SYNTHESIS AND CHARACTERIZATION OF SOME THIONE CONTAINING PLATINUM(II) COMPLEXES AND THEIR ANTICANCER ACTIVITY

BY

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Dedication

It is my genuine gratefulness and warmest regard that I dedicate this thesis work to my parents for their endless love, support and encouragement throughout my entire life, and to my brothers and sisters for supporting me all the way.

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First and foremost, I would like to thank almighty God for letting me alive and giving me the strength and power to complete this work, despite all difficulties.

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LIST OF ABBREVIATIONS

A549	:	Human lung carcinoma
CDDP	:	*Cis*-diamminedichloridoplatinum(II) complex
CTR1	:	Copper transporter
DFT	:	Density functional theory
DMEM	:	Dulbecco's Modified Eagle's Medium
DNA	:	DeoxyriboNucleic Acid
FBS	:	Fetal Bovine Serum
FDA	:	Food and drug administration
FTIR	:	Fourier transform infra-red
GSH	:	Glutathione
Hela	:	Human cervix epitheloid carcinoma
HTC15	:	Human colon carcinoma
IC_{50}	:	Drug concentration needed to inhibit cell growth by 50% Against a single cell line
MCF-7	:	Human breast adenocarcinoma
MT	:	Metallothionein

NMR	:	Nuclear magnetic resonance
RNA	:	RiboNucleic Acid
TMS	:	Tetramethylsilane
Imt	:	R = R$^{/}$ = H; Imidazolidine-2-thione
MeImt	:	R = CH_3, R$^{/}$ = H; N-methylimidazolidine-2-thione
Me$_2$Imt	:	R = R$^{/}$ = CH_3; N,N$^{/}$-dimethylimidazolidine-2-thione
Et$_2$Imt	:	R = R$^{/}$ = C_2H_5; N, N$^{/}$-diethylimidazolidine-2-thione
PrImt	:	R = C_3H_7, R$^{/}$ = H; N-propylimidazolidine-2-thione
***i*-PrImt**	:	R = *i*-C_3H_7, R$^{/}$ = H; N-(*i*-propyl) imidazolidine-2-thione
di*i*-PrImt	:	R = R$^{/}$ = *i*-C_3H_7; N N$^{/}$-(di*i*-propyl)imidazolidine-2-thione
Diaz	:	R = H; 1,3-Diazinane-2-thione
EtDiaz	:	R = C_2H_5; N-ethyl-1,3-Diazinane-2-thione
Diap	:	1, 3-Diazipane-2-thione

ABSTRACT

Full Name : Mohammed Yagoub Ahmed Jomaa

Thesis Title : Synthesis and characterization of some thione containing platinum(II) complexes and their anticancer activity

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Cisplatin is one of the well-known anti-cancer agents it has been used for decades in cancer therapy. However, it has severe side effects. Therefore, it is desirable to design new platinum(II) complexes that would be able to enlarge the biological activity spectrum, improve clinical effectiveness as well as reduced toxicity. A new series of platinum(II) complexes based on thione ligands with general formula, *cis*-$[(Et_3P)_2Pt(L)_2]Cl_2$, *cis*-$[(NH_3)_2Pt(L)_2](NO_3)_2$, *trans*-$[Pt(NH_3)_2(L)_2](NO_3)_2$ and $[Pt(i\text{PrImt})_4](NO_3)_2(H_2O)_{0.6}$ have been synthesized and characterized using CHNS elemental analysis, mid- and far-IR, ^1H and ^{13}C solution NMR as well as ^{13}C solid-state NMR spectroscopy, and X-ray crystallography. The spectroscopic methods reveal that the Pt(II) center coordinates to thione ligands via sulfur donor atoms. The X-ray structures showed a distorted square planar geometry for *trans*-$[Pt(NH_3)_2(Imt)_2](NO_3)_2$ and *trans*-$[Pt(NH_3)_2(Me_2Imt)_2](NO_3)_2$ complexes (**T1** and **T3**), while hydrogen bonding interactions in $[Pt(i\text{PrImt})_4](NO_3)_2.0.6(H_2O)$ complex induce a see-saw distortion relative to the ideal square planar geometry. *In vitro* cytotoxicity studies of complexes (**A1-A7**) on four different cell lines (Hela, A549, MCF7 and HCT15) are promising and make these complexes potential anticancer agents. Complex **A6** was found to be the best and 14 fold better cytotoxic agent than cisplatin against human colon cancer cell line (**HCT15**).

ملخص الرسالة

الاسم الكامل : محمد يعقوب احمد جمعه

عنوان الرسالة : تخليق وتوصيف معقدات البلاتين(II) مع بعض مترابطات الثايون ونشاطيتها كمضادات للسرطان

التخصص : كيمياء

تاريخ الدرجة العلمية : مايو 2015

سيسبلاتن واحدة من العقارات المشهورة ضد السرطان وقد تم استخدامه منذ عقود في علاج السرطان. ومع ذلك له عدة اثارة جانبيه. لذلك، من المستحسن تخليق معقدات جديدة للبلاتين(II) تكون لها القدرة على توسيع طيف النشاط البيولوجي، تحسين الفعاليه السريريه وخفض السميه. سلاسل جديدة من معقدات البلاتين(II) مع بعض مترابطات الثايون تم تخليقها بالصيغ العامه *cis*-$[(Et_3P)_2Pt(L)_2]Cl_2$، *cis*-$[(NH_3)_2Pt(L)_2](NO_3)_2$ ، *trans*-$[Pt(NH_3)_2(L)_2](NO_3)_2$ و $Pt(iPrImt)_4](NO_3)_2(H_2O)_{0.6}$ وتم توصيفها باستخدام تقنيه تحليل العناصر، مطيايفيه الاشعه تحت الحمراء المتوسطه والبعيدة، الرنين النووي المغناطيسي لانويه كل من (^{1}H, ^{13}C, ^{31}P)، وكذلك الرنين النووي المغناطيسي في الحاله الصلبه لنواة (^{13}C) بالاضافه الى الاشعه السينيه للبلورات.

طرق التحليل الطيفي اظهرت ان مترابطات الثايون مرتبطه بالايون المركزي للبلاتين(II) عبر ذرة الكبريت المانحه. باستخدام طيف الاشعه السينيه للبلورات تم التعرف على البنيه التركيبيه لبعض المركبات، حيث اظهرت ان المعقدين *trans*-$[Pt(NH_3)_2(Imt)_2](NO_3)_2$ و *trans*-$[Pt(NH_3)_2(Me_2Imt)_2](NO_3)_2$ (**T3** و**T1**) لها شكل رباعي سطوح مشوه، بينما تفاعلات الرابطه الهيدروجينه في المعقد $[Pt(iPrImt)_4](NO_3)_2.0.6(H_2O)$ حثت وجود تشويه الارجوحه مقارنه بالشكل المثالي لرباعي السطوح.

دراسه الفعاليه الحيويه للمركبات **A7-A1** على اربعه خطوط مختلفه من الخلايا البشريه (خليه سرطان عنق الحم، خليه سرطان الرئه، خليه سرطان الثدي وخليه سرطان القولون)، اظهرت نتائج واعدة مما جعل هذه المركبات محتمله كمضادات للسرطان.

المعقد **A6** وجد انه الافضل بين المركبات وله فعاليه حيويه 14 اضعاف السيسبلاتين ضد خليه سرطان القولون البشري (HCT15).

CHAPTER 1

INTRODUCTION

Bioinorganic chemistry is a rapidly developing area of research with enormous potential applications in medicine [1]. Different metal complexes are presently used to treat various types of human disorders [2]. *cis*-Diammine dichlorido platinum(II) complex, clinically called cisplatin, is one of the most effective anticancer agent [3–5], showed a broad spectrum and high level of activity in the history of a successful antitumor drug [6–8]. It is generally believed that the biological activity of this drug results from its interactions with DNA [9]. It has been used for cancer treatment for the last forty years [10], particularly in treating specific cancers, including small and non-small cell lung, ovarian, testicular, head, neck and bladder tumors [11, 12].

Despite its great success and wide spectrum of applications [13], unfortunately, cisplatin is not effective against some cancer cells. In addition, this drug has several toxic side effects, like nephrotoxicity, neurotoxicity, ototoxicity and emetogenesis [14–16], and also limited by inherent and acquired tumor resistance [17]. In response to the urgent need for new anti-cancer drugs that are capable to overcome these disadvantages, thousands of Pt(II) complexes have been developed [18]. Researchers synthesized cisplatin analogues. However, a few of the developed compounds have entered human clinical testing, for instance carboplatin, which received worldwide registration for clinical use, while others received limited approval, such as oxaliplatin, in some countries, nedaplatin, lobaplatin,

and heptaplatin in Asia, China, Japan and South Korea, respectively [5, 11]. However, since most of these compounds have a common structure as cisplatin, the presence of two good leaving groups and two ammine ligand or two amine donor groups in cis configuration, this produce similar adducts with DNA as cisplatin and some drawbacks associated with cisplatin are consequently inherited [19].

A logical strategy has been directed toward the design of new Pt(II) complexes which interact distinctively in a different manner with DNA. One platinum agent that is known to possess DNA binding mode different from those of cisplatin is the trans isomer [20], While it's thought that the two good leaving groups in cis geometry in the Pt(II) complexes are necessary for the cytotoxic activity, some exceptional cases have been reported recently, where some trans isomers with planar heterocyclic ligands [21], are able to exhibit higher anticancer activity against cisplatin resistant cancer cells [12].

1.1 Statement of the problem

After administration of cisplatin in the human body there are many possibilities for binding, wither interaction of Pt(II) ion with the sulfur containing ligand biomolecules in the form of proteins, enzymes and peptides, such as glutathione (GSH) and L-cysteine, in intracellular, or with nitrogen containing biomolecules such as amino acids or DNA.

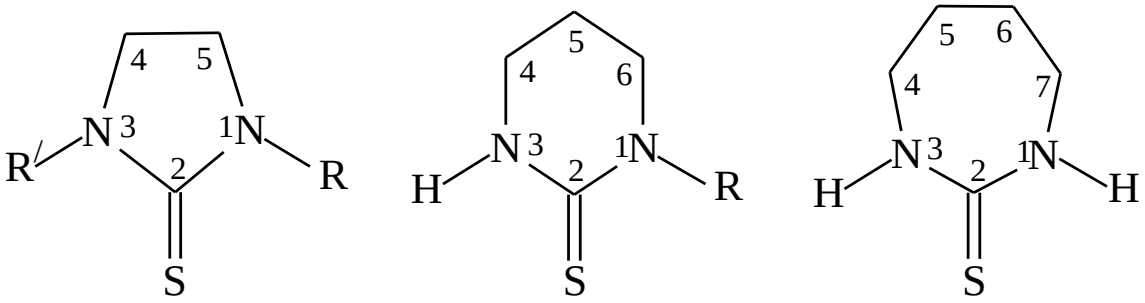
The cytotoxicity of the Pt(II) complexes is generally attributed to the interaction of the platinum metal with nitrogen donor biomolecules in DNA [22]. On the other hand Pt(II) compounds can interact with sulfur donor biomolecules before they reach the DNA, to form Pt-thiol complexes which are thermodynamically stable. These are believed to be responsible of the toxicity occurrence [23].

1.2 Rationale of the current study

The interaction of Pt-S(thiol) can be avoided using some ligands known as rescue or protecting agents, which are compounds containing sulfur and they are very strong nucleophiles. These compounds have been used to control the toxicity of cisplatin, such as thiourea, thiosulfate, diethyldithiocarbamate, thiosemicarbazone, xanthate, cysteine, GSH, etc. [24]. Pt(II) compounds coordinated to sulfur-containing ligands and possessing similar structures to cisplatin, have demonstrated superior or equal effectiveness against some human cancer cell lines and with less toxicity effect than cisplatin [25], The coordination of platinum with thiourea and its derivatives has been known for a long time, even though initial interest of these complexes were not related to their biological activities [26].

The high activity of platinum compounds with sulfur-donor ligands toward some cancer cells encouraged us to prepare some thiones containing platinum(II) complexes. Therefore the synthesis of complexes and studying their cytotoxic effect represents an interesting area of research. Specifically the aims of this work is to synthesize and characterize some thione containing platinum(II) complexes in order to produce non-cisplatin behavior.

In general, thiones are a class of organosulfur ligands [27]. Thione ligands used in this work were prepared according to the reported procedure by the addition of carbon disulfide to diamines in ethyl ether, the resulting adduct was heated, at 100-110 ºC for 2-3 hours, then crystallized in methanol [28]. The general structures of sulfur-donor ligands (Thiones) used in this work are given in, (Fig 1.1).



(1-7) (8-9) (10)

1 R = R/ = H; Imidazolidine-2-thione (Imt).

2 R = CH_3, R/ = H; N-methylimidazolidine-2-thione (MeImt).

3 R = C_3H_7, R/ = H; N-propylimidazolidine-2-thione (PrImt).

4 R = *i*-C_3H_7, R/ = H; N-(*i*-propyl) imidazolidine-2-thione (*i*-PrImt).

5 R = R/ = *i*-C_3H_7; N N/-(di*i*-propyl)imidazolidine-2-thione (di*i*-PrImt).

6 R = R/ = CH_3; N,N/-dimethylimidazolidine-2-thione (Me_2Imt).

7 R = R/ = C_2H_5; N, N/-diethylimidazolidine-2-thione (Et_2Imt).

8 R = H; 1,3-Diazinane-2-thione (Diaz).

9 R = C_2H_5; N-ethyl-1,3-Diazinane-2-thione (EtDiaz).

10 1, 3-Diazipane-2-thione (Diap).

Figure 1.1 General structures of sulfur-donor ligands (thiones) used in this work

1.3 Objectives

The objectives of this work are as follow.

a. Synthesis of complexes with general formula *cis*-[$(NH_3)_2PtL_2$]$(NO_3)_2$ by using *cis*-[$(NH_3)_2PtCl_2$] and a series of thione ligands as precursors.

b. Synthesis of complexes with general formula *trans*-$[(NH_3)_2PtL_2](NO_3)_2$ based on *cis*-$[(NH_3)_2PtCl_2]$ and a series of thione ligands.

c. Synthesis of complexes with general formula *cis*-$[(Et_3P)_2PtL_2](NO_3)_2$ by using the *cis*-$[(Et_3P)_2PtCl_2]$ and a series of thione ligands.

d. Characterization of the synthesized complexes by different techniques like elemental analysis, FT-IR spectroscopy, ^{1}H & ^{13}C solution NMR, and ^{13}C solid state NMR spectroscopy in addition to single-crystal X-ray crystallography.

e. *In vitro* anticancer activity studies of the synthesized complexes against a panel of human cancer cell lines.

1.4 The thesis organization

The work in this thesis has been divided into five main chapters

1.4.1 Chapter one: introduction

A brief introduction is given about the anticancer drugs specially cisplatin and its analogues and their drawbacks. The problem of this study is also stated, followed by the aim and objectives of our work.

1.4.2 Chapter 2: literature review

A short review about cancers, cancer treatment, the proposed mechanism and the mode of action of the most successful anticancer agent cisplatin.

1.4.3 Chapter 3: methodology and techniques

The first part of this chapter describes the methodologies which we followed to synthesize

our complexes. In the second part a detailed description of the characterization techniques used to analyze the prepared compounds is given.

1.4.4 Chapter 4: results and discussions

This chapter shows the significance of our work and results from all characterization techniques and discussions.

1.4.5 Chapter 5: conclusion and recommendations

The general conclusion of the work is summarized in this chapter and describes how this work could be extended in the future.

CHAPTER 2

LITERATURE REVIEW

2.1Cancer

Cancer is a name for a number of many diseases in which the body cells grow abnormal then divide without control and easily invade other tissues. Cancer cells growth and spread through the blood stream and lymph systems to other parts of the body. Lastly the tumor load will cause death in humans [29]. Cancer presents two types of tumors, malignant tumors and benign tumors. Malignant tumors are different from benign tumors as they show uncontrollable growth, invade locally, and metastasize to distant body parts, they are named according to the type of cell or organ in which they start such as breast cancer, lung cancer, colon cancer, etc. Benign tumors grow in one place and lack the ability to metastasize [30].

Cancer is caused by both internal factors such as hormones, inherited mutations and immune conditions or acquired/environmental factors such as radiation, diet, tobacco, infectious organisms [31].

2.2Cancer treatment

There are many methods used for cancer treatment, it can be used alone or in combination, including surgery, biological therapy, immunotherapy, radiation, gene therapy and chemotherapy[32].

2.2.1 Surgery

In this case cancer can be surgically removed from the body and lead to a complete cure. This treatment is often used in the removal of the breast, testicle or prostate. However, it is almost impossible to remove all of the cancer cells after the disease has spread. Surgery can be also by instrumental in helping to control symptoms such as spinal cord compression or bowel obstruction [33].

2.2.2 Radiotherapy

High energy rays, such as gamma rays that are emitted from metals like radium or energy that can be generated from special machines such as X-ray are used or exposing on the cancer cells. These rays cause damage to the molecules that make the cancer cells and lead them to destroy oneself. The energy beams cannot differentiate between normal tissues and carcinogenic tissues this will lead to severe side effect, but technological improvements have been made so the carcinogenic tissues can be targeted accurately. This type of treatment can be used alone or in combination with other treatment methods [33].

2.2.3 Immunotherapy

This method of treatment is aiming to get the body's immune system to fight the cancer. Materials either made by the body or in a laboratory are used to restore or improve the immune system function. Although the mechanism of immunotherapy that treats cancer is still not well understood, it may work by slowing or stopping the cancer cell growth, stopping cancer from spreading all over the body, or helping the immune system to increase its effectiveness at eliminating cancer cells [34].

2.2.4 Gene therapy

Gene therapy is designed to remove the roots that cause cancer by replacing the damaged genes with ones that work. Many research works have been carried out to replace the damaged genes that signals cells to stop dividing with a copy of a working gene [35].

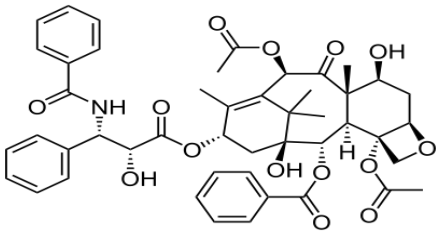
2.2.5 Chemotherapy

Chemotherapy is a type of treatment that uses chemical compounds to damage the cells that are rapidly dividing mainly cancer cells, so chemist can play an important role. Extensive research has been done to develop potent chemotherapeutic agents [32]. Since the drugs travel throughout the entire body, chemotherapy is suitable to treat cancers that have spread or metastasized. The treatment occurs in cycles, so the body has time to heal between doses [36]. However, there are common side effects such as hair loss, nausea, vomiting, and fatigue [37]. Chemotherapy often includes multiple drugs in combination, or it can be also used in combination with another type of treatment [36].

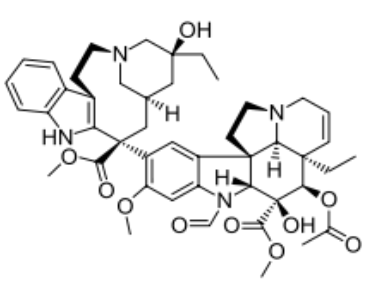
2.3 Non-metal containing cancer drugs

The therapeutic properties of natural products have been used for a long time, the practice is as ancient as human civilization. Natural products have provided all humankind needs in term of food, clothing, shelter, flavors and perfumes and it's the source of most drugs in medicine, and most of these therapeutic agents are provided by the higher plants [38]. Nowadays, natural products, derivatives and their analogues are commonly used as curatives and represents more than 50% of all drugs in the medicinal use worldwide, with higher plant-derived natural products representing approximately 25% of the total [38]. A major group of these products includes the powerful antioxidants, others are phenolic in

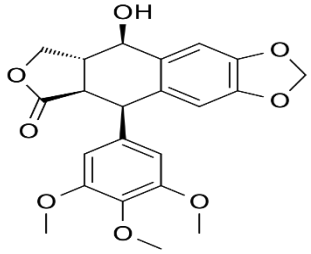
nature, and some important drugs that obtained from plants like quinine and quinidine from *Cinchona* spp., Digoxin from *Digitalis* spp., Vinblastine and vincristine from *Catharanthus roseus*, codeine and morphine from *Papaver*, atropine from *Atropa belladonna* and *somniferum*. [39]. It is estimated that about 60% of antitumor and anti-infective drugs that are available on the market or in the clinical testing's are of natural origin [40]. Besides the natural products that exhibit anticancer activity such as tamoxifen (Nolvadex) paclitaxel (Abraxane or Taxol), vincristine (Oncovin), Podophyllotoxin (Condylox) and camptothecin [41], there are many others that have served as templates or chemical models for the synthesis, semi-synthesis, and design of novel substances for diseases treatment [42].



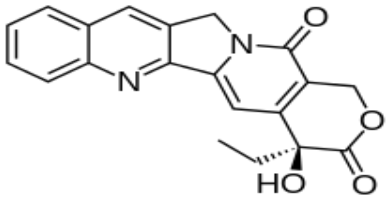
Taxol



Vincristine



Podophyllotoxin



Camptothecin

Figure 2.1 Structures of compounds among the clinically useful drugs

It's worth mentioning that metal-containing drugs (inorganic based drugs) still remain superior to other agent's especially anticancer drugs. This is due to the simple reason that inorganic compounds are synthesized with specific designs for specific targets, as to bind to the DNA; hence their activity as anti-cancer drugs [43]. While, the organic compounds are found in nature in smaller quantities and their synthesis in the laboratory is a big challenge, i.e. Total synthesis of taxol took many years [44]. Even though these drugs can be easily achieved by synthesis of other new metal containing compounds [43].

2.4 Metal and transition metals in medicine

Metal ions are very important for many critical functions in humans. Deficiency of some metal ions can cause a disease. For examples iron deficiency causing pernicious anemia, growth retardation emerging from insufficient dietary zinc, and heart disease in infants due to deficiency of copper. Furthermore, metal ions may also induce toxicity in humans, heavy metal poisons such as lead and mercury being classic examples. Even that are essential can be toxic when present in excess [45]. Some metal complexes showed activity against some human disease such as, Ga^{3+}, Al^{3+} and Fe^{3+} were shown to have activity against malaria [46].

Transition metal complexes have been widely used in medicine as drugs for treating several human diseases, such as carcinomas, antiarthritis, anti-inflammatory, infection control, diabetes, lymphomas, and neurological disorders [47]. Transition metals exhibit unique properties like electrophilicity, Lewis acidity, redox activity, valency, magnetic resonance and radiochemical properties, and can covalently interact with a negatively charged

molecules. Transition metal-based drugs have promising medicinal applications , some has shown unique therapeutic activities [48].

In the early 1960s the anticancer activity of Pt(II) complex, cisplatin, one of the most effective and widely used cytotoxic drug was discovered, other metals such as gold, titanium, ruthenium, and vanadium also showed promising results in this area [49]. One of the interest metal in cancer therapy is silver because its toxicity level in humans is believed to be quite low. Silver is being used in medical applications, including cardiac catheters, coating of heart valves, and urinary catheters to prevent or reduce infection [50]. It is widely known that excessive exposure to silver for long periods causes Argyria, which is permanent skin discoloration [50].

