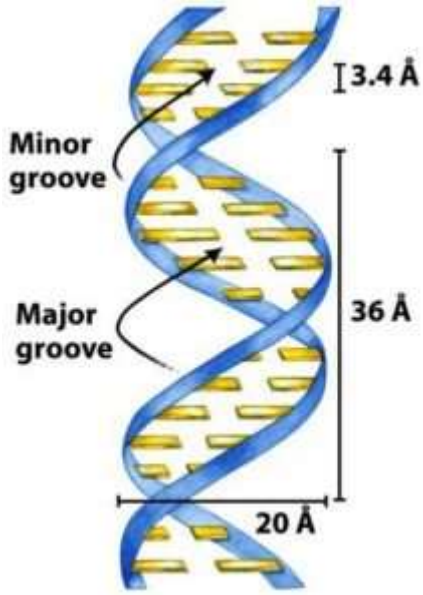
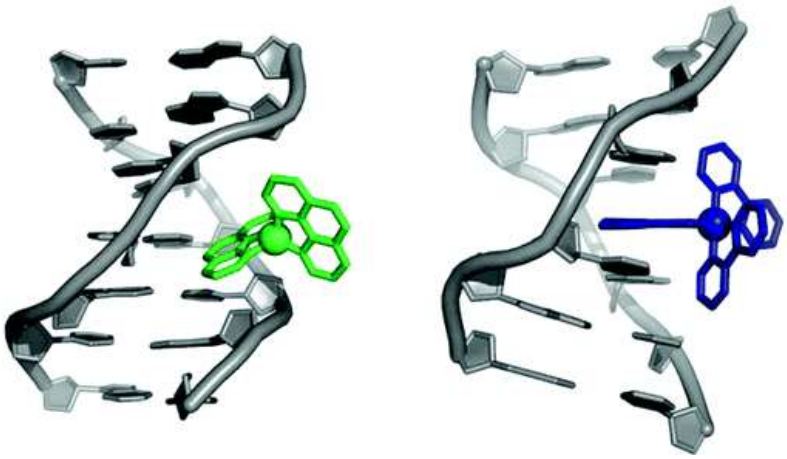


Figure 1.1*: Position of the Minor and Major groove in the DNA double helix.*[15]

The groove binding is stabilised by intermolecular interactions. This kind of binding does not bring large conformational changes in the DNA because it is just like the standard lock-and-key model for ligand-macromolecular binding.[14] The groove binding contains compounds which have unfused aromatic ring systems linked by bonds with a torsional freedom which then allows the molecules to adopt appropriate conformation to fit the helical curvature of the groove without so many changes to the DNA.

Electrostatic interactions on the other hand, typically involve metal cations such as Na^+ or Mg^+ since the stability of the folded DNA confirmations requires the interaction or ion-pairing with such metal from the solution for a process called counterion condensation.[13] The interaction between organic cationic molecules with DNA neutralises phosphate charges which then results in the release of condensed counterions. This is energetically favoured by providing an entropic contribution to the binding free energy.[16] These organic cations have shown increased binding constants with decreasing salt concentration. The classical DNA intercalators, unlike the groove binders (Figure 1.2), are fused-ring aromatic molecules which could have positive charges on an attached chain or the ring system itself.[13]



(a) Groove binder (b) Metallointercalator

Figure1.2*: The two binding modes of metal complexes with DNA: (a) groove binding and (b) intercalation*[17]

The intercalation occurs without even interfering with the hydrogen bonding of the base pairs and obeys the nearest neighbour exclusion principle.[18] Moreover, when DNA is saturated with intercalators, every second potential intercalation site on the helix remains empty. For the intercalation to occur, the DNA base pairs need to separate 3.4 Å to form a cavity for the incoming planar ligands through the localised left-handed unwinding of the duplex. The B-form DNA can twist at 36° (10 base pairs per one turn of 360°) for the DNA to accommodate the ligand, a reduction of this rotation occurs. Intercalation is believed to be due to the results of a hydrophobic interaction, whereby a hydrophobic aromatic molecule is drawn to a hydrophobic environment of the base pairs from the hydrophilic aqueous environment.[19]

The other type of intercalation is the threading intercalation, whereby to the complex with DNA, an aromatic system is inserted between the base pairs, and you find that one cationic substituent binds in a major groove and the other binds in a minor groove.[20] There is also another class of intercalation which is called the non-classical intercalation. This class of intercalation is based on the intercalators that are fused polyaromatic systems that have protonated nitrogen atoms or alternatively have protonated side chains attached to the ring

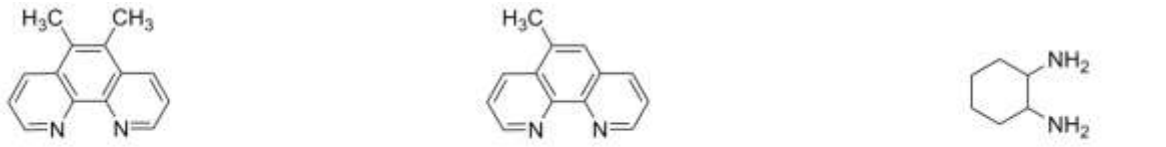
system.[13] This binding is different from groove binding even though they both use polyaromatic molecules, the non-classical uses fused polyaromatic with protonated side chains while the groove binding uses unfused aromatic systems with terminal basic functions. In addition, the non-classical intercalators interact with DNA in a stereoselective manner, whereby the positive part of the polar aromatic system interacts with the DNA base pairs.[21] Recently intercalation has been found to be the mode of binding for many metallodrugs.

1.2 Square-planar metallodrugs

The intercalation of square-planar transition metal complexes as demonstrated by Jennette *et al.* $[Pt(terpy)S(CH_2)_2OH)]^+$ ($[Pt(terpy)(HET)]^+$ where, terpy = 2,2',2"-terpyridine and HET = 2-hydroxythenethiolato ligand, showed that these metallodrugs intercalate double-stranded DNA by using UV-vis, viscosity, circular dischroism, fluorescence displacement and unwinding experiments.[22] Ligands like 1,10-phenanthroline and 2,2'-bipyridine have shown to intercalate DNA when incorporated into square-planar platinum complexes.[22] However, when coordinated to non-square planar metals such as copper, the intercalation with DNA is much weaker due to the size of the intercalating ligand, although $[Ru(phen)_2dppz]$ where dppz=dipyrido[3,2-a:2',3'-c]phenazine do intercalates strongly probably due to the large size of the intercalating ligand.[22] Square–planar metallodrugs are of interest because they can bind DNA in a number of ways which include DNA binding *via* the major or minor grooves or by partial intercalation. *Wang et al*. showed that the platinum complex ($[Pt(terpy)(HET)]^+$) bound the DNA *via* the major groove as shown by the crystal structure of a dinucleotide (CpG) with $[Pt(terpy)(HET)]^+$.[22] Collins *et al*. demonstrated that platinum metallodrugs which contain phenanthroline and phenantheraquinone diamine ligands, bound the oligonucleotide $d(GTCGAC)_2$ *via* the minor groove of the DNA.[22]

Over the years, more attention has been drawn towards metallodrugs since they have shown to be active against cancer at much lower concentration than the alkylating agent, cisplatin.[23] Furthermore, they are also able effective against cisplatin-resistant cancer cell lines. Aldrich-Wright and co-workers studied the structure-activity relationship of platinum intercalators with methylated phenanthroline intercalating ligand and chiral diamino ancillary ligands. They observed that [Pt(5,6-dimethyl-phen)(*S*,*S*-dach)]$^{2+}$ where 5,6-dimethyl-phen = 5,6-dimethyl-1,10-phenanthroline and S,S-dach = 1*S*,2*S*-diaminocyclohexane, was 12 times more cytotoxic than cisplatin for a wide range of cancer lines and showed excellent activity against cisplatin-resistant cancers.[23]

Phenanthroline derivatives Pt complexes against L1210 mouse leukaemia cell line with the substitution of the methyl group in the 5 and 5,6 positions (Figure1.3) were also investigated by Brodie *et al.*; it was observed that these complexes with methyl groups in these positions showed a significant increase in activity.[24] 5,6-dimethyl-1,10 phenanthroline was found to be 100-fold more cytotoxic than cisplatin. This family of DNA intercalators also displayed excellent cytotoxicity in a number of human cancer cell lines, including some that are sensitive to cisplatin treatment and others that have one or more modes of resistance to cisplatin.[24] Before the derivatives of 1,10-phenantroline ligand were discovered to be more active, phenanthroline complexes displayed potent cytotoxicity various cancer cell lines.[23] From these cytotoxicity tests, it was found that for high cytotoxicity the phenanthroline ligands should contain methyl groups in either the five or both five and six positions (Figure 1.3)



5,6-dimethyl-1,10-phenanthroline 5-methyl-1,10-phenanthroline 1S,2S-diaminocyclohexane

Figure 1.3: *1,10-phenathroline derivatives and chiral diimine ligands tested for cancer against L1210 mouse leukaemia.*

Even though these Pt complexes with phenanthroline ligands have high activity against cancer, the aqueous solubility of such platinum(II) complexes is low and aggregate in solution as reported by Kotzé *et al.*[25] Therefore one of the roles of the ancillary ligand is to improve the solubility of the complex. Ancillary ligands play an essential role in the development of the metal compounds as anticancer drugs. Ancillary ligands are incorporated to increase the stability of the metal centre, to change the kinetics of the complexes and also increase the selectivity of the complexes.[26] In the rhodium compounds $[Rh(L)_2chrysi]^{3+}$ where $chrysi^{3+}$ = chrysene-5,6-dione diimine and L- bipyradine and 1,10-phenathroline ligand, the ancillary ligands had a significant effect in targeting DNA mismatches *in vitro* and *in vivo*.[27]. Hence, it is important to carefully choose the ancillary ligand since it plays an important role in the success of the drug design. Acylthioureas have been proved to be an excellent ancillary ligand and can be used to increase the solubility of platinum complexes.

1.3 *N,N*-alkyl-*N*'-acylthiourea as ancillary ligands

Koch *et al*[28] have shown that the inclusion of hydroxy or amino functional groups in acylthioureas such as *N*-benzoyl-*N*'-*N*'-di(2-hydroxyethyl)thiourea and *N*-benzoyl-*N'*-*N*-bis-diethylaminoethylthiourea drastically improves water solubility of the Pt complexes.

N-*N*-alkyl-acylthioureas have gained much attention over the years as ligands for different metallodrugs due to their diverse modes of coordinating to the metal centre and also due to the fact that they are easy to prepare.[29] Thioureas can be described as compounds which contain the (>N-C(S)-N'<) functionality as shown in Figure 1.4, with the presence of O, N, N' and S donor atoms which result in different coordination modes.[30]

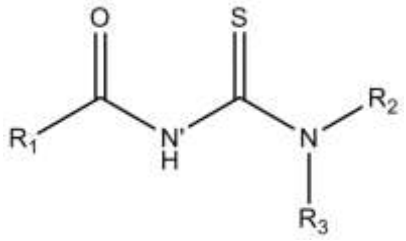
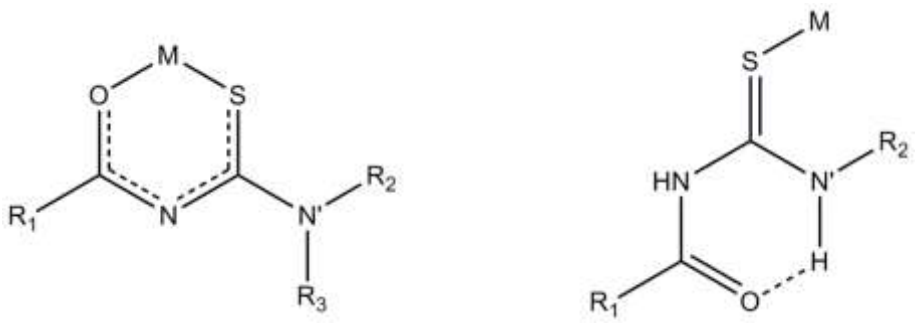


Figure 1.4*: General chemical structure of 1-(acyl/aroyl)-3-substituted thioureas*

These molecules were first prepared by Neucki in 1871,[29] and their coordination chemistry towards first-row transition metals have been investigated ever since. Thioureas may be mono-, di, tri or tetra substituted derivatives depending upon the extent of substitution on the nitrogen atoms. More specifically, acyl/aroyl substituted thioureas at (N') have a carbonyl group bonded to the thiourea core. [29] Moreover, the substitution can also occur on the second nitrogen (N') atom giving 1-(acyl/aroyl)-3(mono-substituted) and 1-(acyl/aroyl)-3,3(di-substituted) thioureas with general formula $R^1C(O)NHC(S)N'R_2R_3$ (Figure 1.4) , where R may be an alkyl, aryl or heterocyclic substituent. These molecules have been found to play an important role in the biological activity of a number of complexes.[31] Moreover, acylthiourea derivatives have been patented as antidiabetic, antiarthritic, antineoplastic and

anticoagulant agents and the potential use for the treatment of cognitive problems and prostrate disorder, herbicidal, fungicidal, bactericidal, insecticidal and plant growth regulator have also been shown.[32]

It has been found that the presence of hard (O, N'and N) and soft donor sites (S) in these compounds offers a variety of coordination potential. The possible coordination modes include the bidentate coordination mode (L-*O,S*) with the loss of the NH proton.[33] The monodentate coordination is *via* the S donor atom (L-*S*) which typically proceeds without the deprotonation (Figure 1.5). There are however, other coordination modes that have been reported which include the doubly deprotonated bridging mode (L-N: O, S).[33]



(A) (B)

Figure 1.5: *Most common coordination modes for monobasic O,S bidentate for dialkyl (A) and neutral monodentate through S atom (B) coordination mode for mono alkyl substituted thiourea.*

The *N*,*N*-alkyl-*N*'-acylthioureas show pronounced affinity to coordinate to different transition metals. Their ability to form metal chelates with various transition metals, specifically Ni(II), Cu(II), Pd(II), Co(II) and Pt(II) metal coordination takes place through the oxygen and sulphur atoms following deprotonation of the central nitrogen atom to yield predominately *cis* square-planar complexes. [34]

Interestingly, the *N,N*-alkyl–*N'*-acylthiourea ligands can be used as an ancillary ligand to increase the water solubility of the insoluble drugs, since these ligands are reasonably soluble in water. Furthermore, hydroxyl groups can be introduced into the amine chain since the functionality of the amine chain can be easily changed, which may result in an increase in solubility of the ligand and the corresponding complex. However, the ancillary ligand is not completely acquitted and does affect the activity of the overall complex. Therefore, in certain cases, the more water soluble variation of the ancillary ligand results in a complex with lower activity and alternatives strategies such as drug delivery systems are required to improve solubility.[35]

1.4 Drug-delivery Systems and Host-guest interactions

Drug-delivery systems have the ability to improve the delivery and effectiveness of drugs and can sometimes reduce the cytotoxicity and improve drug metabolism.[36] They are exerted in a controlled-release manner to prolong *in vivo* drug activity.[36] There are a few molecular hosts that are typically used as effective drug delivery systems, which include cucurbit, calix[n]arenes, dendrimers, micelles and cyclodextrins. However, we will only focus on cyclodextrins and micelles in this study.

1.4.1 Cyclodextrins as a drug delivery vehicle

Cyclodextrins are often preferred due to their ability to form inclusion compounds with a large variety of molecules and also to discern various types of guest molecules by selectively incorporating molecules through size and polarity consideration.[35] Furthermore, cyclodextrins are able to improve the aqueous solubility and chemical reactivity of many lipophilic drugs without changing their intrinsic ability to permeate lipophilic membranes.[35] Cyclodextrins are made up of hydrophobic interior and hydrophilic exterior, which makes it possible for the

water insoluble drugs to insert into the hydrophobic interior via non-covalent association to the cyclodextrin, resulting in a host-guest complex with improved water solubility.

Cyclodextrins possess hydrophobic cavities which can be used as pharmaceutical excipients to improve the solubility, stability and bioavailability of hydrophobic and biomolecular drugs.[37] Due to the lipophilic inner cavities and the hydrophilic outer surface of cyclodextrin (Figure 1.6), they are capable of interacting with a large variety of guest molecules to form non-covalent inclusion complexes.[38]

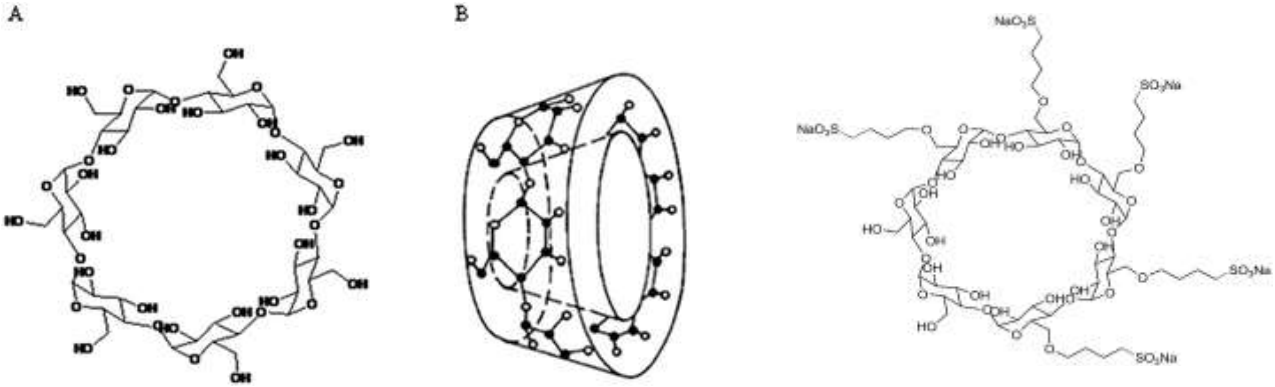


Figure1.6: *The chemical structure (A) and the toroidal shape (B) of the a-cyclodextrin molecule, (C) captisol structure*.[39]

There are three naturally occurring cyclodextrins namely; alpha (α), beta (β) and gamma (δ)-cyclodextrin. These cyclodextrins differ in the ring size and solubility. β-cyclodextrins have been widely used in the early stage of pharmaceutical application because of its readily availability and cavity size suitable for the widest range of drugs.[38] Over the past 30 years, the use of cyclodextrin has been considered to form inclusion complexes in order to increase the solubility of the insoluble drugs.[38] This process is driven by both enthalpic and entropic energy contributions. The inclusion complexation helps to solubilize and stabilise drug molecules. Cyclodextrins that are successful in drug delivery are able to increase the permeability of drugs by increasing drug solubility which improves the bioavailability of the drug.[40] Furthermore, increased drug solubility and mobility with cyclodextrins may reduce

drug toxicity by making the drug effective at lower doses. The cyclodextrin complexation also controls the drug release and in most cases allows the drug to be administered orally. The naturally occurring cyclodextrins namely; the α and β-cyclodextrins are known to be parenterally unsafe due to the nephrotoxicity and low solubility, which led to the use of functionalised cyclodextrins. Functionalised sulfobutyl-ether-β-cyclodextrin commonly known as captisol have shown to overcome the limitations of β-cyclodextrins and have been used frequently in the past few years. Captisol was developed by The University of Kansas' Higuchi Biosciences centre for specific use in drug development and formulation.[41] Captisol is an anionic β-cyclodextrin derivative with a sodium sulfonate salt separated from the hydrophobic cavity by a butyl ether space group. The sulfobutyl ether (SBE) substituent is introduced at the 2, 3 and 6 positions in one or more of the glucopyranose units in the cyclodextrin mixture.[41] Captisol is safe when administered parenterally in stark contrast to the nephrotoxicity exhibited by unmodified natural β-cyclodextrin. In addition, captisol is present in six FDA- approved products and there are also currently more than 50 captisol containing products in clinical development.

Captisol is a successful drug delivery vehicle to many molecules that suffers from poor solubility and stability. Captisol has shown to complex neutral, cationic and anionic drugs and small and large molecules. The aqueous solubility of a complexed insoluble drug can increase by a factor of 10 to 25 000 depending on the compound.[41]

1.4.2 Micelles as drug delivery vehicle

Similarly to cyclodextrins, micelles have the hydrophobic core which is sterically stabilised by a hydrophilic corona.[42] Micelles are formed when surfactant molecules self-assemblies as shown in Figure 1.7.

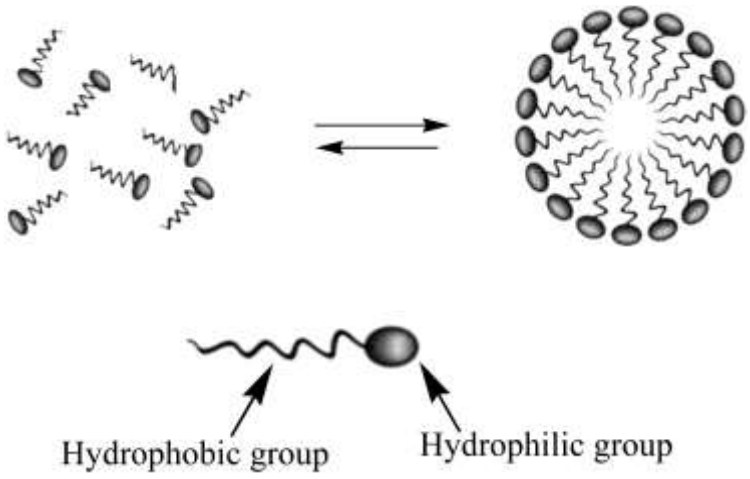


Figure 1.7: *Illustration of the hydrophobic and hydrophilic groups and the micelle formation.*[43]

The core acts as a reservoir in which the drug molecule can be incorporated by different interaction namely the chemical, physical or electrostatic interaction depending on the physicochemical properties of the drug molecules.[42] The micelles ability to solubilise the drug molecules helps in increasing the drug permeability across the physiologic barrier, protect the drug from inactivation by its biological surroundings, lower toxicity, and decrease the incidence and severity of adverse effect.[42]

Micelles do not only solubilise hydrophobic drugs but they may also increase the systemic half-life of drugs and passively enhance their accumulation at the tumour site *via* enhanced permeation and retention (EPR) effect. Furthermore, micelles can also potentially improve the treatment efficacy by bypassing the drug resistance mechanism of multidrug resistance cells. There are two common types of micelles namely the low-molecular-weight surfactant

micelles and polymeric micelles. The micelle formation for low-molecular-weight surfactant may happen spontaneously in aqueous media or with the involvement of energy by heating at a specific temperature. At low concentrations in water (below the critical micellar concentration-CMC) amphiphilic molecules, with a hydrophobic moiety and a hydrophilic moiety orient themselves at the air-water interface and in the bulk disperse phase they exist as monomers.[44] When the concentration of the amphiphiles reaches the CMC, the amphiphilic molecules aggregate in such a way that the hydrophobic moiety is shielded from the polar aqueous medium. In the case where there is no heat applied and the reaction happens at room temperature, the driving force is the entropy which increases from the release of water molecules adjacent to the hydrophobic moiety. In the case where the heat is needed, a specific temperature is required for micelle formation to occur. At the critical micellar temperature, the amphiphile is no longer soluble in the aqueous medium as a monomer. Once the monomers intrinsic solubility is exceeded, then micelle forming molecules proceed to aggregate and shield the hydrophobic moieties in the molecule from the aqueous disperse phase.[44] On the polymeric micelles, the amphiphilic block or graft copolymers behave similarly to the conventional amphiphiles and aqueous solution. However, in polymeric micelles, there is a covalent linkage of individual surfactant molecules with the hydrophobic core in comparison to low-molecular-weight surfactants (Figure 1.8).

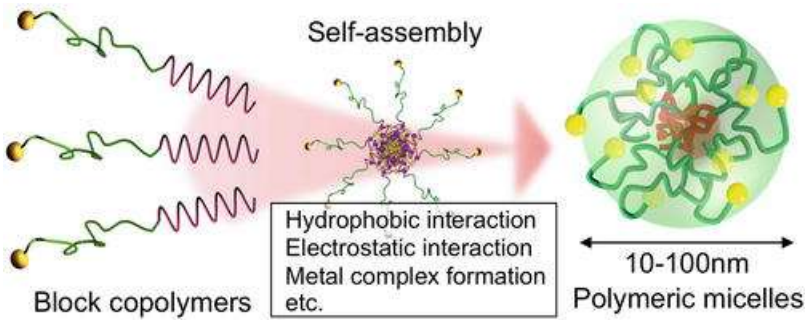


Figure 1.8: *Polymeric micelles showing the linkage present in polymeric micelles*

In addition, the linkage present in polymeric micelles prevents a dynamic exchange of monomers between a free solution and the micellar pseudo-phase.[45] Hence, the rigidity and stability to the polymeric micelles are conferred. There are different types of polymeric micelles which are characterised based on the type of intermolecular forces governing the segregation of the core segment from the aqueous environment. This includes conventional micelle and poly-ion micelles which result from the electrostatic interaction.[45] The conventional micelles are based on the hydrophobic interactions between the core segment and the corona region in the aqueous environment. The poly-ion complex micelles, on the other hand, require an electrostatic interaction between two moieties such as polyelectrolytes that are oppositely charged in order to allow the formation of the polymeric micelles.[45]

As previously stated, the development of micelles based–carriers of cancer chemotherapy have been a growing scientific interest as a result of the characteristics of micelles. Different micelles be it the low-molecular-weight surfactants micelles or polymeric micelles have been used to solubilize a range of anticancer drugs. It is undisputable that micelles offer great potential for the solubilisation and protection of poorly water-soluble anticancer drugs.