In the early 1970's rhodium was discovered to possess antitumor activity against various tumor cell lines, the majority of the rhodium complexes evaluated were found to be active but they are highly toxic and have lesser effectiveness than cisplatin. A class of dirhodium compounds [μ-$(RCO_2)_4Rh_2(H_2O)_2$] (R: Alkyl group), in particular, displayed high activity *in vivo* against many types of tumor, however; several toxic side effects hindered their advancement. Ruthenium complexes were also reported for their antitumor activity. The earliest investigation of ruthenium compounds having antitumor activity discover when Clarke investigated the activity of *fac*-[Ru(III)$Cl_3(NH_3)_3$] in the 1980's. However this drug has a poor water solubility. Up to now there are many of ruthenium compounds have been investigated, but, only a few of them have activity comparable to cisplatin [51].

Copper is an essential trace element and that plays a vital role in the biochemistry of every living organism. The use of copper in cancer treatment was reported in the early 1980s on

the activity of copper thiosemicarbazones. Recently copper complexes of carboxylates and carboxamidrazones have been developed [52].

Rhenium has also potential applications in the field of cancer treatment. Re(IV)-based complexes of the type $[ReCl_4(L)]$ have shown potent anti-proliferative effects *in vitro* against some cancer cell lines. These complexes represent novel, potentially active metallo-drugs based on the satisfactory results [53].

Metallic gold and gold complexes, have been used for a long time in medicine [47]. The value of gold in this field was recognized when the bacteriologist Koch's explored the activity of $K[Au(CN)_2]$ in fighting the bacteria that cause tuberculosis, it was used to treat rheumatoid arthritis, which they believed was related to tuberculosis. gold(I) with phosphine as ligands that can stabilize the +1 oxidation state attract much attention, many gold(I) phosphine compounds have been evaluated, such as triethylphosphine compounds and auranofin, were found to possess excellent activity for rheumatoid arthritis treatment [54].

2.5 Platinum based anticancer drugs

Platinum compounds are well known as successful chemotherapeutic agents such as cisplatin, which indicated considerable activity toward some of the cancer cell lines. It was first synthesized in 1844 by Michel Peyrone and called Peyrone chloride at that time [55]. In 1893 Alfred Warrner deduced the structure of cisplatin. Its biological activity was discovered accidentally in the 1960s by Barnett Rosenberg in United States at University of Michigan, East Lansing [56]. Initially, he designed to study whether magnetic or electric dipole fields contribute to cell division, by applying electromagnetic radiation on

mammalian and bacterial cells. Without intention, a set of platinum electrodes which is known to be inert was involved in the growth chamber of *Escherichia coli* in ammonium chloride buffer [18]. After the electromagnetic field turned on, the filamenation occurs which is a strange growth of the bacteria length; reached up to 300 times of the normal length. This phenomenon could not be attributed to the effect of the applied field. Investigations found that the observation is due to the presence of platinum(II) and

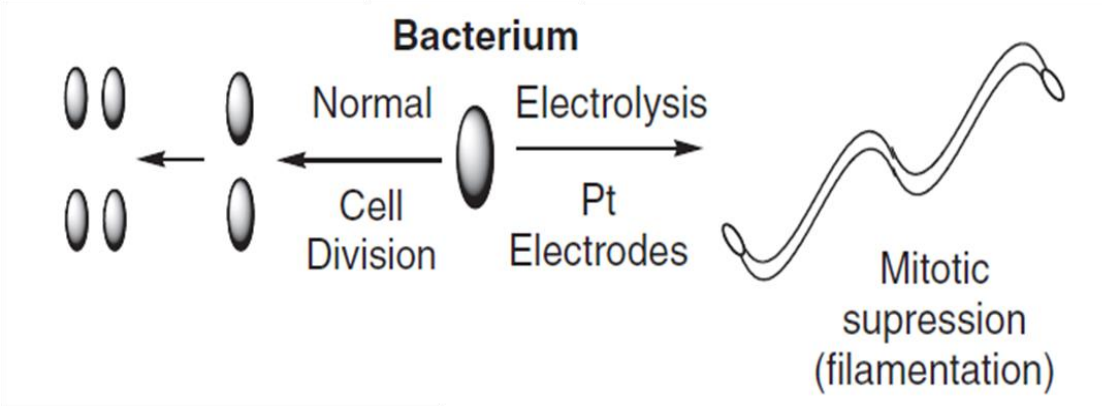


Figure 2.2 Cell division and bacteria filamentation

platinum(IV) ammine chlorido complexes formed *in situ* originating from electrolysis at the platinum electrodes. Further investigations showed that specifically, *cis*-diammine dichlorido platinum(II) is the causing molecule of these biological effects [57].

Several medicinal trials of cisplatin as a drug commenced soon thereafter, and it was used to treat the first patient in 1971. Cisplatin was granted approval against several human cancers in 1978 by the US food and drug administration (FDA) [7]. At present, cisplatin is considered to be one of the most extensively used anticancer drugs [5, 58]. This is due to its activity against a lot of cancer cells like bladder, cervical, ovarian, head and neck,

testicular, small-cell and non-small cell lung cancers. In spite of the mentioned, cisplatin is ineffective in other cancers e.g. Leukemia, gastrointestinal, and renal cancers [3].

Generally it was believed that the relationship between structure and activity of platinum drugs is the cis geometry. Platinum atom should bind to bidentate amine ligands or cis to two amines (at least have one NH group on the amine) and two good leaving groups with an intermediate binding strength, such as chloride, sulfate, oxalate and citrate, until Farrel and his group reported that platinum complexes with trans configuration are also able to show antitumor activity. Examples of such biologically active trans platinum compounds include *tran*-[$PtCl_2$(iminoether)$_2$] and *trans*-[$PtCl_2${$NH_2CH(CH_3)_2$}{NH-$(CH_3)_2$}] [47].

Figure 2.3 Isomeric structure of diamminedichlorido platinum(II) complex

When the two ammine ligands were replaced by a planar pyridine ligand and *trans*-[$PtCl_2$ $(py)_2$,] was formed, the cytotoxic effect of the new complex is dramatically enhanced compared to that of both cisplatin and transplatin isomers [59].

2.5.1 The mechanism of action of cisplatin

After administration of cisplatin usually intravenously through injection, although there is some evidence that uptake may occur by an active transport mechanism, a variety of chemical reactions may take place [60].

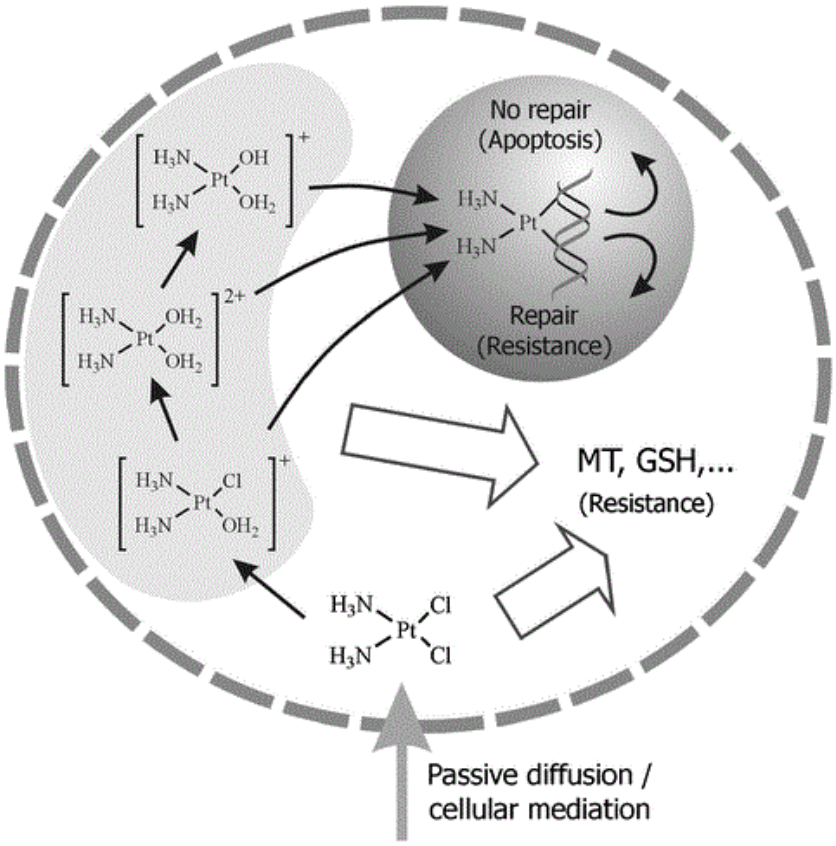


Figure 2.4 Cellular uptake of cisplatin drug

It's generally accepted that the neutrality of cisplatin allows it to enter the cell through passive diffusion, due to the fact that the concentration of chloride ion inside the cell is relatively low, approximately 3–20 mM compared to the extracellular medium, approximately 100 mM. It has to be activated through spontaneous aquation reactions [61], which involve the replacement of one chlorido ligand by water molecule to form of reactive monoaqua adduct or by replacement of the both chlorido ligands and form a diaqua complex. These aquated species are highly active due to positive charge and H_2O molecule is a better leaving group than Cl^-, these species can react with many different cellular targets, including proteins, glutathione or others inside the cell [62].

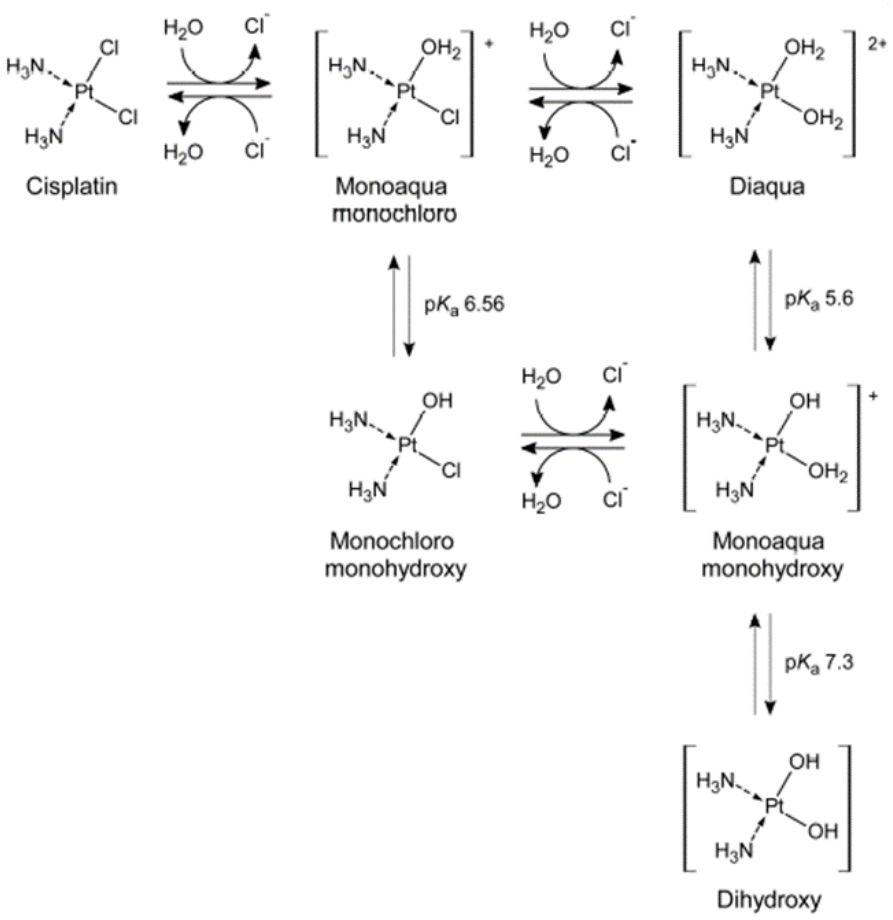


Figure 2.5 Spontaneous hydrolysis of cisplatin in aqueous solutions

2.5.2 Interaction of cisplatin with DNA targets

It's widely believed that the cytotoxicity of CDDP is due to the formation of DNA adducts to induce its significant biological effects, either repair the damage of the DNA and cell survival or activation of the irreversible apoptotic program [63, 64]. Although many components have electron lone pairs in the DNA, where metal ions may covalently bind *(i.e.* The phosphate groups, the sugar, oxygen atoms, and the heterocyclic nucleobases), early investigations showed that N7 position in the imidazole rings of both guanine and adenine are the most accessible and reactive site for platination due to their high

nucleophilicity [60]. Platination may form different DNA adducts such as monofunctional *cis*-[Pt(NH_3)$_2$(H_2O)]-DNA, bifunctional *cis*-[Pt(NH_3)$_2$]-DNA called intrastrand or interstrand cross-links [65]. It was found that 60 - 65% of the formed adducts are 1, 2-d(GpG) intrastrand cross-links binding with two neighboring guanine in the same strand and 20 - 25% d(ApG) cross-links with one adenine and one guanine [9, 66]. It was shown that minor adducts 1, 3-d(GpTpG) intrastrand and interstrand cross-link with two guanine separated by one nucleotide could be also involved in the mechanism of action of cisplatin, and DNA-protein cross-links have also been reported to be induced by cisplatin [67], However, consequently, these cross-links mainly 1, 2-d(GpG) intrastrand lead to either cell repair by removing the adduct or cell destrotion by preventing the replication of the DNA . The mechanism by which these adducts lead to the significant biological effect is not well understood [67].

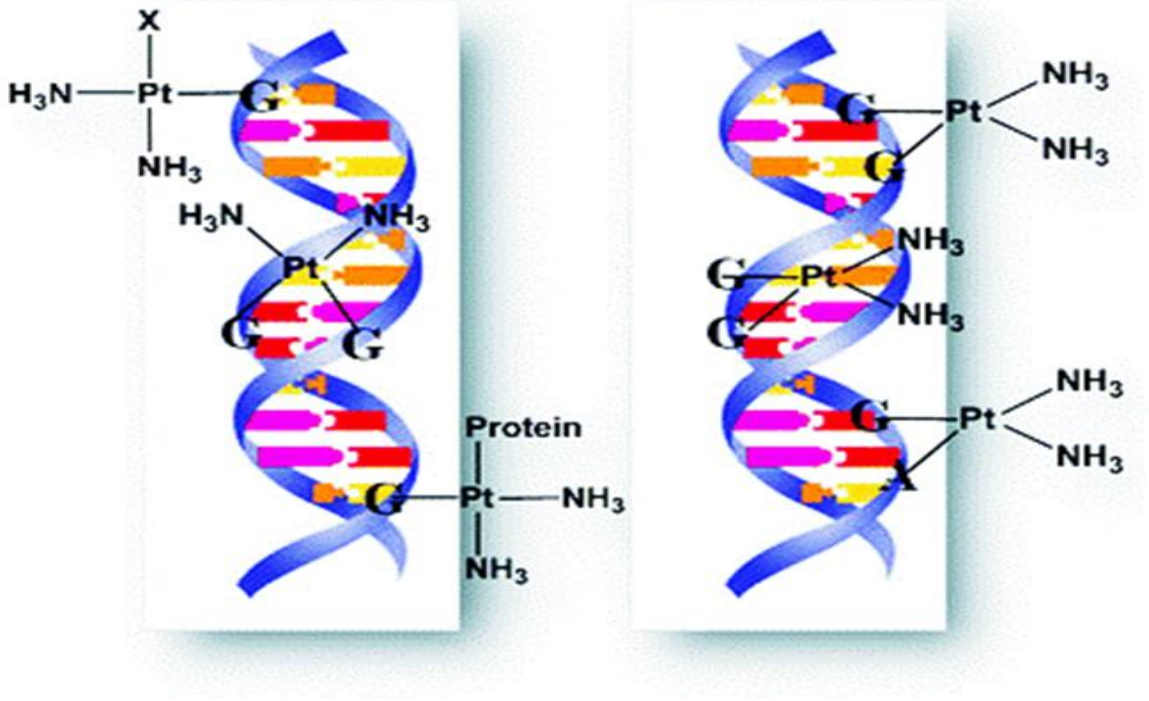


Figure 2.6 Different cisplatin - DNA binding ways

2.5.2.1 Guanine versus adenine

It's believed that the N7 atoms of the imidazole rings in the DNA purine bases guanine and adenine are most nucleophic and accessible sites which can be a major target for platination, mainly through 1, 2-d(GpG) or 1,2-d(ApG) intrastrand cross-links. It was found that N7 of guanine is thermodynamically and kinetically favorable site for binding over adenine that leads to bending and unwinding of DNA [10, 12].

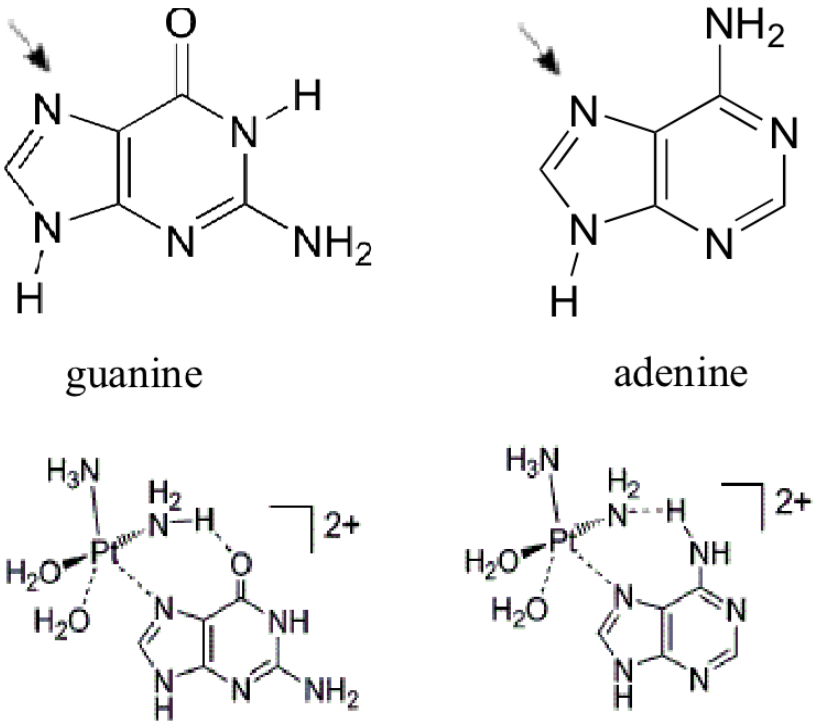


Figure 2.7 Hydrogen bonding in case of guanine and adenine after platination

After the binding of platinum to the nucleophilic site in both structures, the ammine ligands act as hydrogen-bond donors, whereas C6 position in the guanine ring (oxo group) and (amino group) in case of adenine ring, known to be hydrogen-bond acceptors. The hydrogen bond between the N-H….O=C6 in case of guanine is a much stronger than the hydrogen bond forms between the N-H….NH_2-C6 in case of adenine. In addition, MO calculation have identified strong interaction for guanine compared to adenine [10].

In order to confirm that the N7 of guanine is a preferable site for platination over adenine, Lippard and his group performed DFT studies of $[Pt(NH_3)_2]^{2+}$ complexes with guanine and adenine. Thermodynamics and kinetics factors of the complexes were taken into account, verifying that guanine is more reactive 20 times than adenine toward platination [12].

2.5.2.2 DNA repair

DNA repair systems are vital to the survival of all organisms, and can be by four major pathways, including nucleotide-excision repair (NER), base-excision repair (BER), mismatch repair (MMR) and double-strand-break repair, NER is the major pathway which is an ATP-dependent multiprotein complex can recognize the bending that induced on DNA by 1, 2-intrastrand cross-links, and thereafter eliminate the cisplatin lesions from DNA. And then filled the gap that remains by DNA polymerase [63].

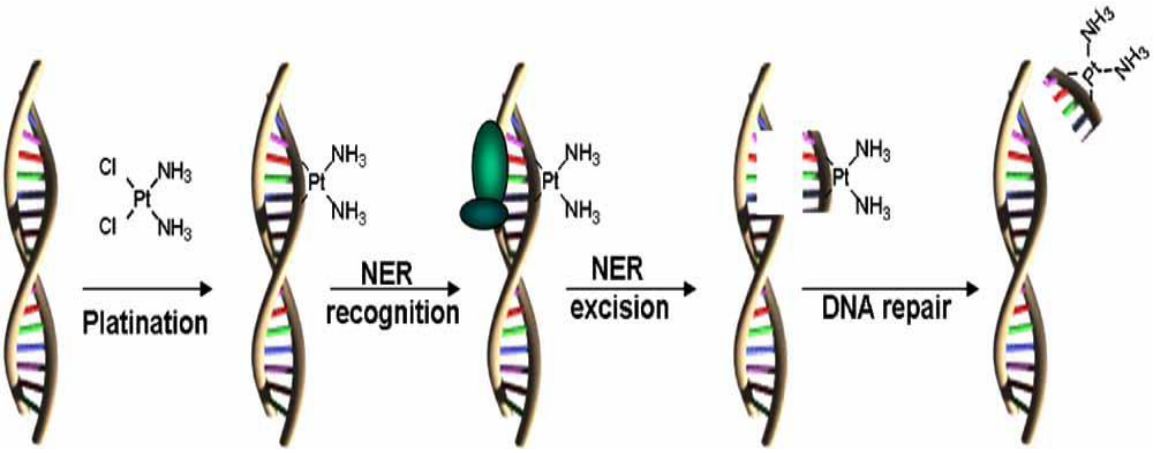


Figure 2.8 The steps involved in the repair of the major cisplatin crosslink by the NER.

2.5.2.3 Tumor resistance to cisplatin

One of the common drawbacks of platinum drugs, occurrence of the drug resistance, moreover, the resistance of cisplatin considerably varies between tumor types. Some tumors, like head and neck cancer, ovarian cancer, and small cell lung cancer, developed

acquired resistance. While others, such as colorectal cancer and non-small cell lung cancer have developed intrinsic resistance to cisplatin chemotherapy [67].

In most preclinical models of cisplatin resistance (either intrinsic or acquired), two mechanisms are mediated to operate. The first one, the failure of a sufficient amount of *cis*-DDP reaching DNA as its main target and the second, a failure to achieve cell death after the formation of the cisplatin-DNA adduct [7].

The acquired resistance to cisplatin is a resistance through insufficient DNA binding, commonly observed over many years that cause reduction in platinum accumulation compared to parental [68]. This is due to the binding of the platinum with highly abundance sulfur containing species such as metallothionein, and glutathione that leads up to detoxification and thus causing resistance to cisplatin [69, 70].

The intrinsic resistance to cisplatin is a resistance mediated after DNA binding, after the formation of platinum-DNA adducts, cellular survival (and therefore, tumor, drug resistance) can occur either by DNA repair or removal of these adducts, or by tolerance mechanisms [71].

2.5.3 Interaction of cisplatin with Non-DNA Targets

It is well known that the activated form of cisplatin also can bind to other biomolecules before accumulation in the cell [72], many components have nucleophilic sites in the cytoplasm e.g., cytoskeletal microfilaments, thiol-containing peptides, sulfur containing amino acids (cysteine, methionine) and proteins, and RNA may react with cisplatin based on hard-soft principle. Pt(II) is a soft acid and has high affinity to S-donor molecules,

glutathione (GSH) and metallothionein (MT) are the most abundant cellular thiol that can strongly bind to the platinum(II) ion, this binding can associate with negative phenomena that can prevent the performance of these proteins and lead to the toxicity [67]. The activity of enzymes, receptors, and other proteins may be affected also through binding of Pt(II) ion to sulfur atoms of methionine and/or cysteine or nitrogen of histidine residues. The resulting functional protein damage may also contribute to the biochemical mechanism of cisplatin cytotoxicity [67].

2.6 Cisplatin drawbacks and side effects

In spite of the great success of cisplatin as drug, it's not effective against some cancer cells (e.g. Breast and colon), and suffers from several side effects [73], such as nephrotoxicity (damage of the kidney), neurotoxicity (damage nervous system), ototoxicity (hear loss) and emetogenesis, as well as drug resistance, which remains one of the most serious and challenging problems to overcome, as mentioned in (2.5.2.2) [74]. Current research and focuses on designing a modified version of cisplatin as new generations that interact differently with the targeted DNA to minimize the negative aspects coupled with cisplatin, and are active towards tumors, which are non-responsive to current cisplatin chemotherapy. Many platinum complexes are currently in clinical testing, but some of them have not yet demonstrated significant advantages over cisplatin[11].

2.7 Second generation carboplatin

cis-diammine(cyclobutane-1,1-dicarboxylato) platinum(II), (**Fig 2.9**) is a new generation known as carboplatin, it differs from the first generation cisplatin by the presence of a bidentate ligand (dicarboxylate) as leaving group instead the labile chlorides of the

cisplatin, it was first discovered in the early 1970s by Rosenberg and his group to enhance the performance of the first generation and to expand the range of useful anticancer activity [75]. It was approved in 1986 under the brand name of paraplatin as a drug. Carboplatin has a slower substitution rate compared to cisplatin, this feature makes it much less chemically reactive (ototoxic, neurotoxic, and nephrotoxic) compared to cisplatin and can be administrated at a higher dose than cisplatin, this prompt researchers to focus and study how this drug can be activated *in vivo* [76]. After passing through the blood, carboplatin shows to enter the cell via passive diffusion, although it may enter through active transport or via ion channels also. A study done by Osella and coworkers suggests that carboplatin enters the cell through a passive diffusion mechanism [77].

The low toxicity has been the feature that enabled carboplatin to receive worldwide clinical use approval. Unfortunately, it's still active only in the same cisplatin range of tumors and still administered intravenously [36]. Carboplatin possesses a similar structures as cisplatin that can form similar DNA adducts. This will lead to the same cisplatin disadvantages. So the researchers focused on seeking on a new platinum compound with improved tolerability profiles that may overcome the side effect of the first generation.

2.8 Third generation oxaliplatin

[(1R, 2R)-cyclohexane-1, 2-diamine](ethanedioato-O,O')platinum(II), is a third generation of platinum(II) complexes known as oxaliplatin which contain a rigid, stable bidentate ligand 1,2-diaminocyclohexane instead of two monodentate ammine ligands and an oxalate as leaving group. It was approved in 1996, under the brand name eloxatin. Inclusion of the

rigid moiety diaminocyclohexane was intended to contribute to a larger cytotoxicity when compared to the first and second generation (more damaging Pt-DNA adducts), as well as to overcome cross-resistance with those widely used drugs [78]. Particularly, the high activity of the oxaliplatin, even in cisplatin-resistant tumor models, coupled to its decreased toxicity, encouraged further investigations on its use as a treatment option after the failure of the first or second generations therapy [79].

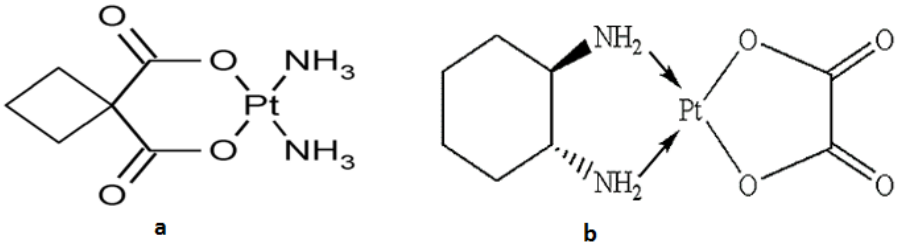


Figure 2.9 Chemical structure of a. carboplatin, b. oxaliplatin

2.9 Sulfur containing Ligands in the synthesis of anticancer drugs

The properties of metal- ligand coordination in classical inorganic coordination compounds or in organometallic and in bioinorganic compounds, are largely determined by the nature of ligands bound to the metal ion [79]. Most ligands are based on sulfur containing heterocyclic compounds moieties such as dimethyl sulfoxide, dimethyl sulfide, and thiosemicarbazones and xanthate have shown a lot of pharmacological benefits [24]. Thiourea and its derivatives, such as 1, 3-imidazolidine-2-thiones and their derivatives are an interesting type of ligands, they possess ambidentate nature, may give a better result if they coordinate with platinum(II) ion. They have been used for a long time as antifungal agents, protecting agent against nephritic side effects during cisplatin administration, and

as inhibitors of HIV-1 and HIV-2 reverse transcriptase [80]. The reactions of thione ligands with transition metals have been well studied in order to find simple model compounds for metalloproteins [81].

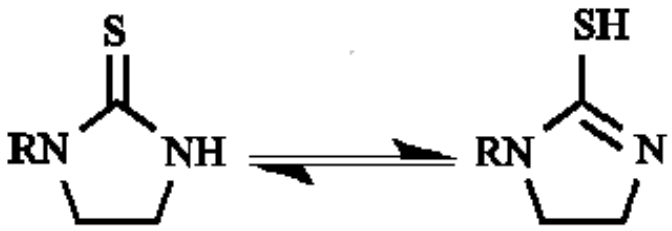


Figure 2.10 Tautomerism structure of Thiol - thione

These ligands can coordinate to a metal through various modes, by their sulfur or nitrogen atoms. Also, they may exist in a thiol-thione equilibrium. Studies have displayed that the thione form is the dominant one in the solid state and in the most common solvents [82].

CHAPTER 3

EXPERIMENTAL WORK

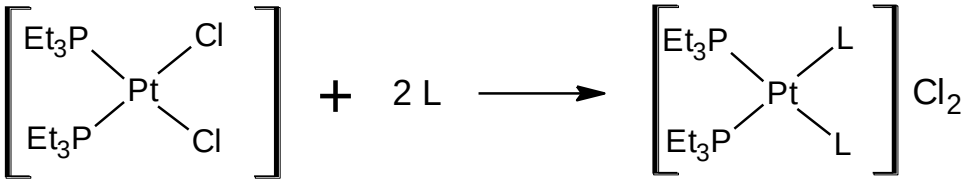
3.1 Materials and methods

Cisplatin and transplatin were obtained from Fluka AG, bis(triethylphosphine) dichloridoplatinum(II) was purchased from STERM Chemicals, silver nitrate was obtained from MERCK Chemicals. The deuterated solvents and thione ligands were purchased from Sigma-Aldrich. All other solvents were obtained from Fluka Chemical Co. And used without further purification.

3.2 Synthesis of the complexes

3.2.1 *cis*-$[(Et_3P)_2PtL_2]Cl_2$ complexes

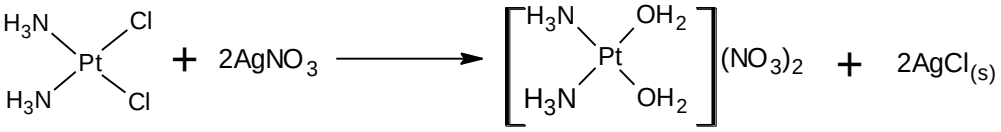
Complexes were synthesized in a same manner, through dropwise addition of a solution containing two equivalents of thione (1.00 mmol) dissolved in 10 ml methanol to 250 mg (0.50 mmol) of bis(triethylphophine)dichlorido platinum(II) dissolved in10 ml dichloromethane. The mixture was stirred for 3h resulting in a colored solution. The solutions were kept at room temperature after filtration, solid powders were obtained on slow evaporation of the solvent.

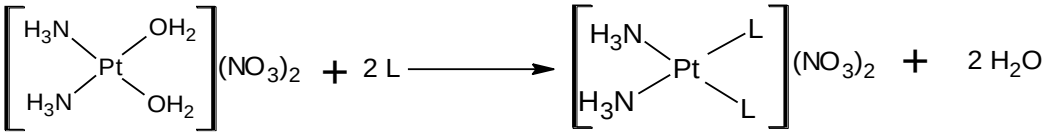


L = Imt; complex **A1**, (MeImt; **A2**), (Me_2Imt; **A3**), (Et_2Imt; **A4**), (Diaz; **A5**), (EtDiaz; **A6**), (Diap; **A7**).

3.2.2 *cis*-$[(NH_3)_2PtL_2](NO_3)_2$ complexes

All compounds were prepared by adding (1.000 mmol) of $AgNO_3$ to solutions containing (0.500 mmol) of *cis*-platin in 10 ml water and stirred for 24 hours in the dark at room temperature. The mixtures were filtered to remove the silver chloride precipitate. (0.500 mmol) of thione ligands dissolved in 10 ml methanol were added dropwise to the filtrates, after stirring for 3 hours. The solutions was refiltered and the kept at room temperature. Solid powders were obtained on slow evaporation of the solvent.



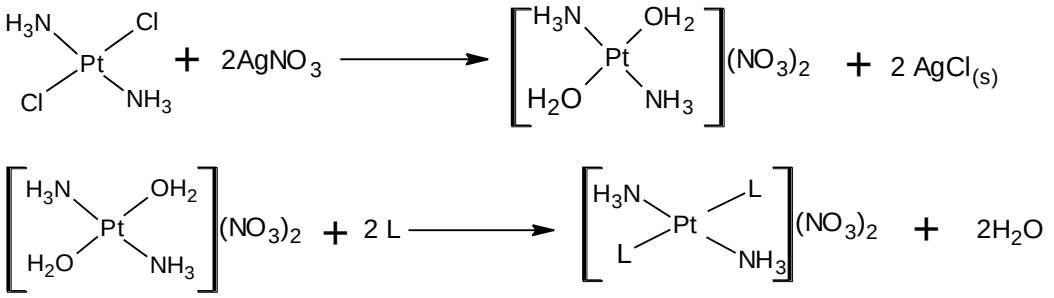


L = Imt; complex **B1**, (MeImt; **B2**), (Me_2Imt; **B3**), (Et_2Imt; **B4**), (PrImt; **B5**), (iPrImt; **B6**), (*i*Pr_2Imt; **B7**), (Diaz; **B8**), (EtDiaz; **B9**), (Diap; **B10**).

3.2.3 Synthesis of *trans*-$[Pt(NH_3)_2L_2](NO_3)_2$ complexes

All compounds were prepared by adding (1.000 mmol) of $AgNO_3$ to solutions containing (0.500 mmol) of *trans*-platin in 10 ml water and stirring for 24 hours in the dark at room temperature. The mixtures were filtered to remove the silver chloride precipitate. (0.500 mmol) of thione ligands dissolved in 10 ml Methanol were added to the filtrates dropwise. The mixtures were stirred to give colored solutions. Filtration and room temperature

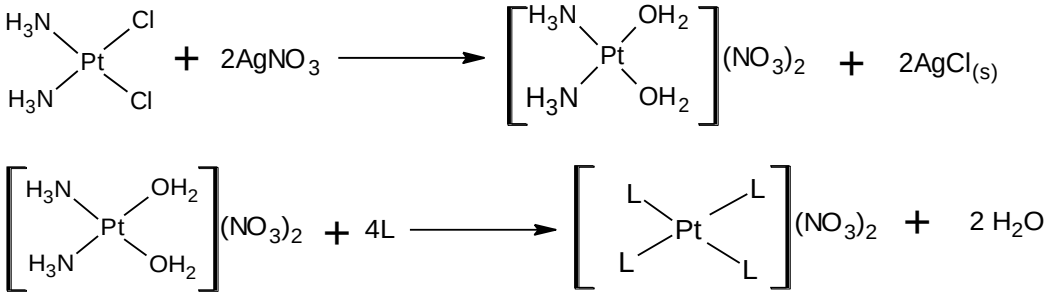
evaporations afforded powders. *trans*-$[Pt(NH_3)_2(Imt)_2](NO_3)_2$ and *trans*-$[Pt(NH_3)_2(Me_2Imt)_2](NO_3)_2$ complexes were crystallized from a mixture of methanol water.



L = Imt; complex **T1**, (MeImt; **T2**), (Me_2Imt; **T3**), (Et_2Imt; **T4**), (PrImt; **T5**), (*i*PrImt; **T6**), (*i*Pr_2Imt; **T7**), (EtDiaz; **T8**), (Diap; **T9**).

3.2.4 Synthesis of $[PtL_4](NO_3)_2$ complex (C1)

The compound was prepared by adding 168.8 mg (1.000 mmol) of $AgNO_3$ to solution contain 150.06 mg (0.500 mmol) of cisplatin in 10 ml water. The mixture was stirred for 24 hours in the dark at room temperature, then filtered. 288.2 mg (2.000 mmol) of N-*i*Propyl-Imt dissolved in 10 ml Methanol was added to the filtrate. After stirring for 3 hours, the brown solution was filtered and kept at room temperature for evaporation to afford X-ray quality crystals.



3.3 Spectroscopic measurements

Melting points of the synthesized complexes were carried out on Electro thermal apparatus. Elemental analysis of carbon, hydrogen, nitrogen and sulfur were performed on Perkin Elmer Series 11(CHNS/O), Analyzer 2400. The solid state FTIR spectra of the ligands and their platinum(II) complexes were performed on a PerkineElmer FTIR180 spectrophotometer using KBr pellets over the range 4000-400 cm^{-1} and solid Far-IR spectra below 400 cm^{-1} of the complexes were recorded at 4 cm^{-1} resolution at room temperature over polyethylene Disk.

The ^{1}H and ^{13}C NMR spectra were carried out on a JEOL JNM-LA 500 NMR spectrometer at 500.00 MHz and 125.65 MHz operating frequency respectively. The spectra of ^{13}C NMR were recorded at the above mentioned frequency with ^{1}H broadband decoupling at 297 K. The conditions of the spectra were 32 K data points, 0.963 acquisition time, 3.2 s pulse delay and a 5.75μs pulse width for ^{1}H NMR, and 32 K data points, 0.963 s acquisition time, 2.5 s pulse delay and a 5.12μs pulse width for ^{13}C NMR. The chemical shifts were measured relative to external reference TMS.

The ^{13}C solid state NMR spectra were performed on a JEOL LAMBDA 500 spectrometer at 125.65 MHz operating frequency corresponding to magnetic strength of 11.74 T, at ambient temperature. Samples were packed into 6mm zirconium oxide rotors. Cross polarization and high power decoupling were employed. Pulse delay of 7.0 s and a con-tact time of 5.0 ms were used in the CPMAS experiments. The magic angle spinning rates were from 2 kHz to 4 kHz. ^{13}C chemical shift were referenced to TMS by setting the high frequency isotropic peak of solid adamantine to 38.56 ppm.

3.4 Single crystal X-ray diffraction analysis

X-ray data were collected at 173K (-100°C) on a Stoe Mark II-Image Plate Diffraction System equipped with a two-circle goniometer and using MoKα graphite monochromated radiation (λ = 0.71073 Å). The structure was solved by direct methods with SHELXS-2014/6. The refinement and all further calculations were carried by SHELXL-2014/6 [83]. The N-bound and C-bound H-atoms were included in calculated positions and treated as riding atoms with N–H = 0.91 Å, C–H = 0.99 and 0.98 Å for CH_2 and CH_3 H atoms, respectively, and with $U_{iso}(H) = 1.5U_{eq}(C)$ for methyl H atoms and = $1.2U_{eq}$(N or C) for other H atoms [84]. The non–H atoms were refined anisotropically, using weighted full-matrix least-squares on F^2. A semi-empirical absorption correction was applied using the MULABS routine in PLATON [85]. The figures were drawn using the programs ORTEP [86] and MERCURY [86].

Table 3.1 Crystallographic characteristics, experimental and structure refinement details of crystal structure of complexes (**T1**) and (**T3**)

Parameter	**Complex T1**	**Complex T3**
Formula	$C_6H_{18}N_8O_6PtS_2$	$C_{10}H_{26}N_8O_6PtS_2$
Formula weight	557.49	613.60
Crystal size/mm	0.41 × 0.34 × 0.21	0.40 × 0.36 × 0.28
Wavelength/Å	0.71073	0.71073
Temperature/K	173	173
Crystal symmetry	Monoclinic	Monoclinic
Space group	P 2_1/n	P 2_1/n
a/Å	5.4002 (4)	7.0160 (5)
b/Å	23.4438 (15)	18.6235 (10)
c/Å	6.6485 (5)	8.0794 (6)
β/°	105.458 (6)	107.228 (6)
V/ Å^3	811.26 (10)	1008.31 (12)
Z	2	2
Dc/Mg m^{-3}	2.282	2.021
μ(Mo-Kα)/mm^{-1}	8.95	7.21
F(000)	536	600
θ Limits/°	1.7–25.6	2.2–26.1
Collected reflections	10466	13070
Unique reflections(R_{int})	1532 (0.087)	1900 (0.046)
Observed reflections [F_o >2σ(F_o)]	1332	1550
Goodness of fit on F^2	1.04	0.98
R_1(F), [I>2σ(I)]	0.023	0.015
wR_2 (F^2), [I>2σ(I)]	0.050	0.035
Largest diff. peak, hole/e Å^{-3}	0.99, −1.86	0.76 and −0.75

Table 3.2 Crystallographic characteristics, experimental and structure refinement details of crystal structure of complex **C1**.

Parameter	**Compound**
CCDC deposit no.	1008031
Empirical formula	$C_{24}\ H_{49.20}\ N_{10}\ O_{6.60}\ Pt\ S_4$
Formula weight	906.86
Temperature (K)	173 (2)
Wavelength (Å)	0.71073
Crystal system	Tetragonal
Space group	$P4_32_12$
Unit cell dimensions	
a (Å)	11.608 (3)
c (Å)	27.182 (1)
Volume ($Å^3$)	3662.7 (2)
Z	4
Calc. density ($g.cm^{-3}$)	1.645
Absorp. coefficient (mm^{-1})	4.11
F(000)	1832
Crystal size (mm)	0.45 × 0.35 × 0.20
θ range (^{o})	1.9–26.1
Limiting indices	$-14 \leq h \leq 14$
	$-14 \leq k \leq 13$
	$-32 \leq l \leq 33$
Max and min transmission	T_{min}= 0.7681, T_{max}= 1.0000
Data/restraints/parameters	3457 / 5 / 230
Goodness-of-fit on F^2	0.875
Final R indices [I >2 σ (I)]	R_1 = 0.0153, wR_2 = 0.0265
R indices (all data)	R_1 = 0.0259, wR_2 = 0.0269
Largest diff. Peak and hole (e $Å^{-3}$)	0.34 and −0.75

3.5 *In vitro* cytotoxic activity against HeLa, A549, MCF7 and HTC15 human cancer cell lines

In the present study, metal precursor, cisplatin and seven synthesized complexes were evaluated for their *in vitro* cytotoxic activity against Hela (human cervical cancer), A549 (human lung cancer), MCF-7 (human breast cancer) and HTC15 (human colon cancer) cell lines [87]. The cells were seeded at 4 x 10^3 cells/well in 100µL DMEM containing 10% FBS in 96-wells tissue culture plate and incubated for 72 h at 37° C, 5% CO_2 in air and 90% relative humidity in CO_2 incubator. After incubation, 100 µL of each sample solution (50, 25, 12.5 and 6.25 µM), prepared in DMEM, were added to cells and the cultures were incubated for 24 h. The medium of wells was discarded and 100 µL DMEM containing MTT (3-(4, 5-Dimethylthiazol-2-Yl)-2, 5-Diphenyltetrazolium Bromide) (5 mg/mL) was added to the wells and incubated in CO_2 incubator at 37° C in dark for 4 h. After incubation, a purple colored formazan (artificial chromogenic dye, product of the reduction of water insoluble tetrazolium salts e.g., MMT by dehydrogenases and reductases) in the cells is produced and appeared as dark crystals in the bottom of the wells.

The medium of culture was discarded from each well carefully to prevent disruption of monolayer. 100 µL of DMSO was added in each well. The solution was thoroughly mixed to dissolve the formazan crystals, producing a purple solution. The absorbance of the 96-wells plate was taken at 570 nm with Labsystems Multiskan EX-Enzyme-linked immunosorbent assay (EX-ELISA) reader against a reagent blank. The IC_{50} values were calculated from three independent experiments by generating an equation of logarithmic trendline of percentage cell viability against concentration compounds in Microsoft excel.

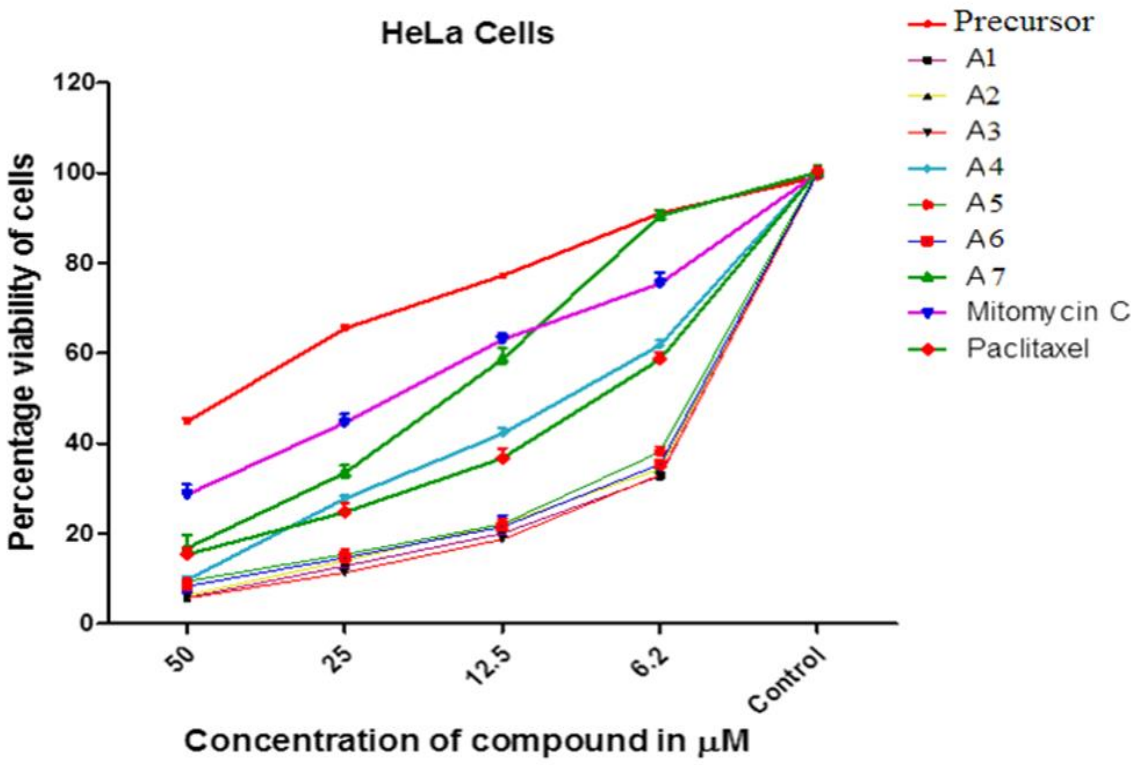


Figure 3.1 Percentage of Hela cell viability against compounds concentration

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Synthesis and structures

The reaction of the platinum(II) precursors with thione ligands afford a group of compounds with general formula of *cis*-$[(Et_3P)_2Pt(L)_2]Cl_2$, *cis*-$[(NH_3)_2Pt(L)_2](NO_3)_2$, *trans*-$[Pt(NH_3)_2(L)_2](NO_3)_2$ and $[Pt(i\text{PrImt})_4](NO_3)_2(0.6H_2O)$ in a good yields with different melting points. The observed elemental analytical data (CHNS)% of these complexes is consistent with their compositions as given below in table **4.1**.