1.4.3 Host-guest interactions involved in drug delivery systems

To develop effective drug delivery systems involves an understanding of the interaction between the guest (drug) and host molecules (delivery system). Generally, a host is a molecule that contains a large cavity volume such as cyclodextrins, micelles, cucurbituril or calixarenes and the guest typically has both a complementary shape and interaction with the host, which allows for selectivity with this host-guest match also known as molecular recognition.[46] There are various non-covalent interactions that take place between the host and the guest and these includes the hydrogen bonding, electrostatic and Van der Waals interaction. Cyclodextrins and micelles form a great variety of host-guest complexes which

are also called an inclusion compound with the hydrophobic guest molecules typically encapsuslated in aqueous solution.

The formation of the host-guest supramolecular complexes with cyclodextrin and an amphiphilic compound is driven by chemical, physical and electrostatic interactions.[47] The formation of these host-guest complexes allows the controlling of the assembly and disassembly of the supramolecular structure, by tuning the amphiphilicity of guest molecule.[47] In aqueous solutions, the inclusion of the (dehydrated) guest into the non-polar cavity of the cyclodextrin is accompanied by the release of water from the cyclodextrin cavity. The latter process is strongly dependent on the interactions between water-water and water-cyclodextrin occurring inside the cyclodextrin cavity and it also depends on the other factors, including the size of the cyclodextrin cavity and guest as well as the structure (geometry) of guest molecules.[48]

Many qualitative and quantitative methods have been used to study the host-guest interactions and these include NMR Spectroscopy, Titration Calorimetry, Potentiometry, UV-Vis Spectrometry and Polarography. NMR Spectroscopy data can be used to determine the thermodynamic parameters, kinetic parameter and the host-guest complex conformation(s) using the two- dimensional (2D) NMR techniques. A correlation of the degree of molecular recognition with a structural feature of the host-guest complex is essential for understanding the origin of molecular recognition. Since molecular recognition involves a complicated steric fit between host and guest molecules, an understanding of molecular recognition requires a knowledge of the conformation of the complex.[49] 2D NOESY and ROESY NMR experiments provide information about the spatial distance between two protons as long as the motion of the molecule are not too fast for observation and the distance between the two hydrogens is shorter than 5Å.[49] NMR is, therefore, an excellent technique to study the host-guest interactions because it doesn't require any external probes or any modifications of

molecules under study and also the measurement are fairly quick and easy to perform since no pre-reaction is required.[50] Diffusion-Ordered Spectroscopy (DOSY) NMR is also a useful to gain insight molecular dynamics, aggregation and conformational changes of the inclusion complex.

1.5 Aims and Objectives of this study

There is a big drive for the development of better anticancer drug candidates that are soluble, more effective, less toxic and more selective. This class of complexes [Pt(diimine)(L-*O*,*S*)]$^{+}$ is believed to interact with DNA, since they have shown antibacterial activity and biomineralisation with DNA hence an investigation on the anticancer activity of these complexes is necessary due to the proposed DNA interactions . Therefore, the aims and objectives of this project were as follows:

Aims:

The aims of the project are:

- To develop novel Platinum(II) complexes as potential anticancer agents.
- To improve the solubility and cytotoxicity of the diimine complexes by using acylthiourea ancillary ligands and drug delivery systems.
- To study the interaction between the drug delivery system and the Platinum(II) complexes.

Objectives:

In order to fulfil the aforementioned aims, the objectives were identified as follows:

- Synthesis and characterization of ancillary ligands (Acylthioureas).
- Synthesis and characterization of Pt(diimine)(L^{n}-*O*,*S*)Cl.

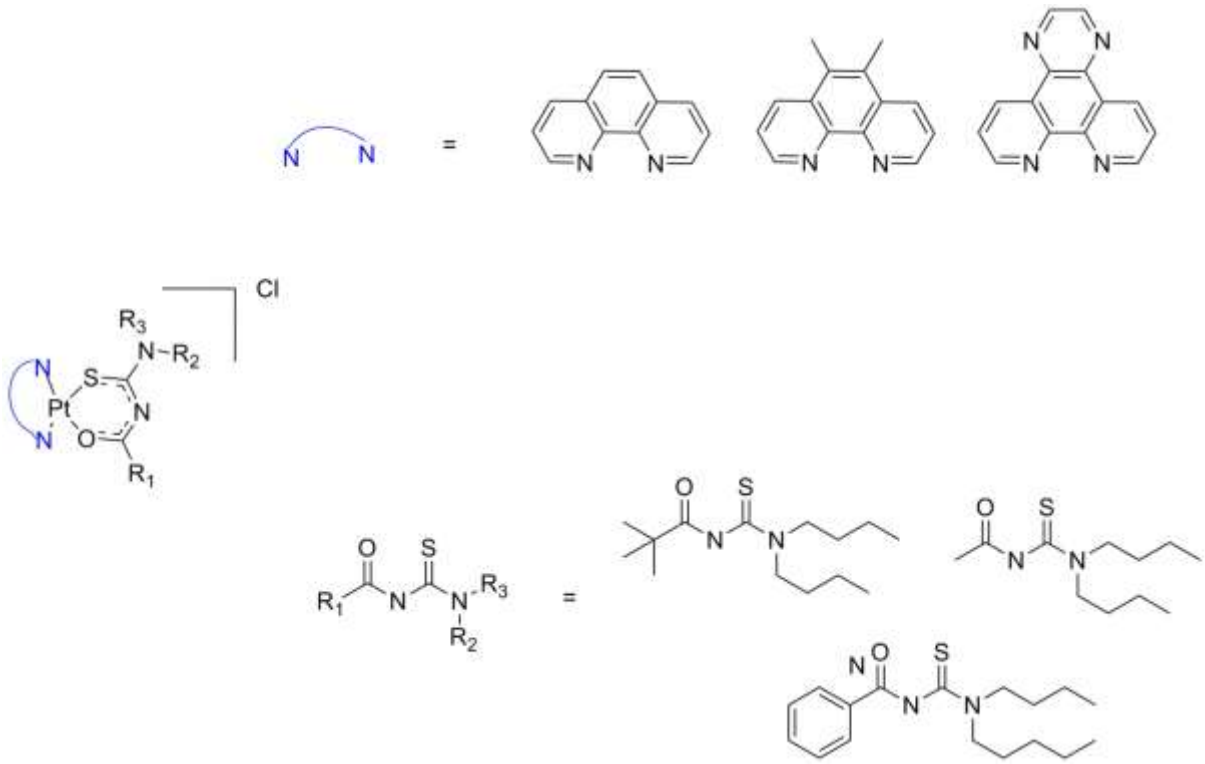


Figure 1.9: *Structural variations of the series of [Pt(diimine)(L^n-O,S)]Cl complexes to be synthesised.*

- *In Vitro* cytotoxicity testing.
- Encapsulation of platinum (II) complexes in Sulphobutyl-ether-β- cyclodextrin.
- NMR Spectroscopy study of the Host-guest interaction between the drug delivery system and the complexes.

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Chapter 2

The synthesis and characterization of the ligands and complexes

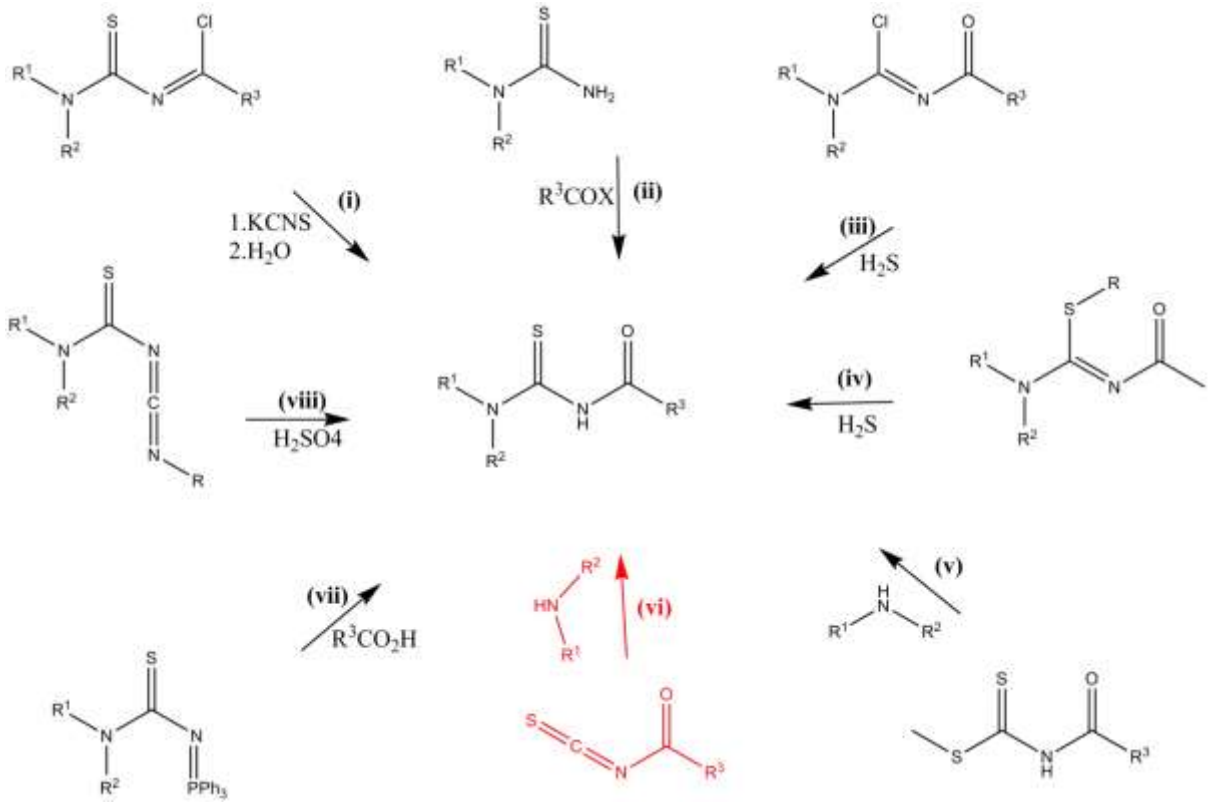
Chapter 2

The synthesis and characterization of the ligands and complexes

2.1 Introduction

In this chapter the synthesis of *N*,*N*-di(alkyl)-*N*'-acylthiourea metal complexes containing Pt(II) metal centre is described. It has been shown by Koch and co-workers that this [Pt(diimine)(L-*O*,*S*]Cl complexes as DNA intercalators have significant antimicrobial activity.[1] Furthermore, this class of complexes have great potential as anticancer compounds since they can ion-pair with the negatively charged phosphate backbone of the DNA double helix and can potentially intercalate or bind to minor or major to the grooves of DNA.

There are several ways in which the *N*,*N*-di(alkyl)-*N*'-acylthioureas can be synthesised of which, the common routes used are shown in Scheme 2.1.



Scheme 2.1: *The eight common synthetic routes for N,N-di (alkyl)-N'- cylthioureas: reaction (i) aninothiocarbonyl chloride with KCNS (ii) acylation of N,N-disubstitued thioureas (ii) N-acyl aminoimidoyl with H_2S (iv) N-acylisothiorea with H_2S (v) methyl-N-acylcarbomodithiated (vi) acyl isothiocynates (vii) aminothiocabonyl with R^3CO_2H (vii) hydrolysis of N-aminothiocarbonylcarbodiimides with mineral acids.* [2]

The most common method that is used and also will be used in this study to make these acylthioureas is the method described by Douglass and Dains,[3] (as highlighted in scheme 2.1) which involves a simple "one-pot" procedure with generally good yields. These ligands tend to coordinate to the platinum group metals in two different modes namely the chelating mode (*O, S*) and the neutral monodentate (*S*).[4] Due to these possible coordination modes, one of the problems with the synthesis of $[Pt(diimine)(L\text{-}O,S)]^+$ complexes is, one is most likely to form a mixture of products even on (1:1 mole ratio) of the $[Pt(diimine)Cl_2]$ and *N,N*-dialkyl-*N*'-acylthiourea. This then requires a careful but tedious purification in order to obtain the

desired [Pt(diimine)(L-*O*,*S*)]Cl complexes. However, these complexes can be successfully synthesised.

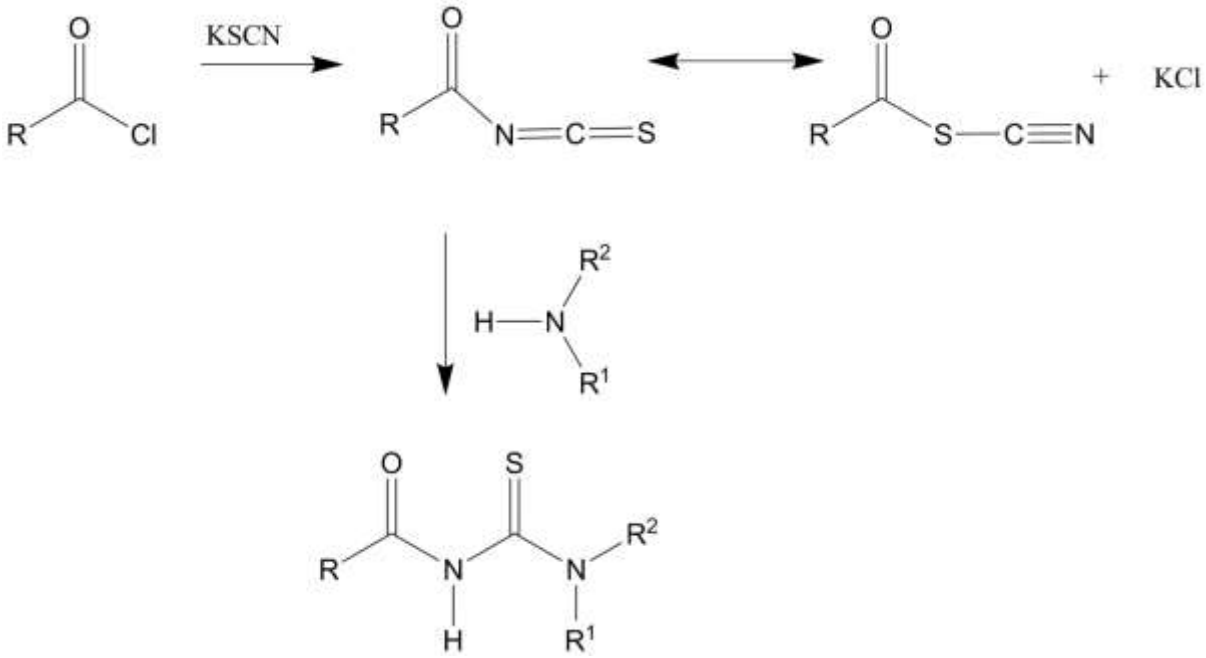
The aim of this study was to synthesize potentially highly active anticancer agents based on Pt complexes, which have the basic structural formula [Pt(diimine)(acylthiourea)]Cl where, diimine represent various diimine ligands as potential intercalating ligands and *N*,*N*-dialkyl-N'-acylthioureas as the ancillary ligand.

The ligands, the metal precursor and the metal complexes were characterised using a range of techniques which includes NMR Spectroscopy, FT-IR Spectroscopy, Mass Spectrometry and Elemental Analysis. Single Crystal x-ray analysis of one of the ligands was also conducted.

2.2 Synthesis and characterization of *N,N*-di(alkyl)-*N'*-acylthioureas

2.2.1 Synthesis of *N,N*-di(alkyl)-*N'*-acylthioureas

The *N,N*–di(alkyl)-*N*'-acylthiourea were synthesised according to a modified procedure to the method described by Douglass and Dains,[3] (Scheme 2.2).



Scheme 2.2*: Reaction scheme of the synthetic procedure used for the synthesis of N,N-di(alkyl)-N'-acylthioureas*[3].

This method involves the addition of acyl chloride to a solution of potassium thiocyanate in dry acetone followed by 3 hours of heating under reflux and cooling to room temperature to yield the crude acyl isothiocyanate. The nucleophilic attack of the thiocyanate can either occur *via* the nitrogen or the sulphur. However, the latter product will revert to the desired acyl isothiocyanate intermediate which will react with the amine for 3 hours to form the desired product. A small volume of water was added to increase the rate of product precipitation and the mixture was placed in the vent of the fumehood to allow for evaporation of acetone to enhance crystal formation. Single product precipitates were recrystallized from

a combination of cyclohexane and ethyl acetate or purely hexane. Where the product failed to crystallise and oil was obtained, extraction into hexane was followed by separation and purification by column chromatography using silica gel. A series of these ligands (Figure 2.1) were synthesised using the above mentioned procedure.

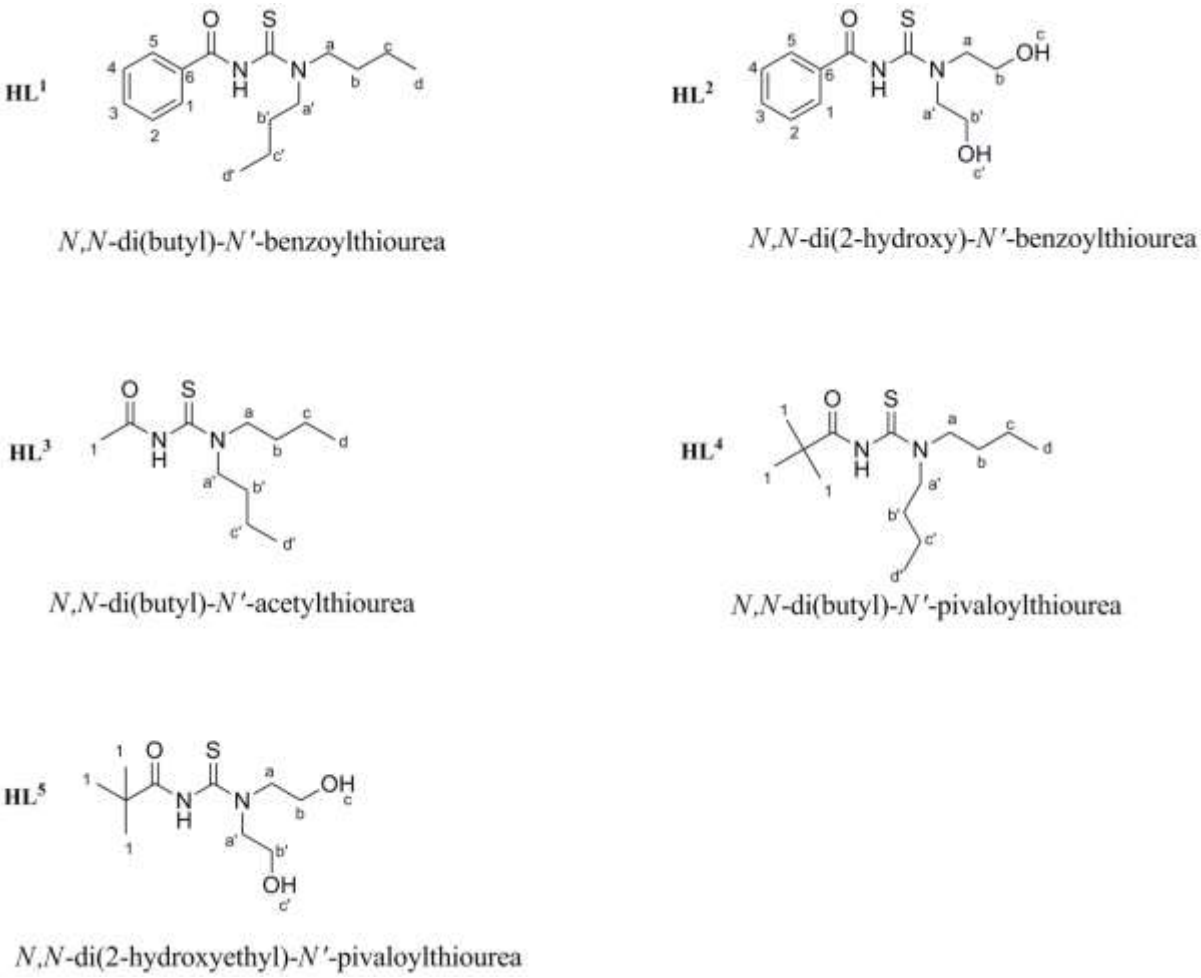


Figure 2.1: *Ligands synthesised with their full and abbreviated names*

The hydroxyl containing ligands (HL^2 & HL^5) could not be easily crystallised and were purified by column chromatography. The pivaloyl containing ligand (HL^4), however, was successfully crystallised from the water in the ice bath. The *N*,*N*-di(2-hydroxyethyl)-*N*'-

acetylthiourea ligand was attempted with changes in the reaction conditions such as the use of hot acetone, acetonitrile and increase in the reaction time but it was found to be unsuccessful.

The synthesised ligands were characterised by NMR Spectroscopy, FT-IR Spectroscopy, Elemental Analysis and Mass Spectrometry (See Experimental Section). One ligand is used to illustrate the characterization methodology.

2.2.2 Characterization of *N,N*-di(alkyl)-*N'*-acylthioureas

The ligand HL^3 was used to illustrate characterization methodology. The NMR assignments of the *N,N*-di(alkyl)-*N*-acylthioureas was relatively simple. The ^1H NMR spectrum of HL^3 exhibited a sharp singlet at 10.23 ppm which was identified as the NH proton. A sharp shielded singlet was observed at 1.95 ppm which integrated for 3 protons and was assigned to H^1.

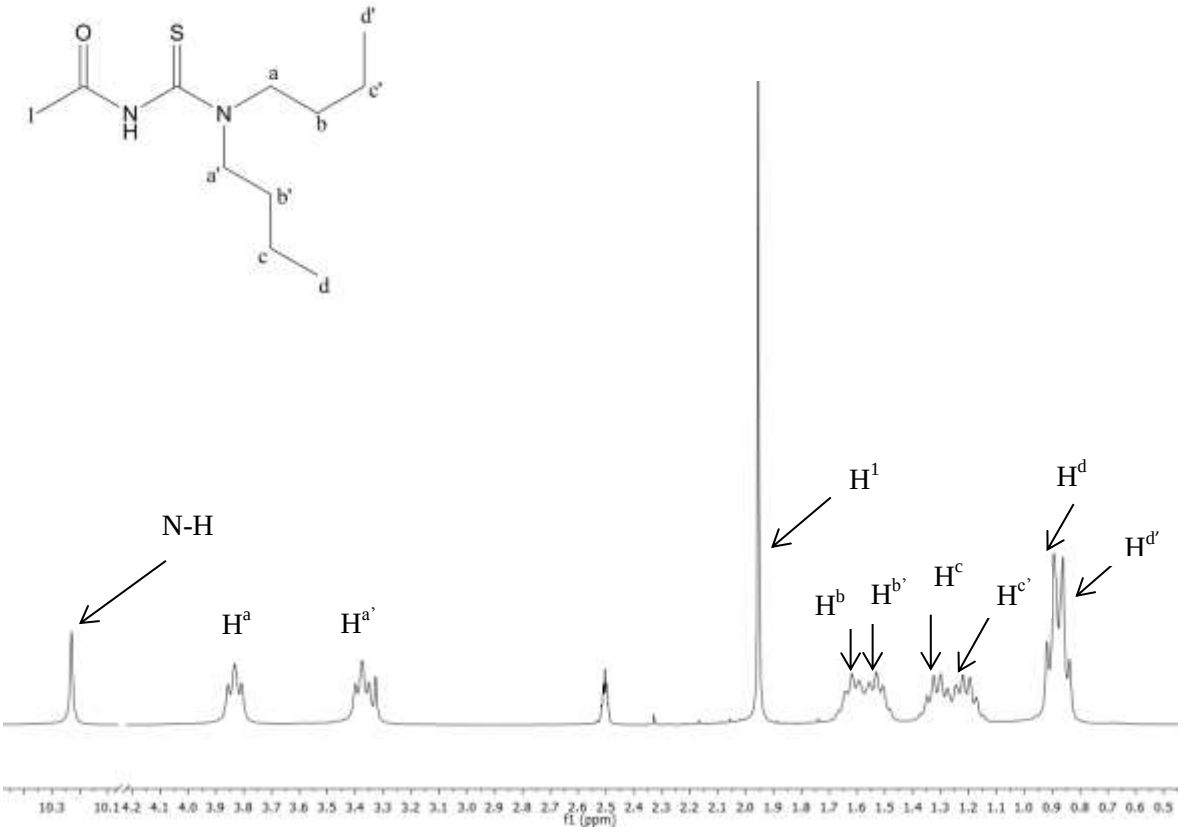


Figure 2.2: *^1H NMR of HL^3 in DMSO-d_6 at 25 °C. (Note: The break in horizontal scale between 10.1 and 4.2 ppm.*

The triplets at 3.83 ppm and 3.35 ppm integrated for 2 protons each were assigned to H^a and $H^{a'}$, due to electron withdrawing nitrogen atom next to them , hence they are expected to be the most deshielded CH_2 group. H^a was assigned as the most dishielded triplet based on the previous published results.[5] The remaining multiplet within the ranges of 1.62-1.21 ppm

which were due to the CH_2's were assigned using the COSY (Figure 2.3).