In order to characterize with the coordination mode of the thione ligand with Pt(II) center atom in these complexes, the structures of *trans*-$[Pt(NH_3)_2(Imt)_2](NO_3)_2$, *trans*-$[Pt(NH_3)_2(Me_2Imt)_2](NO_3)_2$ and $[Pt(i\text{PrImt})_4](NO_3)_2$, have been determined by X-ray crystallography.

Table 4.1 Melting points and elemental analytical data for the synthesized complexes

Species	Chemical composition calculated & (found) %				M.p(ºC)	Color	Yield %
	C	H	N	S			
A1	30.59 (30.49)	6.00 (6.22)	7.93 (703.)	9.07 (8.61)	223 - 225	White	89
A2	32.69 (33.04)	6.32 (6.18)	7.63 (8.11)	8.73 (8.11)	246 - 250	White	82
A3	34.64 (35.84)	6.62 (6.88)	7.35 (8.46)	8.40 (8.46)	214 - 216	Yellow	69

A4	38.13 (37.99)	7.15 (7.36)	6.84 (6.92)	7.83 (8.08)	189 - 191	Yellow	73
A5	32.56 (33.04)	6.30 (6.96)	7.60 (7.63)	8.69 (8.46)	208 - 210	Brown	84
A6	36.45 (36.01)	6.90 (6.66)	7.09 (6.89)	8.11 (9.41)	188 - 190	White	67
A7	34.64 (35.22)	6.62 (7.96)	7.35 (7.40)	8.41 (8.24)	159 - 161	Yellow	77
	12.92	3.26	20.10	11.56	-	-	-
T1	(13.45)	(3.87)	(21.29)	(10.66)	156 - 158	White	92
B1	(12.48)	(3.56)	(19.26)	(12.37)	130 - 132	brown	89
	16.40	3.80	19.14	10.95	-	-	-
T2	(15.86)	(4.22)	(17.15)	(11.96)	140 - 142	Beige	80
B2	(17.01)	(3.78)	(18.33)	(13.06)	126 - 128	yellow	73
	19.57	4.28	18.26	10.45	-	-	-
T3	(20.07)	(4.17)	(16.89)	(11.33)	106 - 108	Beige	81
B3	(18.20)	(4.80)	(16.94)	(10.72)	73 -75	Brown	70
	25.10	5.13	16.73	9.57	-	-	-
T4	(24.07)	(5.88)	(17.62)	(10.72)	83 - 85	Yellow	67
B4	(24.19)	(5.08)	(17.11)	(10.23)	111- 113	orange	64
	22.43	4.72	17.45	9.98	-	-	-
T5	(20.99)	(4.92)	(16.60)	(10.54)	130 - 132	White	77
B5	(21.04)	(4.88)	(16.21)	(10.07)	125 - 127	beige	72
	22.43	4.72	17.45	9.98	-	-	-
T6	(21.05)	(4.46)	(15.65)	(10.79)	124 -126	Yellow	73
B6	(24.01)	(4.95)	(15.77)	(9.68)	138 - 140	white	82
	29.78	5.84	15.44	8.83	-	-	-
T7	(28.55)	(5.14)	(15.43)	(9.04)	132 -134	Orange	58
B7	(28.29)	(5.82)	(14.65)	(9.87)	119 - 121	Orange	49
	16.40	3.80	19.14	10.95	-	-	-

B8	(15.46)	(4.33)	(18.66)	(11.96)	112 - 113	Orange	87
	22.43	4.72	17.45	9.98	-	-	-
T8	(22.70)	(4.90)	(16.58)	(10.30)	92 - 94	Yellow	71
B9	(22.41)	(4.42)	(15.60)	(11.04)	86 - 88	Brown	66
	19.57	4.28	18.26	10.45	-	-	-
T9	(21.96)	(5.78)	(16.38)	(10.86)	164 - 166	Beige	62
B10	(20.22)	(4.50)	(16.37)	(9.49)	155 - 157	Brown	80
	31.78	5.48	15.44	14.14			
C1	(31.04)	(5.18)	(16.42)	13.97	119 – 121	Brown	42.9

4.1.1 Crystal structures of T1 and T3 complexes

Suitable crystals of **T1** and **T3** were obtained as colorless blocks by slow evaporation of a mixture of water methanol. The molecular structures of the two complexes **T1** and **T3** are shown in (Fig 4.1 and Fig 4.3), respectively. The selected bond length and bond angles parameters are reported in (Table 4.2), Both structures are ionic, consisting of *trans*-$[Pt(NH_3)_2(Imt)_2]^{2+}$ and *trans*-$[Pt(NH_3)_2(Me_2Imt)_2]^{2+}$ cations and two uncoordinated nitrate ions.

From the structures, Pt(II) ion is coordinated to two sulfur atoms of thione ligand and two N atoms of ammonia in a *trans* fashion. The Pt(II) ion is located in the inversion center in both structures and adopts nearly square-planar environment lying exactly within the plane defined by the two S and two N atoms.

The *cis* angles around platinum vary between 87.50(9)° and 92.50(9)° in case of **T1** and 88.52(7)° and 91.48(7)° in case of **T3**, while the *trans* angles are 180° in both. In the structure of **T1** The Pt–N and Pt–S bond distances are 2.046(3) and 2.3260(9)Å respectively, while the distances are 2.054(2) and 2.3199(7)Å respectively in **T3** , these

values are in agreement with the average of those reported for similar compounds [88]. Both thione ligands and ammonia molecules coordinated with the central Pt(II) ion are engaged in hydrogen bonding interactions with nitrate counter ions stabilizing the structure and leading to the formation of three-dimensional structure as shown In (Fig 4.2 & 4.4).

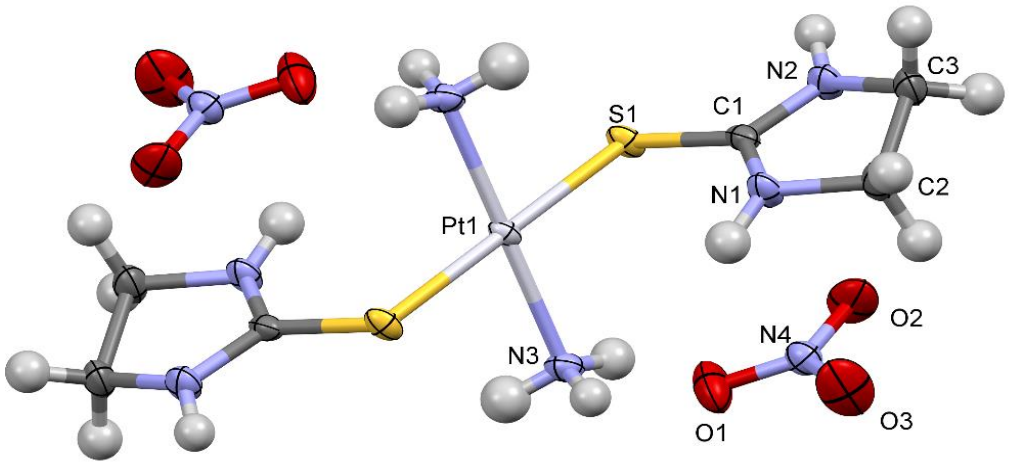


Figure 4.1 Molecular structure of **T1**, showing atomic labeling. Displacement ellipsoids are drawn at the 50% probability level. Atoms not labelled are related by inversion symmetry

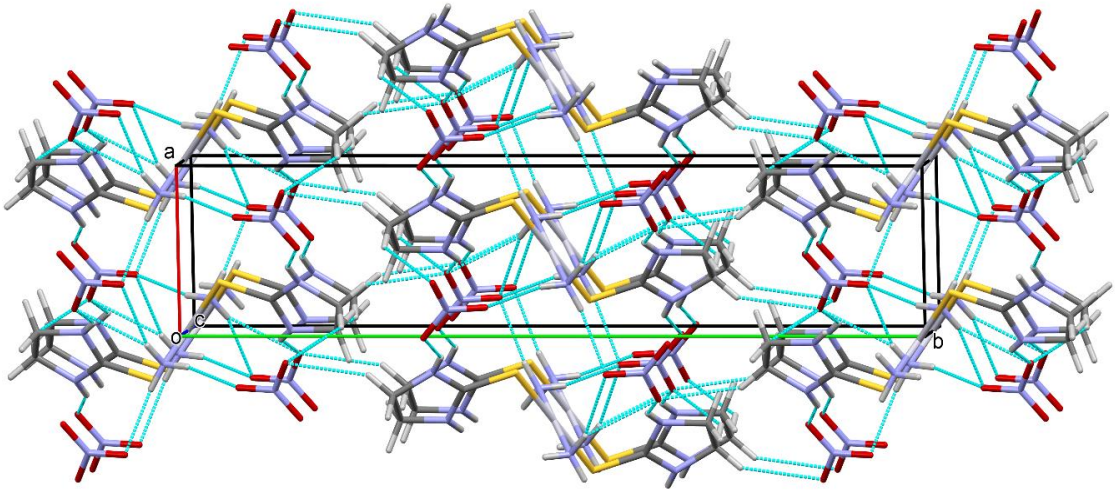


Figure 4.2 Packing diagram of **T1**, along the c axis. The N–H…O, N–H…N and C–H…O hydrogen bonds are shown as dashed lines and lead to the formation of a 3D structure

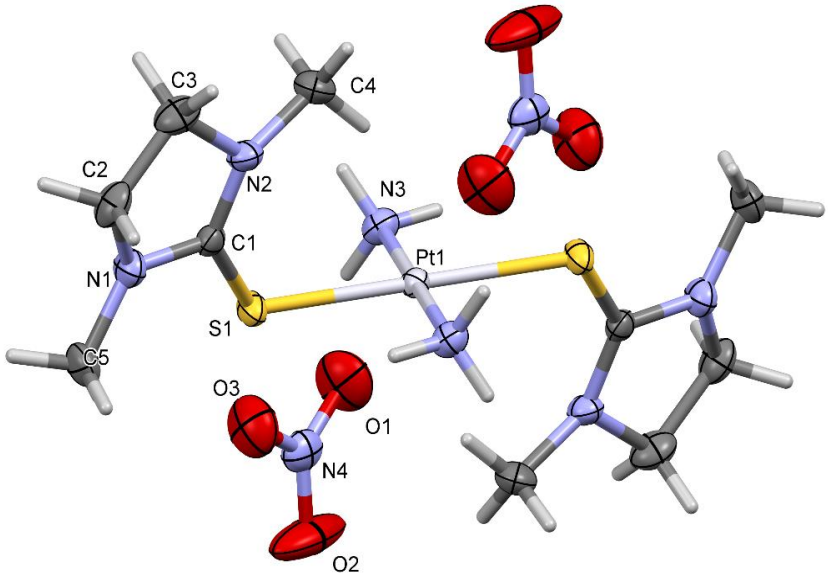


Figure 4.3 Molecular structure of **T3**, showing atomic labeling. Displacement ellipsoids are drawn at the 50% probability level. Atoms not labelled are related by inversion symmetry

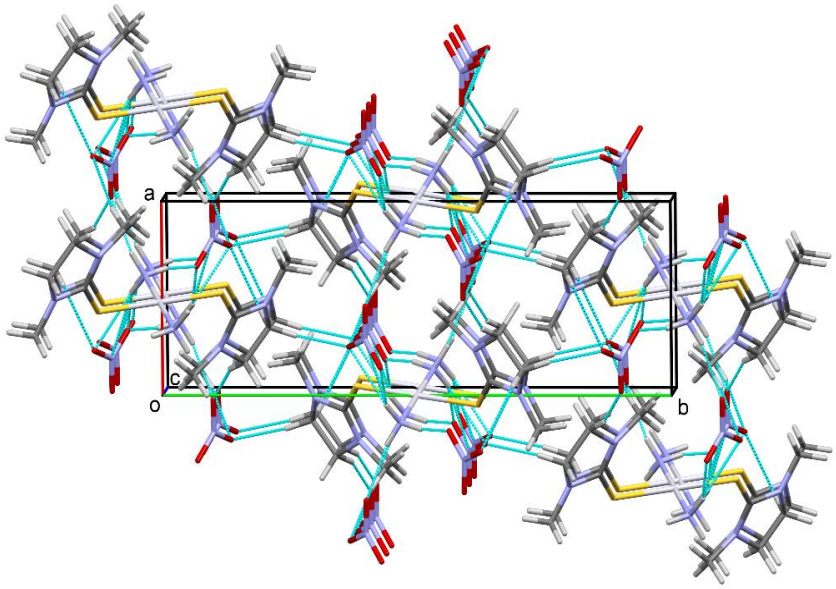


Figure 4.4 Packing diagram of **T3**, along the c axis. The N–H…O, N–H…N and C–H…O hydrogen bonds are shown as dashed lines and lead to the formation of a 3D structure

Table 4.2 Selected bond angles (Å) and bond lengths (°) for complexes **T1** & **T3**

Bond Lengths (Å)			
Complex T1		**Complex T3**	
Pt1—N3	2.046 (3)	Pt1—N3	2.054 (2)
Pt1—S1	2.3260 (9)	Pt1—S1	2.3199 (7)
Bond Angles (°)			
Complex (T1)		**Complex (T3)**	
N3—Pt1—S1	92.50 (9)	N3—Pt1—S1	88.52 (7)
$N3^{i}$—Pt1—S1	87.50 (9)	$N3^{i}$—Pt1—S1	91.48 (7)
$N3^{i}$—Pt1—N3	180	$N3^{i}$—Pt1—N3	180
S1—Pt1—$S1^{i}$	180	S1—Pt1—$S1^{i}$	180

Symmetry code (**T1**): (i) −x, −y, −z+1, Symmetry code (**T3**): (i) −x+1, −y, −z+1.

Table 4.3 Hydrogen-bond geometry (Å, °) in complex **T1**

D*—H···*A	***D*—H**	**H···*A***	***D*···*A***	***D*—H···*A***
N1—H1*N*···$O2^{ii}$	0.87 (2)	2.06 (3)	2.791 (4)	141 (4)
N2—H2*N*···$O3^{iii}$	0.86 (2)	2.13 (3)	2.882 (5)	146 (4)
N3—H3*CN*···$O1^{ii}$	0.87 (2)	2.31 (2)	3.149 (4)	163 (3)
N3—H3*CN*···$O2^{ii}$	0.87 (2)	2.55 (3)	3.270 (4)	140 (3)
N3—H3*AN*···O1	0.88 (2)	2.15 (2)	3.001 (4)	162 (4)
N3—H3*BN*···$O1^{iv}$	0.88 (2)	2.18 (2)	3.044 (4)	167 (6)
C3—H3*A*···$O2^{v}$	0.99	2.60	3.362 (5)	134
C3—H3*B*···$O2^{vi}$	0.99	2.64	3.526 (4)	149

Symmetry codes: (ii) *x*−1, *y*, *z*; (iii) *x*, *y*, *z*−1; (iv) −*x*+1, −*y*, −*z*+2; (v) *x*−1, *y*, *z*−1; (vi) *x*−1/2, −*y*+1/2, *z*−1/2.

Table 4.4 Hydrogen-bond geometry (Å, °) in complex **T3**

D*—H···*A	***D*—H**	**H···*A***	***D*···*A***	***D*—H···*A***
N3—H3*X*···$O2^{ii}$	0.91	2.08	2.956 (3)	161
N3—H3*Y*···O1	0.91	2.22	3.034 (4)	148
N3—H3*Y*···O3	0.91	2.43	3.258 (4)	152
N3—H3*Y*···N4	0.91	2.69	3.561 (4)	160
N3—H3*Z*···$O1^{iii}$	0.91	2.11	2.944 (3)	151
C2—H2*B*···$O3^{iv}$	0.99	2.41	3.222 (4)	139
C3—H3*B*···$O2^{v}$	0.99	2.36	3.317 (4)	161
C4—H4*A*···$O3^{ii}$	0.98	2.51	3.470 (4)	168

Symmetry codes: (ii) *x*+1, *y*, *z*; (iii) −*x*+1, −*y*, −*z*+2; (iv) *x*+1/2, −*y*−1/2, *z*−1/2; (v) *x*+1, *y*, *z*−1.

4.1.2 Crystal structure of complex C1

The molecular structure of compound **C1** is shown in (Fig 4.5) The Pt(II) ion is located on a two fold rotation axis and bound to the sulfur atoms of four N-isopropylimidazolidine-2-thione (*i*PrImt) ligand molecules in a distorted square planar geometry. The Pt-S bond lengths are in the range 2.3035(8) - 2.3222(8) Å, while the S-Pt-S bond angles are in the range 89.34(4) - 92.17(4)°. The bond distances are similar to the related compound tetrakis(1-Methyl-4-imidazoline-2-thione) platinum(II) chloride dehydrate [29]. The SCN_2 moieties of the four ligand molecules are also essentially planar with the S-C and C-N bond lengths in the ranges (1.706(4) - 1.709(4)) Å and (1.321(4)-1.350(4) Å) respectively. However the geometry around the platinum ion exhibits interesting features. The structure presents a deviation from the ideal square planar geometry reminiscent of a bent seesaw distortion where the trans-sulfur atoms S1 and $S1^{i}$ are displaced above the [PtS_4] mean plane by 0.2603(9) Å whereas S2 and $S2^{ii}$ are displaced below the mean plane by 0.2723(8) Å. A closer look to hydrogen bonding interactions reveals that the thione ligands of each of the two trans pairs are engaged in hydrogen bonding interactions with one oxygen atom from each nitrate counter ion, playing the role of a bridge between them: [N-H...O_{NO2}…H-N]. This H-bonding scheme gives rise to two decametallacycles [PtSCNH…O…HNCS] in which the *trans* sulfur atoms are pushed out of the [PtS_4] mean plane and hence resulting in the minor seesaw distortionmean plane by 0.2603(9) Å whereas S2 and $S2^{ii}$ are displaced below the mean plane by 0.2723(8) Å. A closer look to hydrogen bonding interactions reveals that the thione ligands of each of the two trans pairs are engaged in hydrogen bonding interactions with one oxygen atom from each nitrate counter ion, playing the role of a bridge between them: [N-H...O_{NO2}…H-N].

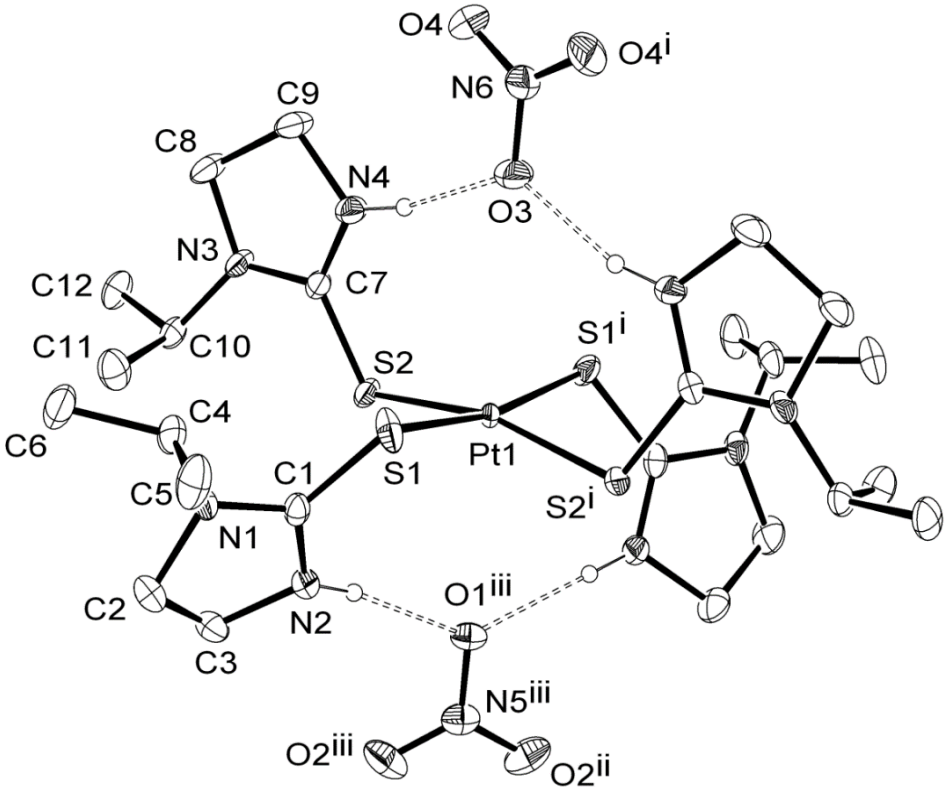


Figure 4.5 ORTEP diagram of complex C1, showing the atomic labeling scheme. Displacement ellipsoids are drawn at the 30 % probability level

The hydration, water molecule and hydrogen atoms other N-H were omitted for clarity.

(Pt1-S1, 2.3035(8) Å; Pt1-S2, 2.3222(8) Å; S1-Pt1-S2, 92.17(4)^{o}; S1-Pt1-S2^{i}, 89.34(4) ^{o}).

Symmetry codes: i = y, x,-z; ii = -y+1/2, x-1/2, z-1/4; iii = x-1/2,-y+1/2,-z+1/4

4.2 Spectral measurements

4.2.1 NMR studies

^{1}H and ^{13}C NMR spectra of the *cis*-[$(Et_3P)_2Pt(L)_2]Cl_2$ complexes have been measured in $CDCl_3$ solution and spectra of the cis-$[(NH_3)_2Pt(L)_2](NO_3)_2$ and trans-$[Pt(NH_3)_2(L)_2](NO_3)_2$ complexes have been recorded in D_2O and DMSO-d_6.

All synthesized complexes show the expected signals. Based on the symmetry of the solid state structures we should expect just a half number of peaks because of chemical environments similarity. All signals assigned to the free thione ligands observed in the ^{1}H & ^{13}C NMR spectra were also found in the Pt(II) complexes spectra. The N–H signals of all thione ligands become less intense upon complexation and shifted slightly downfield toward high frequency by 0.4 - 1.4 ppm from their positions in the free thione ligands. This shift is a good indication of the coordination of ligands to the platinum(II) center atom and the formation of the targeting compounds. The N–H proton deshielding is related to an increase of the π electron density in the C–N bond upon coordination, which indicates that the ligands were coordinated to the centers of platinum(II) through the sulfur atom and not via nitrogen [89].