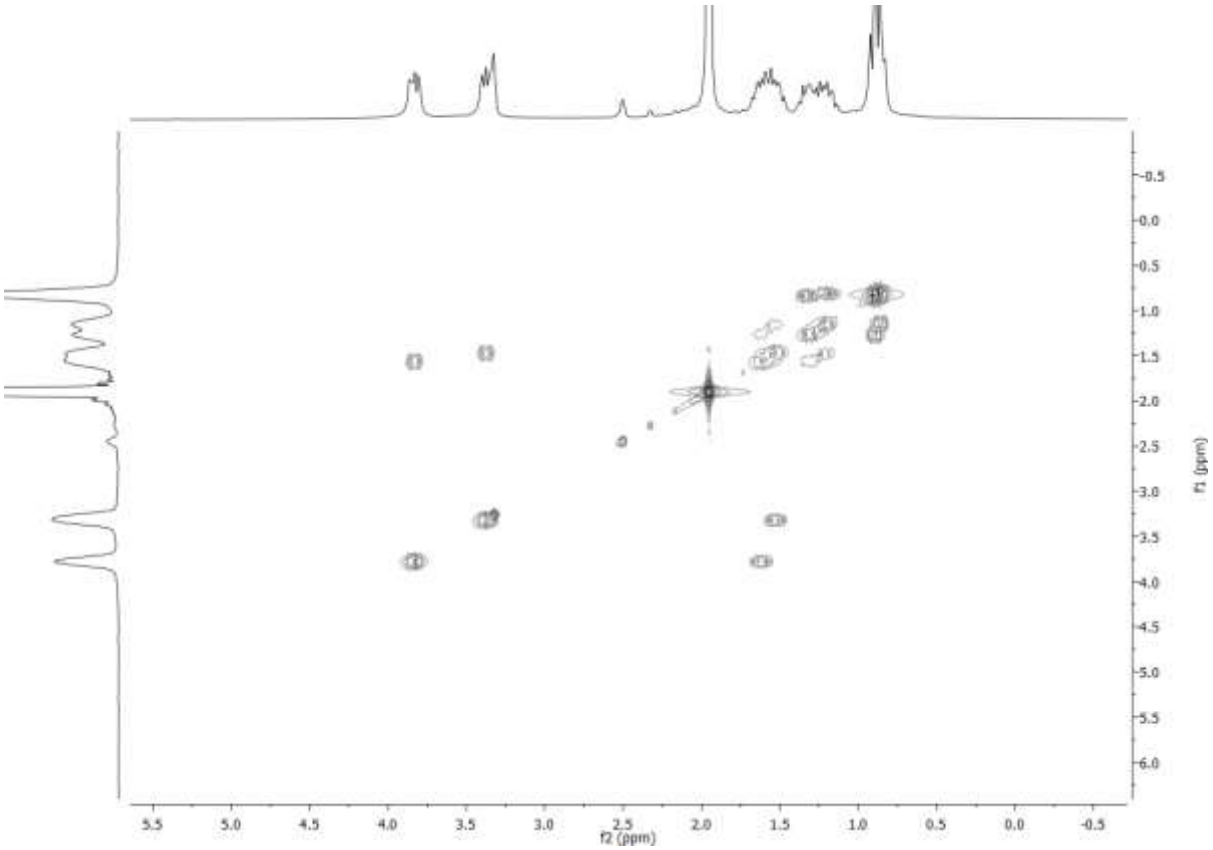


Figure 2.3: *The COSY enlarged in the alphatic region for HL^3 in DMSO-d_6 at 25 °C.*

The proton at 1.62 ppm which was overlapping with the one at 1.52 ppm was found to couple with H^a while the one at 1.52 ppm coupled with $H^{a'}$ which enabled the assignment of H^b and $H^{b'}$. The proton at 1.33 pm coupled with H^b which then enable the assignment of H^c and consequently the assignment of $H^{c'}$. The two overlapping triplets at 0.99 ppm and 0.88 ppm which integrated for 6 protons in total were assigned to H^d and $H^{d'}$. The triplet at 0.99 ppm was found to couple with H^c while the triplet at 0.88 ppm coupled with $H^{c'}$.

The C=S and C=O were assigned as the most deshielded carbons at 180.28 ppm and 166.789 ppm. The C=S could be distinguished from the C=O based on the smaller intensity, which is a result of a broader signal for the C=S due to the quadrupolar relaxation caused by the bound ^{14}N.[5] The carbon that coupled to H^a was identified as the dieshielded carbon at 51.979 ppm and assigned as C^a while $C^{a'}$ was found at 51.636 ppm respectively. The carbons due to the

CH_3 were easily assigned using the DEPT NMR spectrum and also the C-H correlation, C^d was found at 13.83 ppm while $C^{d'}$ was found at 13.68 ppm. The single bond correlation of H^1 with a carbon resonance at 22.982 ppm enabled the assignment of C^1. HSQC was used to assign the remaining carbons. the multiple bond correlation of the protons H^b, H^b and H^c, $H^{c'}$ enabled the assignment of C^b and $C^{b'}$ at 29.543 ppm and 27.874 ppm and also C^c and $C^{c'}$ to be at 19.356 ppm.

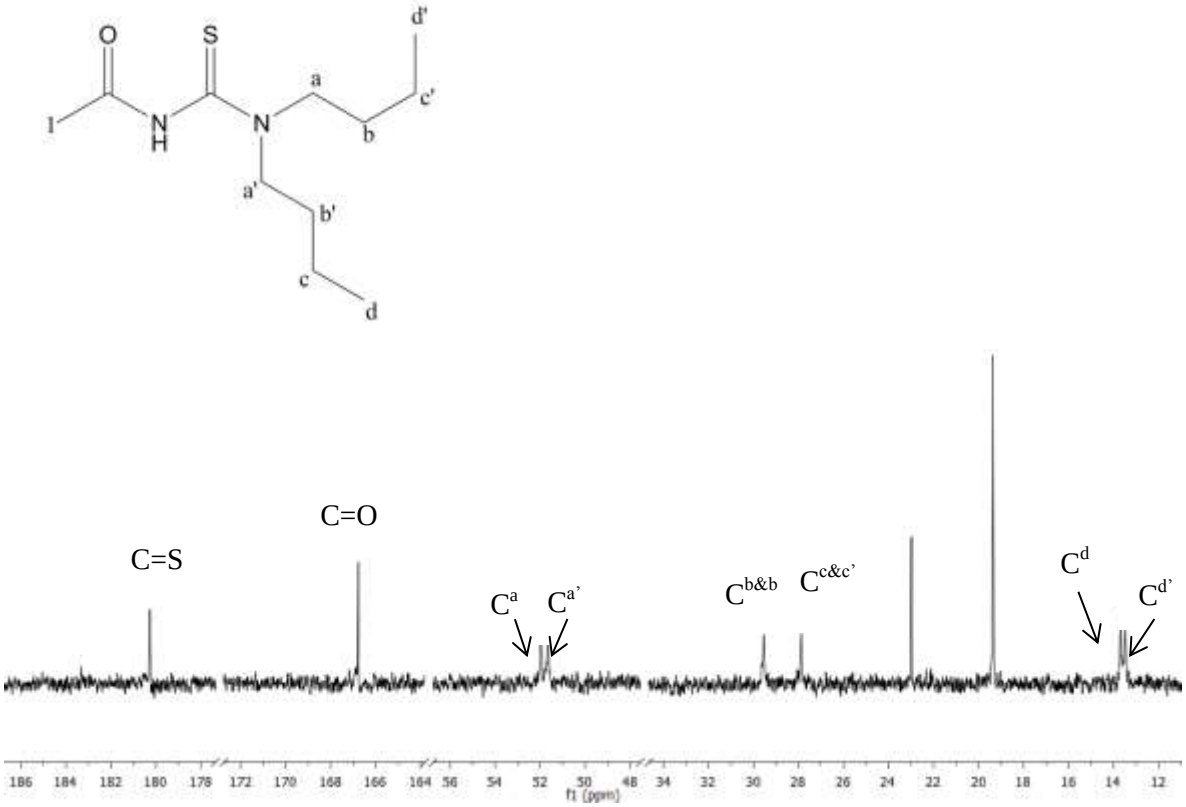


Figure 2.4: *^{13}C NMR spectrum of HL^3 in DMSO-d_6 at 25 °C. (Note: Two breaks in the horizontal scale between 34-48 ppm, 56-164 ppm and 172-178 ppm).*

The formation of the ligand was further confirmed by Infrared Spectroscopy which revealed a strong band at 1661.59 cm^{-1} (see Appendix), due to the C=O. The C=S stretch was observed at 1416.16 cm^{-1} and the NH stretch was found at 3166.93 cm^{-1}. Furthermore, the Elemental Analysis data obtained for the ligands was found to be in agreement with the expected chemical composition. The chemical compositions of C, H, N and S were expected at 57.35,

9.63,12.16 and 13.92% respectively and the analytical results were found to be 56.16 (C), 9.36 (H), 12.25 (N) and 13.59% (S). Several crystals were obtained from the series of ligands but the crystal structure of HL^2 has not been reported in literature before.

2.2.3 X-ray crystallography of *N,N*-di(2-hydroxyethyl)-*N*'-benzoylthiourea (HL^2)

The crystals of HL^2 were isolated from a solvent mixture of cyclohexane and ethylacetate and these crystallised out in the $P2_12_12_1$ space group with three molecular units per unit cell (Table 2.2). The atomic numbering of the structure is given in the ortep diagram below (Diagram 2. 1).

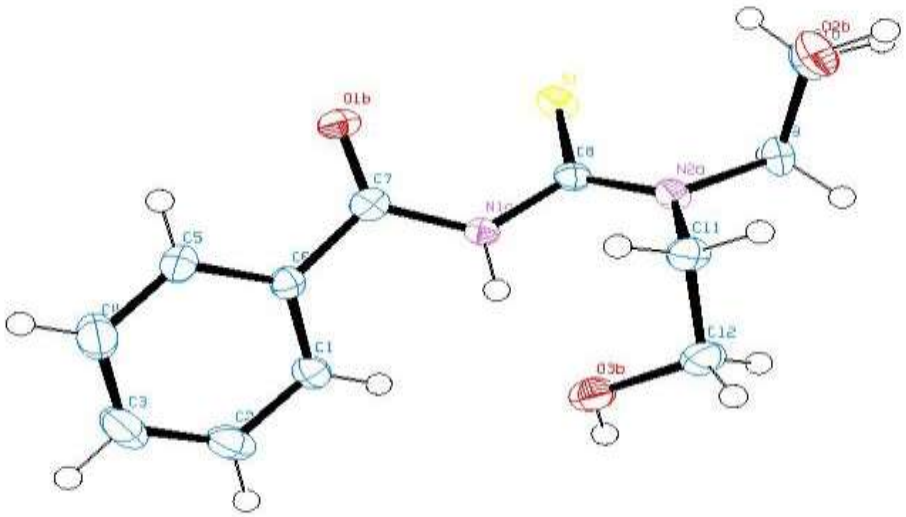


Diagram 2 .1: *ORTEP diagram showing the asymmetric crystal structure of N,N-di(2-hydroxyethyl)-N'-benzoylthiourea.*

The compound exhibited both intramolecular interactions and intermolecular interactions, specifically hydrogen bond.

The hydrogen bonds that resulted from compound included: O2*B*—H2*B*···S1 = 3.117 Å (intermolecular interaction), the above bonds can be confirmed from the Table 2.3 below.

Table 2.1 *Hydrogen-bond geometry (Å, º) for (SHELX)*

D*—H···*A	***D*—H**	**H···*A***	***D*···*A***	***D*—H···*A***
O2*B*—H2*B*···S1^{i}	0.82	2.36	3.177 (3)	178
C11—H11···O2*B*	0.97	2.54	3.104 (4)	117

Symmetry code: (i) *x*−1, *y*, *z*.

Table 2.1 above does confirm that there is intermolecular interaction taking place between S1—H2B-O2B, but again it also shows that there is an interaction between C11—H11---02B. This interaction between C11—H11---02B can be assigned as a weak hydrogen bond (intermolecular interaction) simply because of the directionality it possesses which are; the angle α is in the range between 90 to 180º, and the distance *d* is usually smaller than 3.2 Å.

The unit cell packing of the compound was also investigated and it was extended to 3x3x3 to reveal the packing pattern. Compound 1 shown in Diagram 2.1 showed to have an a ribbon alignment in a herringbone fashion (Diagram 2.2) which is very common in aromatic molecules and this packing was due to the O2B-H2B…S1and C11-H11…O2B interaction.

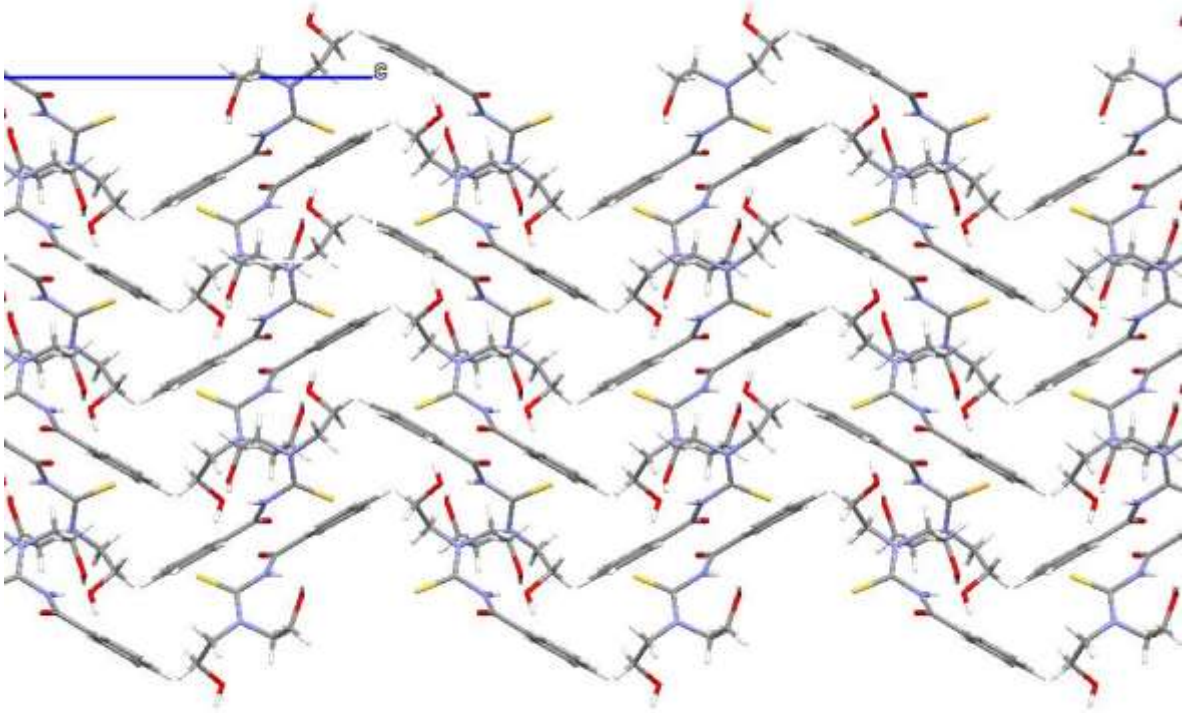
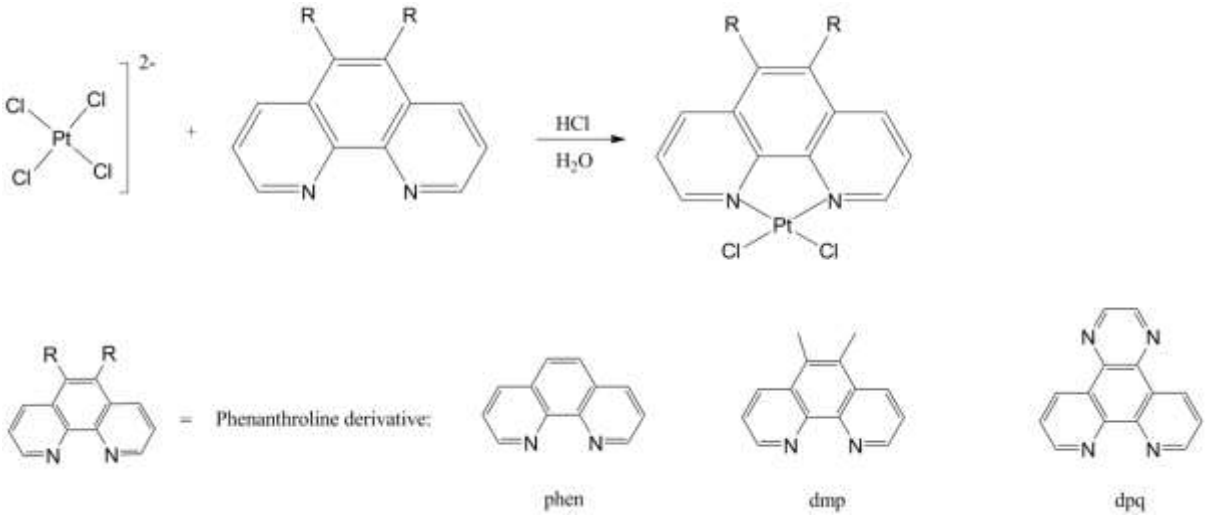


Diagram 2.2: *A view in project down the c axis of the unit-cell contents for compound 1showing the packing*

2.3 Synthesis and characterization of [$PtCl_2$(diimine)]

The [$PtCl_2$(diimine)] precursors were synthesised according to the procedure described by Morgan and Bustall.[6] This method involved the addition of the phenanthroline derivatives to a solution of potassium tetrachloroplatinate in acidic water at room temperature (Scheme 2.3). HCl was added to the reaction mixture to minimise the aquation of the $[PtCl_4]^{2-}$ species. The mixture was heated under reflux for 3 hours. A bright yellow precipitate was obtained for 1,10-phenanthroline and 5,6-dimethyl-1,10-phenanthroline while [pyrazino(2,3-f)(1,10-phenanthroline)] yielded an orange product. The product was washed several times with H_2O to remove any unreacted tetrachloroplatinate followed by 3 aliquots of acetonitrile to remove

any unreacted diimine, using a centrifuge setup. The desired precursors were obtained in good yields (81-91%) and were characterised using ^{1}H NMR Spectroscopy, FT-IR Spectroscopy, Mass Spectrometry and Elemental Analysis.



***Scheme 2.3**: Reaction scheme for the synthesis of [$PtCl_2$(diimine).* [6]

The [$PtCl_2$(dmp)] where dmp= 5,6-dimethyl-1,10-phenanthroline complex is used to illustrate the characterization methodology. The ^{1}H NMR spectrum of [$PtCl_2$(dmp)] revealed 4 sets of signals due to symmetry in the molecule (Figure 2.5).

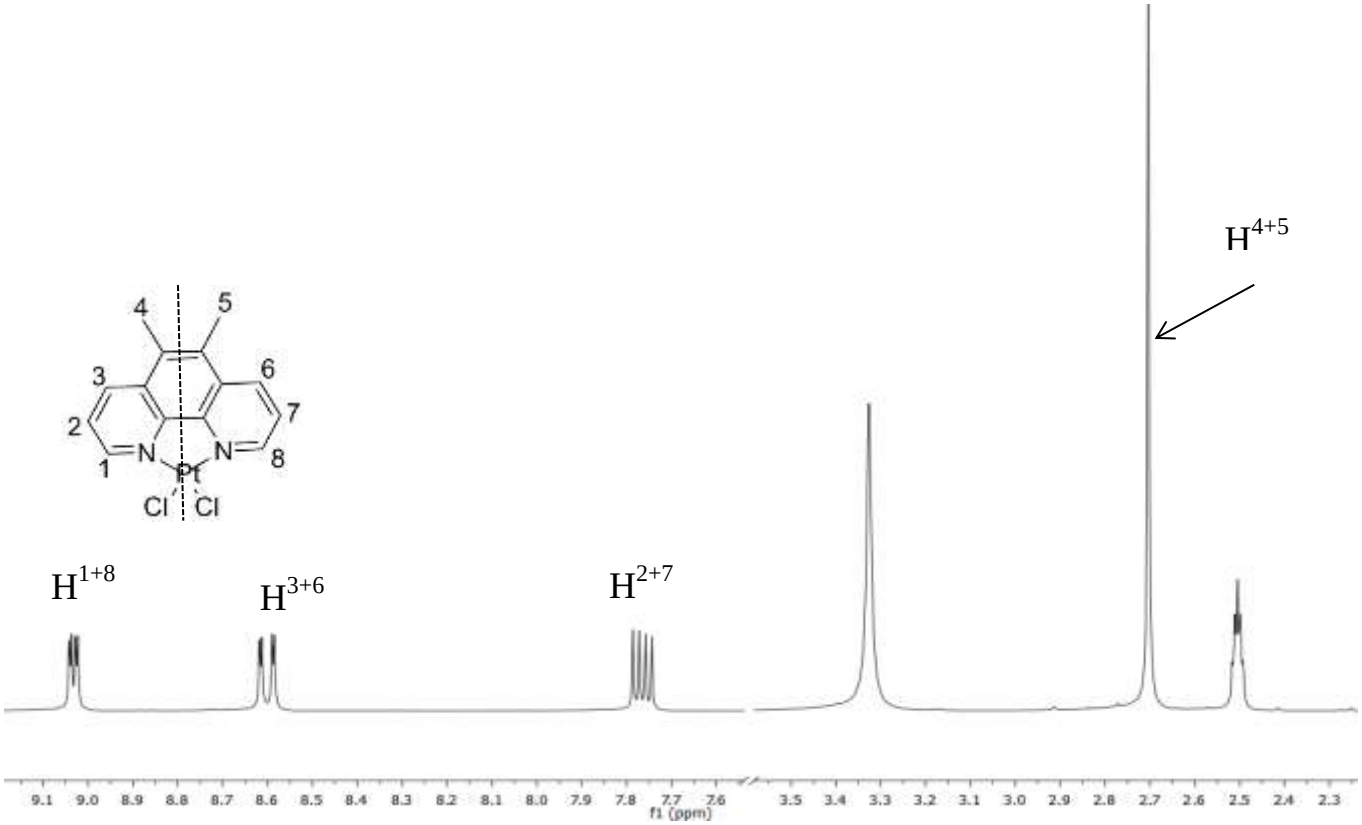


Figure 2.5*: ^{1}H NMR of [$PtCl_2$(dmp]) in DMSO at 25 °C (Note: the break in the horizontal scale between 3.5 and 7.5ppm)*

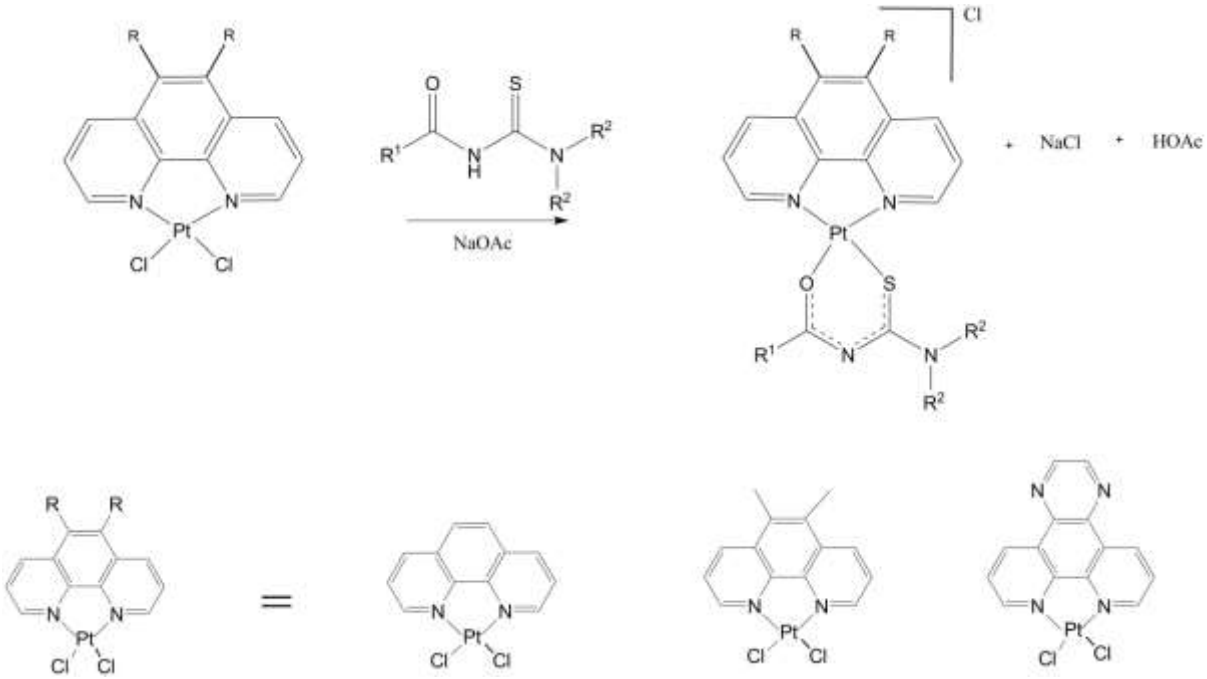
The most deshielded doublet of doublet at 9.10 ppm could be assigned to H^1 and H^8 due to its proximity to the nitrogen atoms. Two doublets of doublet were observed, of which the one have shown a two large coupling constant which was assigned to H^7 and H^2, since they exhibit three-bond coupling (J^3) to proton H^6 and H^3.The last doublet of doublet could be assigned H^3 and H^6 at 8.60 ppm because they will have a J^3 coupling to H^2 and H^7 and a J^4 coupling to H^1 and H^8. The CH_3 (H^4 and H^5) were identified as singlet at 2.75 ppm, which integrated for 6 protons.

The complex was also characterised by FT-IR Spectroscopy and the C=N stretch was found 1616.62 cm^{-1}, while aromatic C-H stretches was found at 122.17 cm^{-1}. The Elemental Analysis of the complex was in agreement with the calculated C (35.46), H (2.55), and N (5.91) %. Analytical results were C (35.12), H (2.05) and N (5.88) %. The complex with the

molecular weight of 474.24 g/mol. Different molecular masses were observed due to the isotopes from the chlorine (^{35}Cl and ^{37}Cl), also platinum (^{195}Pt, ^{197}Pt,^{196}Pt and ^{198}Pt). The masses were ionised by potassium and they were found to be 513.4, 515.2, 516.2, 517.2, 518.2, 520.3 and 522.4 (See Appendix). All these results obtained confirmed the formation of [$PtCl_2$(phen)] complexes.