The ^{13}C NMR chemical shifts of the free ligands and their Pt(II) complexes are listed in (Table 4.5 & Table 4.6). In all platinum(II) compounds, the C-2 signals, corresponding to thiocarbonyl C=S resonance appear (upfield) in the lower frequency regions by 2.0 - 14 ppm as compared to the free thiones. This shift was attributed to the decreasing bond order of a C=S upon complexation which leads to a shift of the electron density, and formation a partial double bond character in the C–N bond. This clearly supports the fact that thiones

are bonding through sulfur [90]. The difference in the thiocarbonyl resonance shift may be related to the platinum–sulfur bond strength as noticed in the other platinum(II) complexes of thiones [81].

Table 4.5 ^{1}H, ^{13}C NMR chemical shifts of the free Ligands and their complexes in $CDCl_3$.

Species	N-H	C-2	C-4	C-5	C-6	C-7	N-C1	N-C2	P-C1	P-C2
Imt	7.98	182.11	45.38	45.38	-	-	-	-	-	-
A1	9.19	174.50	46.60	46.60	-	-	-	-	9.30	14.96
MeImt	7.93	181.38	42.00	51.82	-	-	34.35	-	-	-
A2	8.65	174.32	42.56	52.13	-	-	34.47	-	7.93	13.52
Me_2Imt	-	180.46	48.73	48.73	-	-	34.80	-	-	-
A3	-	174.33	49.71	49.71	-	-	35.16	-	7.89	13.58
Et_2Imt	-	178.74	46.13	46.13	-	-	42.69	11.92	-	-
A4	-	171.94	47.38	47.38	-	-	43.77	12.08	8.84	12.08
Diaz	7.77	173.34	38.36	19.29	38.36	19.29	-	-	-	-
A5	9.13	166.68	41.53	19.92	41.53	19.92	-	-	8.83	14.37
Et-Diaz	7.70	173.36	41.14	20.93	46.14		49.54	12.33	-	-
A6	8.32	168.24	41.35	20.91	47.03		49.66	12.43	8.08	13.33
Diap	7.70	183.99	45.86	26.70	45.86	26.70	-	-	-	-
A7	8.50	175.22	46.44	26.46	46.44	26.46	-	-	7.88	13.50

Table 4.6 ^{1}H and ^{13}C chemical shifts (ppm) for the free Ligands and their Pt(II) complexes in DMSO-d_6, and D_2O

Species	**N–H**	**C-2**	**C-4**	**C-5**	**C-6**	**C-7**	**N-C1**	**N-C2, N-C3**
Imt	7.98	182.11	45.38	45.38	-	-	-	-
B1	9.04	174.89	45.96	45.96	-	-	-	-
T1	9.09	175.74	45.76	45.76	-	-	-	-
MeImt	7.93	181.38	42.00	51.82	-	-	34.35	-
B2	8.82	173.92	42.88	52.50	-	-	34.07	-
T2	8.47	174.90	42.76	52.65	-	-	34.02	-
Me_2Imt	-	180.46	48.73	48.73	-	-	34.80	-
B3	-	172.35	49.75	49.75	-	-	35.80	-
T3	-	166.89	50.29	50.29	-	-	36.07	
Et_2Imt	-	178.74	46.13	46.13	-	-	42.69	11.92
B4	-	171.02	47.45	47.45	-	-	43.78	12.16
T4	-	170.96	47.33	47.33	-	-	43.79	12.11
PrImt	7.99	180.87	49.14	48.86			42.11	20.65, 11.09
B5	8.63	169.74	50.02	48.92	-		42.97	20.64, 10.95
T5	8.81	174.50	50.10	49.10	-		42.82	20.53, 10.95
***i*-PrImt**	7.96	179.70	42.21	43.73	-	-	48.18	19.55

B6	8.57	172.60	42.94	44.69	-	-	48.90	19.23
T6	8.31	174.11	44.05	42.42			48.88	19.18
C1	8.77	174.17	44.65	42.71			48.78	19.20
***i*-Pr$_2$Imt**	-	171.05	48.25	48.25	-	-	41.52	19.10
B7	-	165.55	48.86	48.86	-	-	41.22	18.94
T7	-	169.42	42.30	42.30			49.68	19.26
Diaz	7.77	173.34	41.00	19.25	41.00	-	-	-
B8	8.83	166.78	41.03	18.86	41.03	-	-	-
EtDiaz	7.70	173.36	41.14	20.93	46.14	-	49.54	12.33
B9	8.44	165.54	41.38	20.31	47.53	-	49.61	12.51
T8	8.32	167.43	47.32	20.27	41.09	-	49.67	12.19
Diap	7.70	183.99	45.86	26.99	26.99	45.86	-	-
B10	8.74	177.29	47.40	26.70	26.70	47.40	-	-
T9	8.70	176.53	46.35	26.51	26.51	46.35	-	-

The ^{31}P solution NMR spectra of (**A1**-**A7**) complexes have been carried out in $CDCl_3$ solution. For these complexes three resonances with relative integral values of 1:4:1 around 9.0 ppm, as shown in appendix B. With both active and inactive spins platinum isotopes present, the ^{31}P NMR spectra will consist of signals with intensities corresponding to the

natural abundance of each isotope. Active ^{195}Pt (I = ½) has a natural abundance of 33.8%, while the remaining isotopes are inactive. So the singlet signal resonance peaks, arising from ^{31}P nuclei adjacent to platinum inactive isotopes [91], in contrast the two peaks with ratio 1:1 signals is assigned as doublet due to presence of heteronucelar coupling between $^{31}P - ^{195}Pt$ with high spin coupling constant $^{1}J\ ^{195}Pt - ^{31}P$, This high spin coupling constant is probably due to a high covalency of the platinum-phosphorus bond, which is in agreement with the values reported in the literature for the bisphosphine in the classic example of square-planar platinum(II) complexes between 1462 and 5698 Hz [92].

The slight down field shifts was observed in the all synthesized complexes in compared to the precursor, this shift is likely due to back donation from platinum d-orbital to the empty π* orbital of the thiocarbonyl , which is known to as strong π-accepting ligand [93], leading the lone pair of phosphorus move toward the platinum [94].

Table 4.7 ^{31}P NMR chemical shift of the precursor and synthesized complexes in $CDCl_3$

Species	**∂ ppm**	**$^{1}J(^{195}Pt-^{31}P)$, Hz**
Precursor	s, 7.2 . d, 8.6	2932
A1	s, 9.2 , d, 9.2	2244
A2	s, 9.1 . d, 8.9	2559
A3	s, 9.2 . d, 9.0	2358
A4	s, 8.9 . d, 9.0	2658
A5	s, 9.2 . d, 9.2	2278
A6	s, 9.3 . d, 9.4	2547
A7	s, 8.7 . d 8.6	2300

The shifts were measured relatively to external phosphoric acid (0 ppm) reference.

It was noticed that by increasing of the thione ligands ring size from five membered ring to six and seven (Imt, Diaz and Diap) the hetero nuclear coupling constant between ^{31}P – ^{195}Pt increased, a possible explanation of this is that a big rings have less s-character gaining lesser share of the platinum 6s orbital, thus increasing P – Pt bond covalancy leading to the increase in the coupling constant value as you can see in the below figure

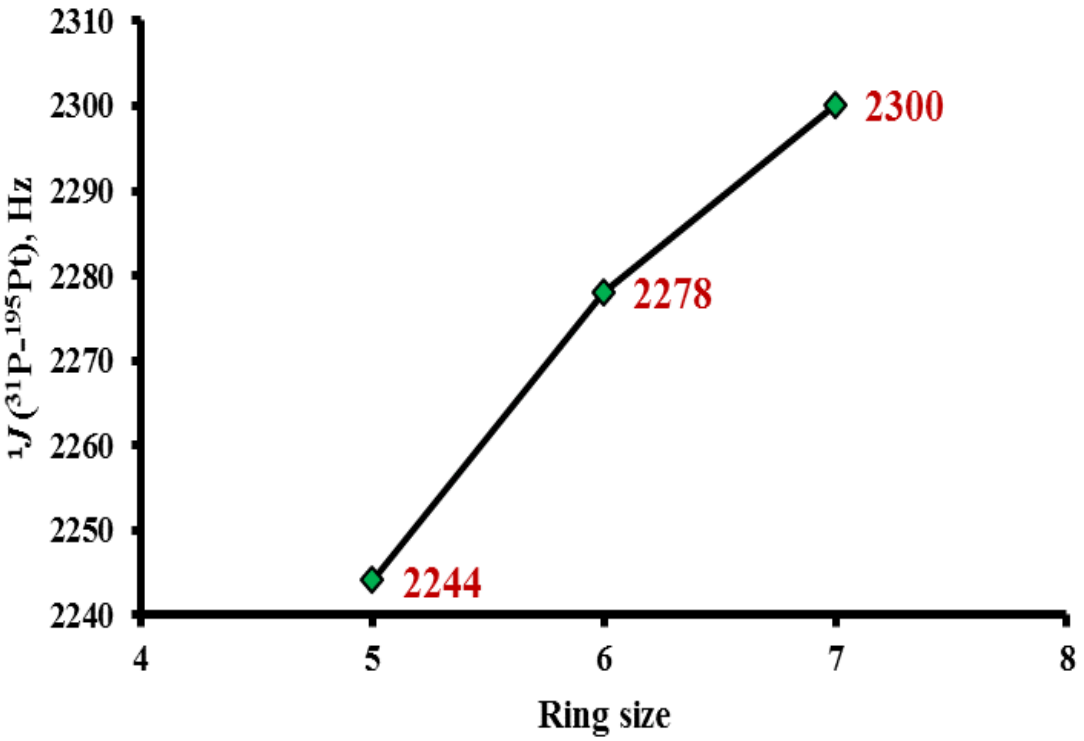


Figure 4.6 Graph shows the relationship between the ligands ring size and hetero nuclear ^{195} Pt-^{31}P coupling constant

4.2.2 Solid State ^{13}C NMR

Solid state ^{13}C NMR data of the complexes are given in table 10. Thiocarbonyl resonances in the free thione ligands, Imt, MeImt, Me_2Imt, Et_2Imt, PrImt, *i*PrImt, Diaz, EtDiaz, Diap appear at 180.6, 181.4, 181.4, 180.1, 180.1, 180.8, 175.6, 176.2, 188.4 ppm respectively, as reported in the literature [82]. Upfield shifts by about 2.4 to 12.59 ppm relative to the

free ligand were observed upon complexation of Pt(II) with the thiones [95]. This is consistent with thione ligand coordination through sulfur atom and also confirm that thione form is dominant after complexation.

Table 4.8 ^{13}C solid state NMR chemical shifts (ppm) of the free Ligands and their platinum(II) complexes

Species	C-2	C-4	C-5	C-6	C-7	N-C1	N-C2	N-C3	P-C1	P-C2
A1	171.50	44.66	44.66	-	-	-	-	-	16.31	12.05
A5	169.42	45.70	20.44	45.70	20.44	-	-	-	16.34	13.17
A7	175.81	51.57	30.57	51.57	30.57	-	-	-	17.88	13.17
T1	172.78	50.05	50.05	-	-	-	-	-	-	-
T2	177.92	47.76	56.4	-	-	39.31		-	-	-
T3	172.72	52.43	52.43	-	-	38.95		-	-	-
T4	174.64	50.04	50.04	-	-	46.36	14.85	-	-	-
T5	176.15	74.11	52.50	-	-	52.50	23.42	16.03	-	-
T6	176.47	46.91	46.91	-	-	50.57	22.29	-	-	-
T8	173.13	43.90	22.00	49.53	-	49.53	15.45	-	-	-
T9	178.18	50.94	31.47	50.94	31.47	-	-	-	-	-

Table 4.9 ^{1}H NMR chemical shift of the free ligand and their complexes

Species	**H-4**	**H-5**	**H-6**	**H-7**	**N-C1**	**N-C2**	**N-C3**	**P-C1**	**P-C2**
Imt	s,4H,3.59	s, 4H,3.59	-	-	-	-	-	-	
A1	s,4H,3.85	s,4H,3.85	-	-	-	-	-	q,12H,2.03	t,18H,1.13
B1	s,4H,3.68	s, 4H,3.68	-	-	-	-	-	-	-
T1	s,4H,3.69	s, 4H,3.69	-	-	-	-	-	-	-
MeImt	t,2H,3.63	t,2H,3.43	-	-	s,3H,2.92	-	-	-	-
A2	t,2H,3.77	t,2H,3.77	-	-	s,3H,2.96	-	-	q,12H,2.06	t,18H,1.15
B2	t,2H,3.75	t,2H,3.58	-	-	s,3H,2.98	-	-	-	-
T2	t,2H,3.71	t,2H,3.56	-	-	s,3H,2.96	-	-	-	-
Me_2Imt	s,4H,3.48	s, 4H,3.48	-	-	s,6H,2.91	-	-	-	-
A3	s,4H,3.55	s, 4H,3.55	-	-	s,6H,3.01	-	-	q,12H,2.02	t,18H,1.11
B3	s,4H,3.67	s, 4H,3.67	-	-	s,6H,3.18	-	-	-	-
T3	s,4H,3.65	s, 4H,3.65	-	-	s,6H,3.29	-	-	-	-
Et_2Imt	s,4H,3.48	s,4H,3.48	-	-	q,4H,3.37	t,6H,0.97	-	-	-

A4	s,4H,3.52	s,4H,3.52	-	-	q,4H,3.51	t,6H,1.08	-	q,12H,1.98	t,18H,1.08
B4	s,4H,3.48	s,4H,3.48	-	-	q,4H,3.37	t,6H,0.97	-	-	-
T4	s,4H,3.48	s,4H,3.48	-	-	q,4H,3.37	t,6H,0.97	-	-	-
PrImt	t,2H,3.58	t,2H,3.41	-	-	t,2H,3.31	m,2H,1.45	t,3H,0.73	-	-
B5	t,2H,3.73	t,2H,3.59	-	-	t,2H,3.36	m,2H,1.52	t,3H,0.76	-	-
T5	t,2H,3.76	t,2H,3.62	-	-	t,2H,3.35	m,2H,1.55	t,3H,0.79	-	-
***i*-PrImt**	t,2H,3.53	t,2H,3.38	-	-	m,1H,4.35	d,6H,1.00	-	-	-
B6	t,2H,3.68	t,2H, 3.57	-	-	m,1H,4.30	d,6H,1.06	-	-	-
T6	t,2H,3.70	t,2H, 3.54	-	-	m,1H,4.25	d,6H,1.07	-	-	-
***i*-Pr$_2$Imt**	s,4H,3.22	s, 4H,3.22	-	-	m,1H,4.48	d,6H,0.99	-	-	-
B7	s,4H,3.41	s, 4H,3.41	-	-	m,1H,4.46	d,6H,0.99	-	-	-
T7	s,4H,3.49	s, 4H,3.49	-	-	m,1H,5.10	d,6H,1.05	-	-	-
Diaz	t,4H,3.15	m,2H,1.75	t,4H,3.15	-	-	-	-	-	-
A5	t,4H,3.60	m,2H,1.67	t,4H,3.60	-	-	-		q,12H,2.12	t,18H,1.21
B8	4H, 3.2	m,2H,1.74	4H, 3.2	-	-	-	-	-	-

EtDiaz	t,2H,3.62	m,2H,1.83	t,2H,3.28	-	q, 2H,3.12	t, 3H,1.02	-	-	-
A6	t,2H,3.68	m,2H,1.93	t,2H,3.36	-	q,2H,3.26	t,3H,1.12		q,12H,2.02	t,18H,1.18
B9	t,2H,3.56	m,2H,1.85	t,2H,3.34	-	q, 2H,3.27	t, 3H,1.05	-	-	-
T8	t,2H,3.59	m,2H,1.84	t,2H,3.33	-	q, 2H,3.21	t, 3H,1.09		-	-
Diap	t,4H,1.67	t,4H,3.18	t,4H,3.18	t,4H,1.67	-	-	-	-	-
A7	t,4H,1.78	t,4H,3.51	t,4H,3.51	t,4H,1.78	-	-	-	q,12H,2.04	t,18H,1.10
B10	t,4H,1.62	t,4H,3.20	t,4H,3.20	t,4H,1.62	-	-	-	-	-
T9	t,4H,1.70	t,4H,3.24	t,4H,3.24	t,4H,1.70	-	-	-	-	-

s: singlet, d: doublet, t: triplet, q: quar

4.2.3 FT-IR spectroscopy

Selected Infrared spectroscopic absorption frequency of the free thione ligands and their corresponding platinum(II) compounds are stated in (Table 4.10). A characteristic band in the spectrum of the free thiones near 3200 cm^{-1} was observed and assigned to N–H stretching vibrations. This band is shifted to a higher frequency in all platinum (II) complexes due to an increase in the double bond character of C-N. This confirms that the thione ligand coordinates to the platinum(II) ion through sulfur of thiocarbonyl group [89].

The presence of (N–H) vibration bands in the complexes consistent with the presence of solid state thione form in the complexes [96]. Another important vibrational band is due to the thiocarbonyl group. This group is less polar than the carbonyl group C=O and has a considerably weak band at lower frequency 1250 – 1020 cm^{-1} this band difficult to identify [97]. In addition, several other bands in the broad region of 1560 – 700 cm^{-1}, are attributed to the strong coupling of C=S stretching with C-N stretching and other vibrational modes [97, 98].

In all, free ligands the band around 1200 cm^{-1}, could be assigned to ν(C=S) stretching vibration based on the reported values in the literature [99]. The shifting of bands towards lower wave number in most platinum(II) complexes, is in agreement with our suggestion that sulfur atom is bonded to platinum center and the double bond character has been reduced. A band around 825 cm^{-1} was observed for the cis-$[Pt(NH_3)_2(L)_2](NO_3)_2$ and trans-$[Pt(NH_3)_2(L)_2](NO_3)_2$ series and does not appear in the free ligands and cis-$[(Et_3P)_2Pt(L)_2)]Cl_2$ series, this band is attributed to NO_3^- bending and is an indication that NO_3^- doesn't coordinate [100].

The far-infrared spectra in the frequency region below 400 cm^{-1} has been recorded to investigate metal–sulfur ν(Pt-S) and metal–phosphorus ν(Pt-P) stretching frequencies, which lie at about 300 cm^{-1} for the transition-metal complexes according to literature [101]. In all complexes a sharp peak around 270 cm^{-1} was observed and assigned to platinum-sulfur bond. In case of the first series another peak around 300 cm^{-1} were observed and assigned for Pt-P bond.

Table 4.10 IR absorption bands (cm^{-1}) assignments for free ligands and their complexes

Species	**IR Frequency (cm^{-1})**				
	ν(C=S)	**ν(N–H)**	**ν(NO_3^-)**	**ν(Pt-S)**	**ν(Pt-P)**
Imt	1199	3200	-	-	-
A1	1034	3446	-	272	305
T1	1042	3310	836	272	-
B1	1036	3376	827	273	-
MeImt	1200	3200	-	-	-
A2	1052	3418	-	280	299
T2	1112	3528	837	274	-
B2	1028	3481	823	287	-
Me_2Imt	1201	-	-	-	-
A3	1048	-	-	281	307
T3	1118	-	825	265	-
B3	1114	3452	825	280	-
Et_2Imt	1199	-	-	-	-
A4	1088	-	-	279	311

T4	1065	-	826	284	-
B4	1079	-	837	268	-
PrImt	1201	3210	-	-	-
T5	1033	3373	824	282	-
B5	1026	3429	827	282	-
***i*-PrImt**	1193	3210	-	-	-
T6	1064	3566	867	279	-
B6	1062	3550	824	274	-
C1	1076	3209	844	269	-
***i*-Pr$_2$Imt**	1198	-	-	-	-
T7	1106	-	856	283	-
B7	1036	-	840	283	-
Diaz	1206	3200	-	-	-
A5	1043	3422	-	282	309
B8	1070	3459	811	268	-
EtDiaz	1217	3210	-	-	-
A6	1038	3448	-	274	303
T8	1074	3446	835	279	-
B9	1039	3465	824	291	-
Diap	1190	3224	-	-	-
A7	1034	3521	-	267	306
T9	1053	3270	822	269	-
B10	1002	3490	808	276	-

4.3 *In vitro* cytotoxicity of *cis*-$[(Et_3P)_2Pt(thione)_2]Cl_2$ compounds against a panel of human cancer cell lines

in vitro cytotoxicity of the compounds **A1- A7** were evaluated against four human cancer cell lines [102], HeLa, A549, MCF7, and HCT15 cell lines [103, 104]. The exposure of the cells to the increase in the concentrations of the complexes resulted in a dose dependent cytotoxic effect. This was obtained by the stipulated increase in the concentrations of the compounds against different number of human cancer cells. The IC_{50} concentration of the complexes was obtained from curves between the complexes concentration and percentage viability of the cells. The IC_{50} values of the complexes ranged between 1.2 and 37 µM as given in (Table 4.11).

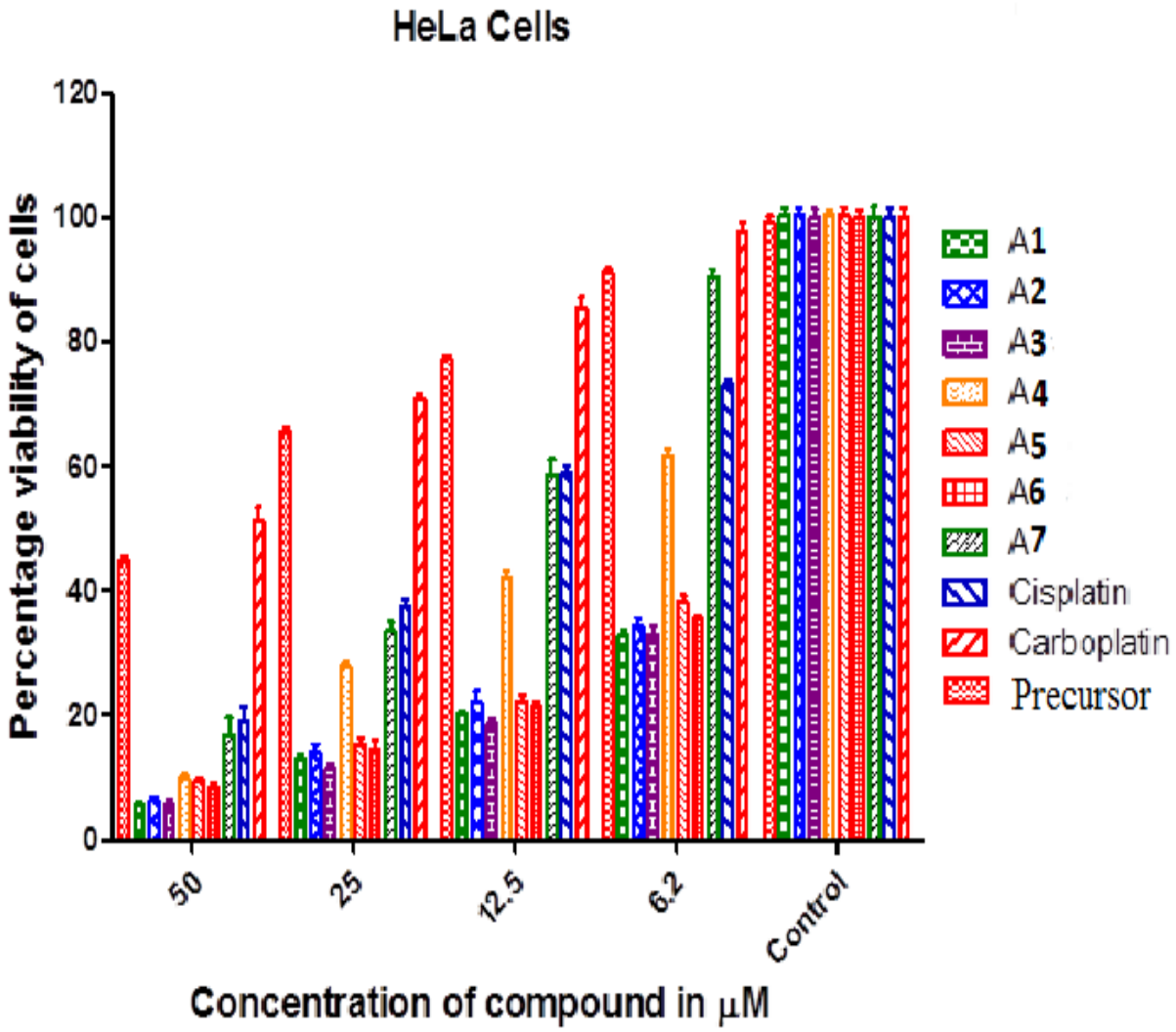


Figure 4.7 Graph showing complexes concentration effect on viability of Hela cell

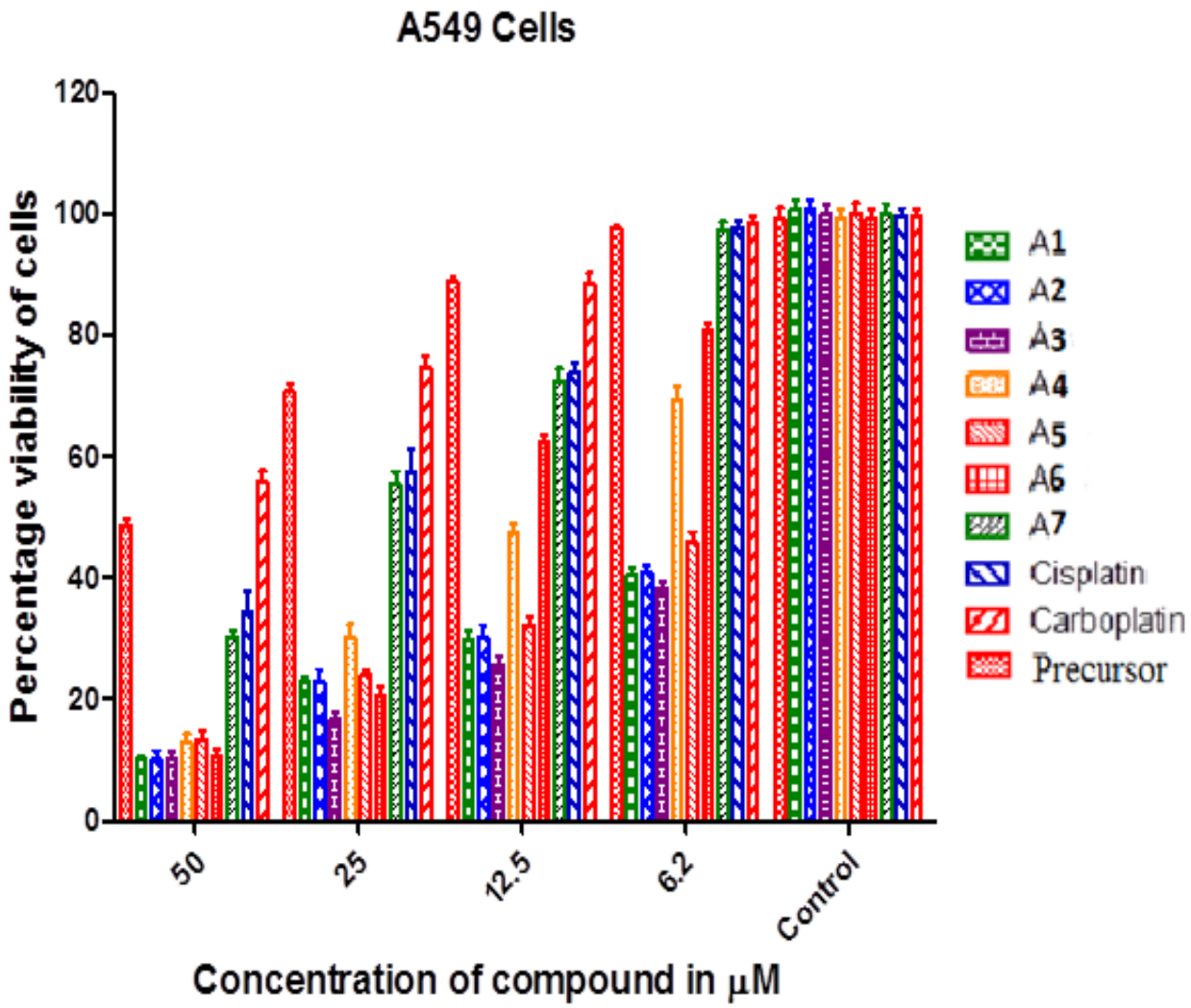


Figure 4.8 Graph showing complexes concentration effect on viability of A549 cell

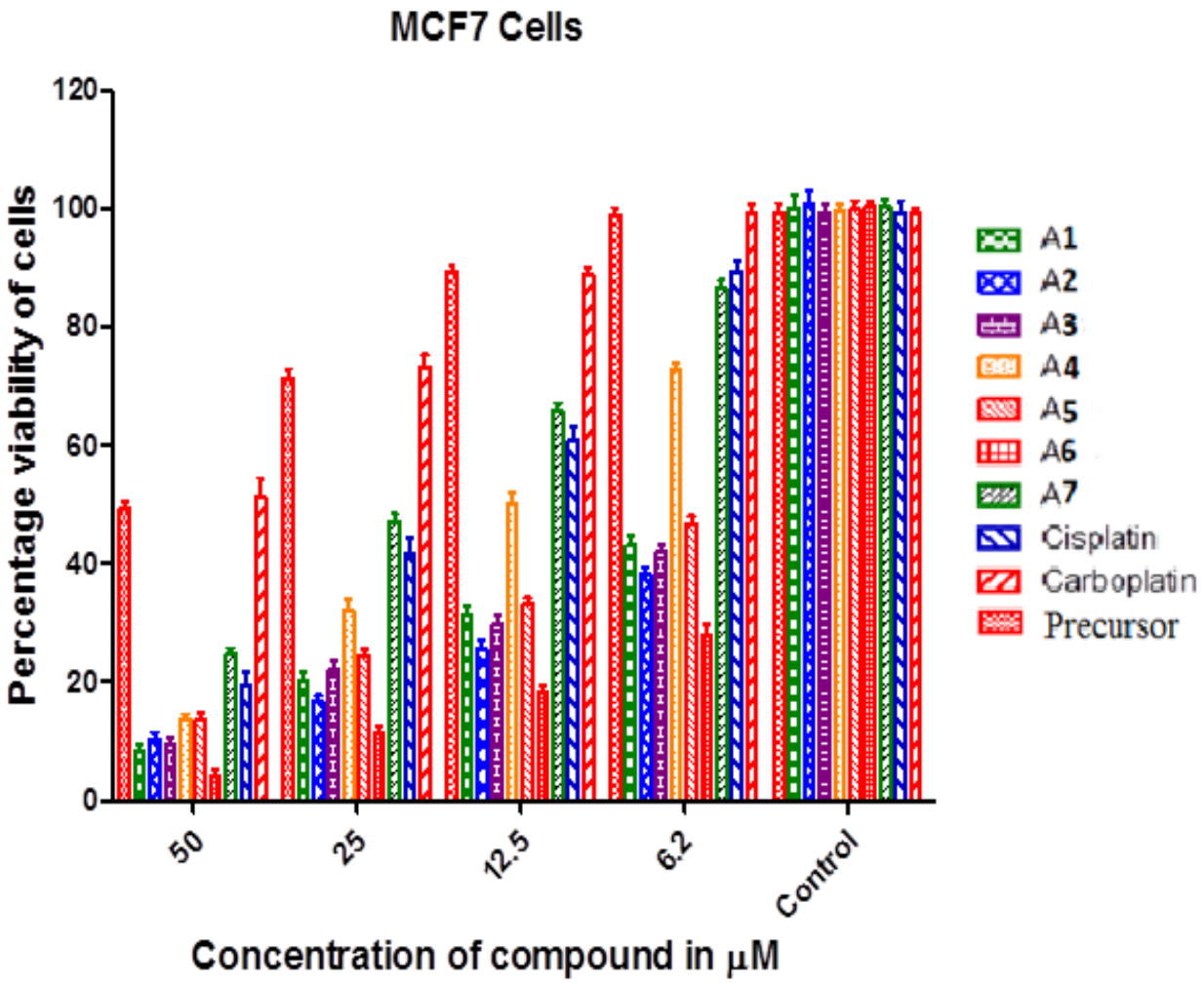


Figure 4.9 Graph showing complexes concentration effect on viability of MCF7 cell

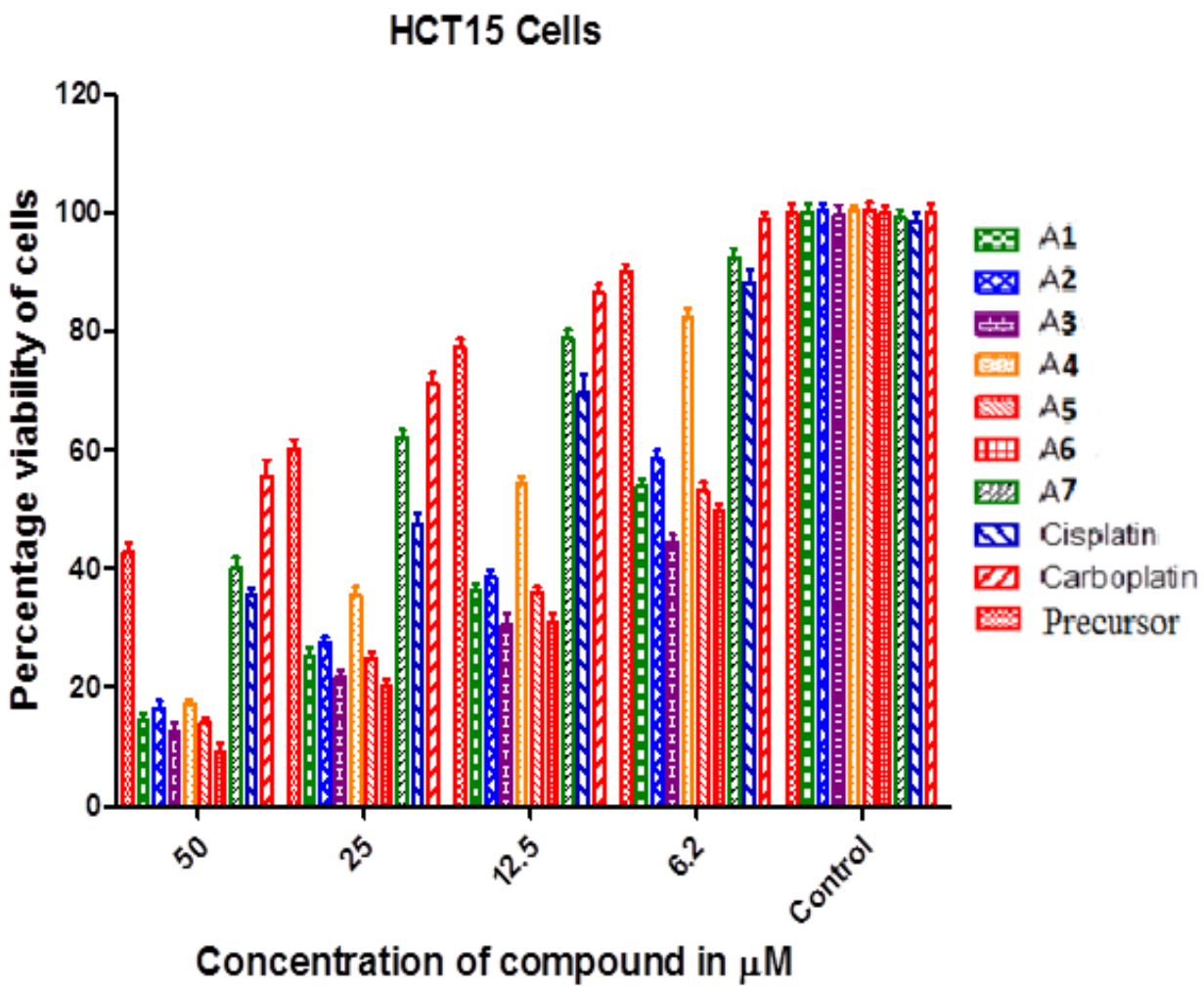


Figure 4.10 Graph showing complexes concentration effect on viability of HCT15 cell

Table 4.11 IC_{50} Values in (μM) of compounds **A1-A7,** against four human tumor cell lines

	IC_{50} ± SEM[a]			
Compounds	**HeLa**	**A549**	**MCF7**	**HCT15**
Cisplatin	16.1 ± 1	30.1 ± 1	24 ± 1	17 ±1
Carboplatin	56 ± 2	71 ± 3	64 ± 2	56 ± 2
Precursor	46 ± 2	56 ±2	39 ± 2	60 ± 2
A1	1.4 ± 0.3	3.5 ±0.7	7 ± 1	4.7 ± 1
A2	1.8 ± 0.3	3.9 ±0.5	8.6 ±0.9	2.6 ± 0.9
A3	1.5 ± 0.2	2.4 ± 0.3	4.2 ± 0.7	3.8 ± 0.6
A4	10 ± 2	13 ± 1	16 ± 1	14 ± 1
A5	2.2 ± 0.5	4.5 ± 0.8	6.9 ± 1	4.7 ± 1
A6	1.8 ± 0.4	3.6 ± 0.7	5.7 ± 0.9	1.2 ± 0.3
A7	17 ± 1	28 ± 1	37 ± 2	22 ± 1

The experimental results are presented as micro-mole concentration of 50% cell growth inhibition (IC_{50}) of each drugs. The MTT assay was performed in three independent experiments, each in triplicate.
[a]Errors are standard deviations determined from at least three independent experiments.

The IC_{50} data in the above table, demonstrated higher cytotoxic activity for our synthesized complexes. Interestingly, all complexes displayed superior cytotoxic effects against Hela cell line, by a factor of 32 to 40 and 27 to 32 higher than carboplatin and the precursor respectively. Complexes **A1 - A6** were showed 1.6 to 11 times higher activity even than

cisplatin in the same cell, with IC_{50} in the range of 1.0 - 10 μM, while the last complex **A7** showed slightly lower cytotoxicity with respect to cisplatin .

For A549 cell; all complexes demonstrated inhibition of the cell growth and showed promising cytotoxic activity. The results showed the complexes have 1.1 to 9.0 time's higher cytotoxicity than cisplatin, 20 to 26 fold higher than carboplatin and 16 to 20 fold higher than the precursor.

The MTT results showed the effect of the complexes **A1 – A6** on human breast cancer cell, the IC_{50} values, in the range of 4.2 – 12 μM, indicate that the complexes are stronger *in vitro* cytotoxic agents than that of carboplatin, precursor and cisplatin, while the complex **A7** was recognized to be less cytotoxic than cisplatin with IC_{50} value of 37 μM, and still with higher cytotoxicity than carboplatin and the precursor.

These complexes were also evaluated against human colon cancer cell line, the complexes **A1 –A6** showed significant cytotoxicity and found to be 1.3 to 14, 4 to 45 and 4.4 to 49 fold higher cytotoxicity than cisplatin, carboplatin and the precursor respectively. Only complex **A7** was found to be less cytotoxic than the parent drug cisplatin, although it's more cytotoxic than carboplatin and the precursor.

The obtained results demonstrate that the cytotoxicity enhances dramatically toward the synthesized compounds. **A1 – A5** have high cytotoxicity against the HeLa cell line [1.4 – 9.9] μM, while **A6** was found to be the most effective complex with IC_{50} value of 1.2 μM against **HCT15** and 14 fold better than cisplatin. These results are consistent with a significant selective cytotoxicity of our complexes against particular cancer cell lines and their tendency to undergo ligand exchange with biomolecules like proteins and DNA.

Even though the exact mechanism on cytotoxicity of these complexes remains inconspicuous, the lowest cytotoxicity of complex **A7** compared to the others, likely due to the ring size of the Diap ligand.

CHAPTER 5

CONCLUSION & RECOMMENDATIONS

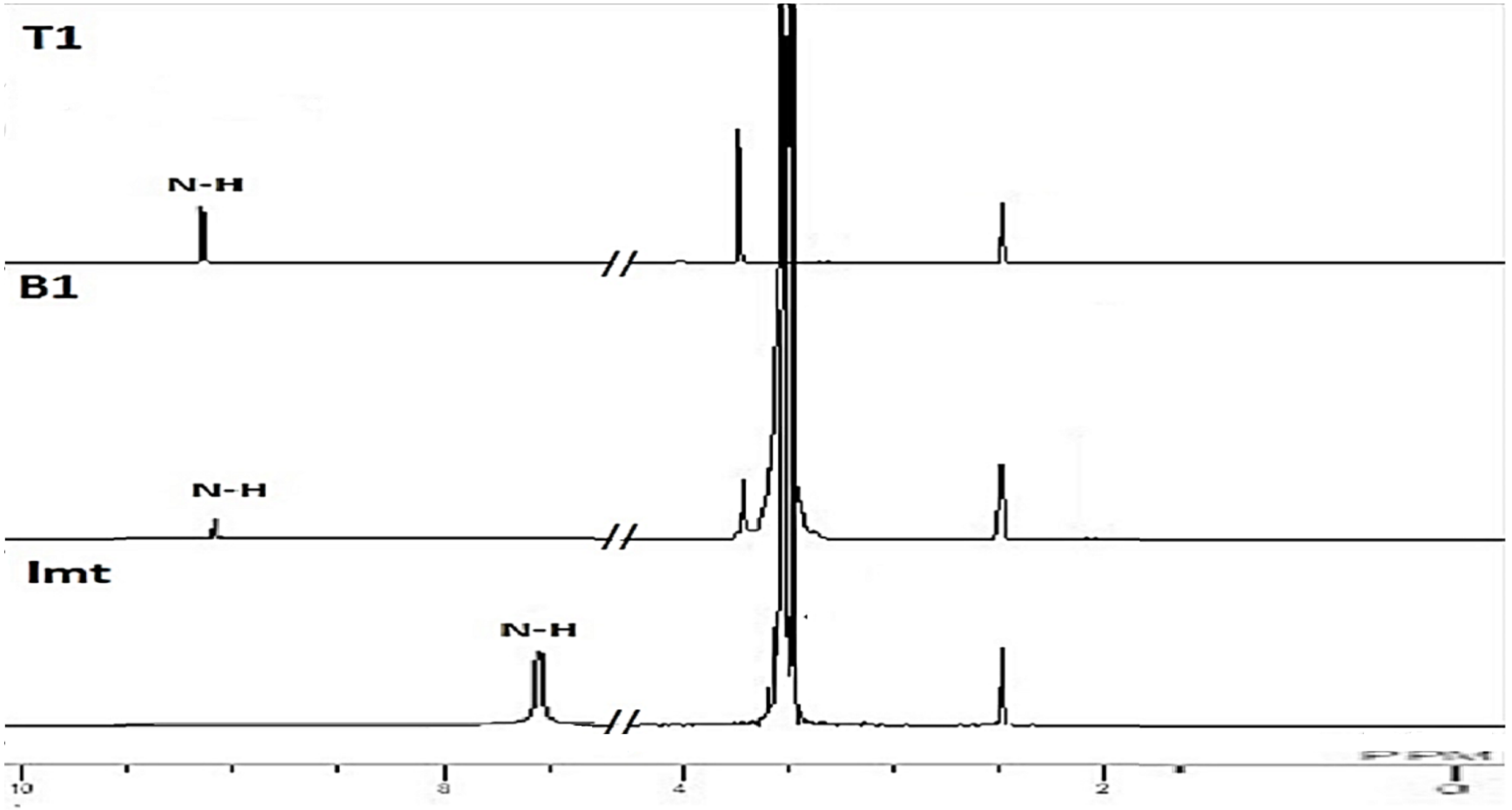
Three new series of platinum(II) complexes with general formula *cis*-[$(Et_3P)_2Pt(L)_2]Cl_2$, *cis*-$[(NH_3)_2Pt(L)_2](NO_3)_2$ and *trans*-$[Pt(NH_3)_2(L)_2](NO_3)_2$ have been successfully synthesized and characterized using both analytical and spectroscopic techniques. The NMR and elemental analysis data strongly support the formation of the products.

In order to characterize the coordination mode of the thione ligand with platinum(II) center ion in these complexes, the structure of *trans*-$[Pt(NH_3)_2(Imt)_2](NO_3)_2$ and *trans*-$[Pt(NH_3)_2(Me_2Imt)_2](NO_3)_2$, in addition to $[Pt(i\text{Pr-Imt})_4](NO_3)_2$ have been determined by X-ray crystallography. The data confirmed that the Pt(II) ion is bonded to the thione ligands through their sulfur atoms. Hydrogen bonding interactions in compound **C1** induce a bent see-saw distortion, relative to the ideal square planar geometry.