2.4 Synthesis and characterization of [Pt(diimine)(L-O,S)]$^+$complexes

The platinum complexes of *N*,*N*-di(alkyl)-*N*-acylthiourea were synthesised using a modification of the method described by Koch *et al.*[7] (Scheme 2.3). One mole equivalent of [$PtCl_2$(diimine)] was dissolved in acetonitrile and mixed with excess of sodium acetate. The reaction was heated under reflux for 30 minutes. One equivalent of the ligand was added in acetonitrile and the reaction mixture was further heated under reflux for 24 hours. The reaction mixture was then filtered in order to remove excess sodium acetate. The filtrate was then subjected to the rotary evaporator in order to remove the solvent until a highly concentrated solution mixture was obtained. The mixture obtained was subjected to diethyl ether to allow for selective precipitation of the complex and it was kept in the freezer overnight to maximise the yield.



***Scheme 2.4**: Reaction scheme of the synthetic procedure of [Pt(diimine)(L-O,S)]$^+$ complexes* [7]

In the case of [$PtCl_2$(dpq)] and [$PtCl_2$(dmp)], the complexes were synthesized with some modifications to the general method. The [Pt(diimine)(L-*O*,*S*)]$^+$ complexes were obtained by reacting the ligand and sodium acetate (1:2) in acetonitrile first to deprotonate the ligand. The reaction mixture was heated under reflux for one hour. One equivalent of the [$PtCl_2$(diimine)] in acetonitrile was added and the reaction mixture was heated under reflux for 8 hours or longer depending on the ligand and the precursor. The solution was concentrated with a rotary evaporation and added to diethyl ether or hexane at -20 °C and stored in the freezer to allow for selective precipitation of the desired product. In the case of [Pt(dpq)(*N*,*N*-dibutyl-*N'*-acylthiourea)] complex, a stronger base than sodium acetate, trimethylamine was used to deprotonate the ligand. In an attempt to synthesis the [Pt(phen)(*N*,*N*-di(2-hydroxyethyl)-*N*'-acylthiourea)]$^+$using different bases (Sodium acetate, Triethylamine) to deprotonate the ligands, and using different solvent combinations (diethyl ether, hexane, dichloromethane, chloroform and ethanol) to crystallise the desired complex (see Experimental Section), the synthesis was still found unsuccessful. The [Pt(phen)(*N*,*N*-di(2-hydroxyethyl)-*N*'-

acylthiourea)] was attempted several times, even when the ligand was reacted with the base for 3 hours then adding the [$PtCl_2$(phen)] to the reaction and running the reaction for 8 hours instead of overnight without success and is still subject to future study.

The complexes that were successfully synthesised and characterised are shown in Figure 2.6.

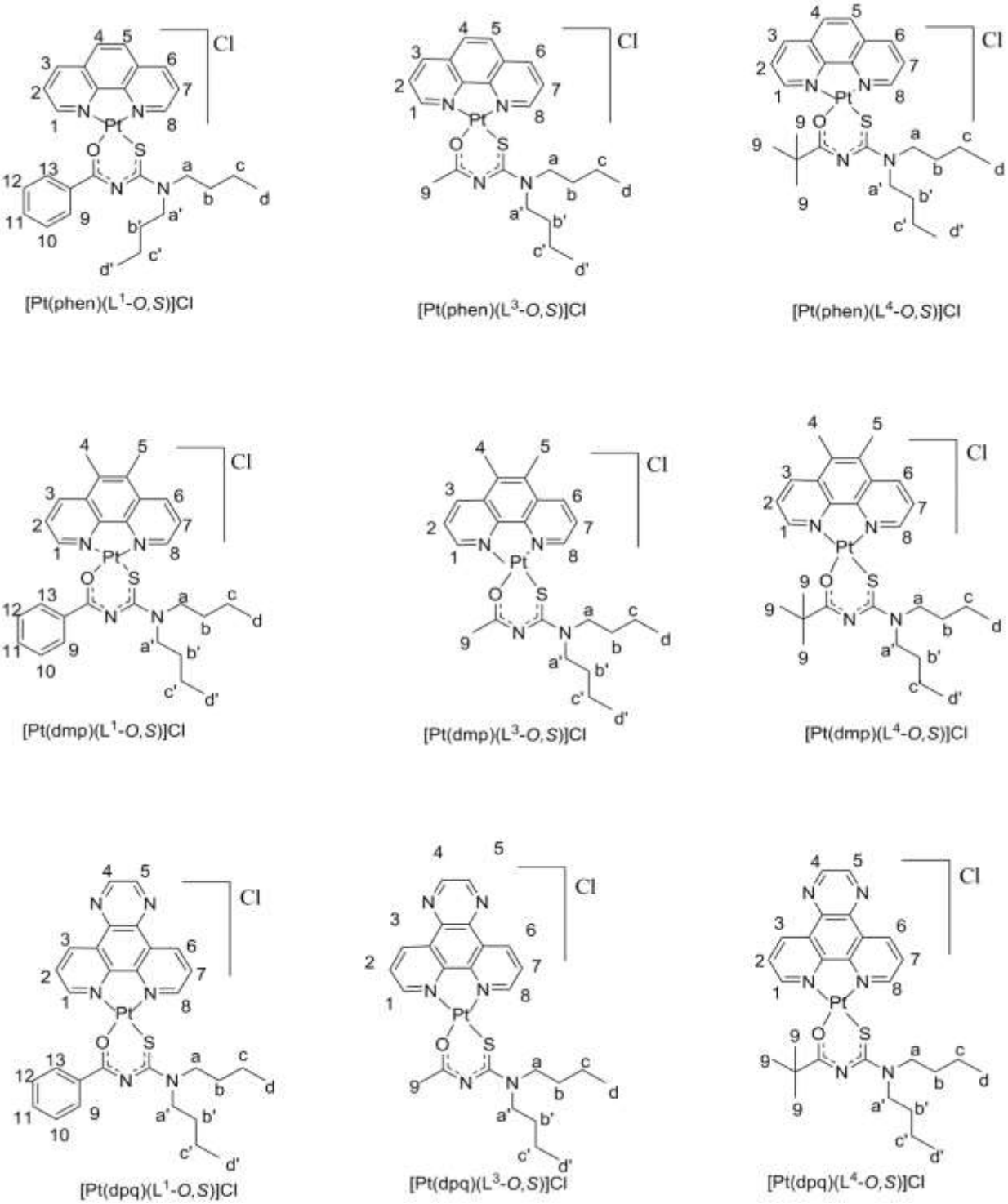


Figure 2.6: *[Pt(diimine)(L^n-O,S)]Cl Complexes synthesised with abbreviation of the names.*

2.2.3.1 ^{1}H–NMR, FT-IR and Mass spectrometry characterization of [Pt(phen)(L-*O*,*S*)]$^{+}$ complexes

The structural characterization of the [Pt(diimine)(L-*O*,*S*)]$^{+}$ included NMR Spectroscopy, FT-IR Spectroscopy, Mass Spectrometry. The ^{1}H NMR showed the absence of the hydrogen due to the NH which indicated that the ligand was deprotonated. The proton due to the Pt precursor showed four sets of resonance signals due to the symmetry; however the synthesised complex showed all 8 sets resonance signals simply because the synthesised complexes had no plane of symmetry (Figure 2.7).

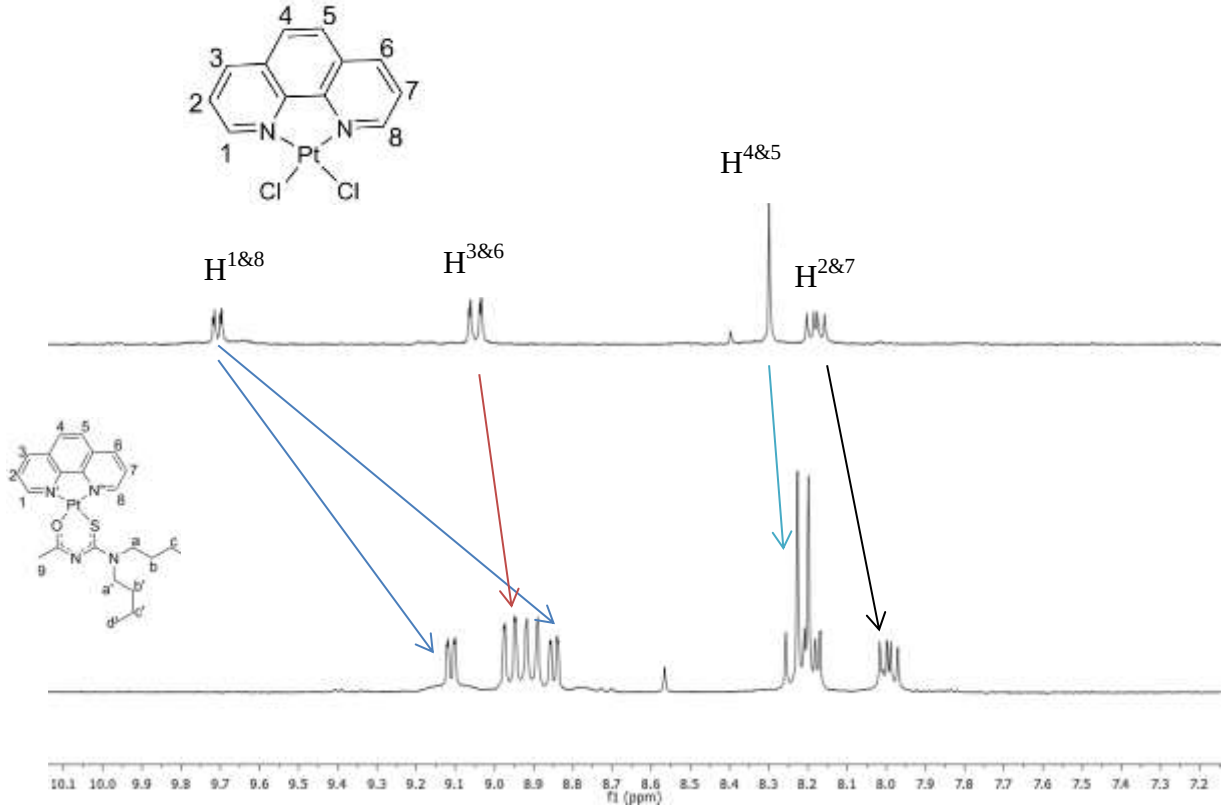


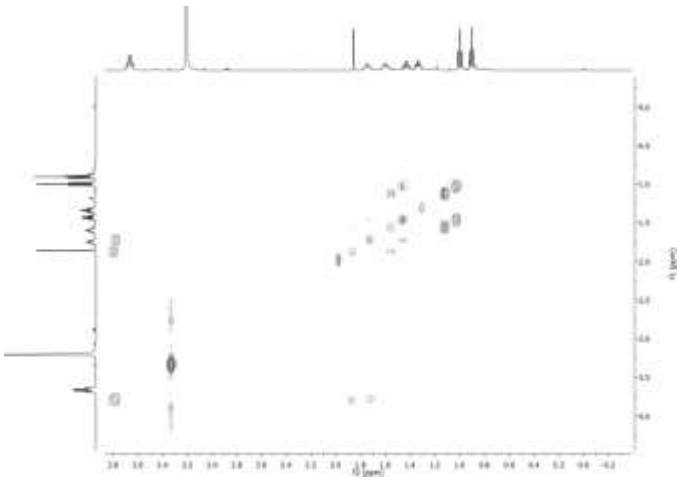
Figure 2.7: *The aromatic region ^{1}H NMR of [Pt(phen)(L^{3}-O,S)]Cl and the precursor [PtCl$_2$(phen)] showing the loss of symmetry upon coordination of HL^{3}.*

In order to assign the protons on the spectra obtained, several two-dimensional NMR experiments were conducted. The complex [Pt (phen)(L^{3}-*O*,*S*)]Cl will be used to illustrate the assignment of the ^{1}H NMR. For the assignment to be made, an assumption that the

nitrogen trans to the sulphur atom is N' and the most deshielded proton resonance signal is H^1 as proved by NOE data by Kotze *et al.*[8]

Given the assumption made, the assignment of all the protons of $[Pt^{II}(phen)(L^3\text{-}O,S)]Cl$ are possible using the correlations obtained from the COSY Spectrum (Figure 2.8). For clarity the aliphatic and aromatic region were enlarged in Figure 2.8a&b

(a)



(b)

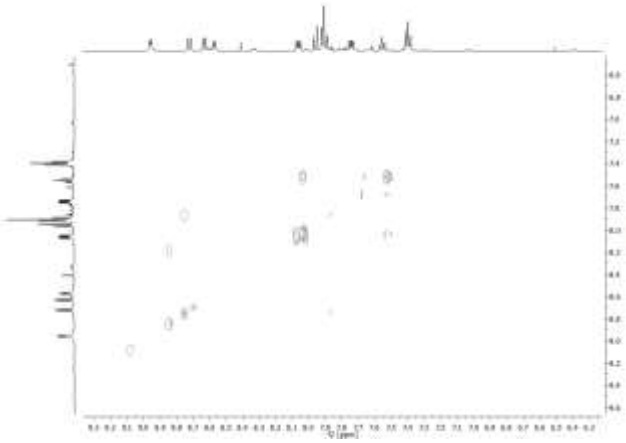


Figure 2.8: *^1H COSY of $[Pt(phen)(L^3\text{-}O,S)]Cl$ in methanol-d_4 at 25 °C where (**a**) aliphatic region (**b**) aromatic region.*

The protons H^4 and H^5 at 8.01 ppm were observed closely to each other but not overlapping due to the second order coupling were found and they were labelled $H^{4+5.}$ Furthermore, there were no overlapping peaks for the phenanthroline that was observed. In order to make the full assignment of the complex [Pt (phen)(L^3-*O,S*)]Cl, an assumption had to be made regarding the most deshielded proton. With these assumptions, full assignment of the proton spectrum were done as explained previously on the $[PtCl_2(diimine)]$ complexes using the ^1H NMR and COSY correlation.

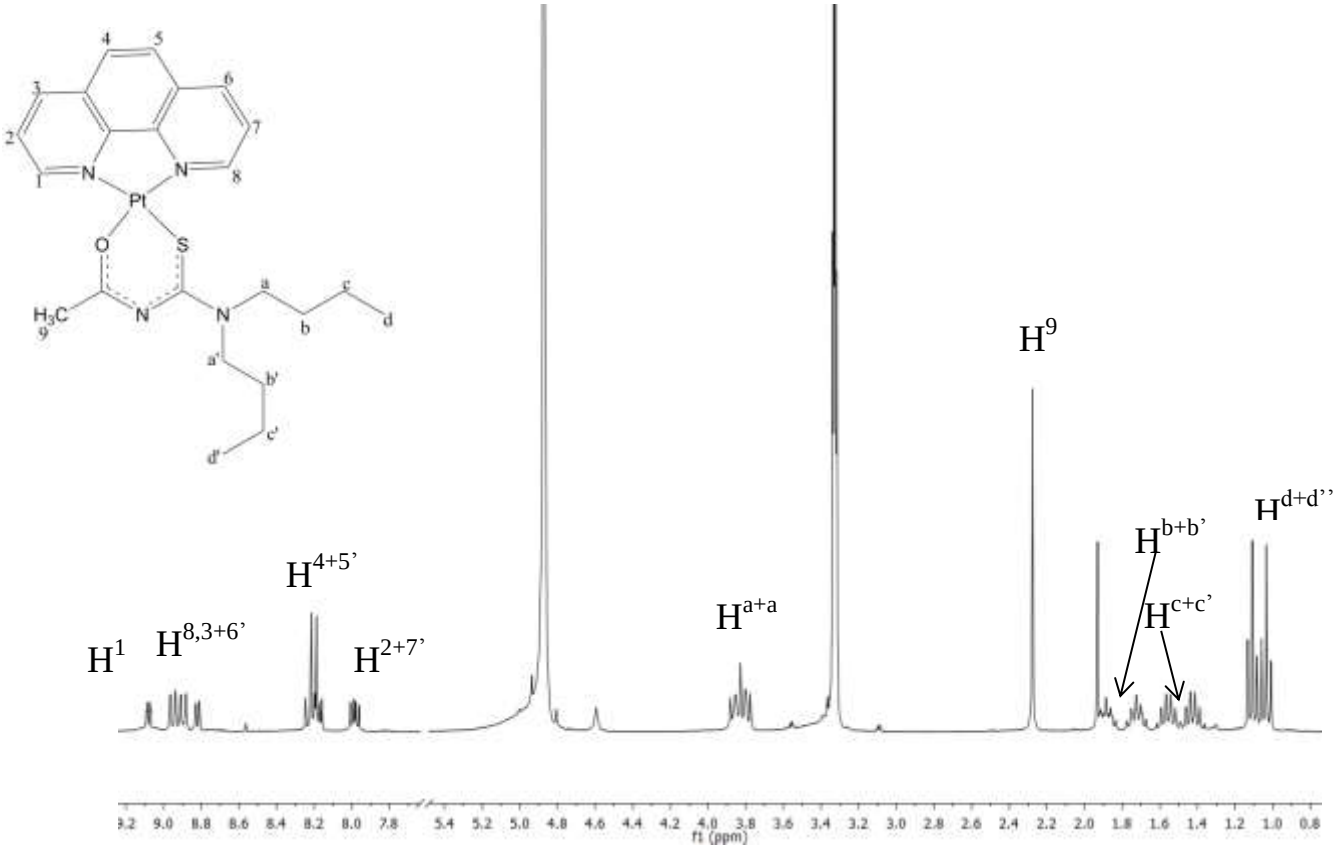


Figure 2.9: *^{1}H NMR spectrum full assignment of [Pt(phen)(L^{3}-O,S)]Cl in acetonitrile-d_3 at 25 °C. (Note: the break on the horizontal scale between 5.4 and 7.7 ppm.*

The complexes were further characterised by FT-IR Spectroscopy, when characterised by FT-IR it was observed that the full C=O and C=S stretching modes were no longer observed in the higher frequency stretching mode as they shift to lower frequency stretching modes. A typical semi C=O and C=S stretching modes were observed. The absence of the NH band in the FT-IR spectra of the metal complexes provided evidence that a bindate cationic complex was obtained. In the case of Mass Spectrometry (see apendix), it was observed that the parent ions for the complexes were observed as $[M+K]^{+}$. In addition, all the isotopes of platinum and chlorine were observed

2.5 Experimental Section

2.5.1 Materials and Instrumentation

All reagents were commercially available and used without further purification. Acetone was dried with K_2CO_3 as a drying agent and distilled prior to use. Commercially available acyl chlorides, amines, and potassium thiocyanate were used to synthesise *N*,*N*-di(alkyl)-*N'*-acylthiourea ligands. $K_2[PtCl_4]$ from Sigma-Aldrich and SA Precious Metals with corresponding phenanthroline derivatives were used to synthesise the complexes. NMR experiments were performed on the Bruker Avance 300MHz and Bruker Avance III 500MHz respectively. The Infrared spectra were recorded on a Bruker FT-IR Spectrophotometer. Mass Spectrometry was performed using a Advion Expression L Compact. Elemental Analysis was performed using a Flash 2000 CHNS-O Analyser fitted with an auto sampler.

2.5.2 General procedure for the synthesis of *N*,*N*-di(alkyl)-*N'*-acylthiourea

The N,N-di(alkyl)-N'-acylthiourea were synthesised according to the method described by Douglass and Dains.[3] The synthetic procedure is described using the synthesis of HL^1 as an example. To a round-bottomed flask containing potassium thiocyanate (31.8 mmol) and dry acetone (75 ml), benzoyl chloride (30.8 mmol) was added. The resultant cloudy-white reaction mixture was heated to reflux for 3 hours and cooled to room temperature. A slow-dropwise addition of dibutylamime (30.8 mmol) followed and the resultant solution was heated under reflux for 3 hours. During this time a white-yellow solution formed. The reaction mixture was poured into 100 ml distilled water and kept overnight in a fumehood to evaporate some of the acetone to allow for crystallisation of the product. The *N*,*N*-di(alkyl)-*N'*-acylthioureas were obtained as white to colourless solids in yields of 76- 96%. Several hydroxyl ligands were attempted with changes in the reaction conditions such as the use of hot acetone, acetonitrile and increase in the reaction time but it was found to be unsuccessful,

possibly due to several donor sites which could have led to different/ unwanted coordination product. In attempting these ligands, different solvents were used; the reaction time was increased from 3hours to overnight reflux. When the reaction mixture was obtained, different ways to crystallise the ligands were attempted, however thick orange oil was obtained. When purified by column chromatography, the oil was still found not to be the desired compound. However, HL^2 and HL^5 were successfully synthesised but could not be crystallised hence they were purified by column chromatography (silica gel). Pivaloyl ligand (HL^4), on the other hand, were successfully crystallised from the ice bath.

The ligands were characterised using ^1H, ^{13}C NMR Spectroscopy, FT-IR Spectroscopy, Mass Spectrometry and Elemental Analysis.

***N, N*-di(butyl)-*N'*-benzoylthiourea (HL^1)** : Yield 76% , m.p. 93-95 °C, ^1H NMR (300 MHz, [d_6] DMSO, 25 °C), δ(ppm) = 10.59 (s, 1H: NH),7.90 (m, 2H: H^1,H^5), 7.51(m, 2H : H^2,H^4), 7.60 (tt, 1H: H^3), 3.92 (t, 2H: H^a), 3.44 (t, 2H: $H^{a'}$), 1.70 (m(q), 2H: H^b), 1.61 (m, 2H: $H^{b'}$), 1.37(m(h), 2H: H^c), 1.21 (m(h), 2H: $H^{c'}$), 0.94 (t, 3H: H^d), 0.82(t, 3H: $H^{d'}$) ; ^{13}C NMR (300 MHz, DMSO, 25 °C) δ(ppm) = 180.93 (C=S), 163.80 (C=O), 128.40 (C^1,C^5), 127.97 (C^2,C^4), 132.19 (C^3), 52.41 (C^a), 51.60 ($C^{a'}$), 29.74 (C^b), 27.80 ($C^{b'}$), 19.47 (C^c), 19.35 ($C^{c'}$), 13.75 (C^d), 13.45 ($C^{d'}$), 132.96 (C^6). IR : ν(cm^{-1}) 1372 (C=S), 1685.78 (C=O), 3166.93 (NH); elemental analysis calculated (%) for $C_{16}H_{24}N_2OS$: C 65.71 H 8.27 N 9.58 S 10.96 , found : C 65.72 H 8.19 N 9.61 S 10.73.

***N, N*-di(hydroxy)-*N'*-benzoylthiourea (HL^2)** : Yield 78% , m.p. 118-120 °C, ^1H NMR (300 MHz, [d_6] DMSO, 25 °C), δ(ppm) = 10.87 (s, 1H: NH), 7.87(m, 2H: H^1,H^5), 7.50(m, 2H : H^2,H^4), 7.60 (tt, 1H: H^3), 3.99 (t, 2H: H^a), 3.77 (unres m , 6H: $H^{a'}$, H^b, $H^{b'}$), H^b), 5.66 (s, 1H: H^c), 4.88 (t, 1H: $H^{c'}$); ^{13}C NMR (300 MHz, DMSO, 25 °C) δ(ppm) = 181.44 (C=S), 164.465(C=O), 120.51 (C^1,C^5), 127.79 (C^2,C^4), 132.25 (C^3), 55.03 (C^a), 54.95 ($C^{a'}$),

59.17(C^{b}), 57.52 ($C^{b'}$), 133.48 (C^{6}). IR : ν(cm^{-1}) 1300.80 (C=S), 1693.83 (C=O), 3165.83 (NH,OH); elemental analysis calculated (%) for $C_{10}H_{20}N_2O_3S$: C 53.71 H 6.01 N 10.44, found : C 53.69 H 5.93 N 10.39.

***N, N*-di(butyl)-*N*'-acetylthiourea (HL^3)** : Yield 84% , m.p. 56-58 °C, ^{1}H NMR (300 MHz, [d_6] DMSO, 25 °C), δ(ppm) = 10.23 (s, 1H: NH),1.95 (s, 3H: H^1), 3.83 (t, 2H: H^a), 3.35 (t, 2H: $H^{a'}$), 1.62 (m(q), 2H: H^b), 1.52 (m(q), 2H : $H^{b'}$), 1.33(m, 2H: H^c), 1.21 (m, 2H: $H^{c'}$), 0.88 (dt, 6H: H^d ,$H^{d'}$); ^{13}C NMR (300 MHz, DMSO, 25 °C) δ(ppm) = 180.28 (C=S), 166.79 (C=O), 22.982 (C^1), 51.979 (C^a), 51.636 ($C^{a'}$), 29.543 (C^b), 27.874 ($C^{b'}$), 19.470 (C^c), 19.356 ($C^{c'}$), 13.678 (C^d), 13.467 ($C^{d'}$), 132.958 (C^6). IR : ν(cm^{-1}) 1416.16 (C=S), 1661.59 (C=O), 3166.93 (NH); elemental analysis calculated (%) for $C_{11}H_{22}N_2OS$: C 57.35 H 9.63 N 12.16 S 13.92 , found : C 56.61 H 9.36 N 12.25 S 13.59.

***N, N*-di(butyl)-*N*'-pivaloylthiourea (HL^4)** : Yield 96% , m.p. 88-90 °C, ^{1}H NMR (300 MHz, [D6] DMSO, 25 °C), δ(ppm) = 10.23 (s, 1H: NH),1.95 (s, 9H: H^1), 3.85 (t, 2H: H^a), 3.31 (q, 2H: $H^{a'}$), 1.63 (m(q), 2H: H^b), 1.54 (m(q), 2H : $H^{b'}$), 1.33(m, 2H: H^c), 1.21 (m, 2H: $H^{c'}$), 0.88 (dt, 6H: H^d ,$H^{d'}$); ^{13}C NMR (300 MHz, DMSO, 25 °C) δ(ppm) = 181.462 (C=S), 174.7373 (C=O), 26.518 (C^1), 52.451 (C^a), 51.575 ($C^{a'}$), 29.770 (C^b), 27.814 ($C^{b'}$), 19.421 (C^c, $C^{c'}$), 13.730 (C^d), 13.512 ($C^{d'}$), 132.958 (C^6). IR : ν(cm^{-1}) 1368.19 (C=S), 1654.13 (C=O), 3302.58 (NH); elemental analysis calculated (%) for $C_{14}H_{28}N_2OS$: C 61.72 H 10.36 N 8.78 , found : C 61.73 H 10.72 N 10.28.