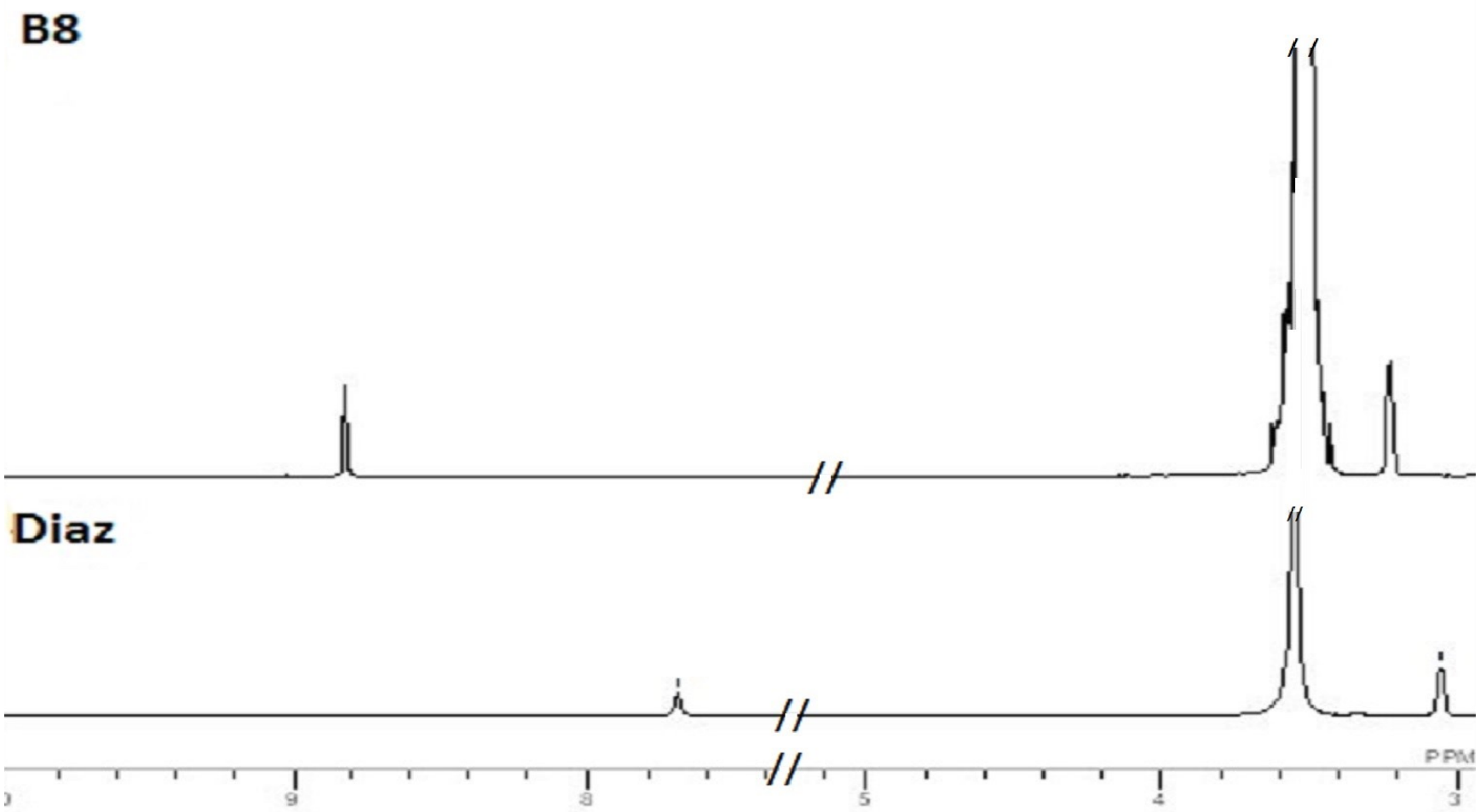
In vitro cytotoxicity studies demonstrated a significant activity of the synthesized complexes against the selected cell lines; *cis*-$[(Et_3P)_2Pt(\text{Et-Diaz})_2]Cl_2$ complexes was found to be the most effective and 14 times better cytotoxic agent than cisplatin against human colon cancer (**HCT15**). The rest of the synthesized compound should evaluate *in vitro* cytotoxic activity. For further studies we recommend to study the interaction of these compounds with DNA as well as the electronic effect of the ligand.

Appendix A

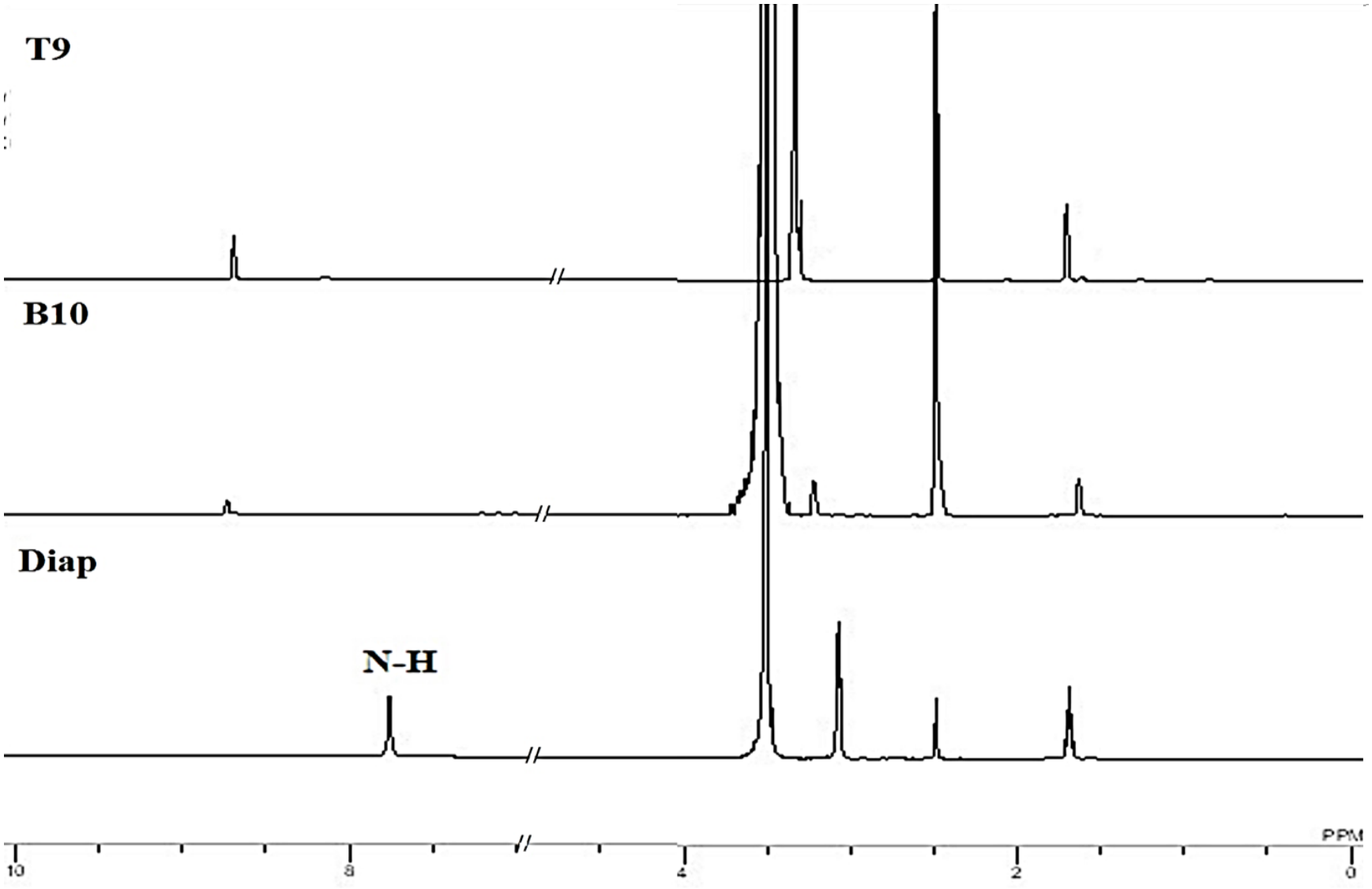
^{1}H NMR



^{1}H NMR Chemical shift (ppm) of the free Imt ligand and its platinum(II) complexes in DMSO–d_6



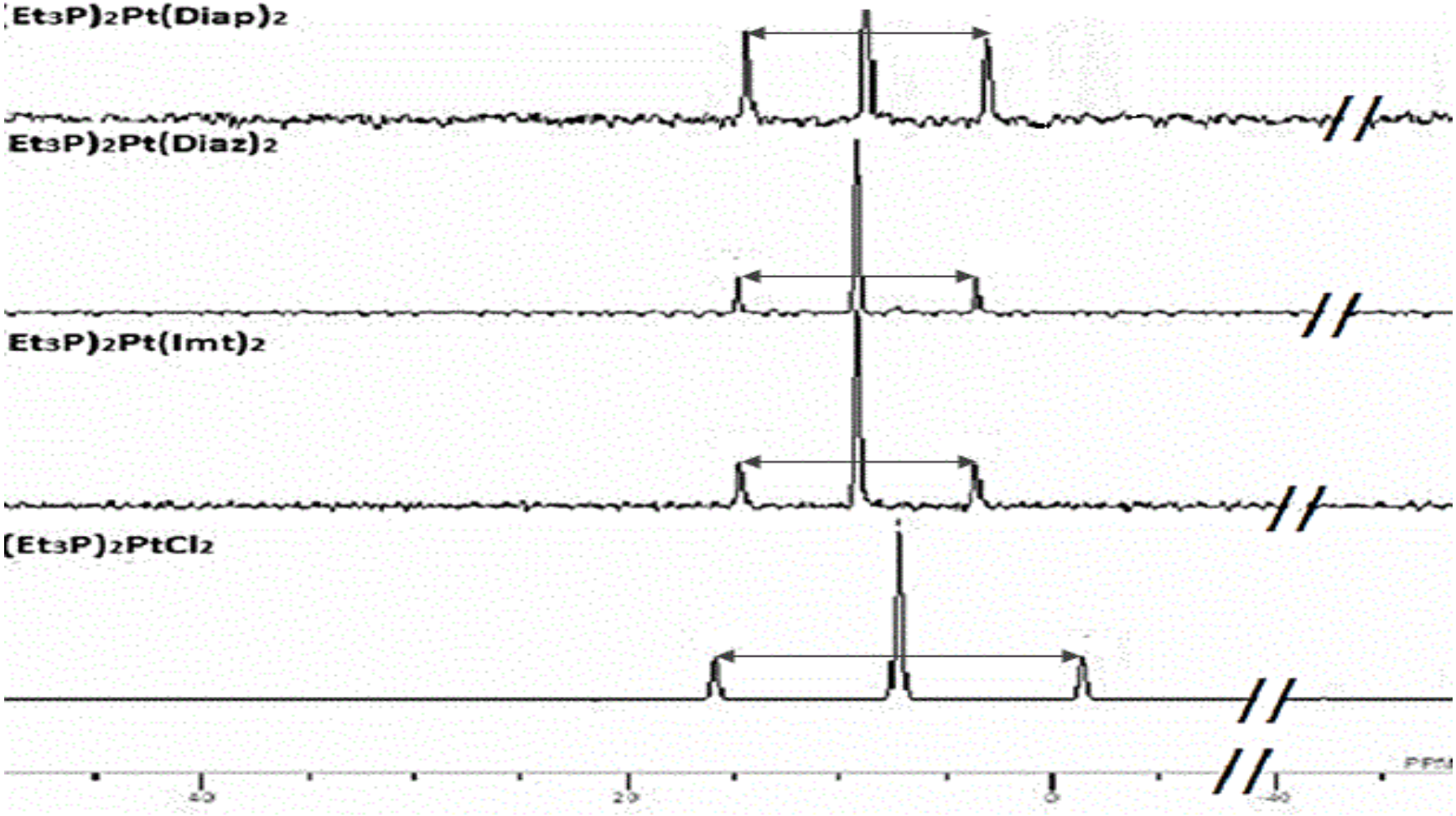
^{1}H NMR Chemical shift (ppm) of the free Diaz ligand and its platinum(II) complexes in DMSO–d_6



^{1}H NMR Chemical shift (ppm) of the free Diap ligand and its platinum(II) complexes in DMSO–d_6

Appendix B

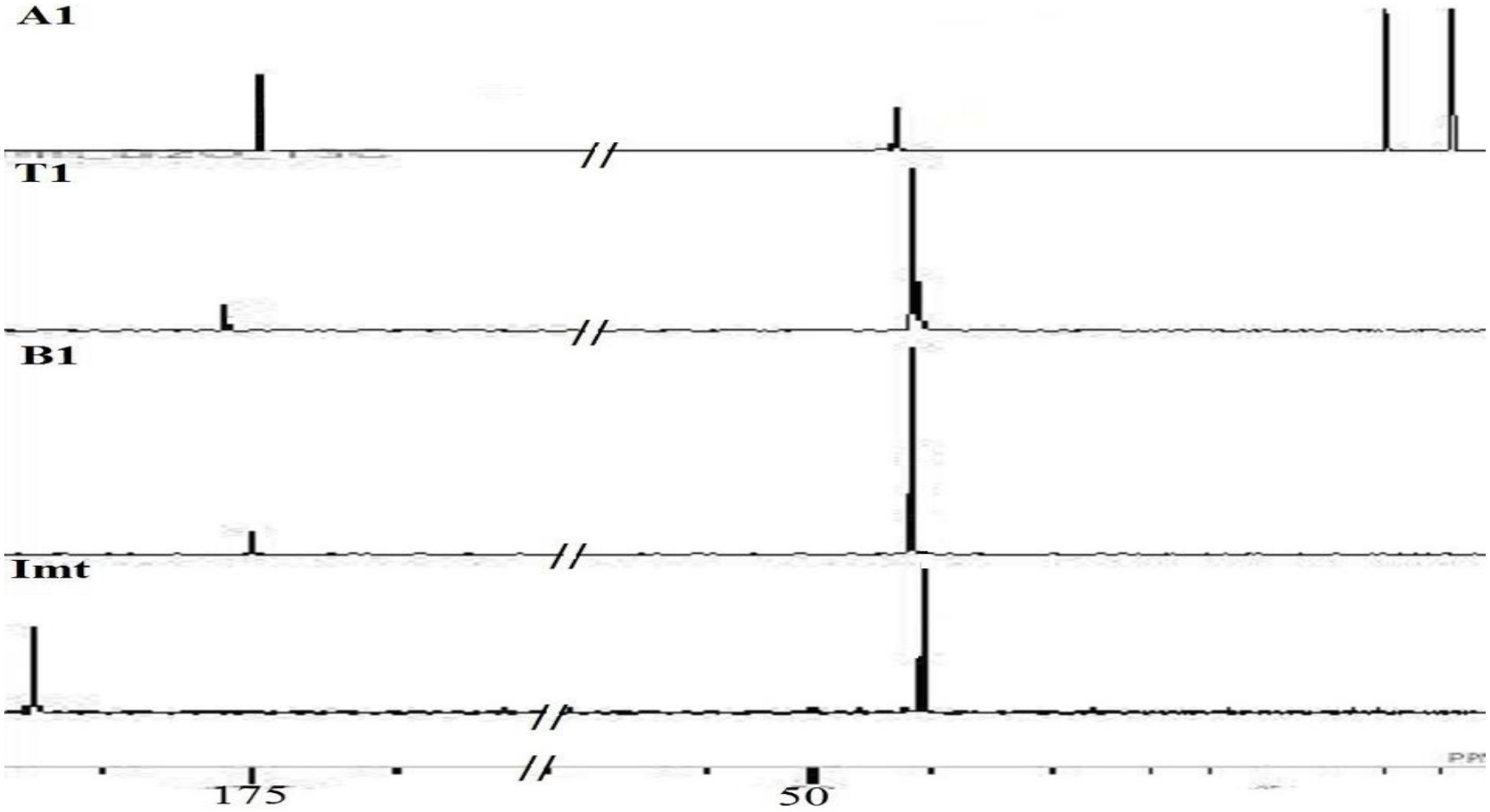
^{31}P solution NMR



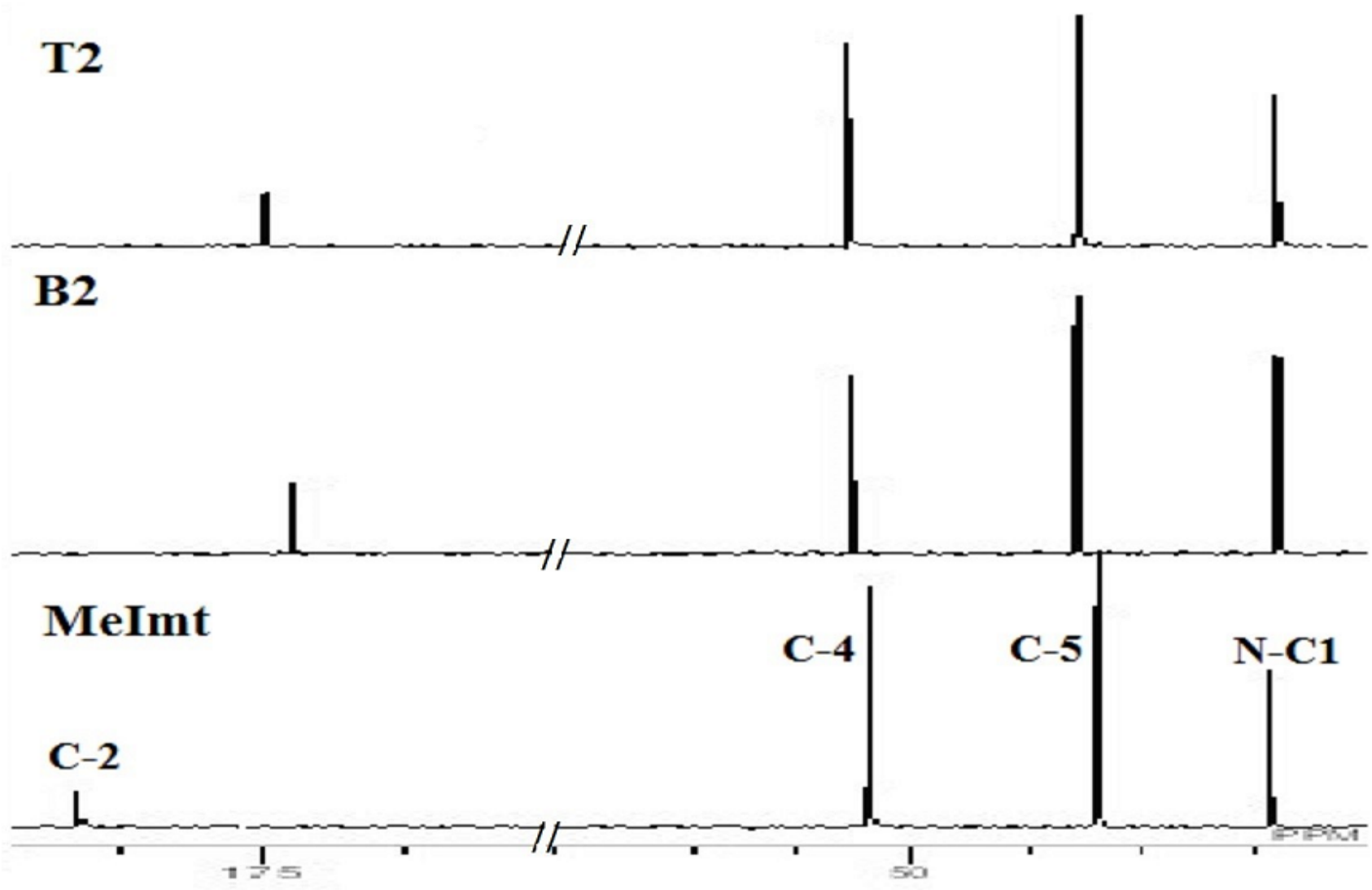
^{31}P Chemical shift (ppm) of the precursor and three platinum(II) complexes in $CDCl_3$

Appendix C

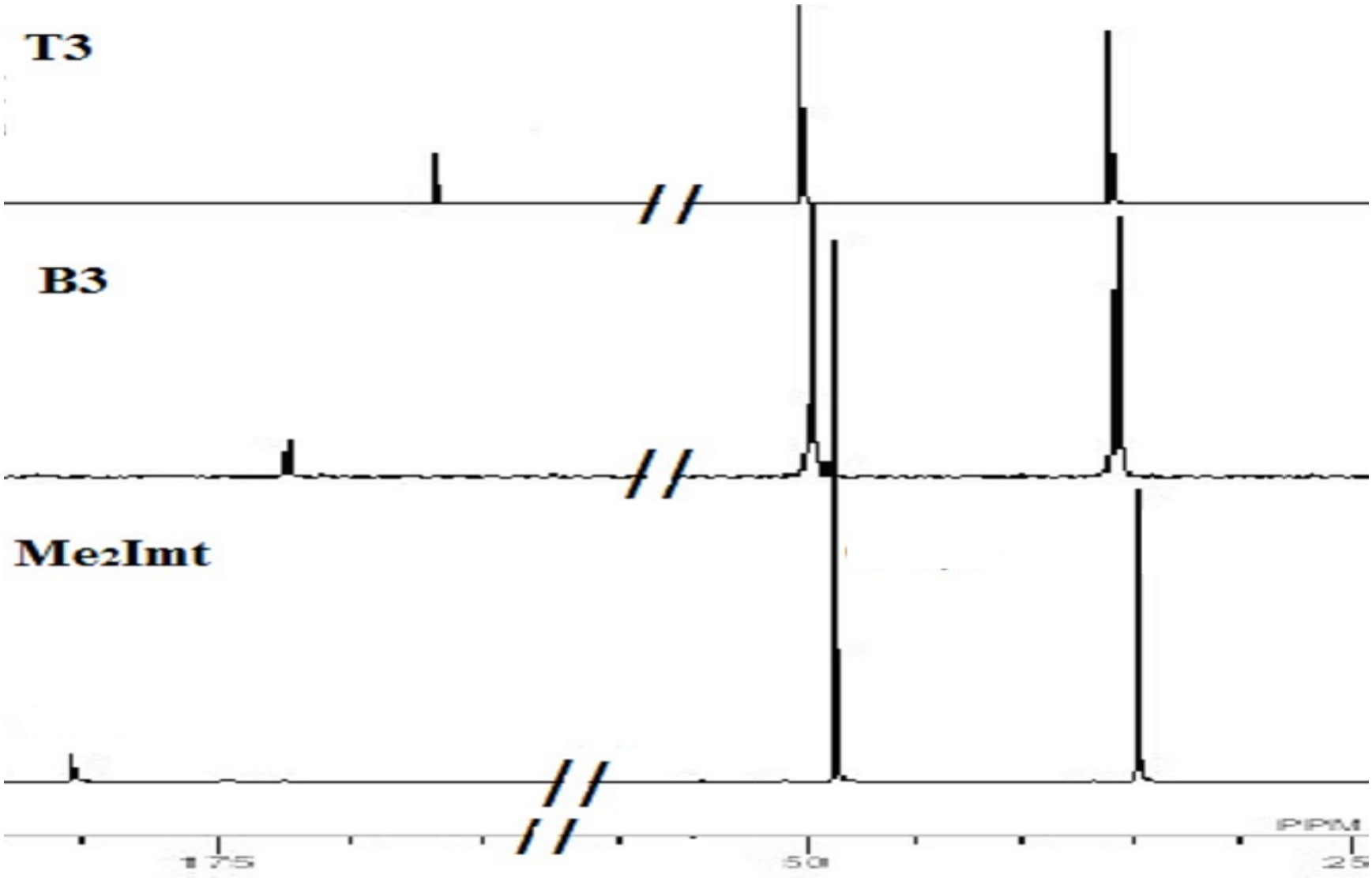
^{13}C solution NMR



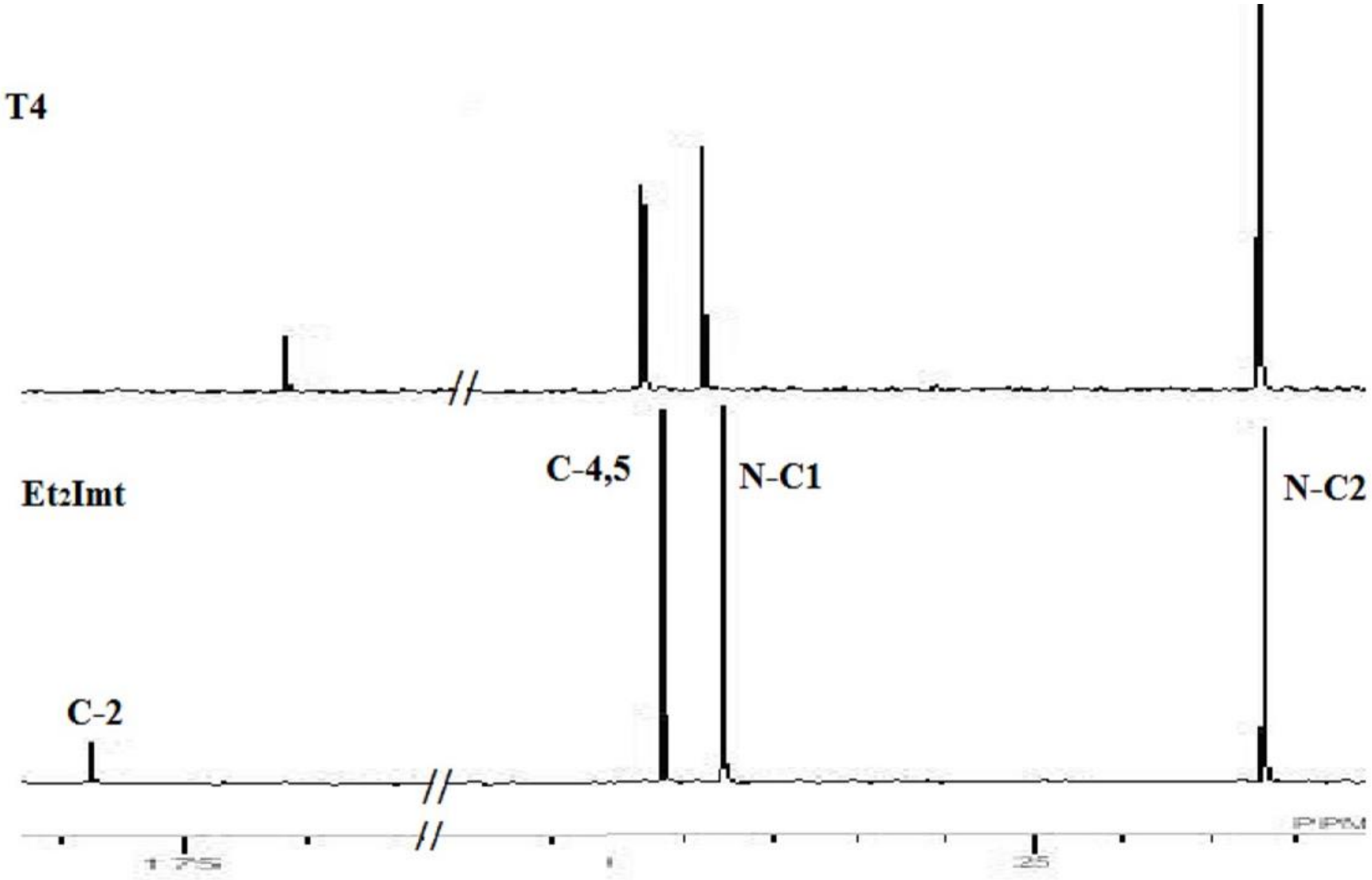
^{13}C Chemical shift (ppm) of the free Imt ligand and its platinum(II) complexes in D_2O