2.5.3 General procedure for the synthesis of [$PtCl_2$(diimine)]

The platinum precursors were synthesised according to the method described by Morgan and Bustall *et al*.[6] The synthetic procedure is described using the first precursor [Pt(1,10-phenanthroline)Cl_2] as an example. To a round-bottomed flask containing a solution of K_2PtCl_4 (0.3614 mmol) in 40 ml distilled water, an equivalent one molar of 1,10-

phenanthroline was added and 6 drops of HCl were added. The reaction mixture was heated under reflux for 2 hours until a yellow precipitate was obtained. The reaction mixture was cooled to room temperature. The desired precursor was washed with acetonitrile and water via centrifuge system and dried under vacuum.

The resultant products ware characterised by ^{1}H NMR Spectroscopy, FT-IR Spectroscopy, Mass Spectrometry and Elemental Analysis.

[$PtCl_2$(1,10-phenanthroline)]: Yield 91.1%. ^{1}H NMR (300MHz, [d_6] DMSO, 25 °C) δ (ppm) = 9.71 (dd, 2H: H^1, H^8), 9.05 (dd, 2H: H^3,H^6), 8.30 (s, 2H: H^4,H^5), 8.17 (dd, 2H: H^2,H^7); IR: ν(cm-^{1}) 3057.16 (arom C-H), 1208.02 (arom C-C), 1627.05 (arom C=N); EIS (+) Mass Spectrometry ,485.1,[$PtCl_2$(phen)]; elemental analysis (%) calculated for $C_{12}H_8Cl_2N_2Pt$ C 32.30 H 1.81 N 6.28 ; found : C 32.16 H 1.72 N 6.27.

[$PtCl_2$(5,6-dimethy-1,10-phenanthroline)]: Yield 81.8%. ^{1}H NMR (300MHz, [d_6] DMSO, 25 °C) δ (ppm) = 9.10 (dd, 2H: H^1, H^8), 8.70 (dd, 2H: H^3,H^6), 7.75(dd, 2H: H^2,H^7), 2.75 (s, 6H: H^4,H^5); IR: ν(cm^{-1}) 3077.46 (arom C-H), 1222.17 (arom C-C), 1616.62 (arom C=N); EIS (+) Mass Spectrometry ,513,[$PtCl_2$(dmp)]; elemental analysis (%) calculated for $C_{14}H_{12}Cl_2N_2Pt$ C 35.46 H 2.55 N 5.91 ; found : C 35.12 H 2.05 N 5.88.

[$PtCl_2$(pyrazino(2,3-f)(1,10-phenanthroline)]: Yield 90.4%. ^{1}H NMR (300MHz, [d_6] DMSO, 25 °C) δ (ppm) = 9.50 (dd, 2H: H^1, H^8), 9.25 (dd, 2H: H^3,H^6), 9.20 (s, 2H: H^4,H^5), 8.00 (dd, 2H: H^2,H^7); IR: ν(cm^{-1}) 3078.63 (arom C-H), 1213.99 (arom C-C), 1613.37 (arom C=N); EIS (+) Mass Spectrometry,537.0,[$PtCl_2$(dpp)] elemental analysis (%) calculated for $C_{14}H_{12}Cl_2N_2Pt$ C 33.75 H 1.62 N 11.24 ; found : C 34.62 H 1.65 N 11.28.

2.5.4 General procedure for the synthesis of [Pt(diimine)(L-O,S)]$^{+}$complexes

A suspension of [Pt(diimine)Cl_2] (33.6 mmol) and excess sodium acetate (67.2 mmol) in 40 ml acetonitrile was heated under reflux for 30 minutes . An appropriate *N*,*N*-di(alkyl)-*N*'-acylthiourea in 10 ml of acetonitrile was heated under reflux overnight. The resultant yellow-orange mixture was filtered to remove any unreacted sodium acetate. The filtrate was concentrated by the evaporation of the solvent. The concentrated solution was added to diethyl ether (20 ml) and left in the freezer overnight. The resultant precipitate from diethyl ether was added to dichloromethane and filtered to remove unreacted sodium acetate that may be remaining. The filtrate was again concentrated and added to diethyl ether and re-suspended several times. The yellow product was dried for 5-10 hours under vacuum.

The successful resultant products were characterised by ^{1}H NMR Spectroscopy, 2D NMR Spectroscopy, FT-IR Spectroscopy and Mass Spectrometry.

[Pt(phen)(L^{1}-*O,S*)]Cl : ^{1}H NMR (300MHz,[d_3]CD_3CN, 25 °C) δ(ppm) = 9.28 (d, 1H: H^{1}), 8.95 (d, 1H: H^{8}), 8.87(td, 2H: H^{4},H^{5}), 8.20 (unres aromatic, 5H: H^{9},H^{10},H^{11},H^{12},H^{13}), 7.96(m(ddd) ,1H: H^{3}), 7.69(m, 1H: H^{6}), 7.55(td, 2H: H^{2},H^{7}), 3.86(m, 4H: H^{a},$H^{a'}$), 1.90(m, 2H: H^{b}), 1.76(m, 2H: $H^{b'}$), 1.57(m, 2H: H^{c}), 1.47(m, 2H: $H^{c'}$), 1.12(td, 3H: H^{d}), 1.02(td, 3H: $H^{d'}$); IR:ν(cm^{-1}) 1203.98 (C-S), 1368.96 (C-O); ESI (+) Mass Spectromentry,667.3,[Pt(phen)(L^{1}-*O,S*)]$^{+}$

[Pt(phen)(L^{3}-*O,S*)]Cl : ^{1}H NMR (300MHz,[d_3]CD_3CN, 25 °C) δ(ppm) = 9.07(dd, 1H: H^{1}), 8.95 (dd, 1H: H^{8}), 8.90 (dd, 1H: H^{3}), 8.82 (dd , 1H: H^{6}),8.20(m, 3H: H^{4},H^{5},H^{2}), 7.99(dd, 1H: H^{7}), 2.28 (s, 3H: H^{9}), 3.83(m, 2H: H^{a}), 3.83(m, 2H: $H^{a'}$) 1.87 (m, 2H: H^{b}), 1.72 (m, 2H: $H^{b'}$), 1.55(m(h), 2H: H^{c}), 1.44 (m, 2H: $H^{c'}$), 1.11(t, 3H: H^{d}), 1.03(t, 3H: $H^{d'}$); IR:ν(cm^{-1}) 1259.00 (C-S), 1369.00 (C-O); ESI (+) Mass Spectromentry,605.3,[Pt(phen)(L^{3}-*O,S*)]$^{+}$

[Pt(phen)(L^4-*O,S*)]Cl : ^{1}H NMR (300MHz,[d_3]CD_3CN, 25 °C) δ(ppm) = 9.35 (dd, 1H: H^1), 9.27 (m, 1H: H^8), 8.97(m, 2H: H^4,H^5), 8.25(m, 3H: H^3,H^6,H^2), 8.03(m, 1H: H^7), 1.90(s, 9H: H^9), 3.93(m, 2H: H^a), 3.84(m, 2H: $H^{a'}$) 1.73(m, 2H: H^b), 1.56(m, 2H: $H^{b'}$), (m, 2H: H^c), (m, 2H: $H^{c'}$), 1.10(td, 3H: H^d), 1.02(td, 3H: $H^{d'}$); IR:ν(cm^{-1}) 1220.00 (C-S), 1431.00 (C-O); ESI (+) Mass Spectromentry,647.2,[Pt(phen)(L^4-*O,S*)]$^+$

[Pt(dmp)(L^1-*O,S*)]Cl : ^{1}H NMR (300MHz,[d_3]CD_3CN, 25 °C) δ(ppm) = 9.15 (d, 2H: H^1,H^8),9.06(d, 1H: H^3),8.83 (d, 1H: H^6), 8.34(dd, 1H: H^2), 8.15(m, 2H: H^9,H^{13}), 8.03(dd, 1H: H^7), 7.72(t, 1H: H^{11}), 7.58(t, 2H: H^{10},H^{12}) 2.74(d, 6H: H^4,H^5) 3.85(m(q), 4H: H^a,$H^{a'}$), 1.84(m, 2H: H^b), 1.67(m, 2H: $H^{b'}$), 1.50(m(q), 2H: H^c), 1.39(m(q), 2H: $H^{c'}$), 1.05(t, 3H: H^d), 0.95(t,3H: $H^{d'}$); IR:ν(cm^{-1}) 1216.99 (C-S), 1397.17 (C-O); ESI (+) Mass Spectromentry,695.3,[Pt(dmp)(L^1-*O,S*)]$^+$

[Pt(dmp)(L^3-*O,S*)]Cl : ^{1}H NMR (300MHz,[d_3]CD_3CN, 25 °C) δ(ppm) = 9.08 (m, 2H: H^1, H^8), 8.99 (d, 1H: H^3), 8.73(d, 1H: H^6), 8.20(dd, 1H: H^2), 8.02(dd, 1H: H^7), 2.80(d, 6H: H^4,H^5) , 2.29(s,3H: H^9), 3.84(t, 2H: H^a),3.75(t, 2H: $H^{a'}$) 1.81(m, 2H: H^b), 1.64(m(p), 2H: $H^{b'}$), 1.50(m(p), 2H: H^c), 1.34(m(q), 2H: $H^{c'}$), 1.03(t, 3H: H^d), 0.95(t, 3H: $H^{d'}$); IR:ν(cm^{-1}) 1208.13 (C-S), 1422.96 (C-O); ESI (+) Mass Spectromentry,633.1,[Pt(dmp)(L^3-*O,S*)]$^+$

[Pt(dmp)(L^4-*O,S*)]Cl : ^{1}H NMR (300MHz,[d_3]CD_3CN, 25 °C) δ(ppm) = 9.12 (d,1H: H^1), 8.99 (d,1H: H^8), 8.91(d,1H: H^3) 8.81(d,1H: H^6), 8.15(dd,1H: H^2), 7.89(dd,1H:H^7), 2.80(d,6H: H^4,H^5) , 1.39(s,9H: H^9), 3.88(m,2H: H^a),3.80(m,2H:$H^{a'}$) 1.75(m,2H: H^b), 1.75(m,2H: $H^{b'}$), 1.54(m,2H: H^c), 1.54(m,2H: $H^{c'}$), 1.09(t,3H:H^d), 0.99(t,3H: $H^{d'}$); IR:ν(cm^{-1}) 1221.76 (C-S), 1359.13 (C-O); ESI (+) Mass Spectromentry,675.3,[Pt(dmp)(L^4-*O,S*)]$^+$

[Pt(dpq)(L^1-*O,S*)]Cl : ^{1}H NMR (300MHz,[d_3]CD_3CN, 25 °C) δ(ppm) = 9.70 (d, 1H: H^1), 9.61(d, 1H: H^8), 9.14(s, 2H: H^4,H^5), 8.87 (d, 1H: H^3), 8.37(m, 2H: H^2,H^7), 8.00(d, 3H: H^6,H^9,H^{13}), 7.64(d, 1H: H^{11}), 7.48(t, 2H: H^{10},H^{12}) ,3.78(m, 4H: H^a,$H^{a'}$), 1.70(m, 4H: H^b,$H^{b'}$),

1.57(m, 4H: H^{c},$H^{c'}$), 1.11(t, 3H: H^{d}), 1.01(t, 3H: $H^{d'}$); IR:ν(cm^{-1}) 1222.00 (C-S), 1383.12 (C-O); ESI (+) Mass Spectromentry,719.2,[Pt(dpq)(L^{1}-*O*,*S*)]$^{+}$

[Pt(dpq)(L^{3}-*O*,*S*)]Cl : ^{1}H NMR (300MHz,[d_3]CD_3CN, 25 °C) δ(ppm) = 9.81 (dd, 1H: H^{1}), 9.75(dd, 1H: H^{8}), 9.26(dd, 2H: H^{4},H^{5}), 8.91 (dd, 1H: H^{3}), 8.33(dd, 1H:H^{6}), 8.10(dd ,2H: H^{2},H^{7}) 3.65(m, 4H: H^{a},$H^{a'}$), 1.70(m, 4H: H^{b},$H^{b'}$), 1.50(m, 2H: H^{c}), 1.40(m, 2H: $H^{c'}$), 1.11(t, 3H: H^{d}), 1.00(t, 3H: $H^{d'}$); IR:ν(cm^{-1}) 1304.08 (C-S), 1381.53 (C-O); ESI (+) Mass Spectromentry,657.3,[Pt(dpq)(L^{3}-*O*,*S*)]$^{+}$

[Pt(dpq)(L^{4}-*O*,*S*)]Cl : ^{1}H NMR (300MHz,[d_3]CD_3CN, 25 °C) δ(ppm) = 9.82 (d, 1H: H^{1}), 9.76(d, 1H: H^{8}), 9.47(dd, 1H: H^{3}), 9.39(s, 2H: H^{4},H^{5}), 9.24(dd, 2H: H^{2},H^{7}), 7.96(dd, 1H: H^{6}), 1.35(s, 9H: H^{9}) 3.89(m, 2H: H^{a}), 3.78(m, 2H: $H^{a'}$), 1.83(m, 2H: H^{b}), 1.66(m, 2H: $H^{b'}$), 1.50(m(q), 2H: H^{c}), 1.50(m(q), 2H: $H^{c'}$), 1.03(t, 3H:H^{d}), 0.94(t, 3H: $H^{d'}$); IR:ν(cm^{-1}) 1222.73 (C-S), 1382.08 (C-O); ESI (+) Mass Spectromentry,699.1,[Pt(dpq)(L^{4}-*O*,*S*)]$^{+}$

2.5.5 X-ray crystallography of HL^2

The Bruker SMART II CCD area detector diffractometer with a graphite monochromated Mok/a radiation (50Kv,30 m A) was used to collect all the intensity data. Crystal structures of *N,N*-di(2-hydroxyethyl)-*N'*-benzoylthiourea were collected at 296 K. The collection method involved ω- scans of width 0.3. Data reduction was carried out using the program SAINT. The crystal structures were solved in the WINGX suite of programs by direct methods using SHELXS- Gui Control. The structures were then refined by least-squares on weighted F2 value for all reflections. All non-hydrogen atoms were refined with anisotropic displacement parameters hence the hydrogen atoms attached to N and O atoms were located in the difference Fourier map and their coordinates refined freely with isotropic parameters 1.5 times those of the "heavy" atoms to which they are attached. All the C-H hydrogen atoms were placed at an idealised position and refined as riding atoms with isotropic parameters 1,2 times those of the "heavy" atoms to which they are attached. Diagrams and publication material were generated using ORTEP-3, and PLATON (11 & 12). Experimental details of the X-ray analysis are provided in Table 2.2.

***Table 2.2**: Crystal experimental details of HL*[2]

Crystal data	
Chemical formula	$C_{12}H_{16}N_2O3S$
M_r	268.33
Crystal system, space group	Orthorhombic, $P2_12_12_1$
Temperature (K)	296
a, *b*, *c* (Å)	7.3353 (5), 10.4232 (6), 17.4154 (11)
V (Å^3)	1331.53 (15)
Z	4
Radiation type	Mo *K*α
μ (mm^{-1})	0.25
Crystal size (mm)	0.58 × 0.42 × 0.09
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	6852, 3292, 2422
R_{int}	0.094
$(\sin \theta/\lambda)_{max}$ (Å^{-1})	0.667
Refinement	
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, *S*	0.049, 0.107, 0.93
No. of reflections	3292
No. of parameters	169
No. of restraints	0
H-atom treatment	H atoms treated by a mixture of independent and constrained refinement
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.41, −0.29
Absolute structure	Flack x determined using 751 quotients [(I+)-(I-)]/[(I+)+(I-)] (Parsons, Flack and Wagner, Acta Cryst. B69 (2013) 249-259).
Absolute structure parameter	0.09 (13)

Computer programs: *SHELXL2014*/7 (Sheldrick, 2014).

2.6 References

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Chapter 3

In Vitro Cytotoxicity Testing

Chapter 3

In Vitro Cytotoxicity Testing

3.1 Introduction

The success and limitations of cisplatin have sparked a considerable interest and research in the search for improved platinum-based drugs for the use of chemotherapeutic agents. Metallointercalators has been considered as one of the potential replacement, simply because they have shown to be active against cancer at much lower concentrations than cisplatin and they are also effective against cisplatin–resistant cancer cell lines.[1] The phenanthroline-based platinum complexes have shown reasonable cytotoxicity against an L1210 mouse leukaemia cell line. Even though the metallointercalors are thought to be very active against cancer, ancillary ligands do have some effect on the activity of the complex. *N*-*N*-di(alkyl)-*N'*-acylthioureas are thought to be an excellent ancillary ligand.[2]

It is therefore expected that this class of [Pt (diimine)(L^n-*O*,*S*)]Cl complexes to have activity against cancer because these complexes have structural features which allow for binding to the DNA in a number of ways which include DNA binding *via* the major / minor grooves or by partial intercalation.[1] The activity of these complexes may be assessed by various assays that provide information on cellular activity. One common method for screening potential anticancer activity is a simple MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide). The MTT is a colorimetric assay which measures the reduction of the yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase.[3] The MTT enters the cell and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilized with an organic solvent (e.g isopropanol) and released;

solubilised formazan reagent is measured spectrophotometrically. Since the reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells. The MTT is widely used in cytotoxicity studies for screening new anticancer compounds, due to its accuracy and relative simplicity. It is worth noting though that the MTT does not provide any information on the molecular mechanism of the cytotoxic activity of the drug.[3]

This chapter describes the pre-screens (2 doses) testing of all the complexes synthesised in this study for in *vitro* cytotoxicity. The testing was done at University of the Witwatersrand medical school by Dr Lionie Harmse using the MTT assay and was tested against the H1975 lung cancer cells. This cell line carries a double mutation that harbours the epidermal growth factor receptor (EGFR(T790M)) ,where the T790M mediates resistance to EGFR inhibitors by acting as a "gatekeeper" mutation bringing about the steric hindrance in the Adenosine Triphosphate (ATP) –binding pocket and preventing inhibitor binding.

3.2 MTT Assay to probe cytotoxicity

The standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was used to assess the cell metabolic activity of the series of [Pt(diimine)(L^n-*O*,*S*)]Cl complexes whose synthesis is described in Chapter 2. The experiments were carried out in 96 well plates with 8 rows and 12 columns and all addition and serial dilutions were made by hand using a multi-channel pipette. The cells were incubated overnight at 37 °C in 5% CO_2 for attachment. Thereafter, the cells were treated with MTT, and further incubated for 2 to 4 hours until purple precipitate visible. The cells were then solubilised with an organic solvent (isopropanol) and the plates were left out at room temperature in the dark for 2 hours. The absorbance was recorded at 570 mm. Each sample was assayed at least four times. The cytotoxicity index was determined using the untreated cells as negative control. The cell

viability was calculated using a background-corrected absorbance according to the following equation:

$$\% \text{ cell viability} = \frac{(1 - absorbance\ of\ experimental\ wells)}{absorbance\ of\ negative\ control\ wells} \times 100$$

The resultant plates of the compounds tested are shown in Figure 3.1.

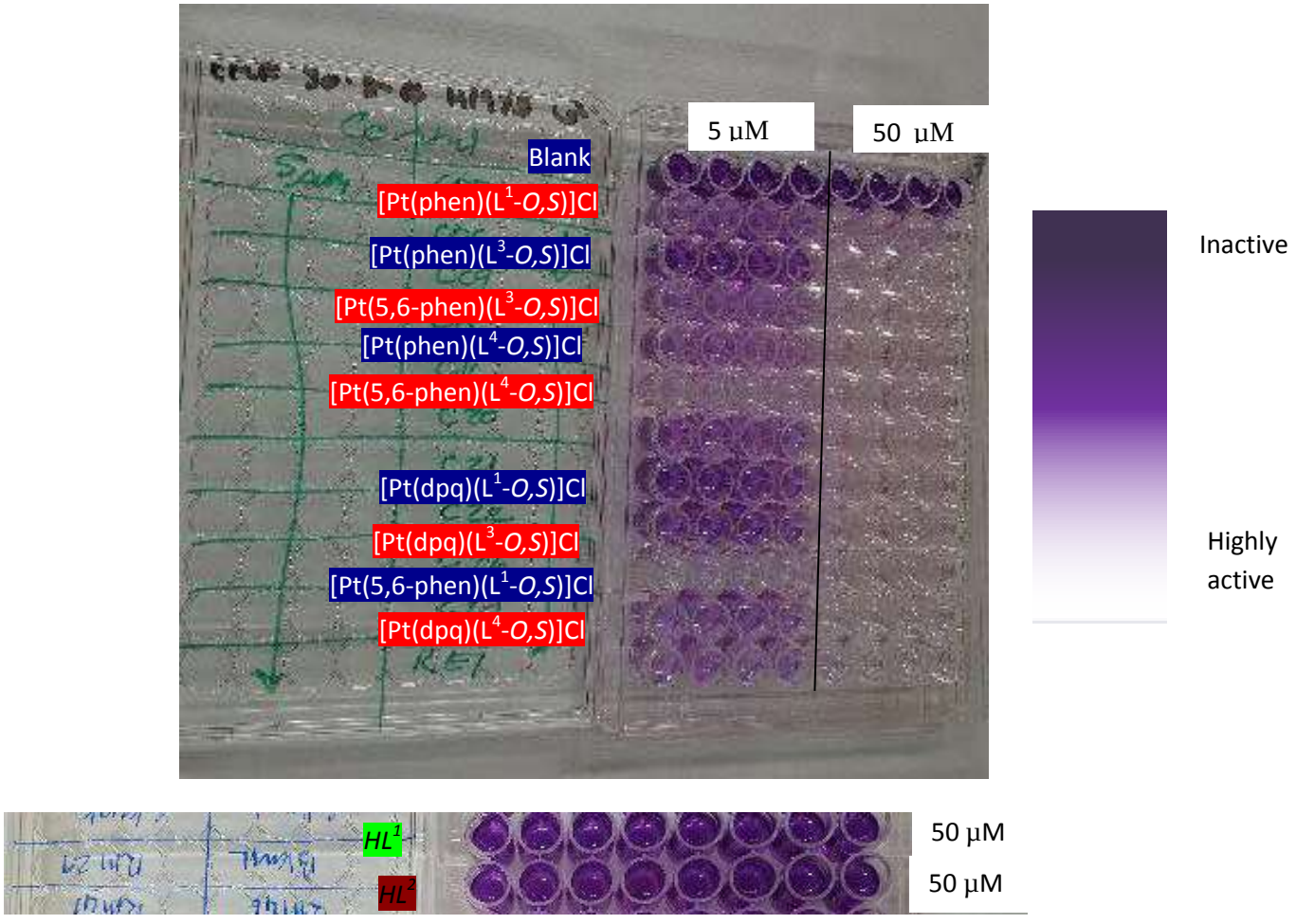


Figure 3.1: *The 96 well plates of the compounds tested with MTT assay at 50 µM (left) and 5µM (right). Refer to the table 3.1 and 3.2 in the experimental section.*

The dark purple on the first row and first column indicate the negative blank while the others are for all the complexes that were tested at 50µM and 5µM respectively.

3.3 Results and Discussion

The results of this study show that all the [Pt(diimine)(L^n-*O*,*S*)]Cl complexes are highly cytotoxic to H1975 lung cancer cell line at 50µM. It is clear from Figure 3.1 that these complexes were highly cytotoxic at 50 µM such that all the cancer cells were dead after 48 hours. Interestingly, even at lower concentrations (5 µM), the complexes still showed more than 50% cell death against the H1975 cancer cell lines as shown in Figure 3.2

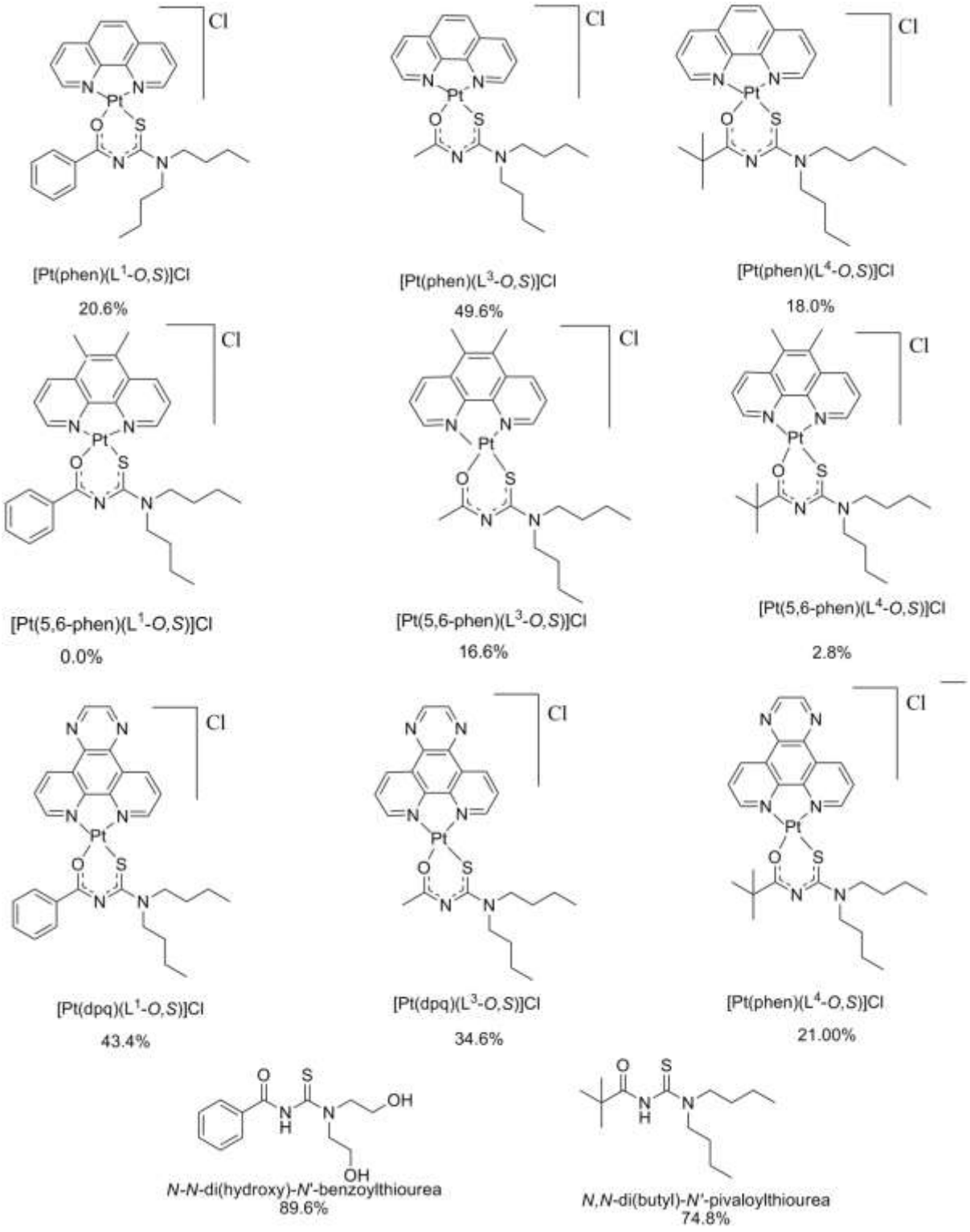


Figure 3.2: MTT assay results for *compounds tested with MTT assay and the % cell viability at 5 µM.*

The 5,6–dimethyl phenanthroline (dmp) based platinum complexes on the other hand still showed almost similar cytotoxicity at 5 µM when compared to 50 µM as early as 12 hours of incubation.

On the other hand, all the dpq complexes had some serious solubility problems; it is therefore very difficult to use these results to fully explain the structural activity relationship. Nevertheless, we can clearly see that the dpq complexes are not as active which is probably attributed to poor solubility. However, if we consider other complexes which water solubility was also a problem but much better than the dpq complexes we can clearly see that the dmp complexes are much more active and this is possibly due to the fact that the methyl-substituted 1,10-phenanthroline bind more strongly to the DNA than the 1,10-phenanthroline, due to the strong hydrophobic interaction of the methyl-substituted ligand with DNA surface.[4] The 5,6-dimethyl groups are thought to be involved in hydrophobic interaction with the hydrophobic DNA surface leading to enhancement in DNA binding affinity suggesting that the 5,6-dimethyl groups might interact in the groove of the DNA.[5]

It has been established by *Ganot and co-workers* that the $[Ru(NH_3)(diimine)]Cl$ complexes where the diimine = bipyridine(bpy); 1,10-phenanthroline (phen) etc, interact with DNA through their diimine face, which is supported by the hydrogen bonding.[4] In addition, if the diimines in these complexes are di/ tetra methyl-substituted-1,10-phenanthroline, the DNA binding affinities are higher than those with just 1,10-phenanthroline and bipyridine co-ligand because the methyl substituted ligands enhances hydrophobic interaction with DNA leading to the release of water molecules of solvation entropically favouring the formation of the DNA- bound complex.

When trying to further establish the structure-activity relationships, the ligands were assayed. It was found that the acetyl thioureas was least cytotoxic than the benzoyl and pivaloyl

variations in the complexes, which proved that these ancillary ligands are not completely acquitted because they do affect the activity of the complexes. These ligands were then tested alone and they showed to have some activity even though it only inhibited 20% of the cell growth; nevertheless, this clearly indicates that the ligands themselves have some activity against H1975 cancer cell lines.

3.4 Conclusion

It was found that all the [Pt(diimine)(L^n-*O*,*S*)]$^+$ complexes displayed significant activity against H1975 lung cancer cell lines at 50 µM and at 5 µM. The complexes containing methyl substitution at the 5 and 6 position of 1,10-phenanthroline ([Pt(dmp)(L^3-*O*,*S*)]Cl and [Pt(dmp)(L^1-*O*,*S*)]Cl) were highly cytotoxic with cell death of greater than 98% even at 5 µM and are expected to have low nano molar IC_{50} values. However, the complexes that contained the pyrazine group were found to be the least active which was attributed to the low solubility of these complexes. Currently, the IC_{50} of these complexes are still being determined, which will give sufficient information to carefully consider the structural activity relationship. Furthermore, the testing of these complexes will be extended to 4 cancer cell lines and mechanistic studies will be are conducted.

The poor water solubility of these complexes remains one of the major challenges when considering them as potential anticancer agents. Variations of the ancillary ligand have proved to improve solubility. However, for some of the diimine ligands especially dpq, solubility was not improved and we have to consider drug delivery system in an attempt to address this problem.

3.5 Experimental Section

The percentage of the activity of the compounds tested for MTT assay at 50 µM and 5 µM is shown Table 3.1 & 3.2 with their average mean of cell viability percentage:

Table 3.1: *Percentage cell viability of compound tested with MTT assay at 50µM, along with the average mean and standard error*

	% cell viability at 50 µM H1975 cells				
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Average%**
[Pt(phen)(L^1-O,S)]Cl	1.7	2.0	2.2	2.2	**2.0 ± 0.1**
[Pt(phen)(L^3-O,S)]Cl	0.2	0.3	0.7	1.0	**0.6 ± 0.2**
[Pt(phen)(L^4-O,S)]Cl	2.5	2.7	2.0	3.4	**2.7 ± 0.3**
[Pt(5,6-phen)(L^1-O,S)]Cl	1.1	0.9	0.7	1.6	**1.1 ± 0.2**
[Pt(5,6-phen)(L^3-O,S)]Cl	1.7	2.1	1.9	1.6	**1.8 ± 0.1**
[Pt(5,6-phen)(L^4-O,S)]Cl	1.1	1.4	0.9	0.9	**1.1± 0.1**
[Pt(dpq)(L^1-O,S)]Cl	2.3	2.5	2.1	1.5	**2.1 ± 0.2**
[Pt(dpq)(L^3-O,S)]Cl	0.6	1.0	0.9	0.3	**0.7 ± 0.1**
[Pt(dpq)(L^4-O,S)]Cl	0.6	1.6	0.7	1.1	**1.0 ± 0.2**
***N,N*-di(butyl)-*N*'-benzoylthiourea**	67.2	67.9	71.7	75.2	**70.5 ±1.6**
***N,N*-di(hydroxy)-*N*'-benzoylthiourea**	90.6	88.8	89.0	89.9	**89.6 ± 0.37**
***N,N*-di(butyl)-*N*'-pivaloylthiourea**	77.2	74.1	74.4	73.4	**74.8 ± 0.7**

Table 3.2: *cell viability of compound tested with MTT assay at 5μM, along with the average mean and standard error*

	% cell viability at 5 μM				
	H1975 cells				
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Average**
[Pt(phen)(L^1-O,S)]Cl	21.1	23.9	18.5	19.0	**20.6 ± 1.60**
[Pt(phen)(L^3-O,S)]Cl	38.5	46.4	57.6	55.8	**49.6 ± 3.8**
[Pt(phen)(L^4-O,S)]Cl	14.9	15.9	21.5	19.6	**18.0 ± 1.4**
[Pt(5,6-phen)(L^1-O,S)]Cl	16.5	16.0	17.4	16.3	**16.6 ± 0.3**
[Pt(5,6-phen)(L^3-O,S)]Cl	2.8	2.2	2.8	3.3	**2.8 ± 0.2**
[Pt(5,6-phen)(L^4-O,S)]Cl	0.0	0.1	0.0	0.0	**0.0 ± 0.0**
[Pt(dpq)(L^1-O,S)]Cl	33.4	43.4	46.1	50.7	**43.4 ± 3.1**
[Pt(dpq)(L^3-O,S)]Cl	29.6	40.5	36.8	31.5	**34.6 ± 2.1**
[Pt(dpq)(L^4-O,S)]Cl	20.5	25.1	22.5	16.1	**21.1 ± 1.7**

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Chapter 4

Encapsulation of the [Pt(diimine)(L-O,S)]$^{+}$ complexes with the Drug-delivery systems.

Chapter 4

Encapsulation of the [Pt(diimine)(L-O,S)]$^+$ complexes with the Drug-delivery systems.

4.1 Introduction

The [Pt(diimine)(L-*O*,*S*)]$^+$ complexes have been found to be highly active against the H1975 lung cancer cell line (*in* vitro). However, one major drawback of [Pt(diimine)(L-*O*,*S*)]$^+$ as potential metallodrugs is their poor solubility, which has an effect on the anticancer activity as shown in chapter 3. The strategy to address this problem of poor solubility typically involves the use of drug-delivery systems such as cyclodextrins, liposomes or micelles to host potential anticancer drugs. Furthermore, smart drug delivery systems have the ability to not only improve the delivery and effectiveness of metallodrugs but also can sometimes reduce the cytotoxicity and improve drug metabolism.[1] Functionalised cyclodextrin such as sulphurbutyl-β-ether cyclodextrin (Captisol) have been widely used in the early stages of pharmaceutical application because of its commercial availability and cavity size suitable for the wide range of drugs.[2] Captisol is made of a hydrophilic exterior and hydrophobic interior which makes it possible for water-insoluble drugs to insert into the hydrophobic cavity *via* non-covalent association to the captisol which results in a host-guest complex with improved water solubility.[3] Wen *et al.* were able to show that the β-cyclodectrins can improve the solubility of Carvedilol({1-[carbazolyl-(4)-oxy]-3-[2-methoxyphenoxyethylamino]propanol-(2)}) compound, where he found that the guest solubility increases linearly with cyclodextrin concentration inferring the formation of a soluble inclusion complex between the drug and β-cyclodextrin.[4]

Micelles have also shown to be excellent drug delivery vehicles. This stems from their unique structural composition, which is characterised by a hydrophobic inner cavity sterically stabilised by a hydrophilic outer surface. The former serves as a reservoir in which the drug molecule can be incorporated by means of chemical, physical or electrostatic interaction, depending on their physicochemical properties. The utility of micelles in the field of drug delivery is based on their characteristic self-assembly into core-shell nanostructure in aqueous solutions. Hence, its formation is not dependent on the drug molecules as other drug delivery systems and could potentially deliver a large variety of therapeutic compounds. Another advantages of using micelles as a drug delivery system is other than solubilizing the drug, they can also target their payload to specific tissues through either passive or active means. They can employ biologically inert macromolecules to direct the drug to its target site in the body.

Micelles can be subdivided into two different groups according to their molecular weight: low- molecular -weight surfactant micelles and polymeric micelles.[5] We opted for using both low-molecular–weight surfactant micelles specifically polysorbate 80 (Tween 80), polysorbate 85(Tween 85) and polymeric micelle namely poloxamer 407. Tween 80 has been used to solubilize different anticancer drugs including cisplatin, docetaxel, etoposide, paclitaxel, carcelesin , bisnatide derivates and several photosensitizers.[5] Furthermore, Tween 80 is biologically active and pharmacologically compatible. Poloxamers known as polyethylene-propylene glycol polymer has been well known for its diverse pharmaceutical applications.[6]

This chapter describe the attempt to encapsulate of [Pt(diimine)(L-*O*,*S*)]$^{+}$ in sulphobutyl-ether-β-cyclodextrin (captisol) and low-molecular-weight surfactant micelles. The inclusion complexes were characterized by ^{1}H-NMR and DOSY NMR Spectroscopy.

4.2 Encapsulation of [Pt(diimine)(L-*O,S*)]$^{+}$complexes with captisol

There are several methods for the synthesis of cyclodextrin–guest complexes depending on the physical properties of the guest molecules. These methods include: kneading, neutralisation, grinding, co-precipitation and lyophilization. The different methods were attempted in order to encapsulate the complexes (see Experimental Section 4.3). The method that seemed to work best was method 2 which involves a solution of complex and captisol (one mole equivalence) in MeOH, heating under reflux for 3 hours, followed by stirring overnight at room temperature. The solvent was removed by rotary evaporation and the product was left to dry in the fumehood. In order to ascertain the structure of the inclusion complex, ^{1}H NMR Spectroscopy studies of the free complex; free captisol and inclusion complex were therefore undertaken.

4.2.1 ^{1}H-NMR Spectroscopy studies of inclusion complex formation

Sulphurbutyl-β-ether cyclodextrin (captisol) is generally known to have primary and secondary OH groups crowning opposite ends of its torus, where 3 hydrogens are located on the exterior of the cyclodextrin and 2 hydrogens are located on the interior with one hydrogen on the edge of the cyclodextrin as shown in Figure 4.1.[7]

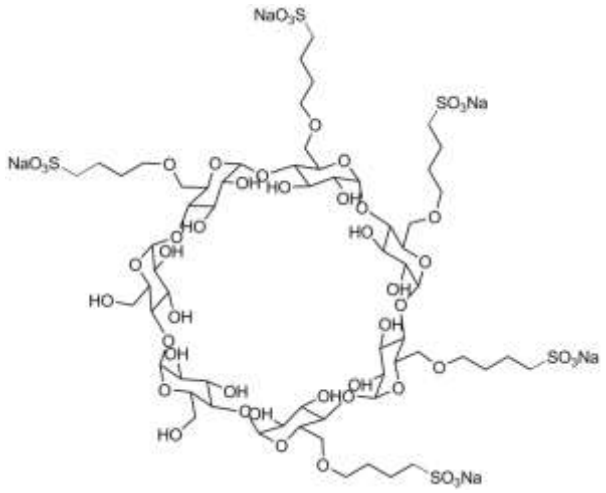


Figure 4.1: *Representation of the structure of captisol.*

If inclusion complexation took place, we expected that the protons located on the interior should be strongly shielded due to the anisotropy of the aromatic moiety, while the protons on the exterior should not be affected. However, this cannot be observed in this case since the protons on the interior will exchange with MeOD. Furthermore, where inclusion does not take place but rather adsorption or ion-pairing, then the protons on the exterior should be the most affected.

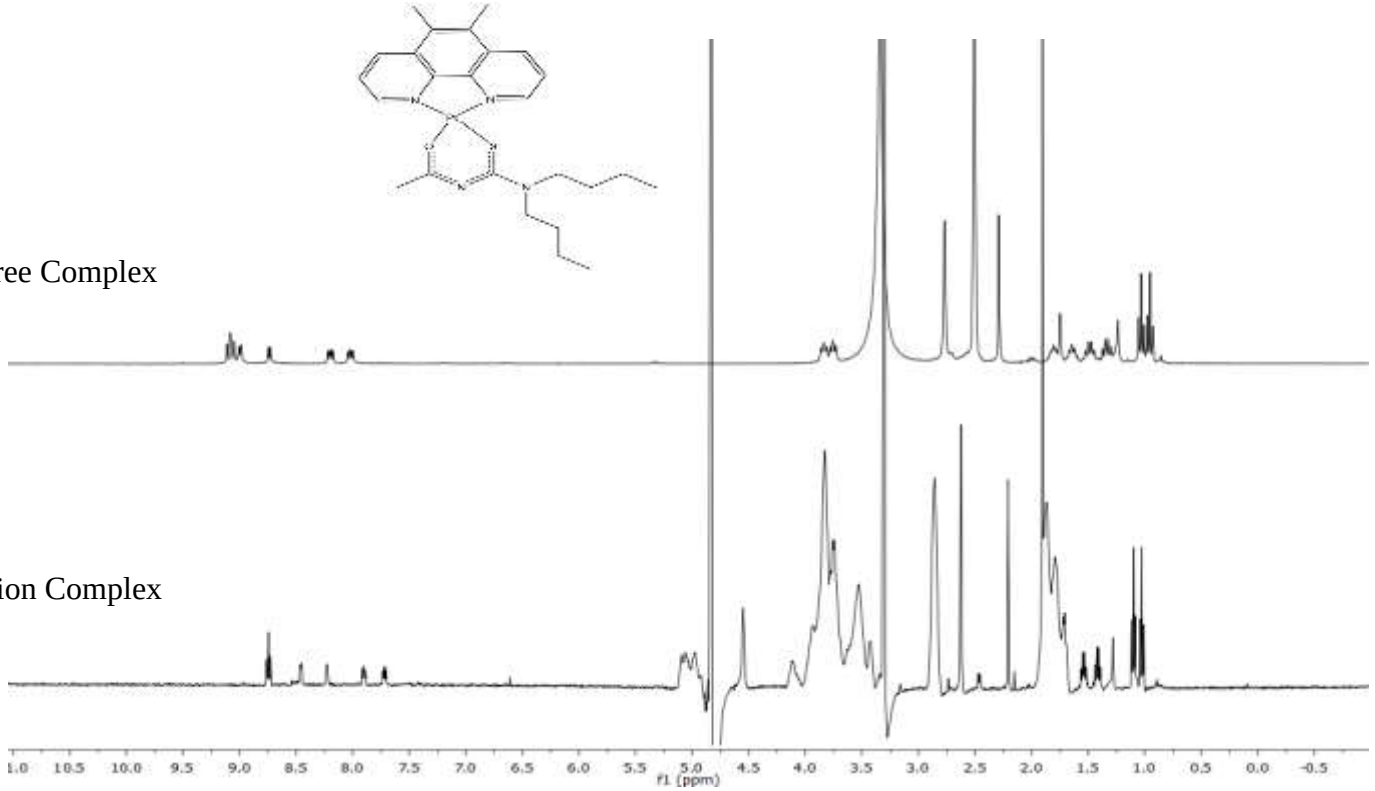
The NMR characteristic protons of the complexes and the protons of the cavity of captisol are different; hence one is able to monitor the interaction between the complex and captisol. Significant shifts were observed on the aromatic protons of the free complex and inclusion complex as shown in Figure 4.2 and summarised in Table 4.1.

***Table 4.1**: NMR chemical shift difference between the free complex and the mixture between complex and captisol.*

Complex	**Inclusion complex**	**Δδ**
H1&8 (9.11)	H1&8 (8.86)	0.25
H3 (9.06)	H3 (8.62)	0.44
H6 (8.84)	H6 (8.35)	0.49
H2 (8.21)	H2 (8.00)	0.21
H7 (7.98)	H7 (7.80)	0.18

All the protons on the aromatic group experienced a shielding effect, after encapsulation as shown in Figure 4.2 (a) and (b).

(a)



(b)

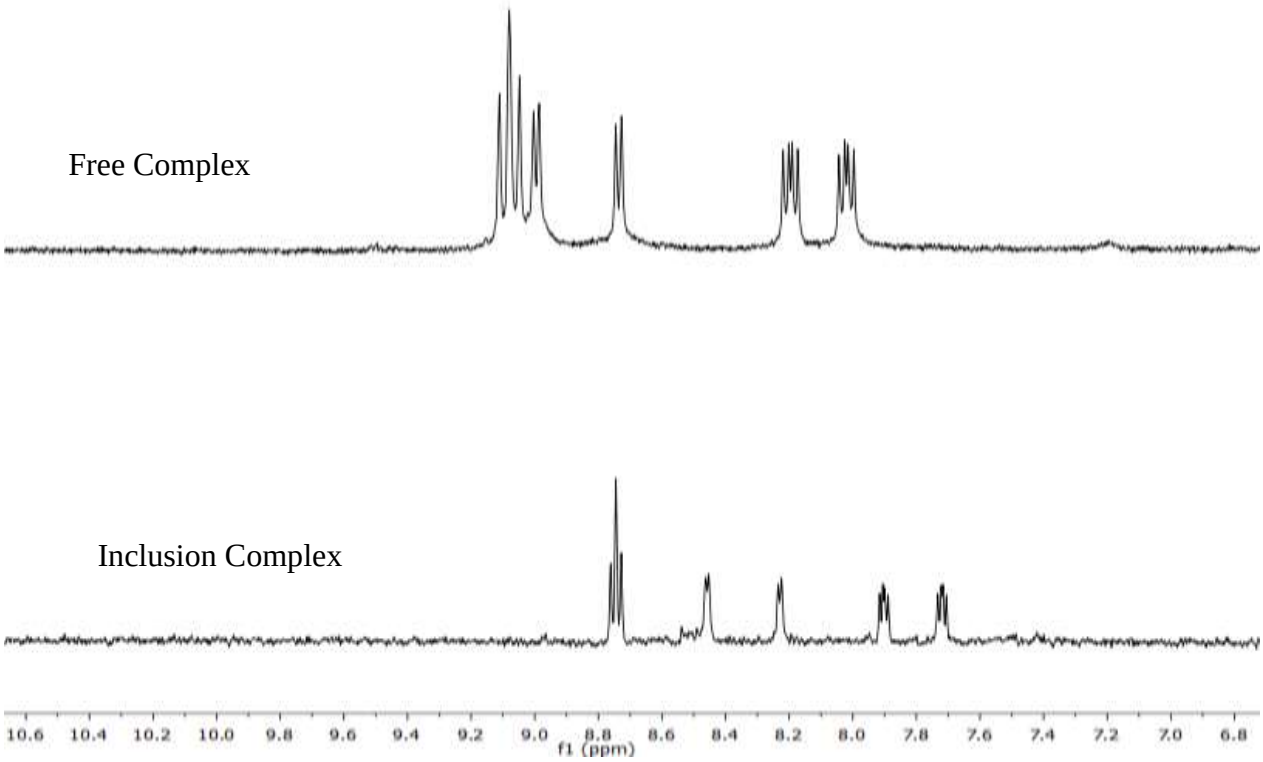


Figure 4.2: (a) *The full spectrum ^{1}H NMR of the free complex (top) and inclusion complex (bottom).* ***(b)*** *Aromatic region of the free and inclusion complex.*

This uplift shift of the aromatic protons might be induced by diamagnetic anisotropy of particular bonds of β-cyclodextrin and van der waals shift. The OH group chemical shifts of captisol could not be established since the OH's from the captisol exchanges with the OH from the deuterated methanol. This suggests that there might be some interaction, whether it is ion-pairing or encapsulation.

However, due to difficulties to pinpoint what is causing the changes in the chemical shifts, elaborate characteristics of the interaction cannot be inferred. Hence the DOSY NMR was used to study this, since it is independent from chemical shift changes and it is related to the diffusion of the molecule in solution.

4.2.2 DOSY NMR

Diffusion-Ordered Spectroscopy (DOSY) NMR can be described as a two-dimensional NMR experiment, in which the signal decays exponentially according to the self-diffusion behaviour of individual molecules.[8] There are two dimensions in the DOSY NMR, the first one is for conventional chemical shift and the second one is for diffusion dimension.[9] The diffusion behaviour is dependent on the properties of an individual molecule, which include the size, shape, mass, charge as well as its surrounding environment, which include solution, viscosity, temperature and aggregation state. Due to these properties of the molecules, each component in a mixture can be pseudo-separated, based on its own diffusion coefficient on the diffusion dimension.[8] DOSY NMR has been known for its advantages, it can be used as a non-invasive method for both physical and chemical information. Diffusion coefficients can be obtained from measuring the attenuation of the NMR signals (Figure 4.3) during a pulsed field gradient (PFG) experiment and are easy to determine using the 2D processing.[10]

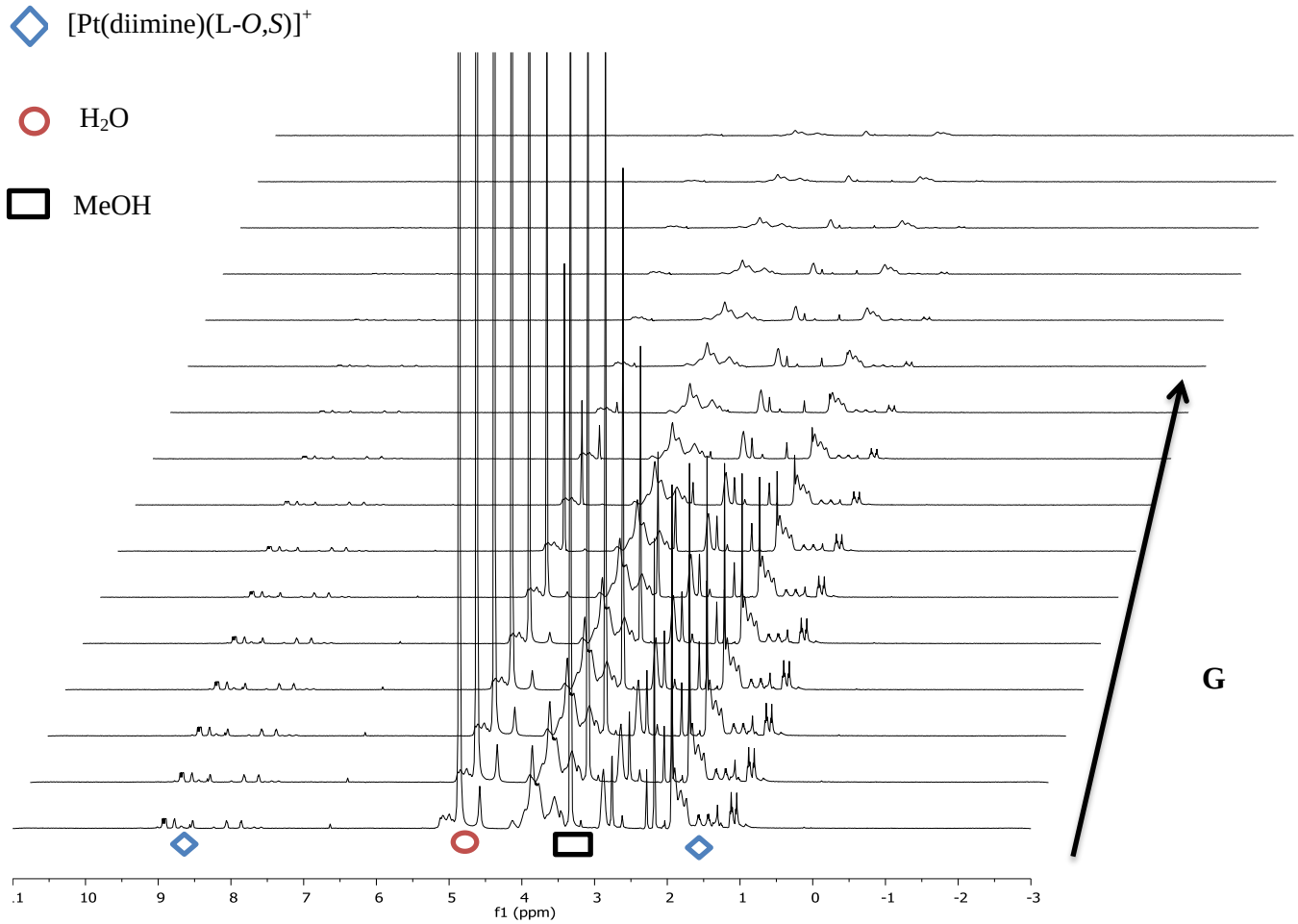
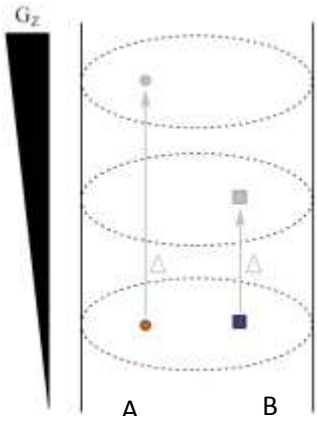


Figure 4.3: *Attenuation of the ^{1}H NMR of the mixture of inclusion complex, methanol, water during a typical DOSY experiment with the increasing gradient strength* (G).

In order to explain the attenuation of the resonance signal and PFG experiment, the Scheme 4.1 below is used.



Scheme 4.1: *Representation of the translation of molecule A and B.*

Hypothetically Scheme 4.1 represents the translation of the molecules A and B in z-axis direction. Furthermore, the molecules A diffuses faster than the molecule B during the same diffusion delay time interval (Δ). The experiment therefore consists of two pulsed field gradient (PFG) where there is a diffusion delay Δ between the two PFG's and the second PFG is the inverse of the first PFG. The complete refocussing of the magnetization components will occur when the local magnitude field of a nucleus is identical during the two gradient pulses.[10] The local magnetic field is spatially dependent because of the field gradient (G_z) that is used. This indicates that a molecule that diffuses at the diffusion delay between the two gradient pulse will experience a different local magnitude field when the second PFG is applied which leads to only partial refocussing of the magnetization, resulting in the attenuation of the resonance signal.

DOSY measurements are attained by means of either gradient in the main magnetic field, or by the gradient in radio frequency fields. The signal contribution of each component from the DOSY experiment is described by equation (1).[9]

$$I(i,g^2) = I_O(i) \exp[-D(i)(\Delta-\delta/3)K^2] \qquad \text{eq. (1)}$$

$$K = \gamma g \delta$$

where I(*i*) is the signal amplitude of component *i*, $I_o(i)$ is the amplitude with no gradient applied,γ is the gyromagnetic ratio of the ^1H nucleus (rad $S^{-1}T^{-1}$), g the gradient strength (T), δ the duration of gradient pulse (S), Δ the diffusion time (s) and D(*i*) is the diffusion coefficient of *i*th component (m^2/s). In eq.(1), if Δ and δ are the experimental constants, then the signal of a DOSY experiment attenuates depends on the gradient strength (g^2) and diffusion coefficient (D) of individual components. In simple terms molecules in liquid or solution state naturally move around. This translational motion is in contrast with rotational motion, known as Brownian molecular motion and is often called diffusion or self-diffusion.[11] Assuming a spherical size of the molecule, the diffusion coefficient D is described by the Stokes-Einstein equation:

$$D = kT/6\pi\eta\gamma_s \qquad \text{eq.(2)}$$

where k is the Boltzmann constant , T is the temperature ,Ƞ the viscosity of the liquid and γ_s, is the (hydrodynamic) radius of the molecule. A least-square fit of the equation 1 to the signal attenuation data allows for the calculation of diffusion coefficient, D. The calculated diffusion coefficients are displayed as a 2D- plot as shown below.

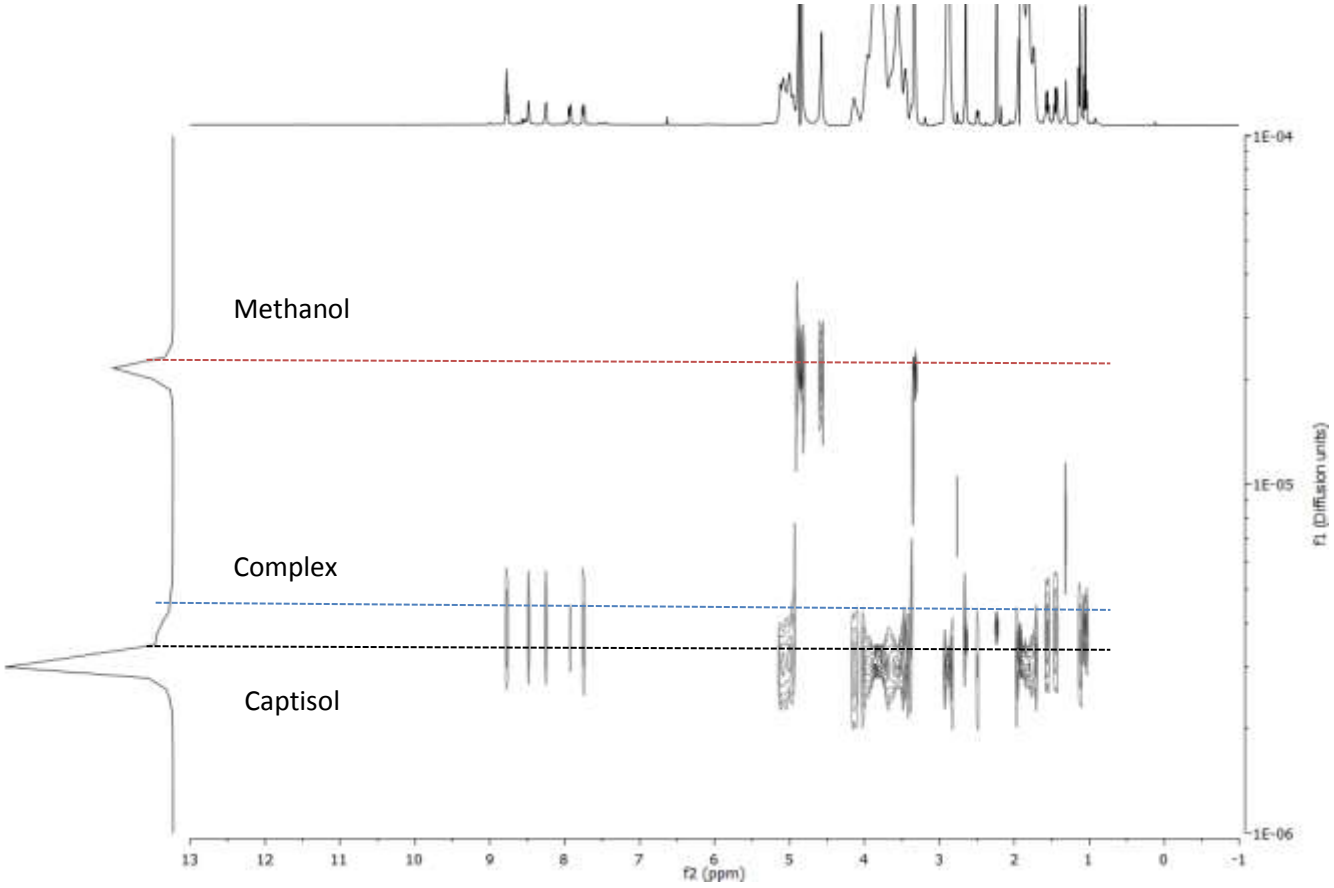


Figure 4.4*: DOSY plot of a mixture of [Pt(dmp)(L^3-O,S)]$^+$, methanol and water showing the diffusion coefficients*.

Captisol has the molecular weight of 2163g/mol while the molecular weight of the complexes range from 604g/mol to 718g/mol. We therefore expect the inclusion complex to have a smaller diffusion coefficient as compared to the diffusion coefficient of the free complex and captisol. Interestingly the opposite was observed even in the cases where the concentration of captisol was changed. The diffusion coefficient of the inclusion complex was found to be larger than that of the free complex and the free captisol. This clearly suggests that the encapsulation was not successful. Furthermore, when the concentration of the complex was increased, similar results were observed as shown in Table 4.2.

Table 4.2: *The Diffusion Coefficients of the inclusion complexes.*

	Complex ratio : Captisol	Diffusion coefficient
MeOH		$2.058x10^{-9}$
$[Pt(dmp)(L^3\text{-}O,S)]^+$only		$3.495x10^{-10}$
Captisol only		$2.906x10^{-10}$
Mixture 1	2:1	$3.970x10^{-10}$
Mixture 2	1:1	$3.911x10^{-10}$
Mixture 3	1:2	$3.556x10^{-10}$

Having the unexpected results from the ^{1}H NMR and DOSY NMR, preliminary solubility studies were performed to get more information on whether the inclusion complexation took place or not. When the solubility studies were performed on the inclusion complexes, it was observed that they were less soluble as compared to the free complexes. What was expected was if the inclusion complexation was successful the inclusion complexes should be very soluble in aqueous solvents. Captisol's solubility in water is greater than 1500mg/ ml, which means that if the encapsulation was successful then the inclusion complex should be soluble in water. This was found not to be the case, in addition, even when the concentration of captisol was increased still the solubility was still low, suggesting that the complex might be aggregating in solution as observed by Kotzé *et al.*[12] Furthermore, the captisol might also be aggregating in methanol and/or ion-pairing with the complex to yield a less soluble product.

Usually, complexation with captisol typically occurs best with neutral molecules. However, many cationic complexes have shown to complex well with captisol because of the electrostatic interaction between the charges. The complexes are cationic and hence what was

expected was the presence of the positive charge on the drug can assist inclusion complexation *via* charge attraction. With all the information gathered it was evident that the complexes were not encapsulated. Micelle formation as a drug delivery system was then used as an alternative in attempt to address the solubility problem.

4.2.3 Micelles as a drug-delivery system

Similarly to the cyclodextrin, the micelles possess the characteristic of having a hydrophobic core sterically stabilized by a hydrophilic corona.[13] We opted for using both low-molecular – weight surfactant micelles specifically polysorbate 80 (Tween 80), polysorbate 85(Tween 85) and polymeric micelle namely poloxamer 407.

4.2.3.1 Encapsulation of [Pt(diimine)(L-O,S)]$^+$complexes in Micelles

Two methods of encapsulating the complex in the micelles were explored in attempt to form the micelles. The first method included dissolving polyoxyethylene- polypropylene block copolymer (poloxamer 407), polyoxyethylene sorbitanmonostearatte (Tween 80), polyoxyethylene sorbitantrioleate (Tween 85) in water at the concentration of 5%(w/v). An excess of [Pt(phen)(L^1-*O,S*]$^+$ was added to obtain a saturated micellar solution and magnetically stirred overnight at room temperature. The [Pt(phen)(L^1-*O,S*]$^+$ complex was used for this study because it is easy to made but relatively insoluble. Afterwards, the resulting saturated solution was filtered in order to room any insoluble complex. The second method was based on dissolving the Tween 80 and Tween 85 in water. A one mole equivalent of the [Pt(phen)(L-S,O)]$^+$ was then added. The reaction was magnetically stirred for 24 hours. The solvent was removed by rotary evaporation to yield the product.

When the preliminary of the products was tested in water, they were found to form stable colloidal solution, suggesting that possibly aggregation is not taking place but rather encapsulation is taking place to some extent . Even though this was not the desired results, but it was a good indication that the micelles can be used as a drug delivery systems for these complexes.

4.3 Experimental Section

4.3.1 Instrumentation and Material

All the reagents were commercially available and used as received. The captisol was kept in a sealed container with desiccants in order to avoid any contact with the moisture from the air. The ^{1}H NMR and DOSY experiments were recorded on a Bruker Avance III 500MHz. The DOSY experimental parameters were as follows: ^{1}H spectral width 14.0019ppm; Number of acquisitions varied from the sample; Recycling delay 2s; Diffusion delay 30ms and Gradient pulse duration 2ms.

4.3.2 Sample preparation for DOSY Spectroscopy

The DOSY experiments were done in 5mm tubes, where the inclusion complexes were dissolved in respective solvents to obtain the desired concentration. The samples were left in the spectrometer for about 10 to 15 minutes in order to make sure that there was temperature equilibrium before the measurements were done.

4.3.3 Methods of encapsulating the drug with the Captisol

Method 1

A solution of [Pt(phen)(L-*O*,*S*)]$^{+}$(10mg), captisol (1 mole equivalent) and NaCl (1.5 mole equivalent) in H_2O (20 ml) was heated under reflux for 6 hours, then stirred overnight at room temperature. The solvent was removed by rotary evaporation to yield the product as a yellow solid.

Method 2

$[Pt(phen)(L\text{-}O,S)]^+$ (4mg) and captisol (1 mole equivalent) in MeOH was heated under reflux for 3 hours, and then stirred overnight at room temperature. The solvent was removed by rotary evaporation and the mixture was left to dry in the fume hood overnight.

Method 3

A mixture of $[Pt(phen)(L\text{-}O,S)]^+$ (10mg) in MeOH and (16.72mg) of captisol in H_2O (50mL),was heated for 3 hours and left to stir overnight at room temperature. The two solvent layers were isolated and the solvent from each layer was removed by means of the rotary evaporator and the product from each layer was dried under vacuum.

Method 4 (Physical Mixtures)

A 20mg sample of solid containing $[Pt(phen)(L\text{-}O,S)]^+$ and 1 mole equivalent of captisol was blended in a mortar and pestle until a homogeneous mixture was obtained .

Method 5 (Lyophilization)

The inclusion complex was prepared by the lyophilisation method. The aqueous solution of $[Pt(phen)(L\text{-}O,S)]^+$ inclusion complex was prepared by dissolving 10mg of $[Pt(phen)(L\text{-}O,S)]^+$ and 17.6mg of captisol in 50mL ethanol. The reaction mixture was heated under reflux for 6 hours and stirred at room temperature overnight. The resultant solution was the subjected to freeze-dying for about 48 hours to obtain a yellow powder. In this method, different molar equivalence was considered, where at some cases the $[Pt(phen)(L\text{-}O,S)]^+$ complex was in excess and in some cases, the captisol was in excess.

4.3.4 Methods of micellar formation

To a glass polytude , 3.99 mg of Tween 80 and Tween 85 were added. 2 mg of the complex was added to the saturated solution. A small volume of water was further added for the micellar saturated solution. The mixture was stirred for 2 days at room temperature. A pale yellow resultant mixture was obtained and preliminary studies were done on it. In the case where polaxamer 407 was used, an equivalent mol of it was added along with Tween 85 and Tween 80 before the addition of the complex and similar results were obtained.

4.4 Conclusion

It was found that the captisol does not encapsulate the complexes. However, significant shifts were observed on the aromatic protons suggesting that there is some interaction that is taking place. Due to the difficulties to pinpoint what was causing the shifts on the aromatic protons, DOSY NMR was considered as an excellent tool to study this, which revealed that the encapsulation was not successful. When preliminary solubility studies were performed, they also showed that the encapsulation was not successful, which might have been due to the size of the cavity of the captisol such that the coordination compound was too big to fit in the cavity.

Low-molecular-weight surfactant micelle was used as an alternative in attempt to address the solubility problem, which was found to show some interaction. The inclusion complex formed colloidal suspension suggesting that aggregation is not taking place but rather encapsulation is taking place to some extent. However, future studies are aimed at exploring the micellar formation as a drug delivery system and trying to encapsulate the complexes into the micelles in order to address the solubility problem.

4.5 References

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Chapter 5

Summary and Conclusions

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Summary and Conclusion

A series of *N*,*N*-dialkyl-*N'*-acylthiourea ligands was successfully synthesised and characterised. The hydroxyl containing ligands were found to be a challenge to synthesise, however *N*,*N*-di(2-hydroxyethyl)-*N'*-acylthiourea was successfully synthesised and we report a crystal structure that has never been published before. The ligand exhibited intermolecular and intramolecular interaction and it was found to have a herringbone packing pattern. A series of novel mixed-ligand cationic $[Pt(diimine)(L^n\text{-}O,S)]^+$ complexes where diimine represents 1,10-phenanthroline; 5,6-dimethyl-1,10-phenanthroline and [3,2-d:2',3'-f] quinoxaline and (L^n-*O*,*S*) represents various *N*,*N*-dialkyl-*N'*-acylthioureas were successfully synthesised. The synthesised ligands and complexes where characterised by FTIR Spectroscopy, NMR Spectroscopy, Mass Spectrometry and Elemental Analysis.

The $[Pt(diimine)(L^n\text{-}O,S)]^+$ compounds were tested against H1975 lung cancer cell line using a MTT assay. The compounds were assayed at 50µM and 5µM. All the $[Pt(diimine)(L^n\text{-}O,S)]^+$ compounds showed significant cytotoxicity with cell death of 90-98% at 50µM. Interestingly, the complexes containing methyl substituents at 5 and 6 position of 1,10-phenanthroline (dmp) were found to be very cytotoxic even at 5µM and it has been shown in literature that this ligand system is considered a good groove binder because of its hydrophobicity. The [3,2-d:2',3'-f]-quinoxaline (dpq)containing complexes were found to be cytotoxic at 50µM, but were least active of all the complexes at 5µM which might have attributed to the low solubility of these complexes. The structure activity relationship could not be fully established from the single concentration results and IC_{50} values are required

which form part of future study which is currently underway. However, from the screening results it is clear that the $[Pt(dmp)(L^n\text{-}O,S)]^+$ complexes are very cytotoxic and are expected to have low nano molar IC_{50} values.

In addition, three ancillary ligands (HL^1 and HL^2, HL^3) were assayed at 50µM and 5µM showed some weak cytotoxicity with less than 10% cell death. However, this proves that these ligands are not innocent and do have an influence on the cytotoxicity of the complexes. This is also evident even on the variations observed in viability percentages when the only the ancillary ligand is varied. Nevertheless, the problem of poor solubility of these complexes still remains, even though the variation of the ancillary ligands did improve the solubility of the complexes. The Sulphurbutyl-β-ether cyclodextrin (captisol) is a good drug delivery system for many drugs due to its hydrophilic exterior and hydrophobic interior which makes it possible for water-insoluble drugs to insert into the hydrophobic cavity. However, the solubility of $[Pt(diimine)(L^n\text{-}O,S)]^+$ did not improve even though the ^1H NMR spectra suggested significant changes in the chemical shift of the aromatic proton of the complex which suggest some interactions or aggregation. All the protons on the aromatic group suddenly experience a shielding effect after encapsulation which might have attributed to diamagnetic anisotropy of particular bonds of β- cyclodextrin. These shifts indicated some sort of interaction between the complex and captisol but that was not sufficient information to conclude whether the complex is encapsulated or not. DOSY NMR was found to be an excellent tool to study this since it does not depend on the chemical shift but rather the diffusion properties of the molecule in the solvent.

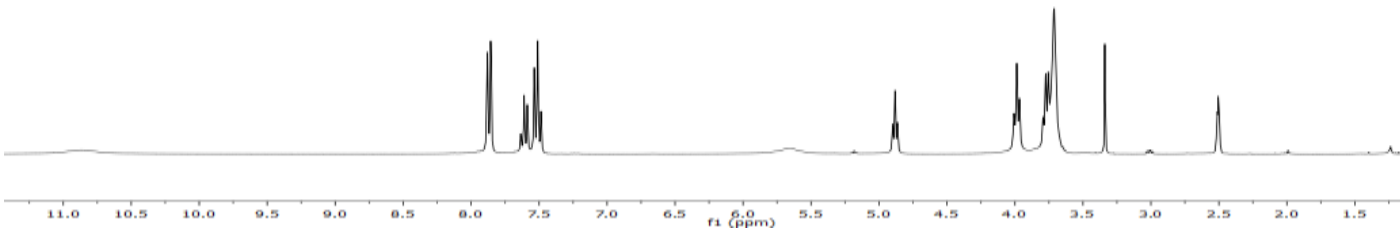
It was expected that if encapsulation was successful, the diffusion coefficient of the complex and captisol should be equal (for stable inclusion complex) or at least smaller than the diffusion coefficient of the free molecules (for a fast exchange system). The DOSY NMR clearly suggested that the encapsulation was not successful in methanol and solubility studies

revealed that the aqueous solubility did not improve. Therefore the low-molecular-weight micelles (Tween 80 and Tween 85) were considered as a drug delivery system in attempt to improve solubility. Preliminary solubility studies were investigated on the inclusion complex upon micellar formation and it was found that the complexes are not completely soluble but rather form a stable colloidal suspension which suggests that this micellar formation can possibly be used as a drug delivery system for these complexes.

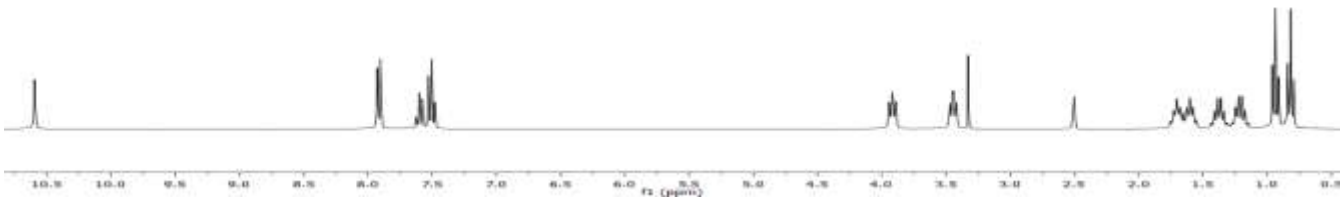
Future studies are aimed at exploring the micellar formation as a drug delivery system, and trying to encapsulate the complexes into the micelles in order to address the problem of solubility. Future investigations also include expanding the *in vitro* cytotoxicity testing to four cancer cell lines, establish the IC_{50} values and do mechanistic studies to explain the cytotoxicity observed. Furthermore, binding between $[Pt(diimine)(L^n\text{-}O,S)]^+$ complexes and the DNA will be studied to establish whether the complexes interact with DNA via intercalation or groove binding.

APPENDIX

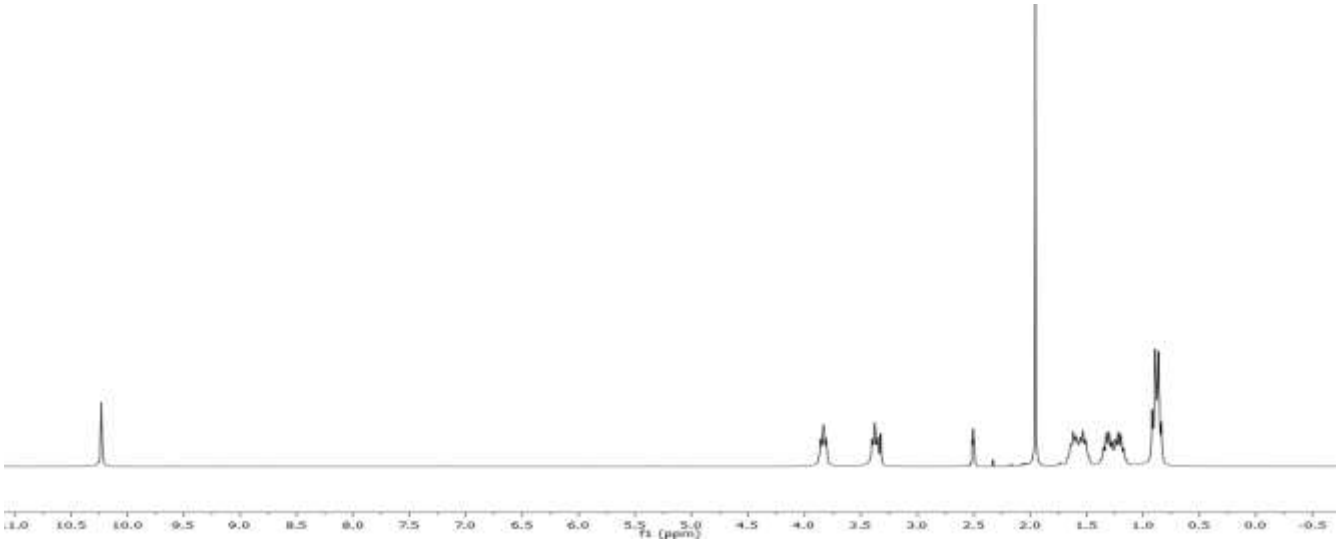
NMR Spectroscopy



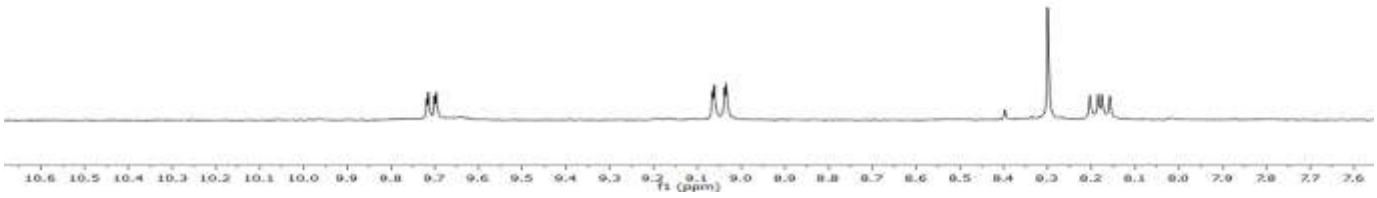
^{1}H NMR spectrum of HL^{2} in DMSO-d6 at 25 °C.



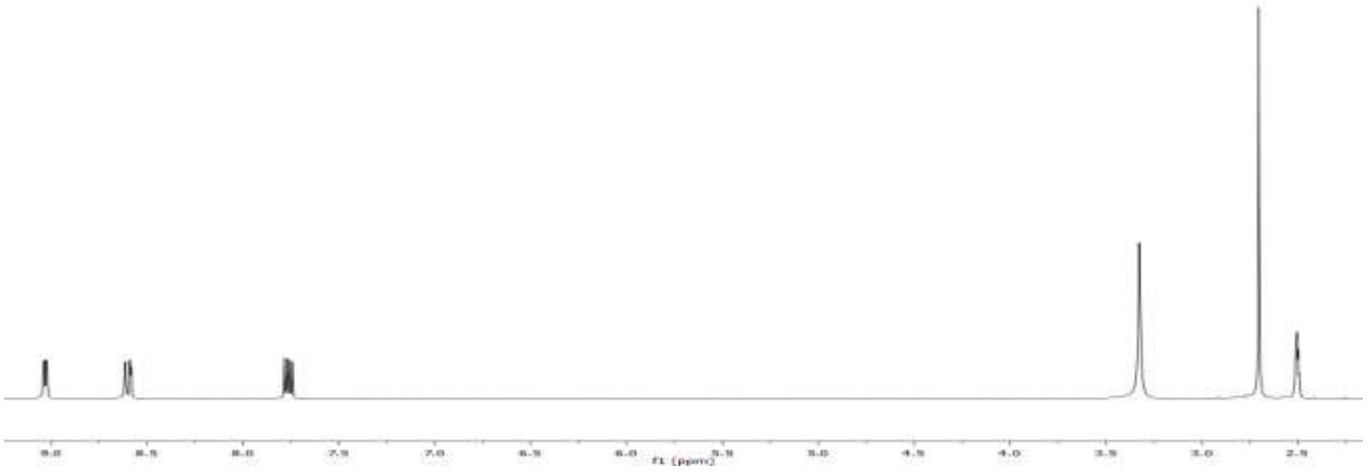
^{1}H NMR spectrum of HL^{1} in DMSO-d6 at 25 °C.



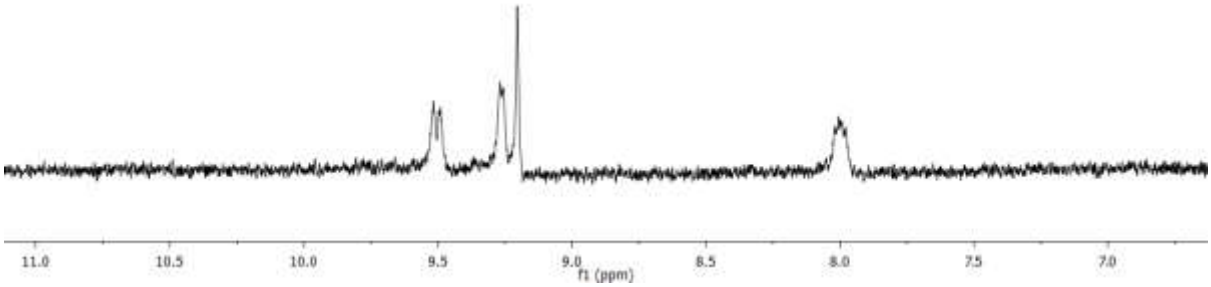
^{1}H NMR spectrum of HL^{3} in DMSO-d6 at 25 °C.



^{1}H NMR spectrum of [$PtCl_2$(1,10-phenanthroline)]in DMSO-d6 at 25 °C.

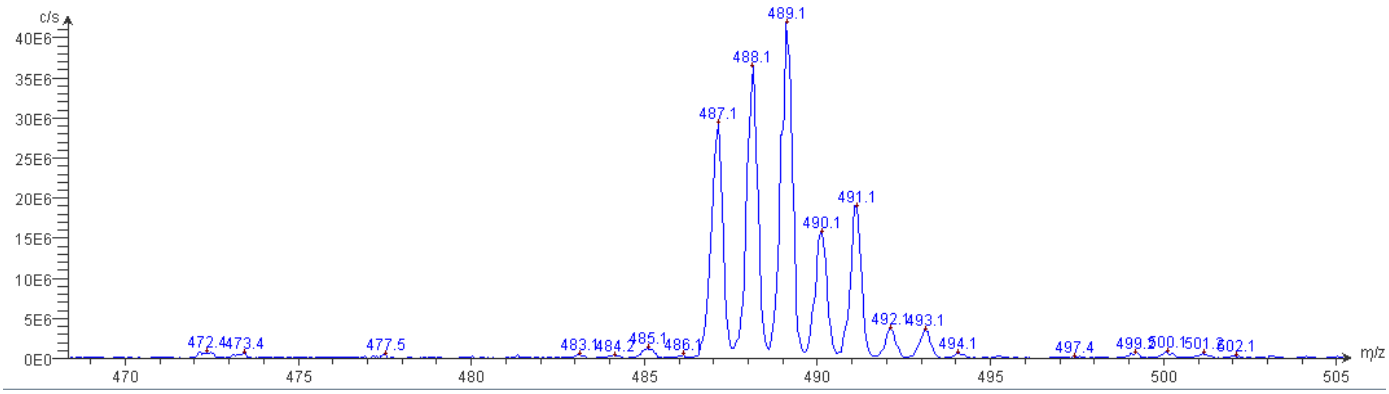


^{1}H NMR spectrum of [$PtCl_2$(5,6-dimethyl-1,10-phenanthroline)]in DMSO-d6 at 25 °C.

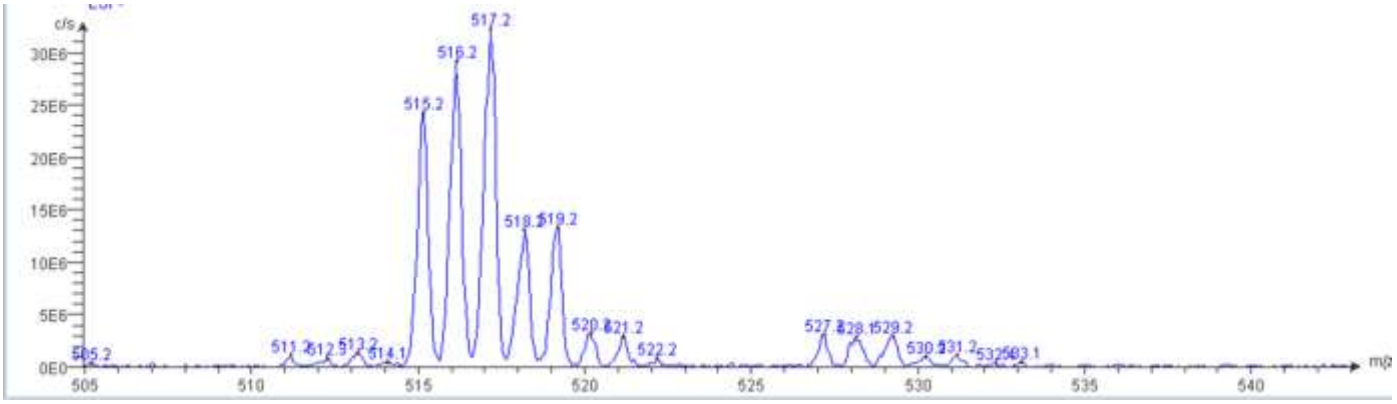


^{1}H NMR spectrum of [$PtCl_2$(pyrazino(2,3-f)(1,10-phenanthroline)] in DMSO-d6 at 25 °C

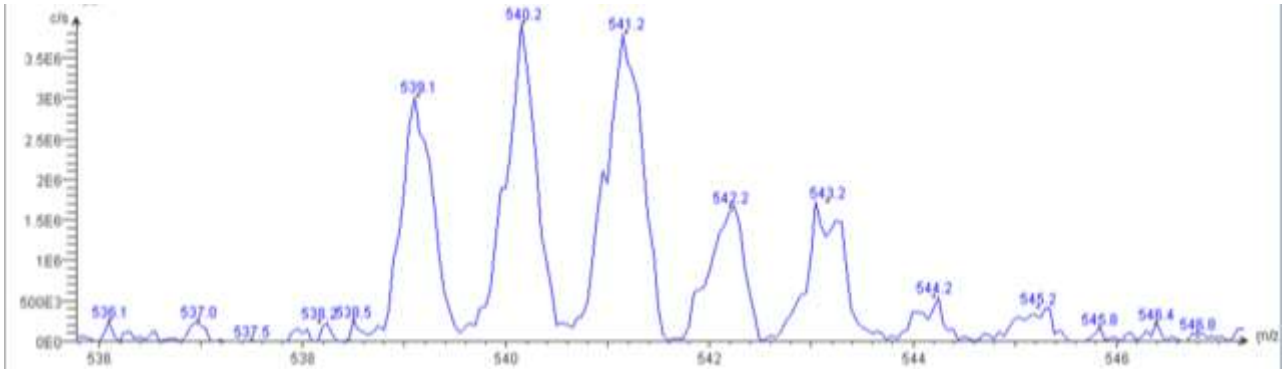
Mass Spectrometry



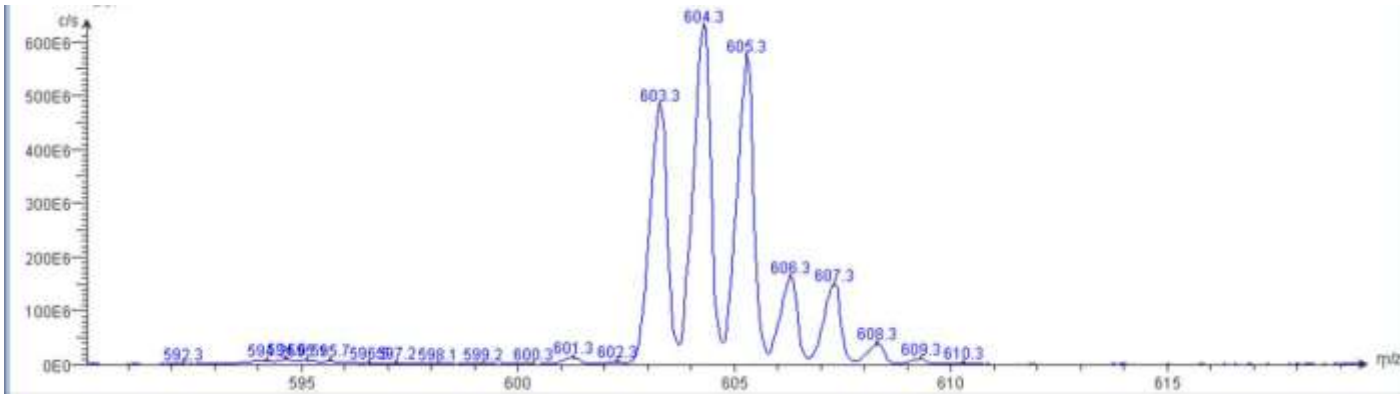
Mass Spectroscopy spectrum of [$PtCl_2$(1,10-phenanthroline)].



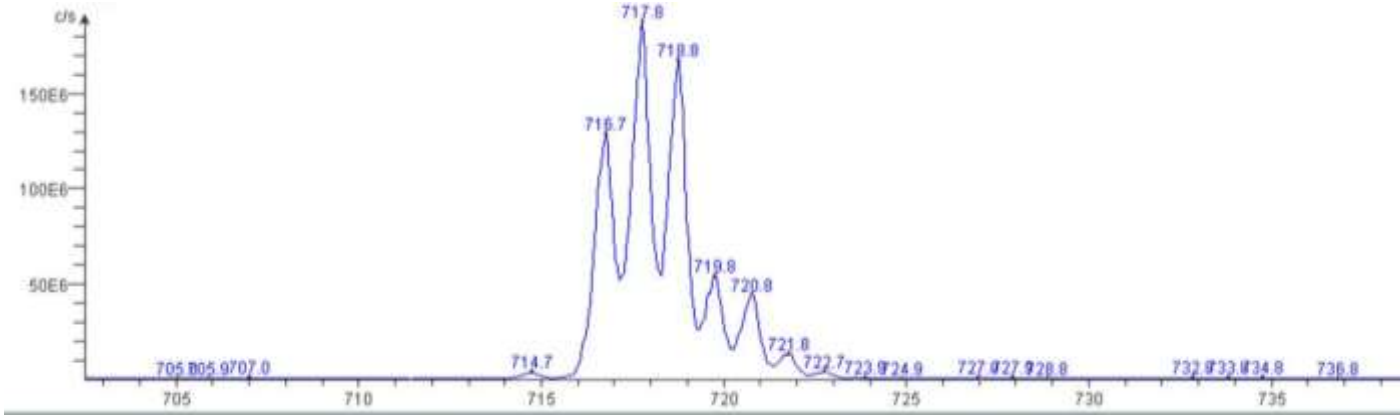
Mass Spectroscopy spectrum of [$PtCl_2$(5,6-dimethyl-1,10-phenanthroline)].



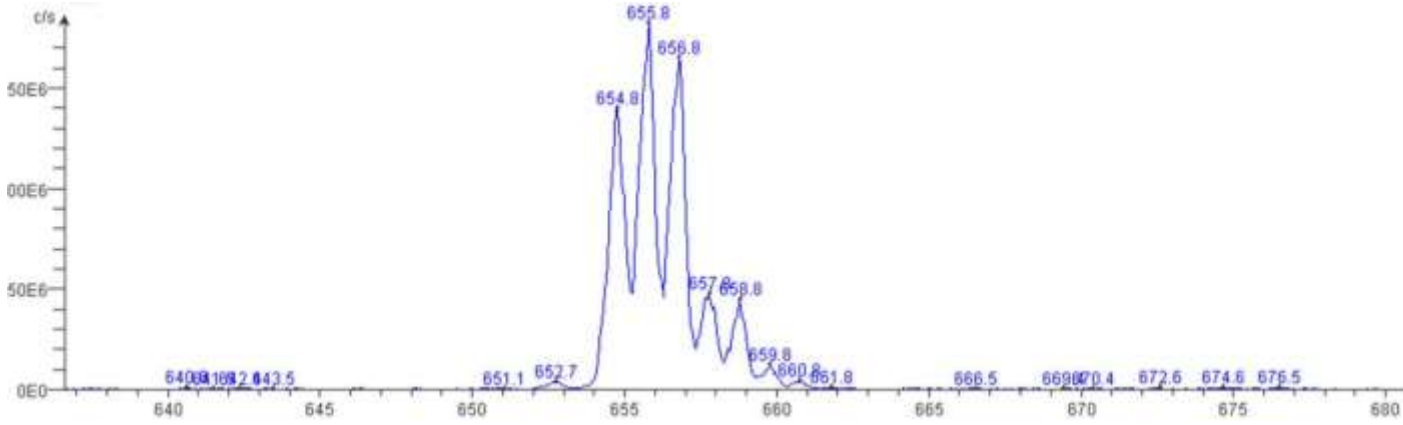
Mass Spectroscopy spectrum of [$PtCl_2$(pyrazino(2,3-f)(1,10-phenanthroline)].



Mass Spectroscopy spectrum of [Pt(dmp)(L^3-O,S)]Cl.

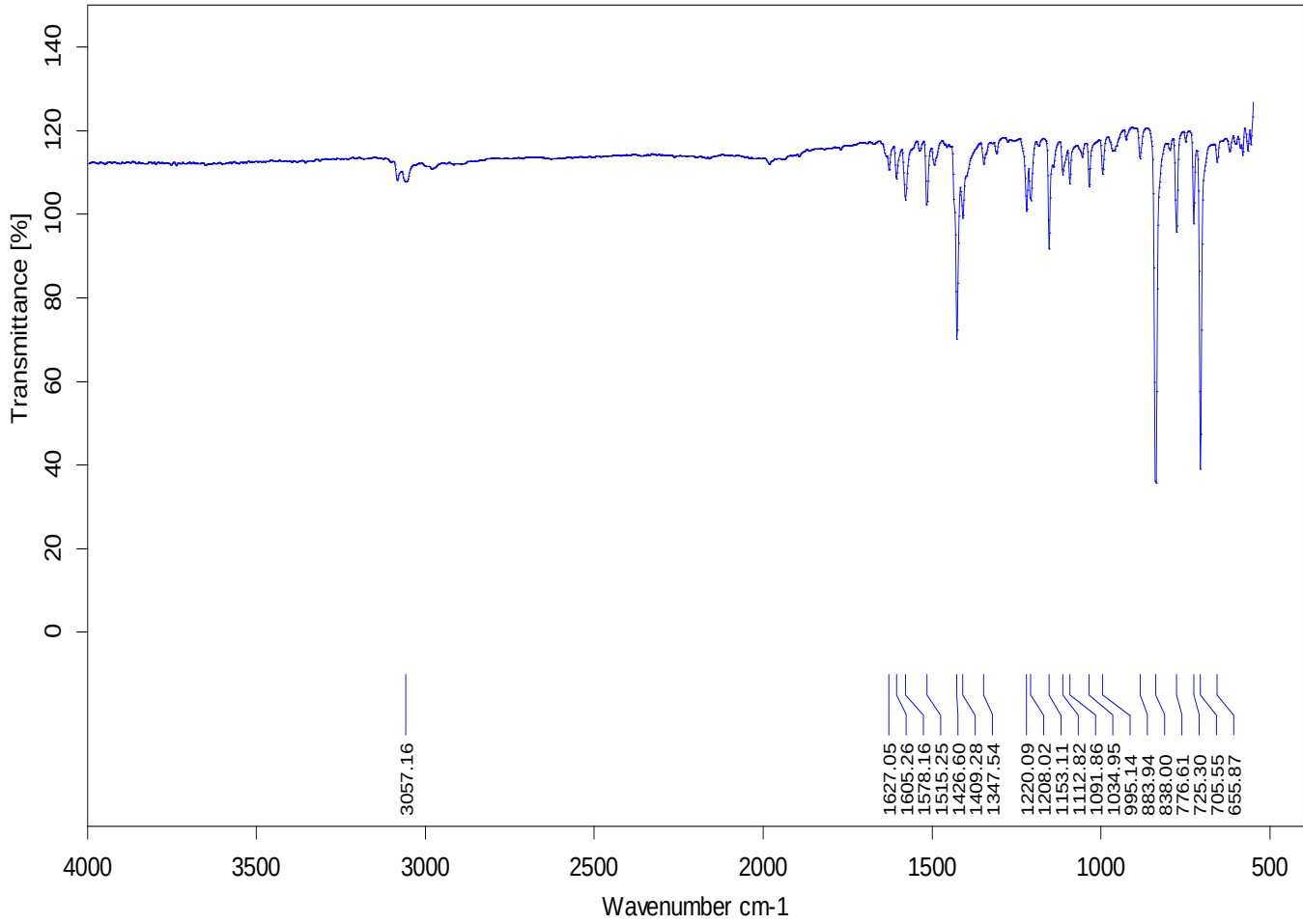


Mass Spectroscopy spectrum of [Pt(dpq)(L^1-O,S)]Cl.

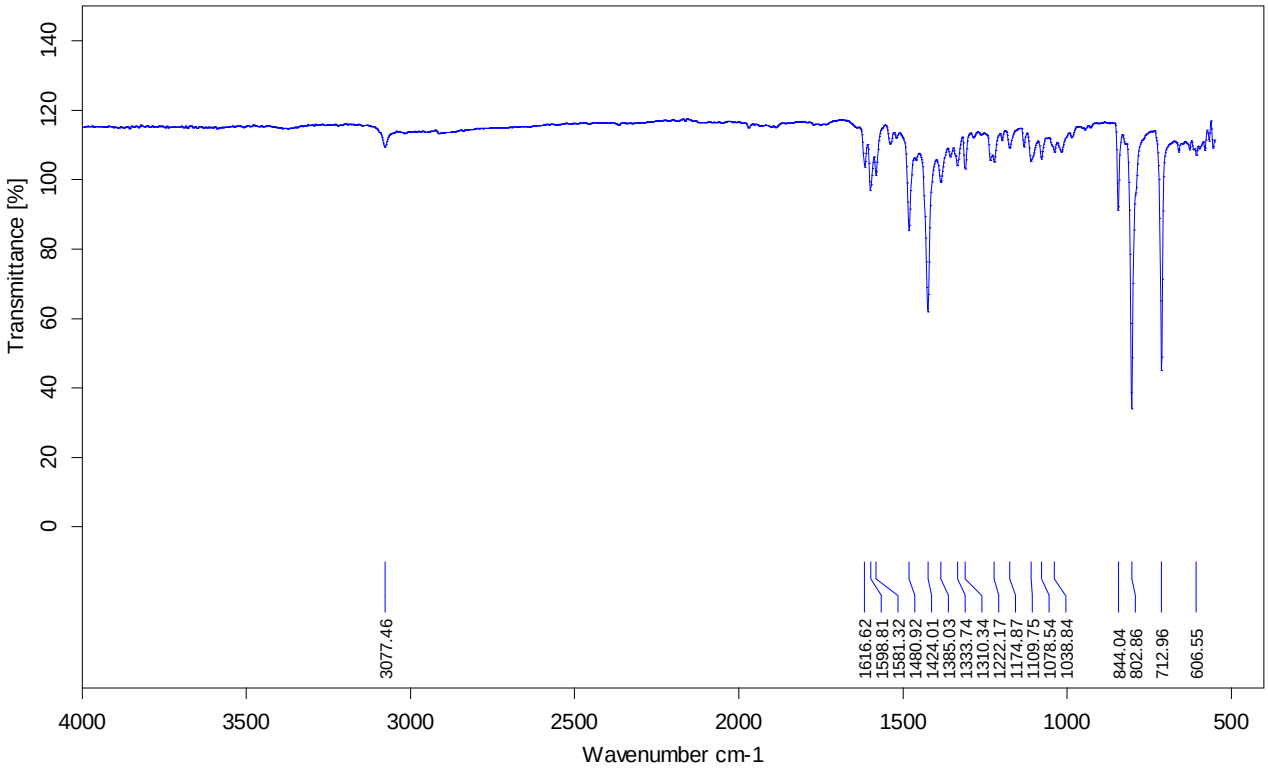


Mass Spectroscopy spectrum of [Pt(dpq)(L^3-O,S)]Cl.

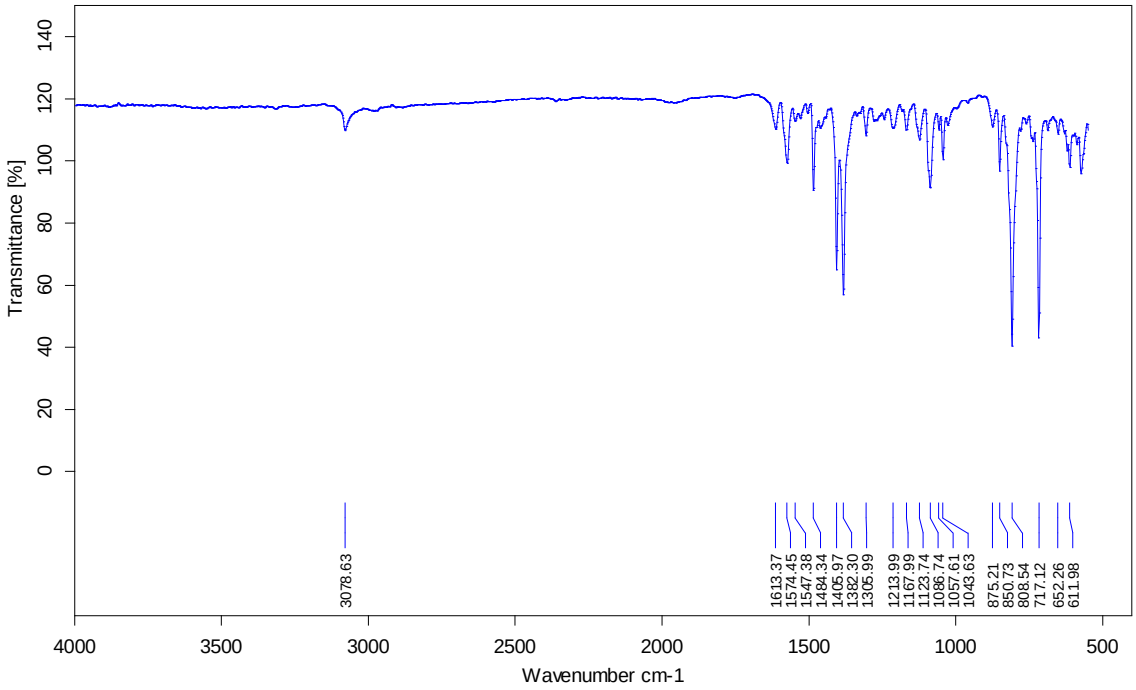
FTIR Spectroscopy



FTIR Spectroscopy spectrum of [$PtCl_2$(1,10-phenanthroline)].



FTIR Spectroscopy spectrum of [$PtCl_2$(5,6-dimethyl-1,10-phenanthroline)].



FTIR Spectroscopy spectrum of [$PtCl_2$(pyrazino(2,3-f)(1,10-phenanthroline)].