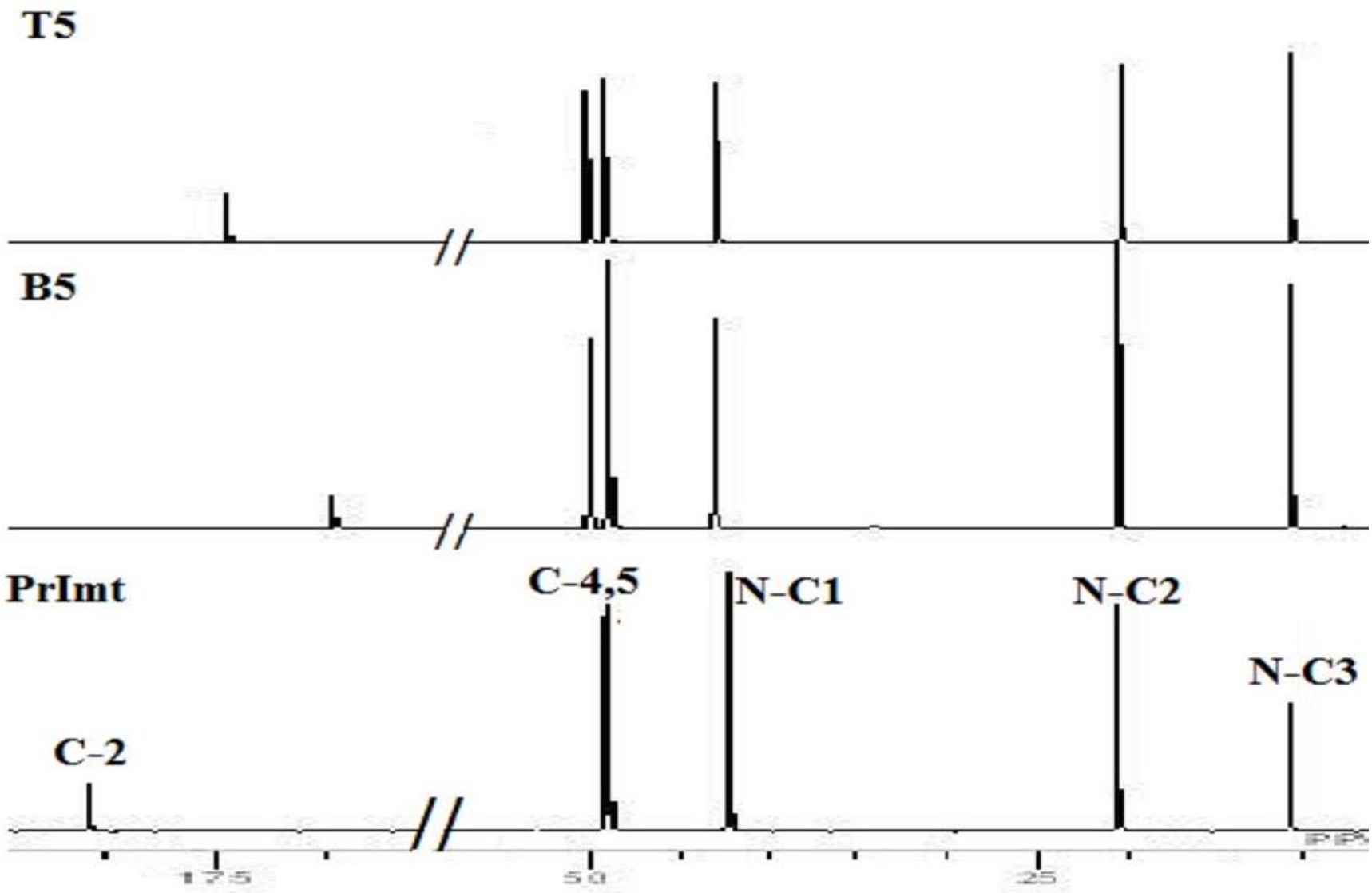
^{13}C Chemical shift (ppm) of the free MeImt ligand and its platinum(II) complexes in D_2O



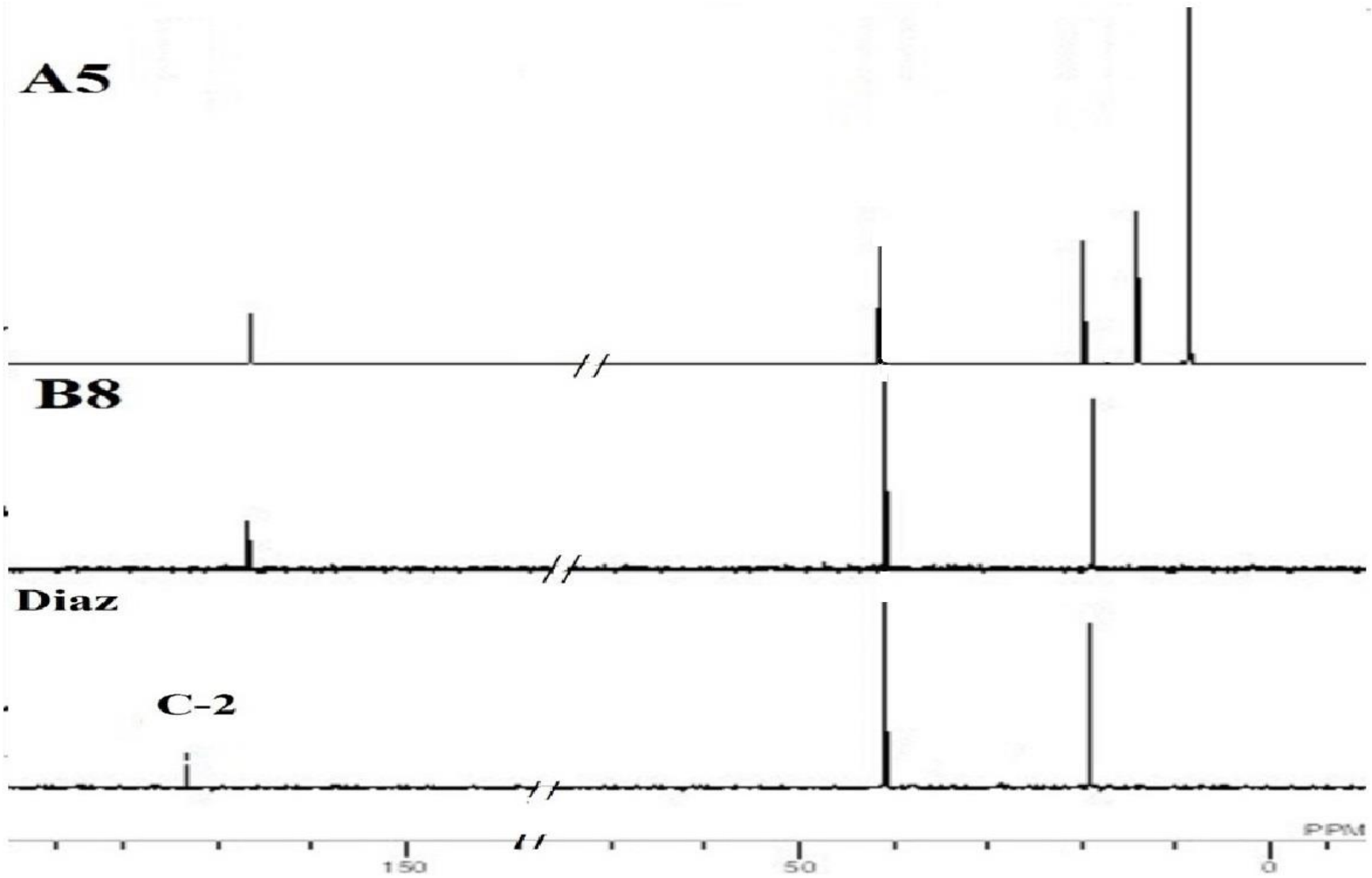
^{13}C Chemical shift (ppm) of the free Me_2Imt ligand and its platinum(II) complexes in D_2O



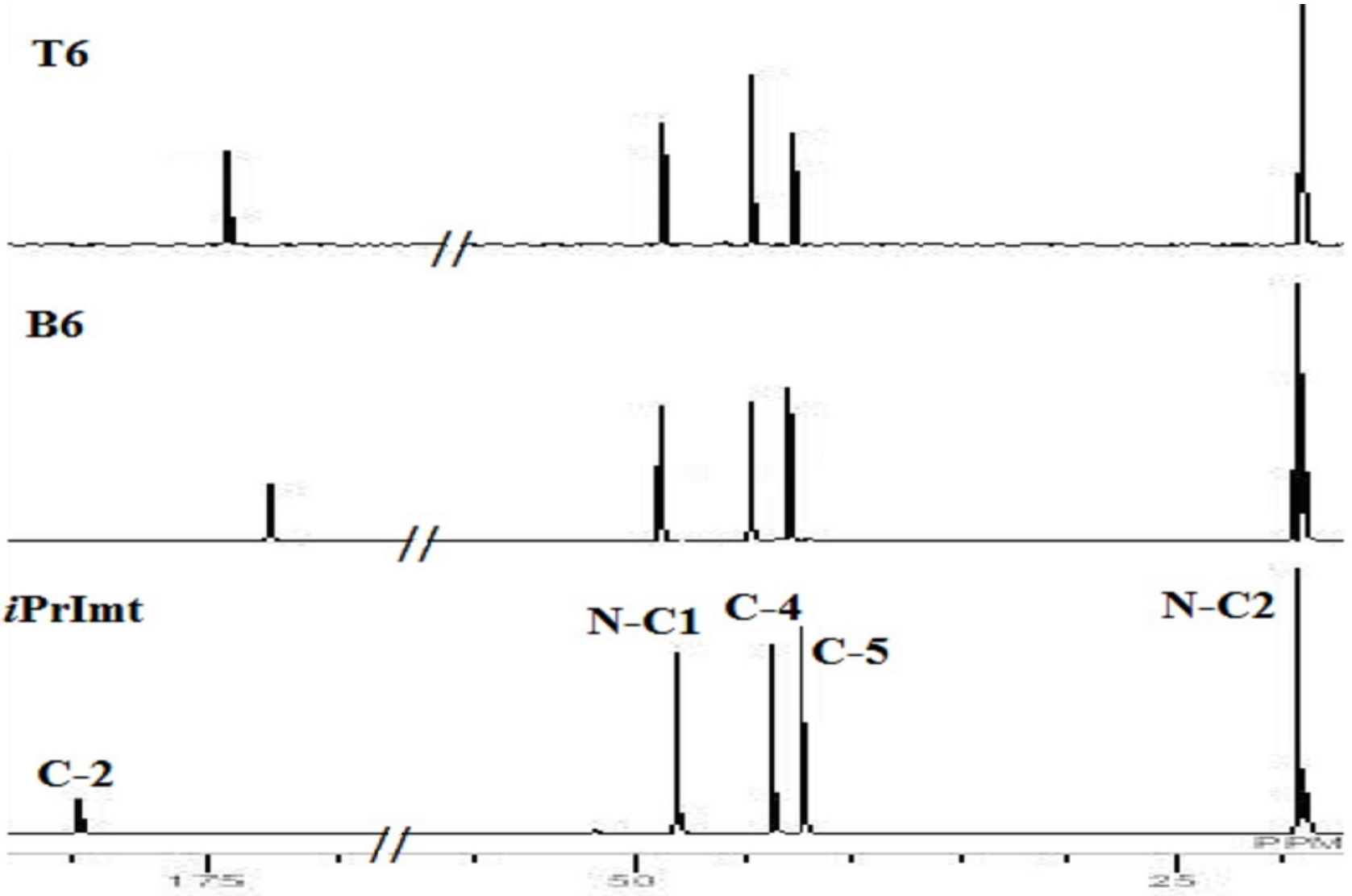
^{13}C Chemical shift (ppm) of the free Et_2Imt ligand and its platinum(II) complexes in D_2O



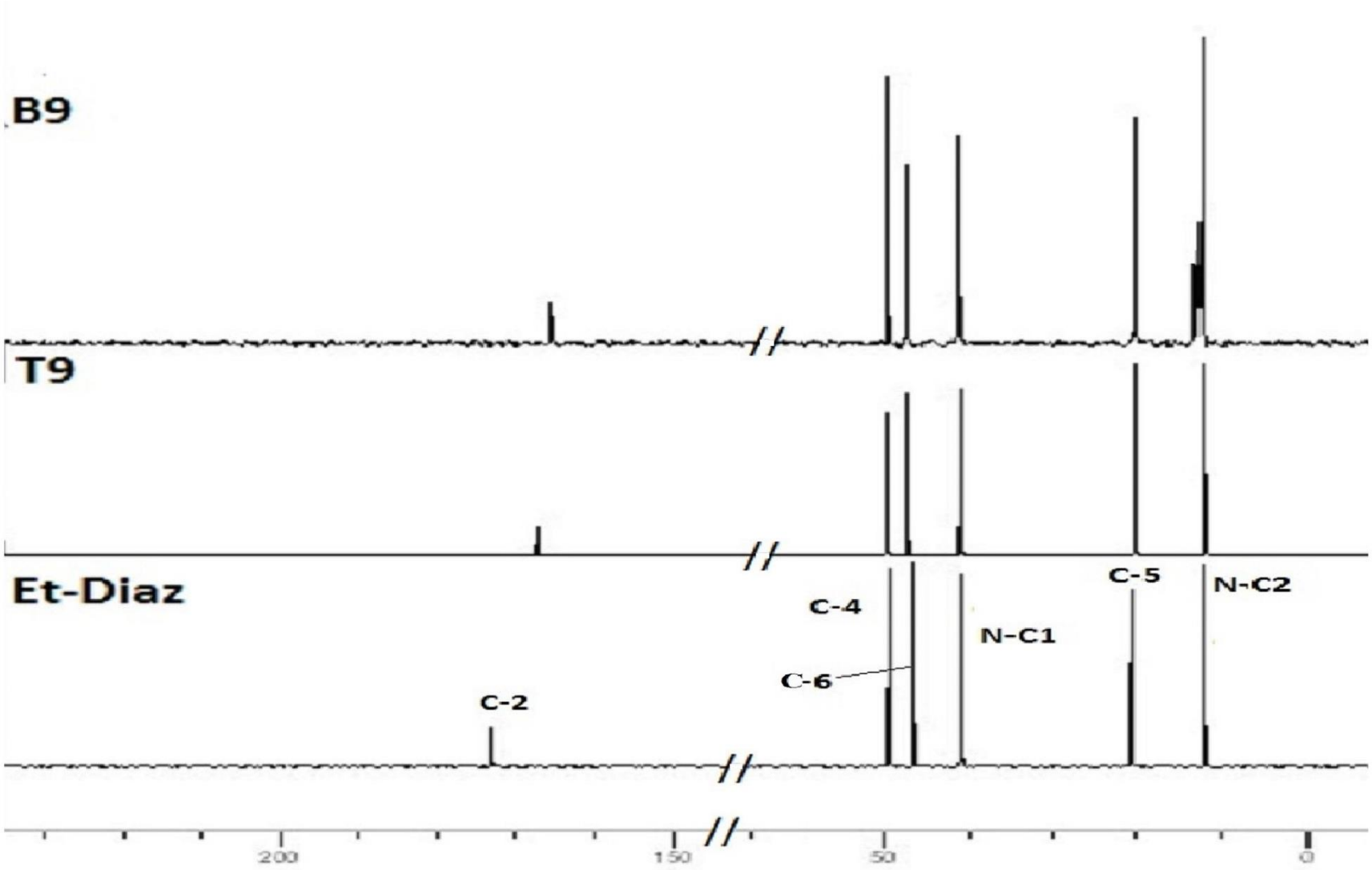
^{13}C Chemical shift (ppm) of the free PrImt ligand and its platinum(II) complexes in D_2O



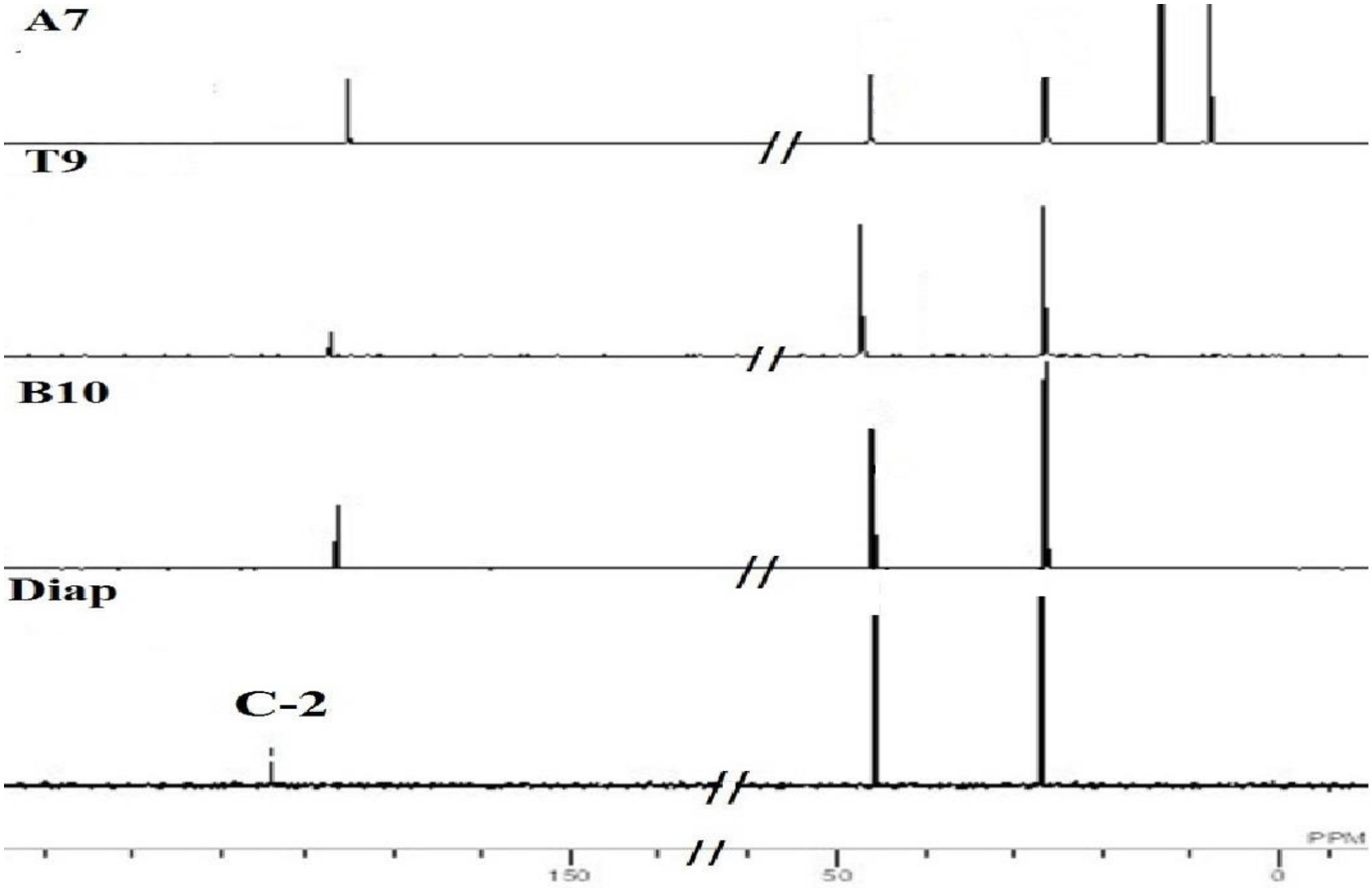
^{13}C Chemical shift(ppm) of the free *i*PrImt ligand and its platinum(II) complexes in D_2O



^{13}C Chemical shift (ppm) of the free Diaz ligand and its platinum(II) complexes in D_2O



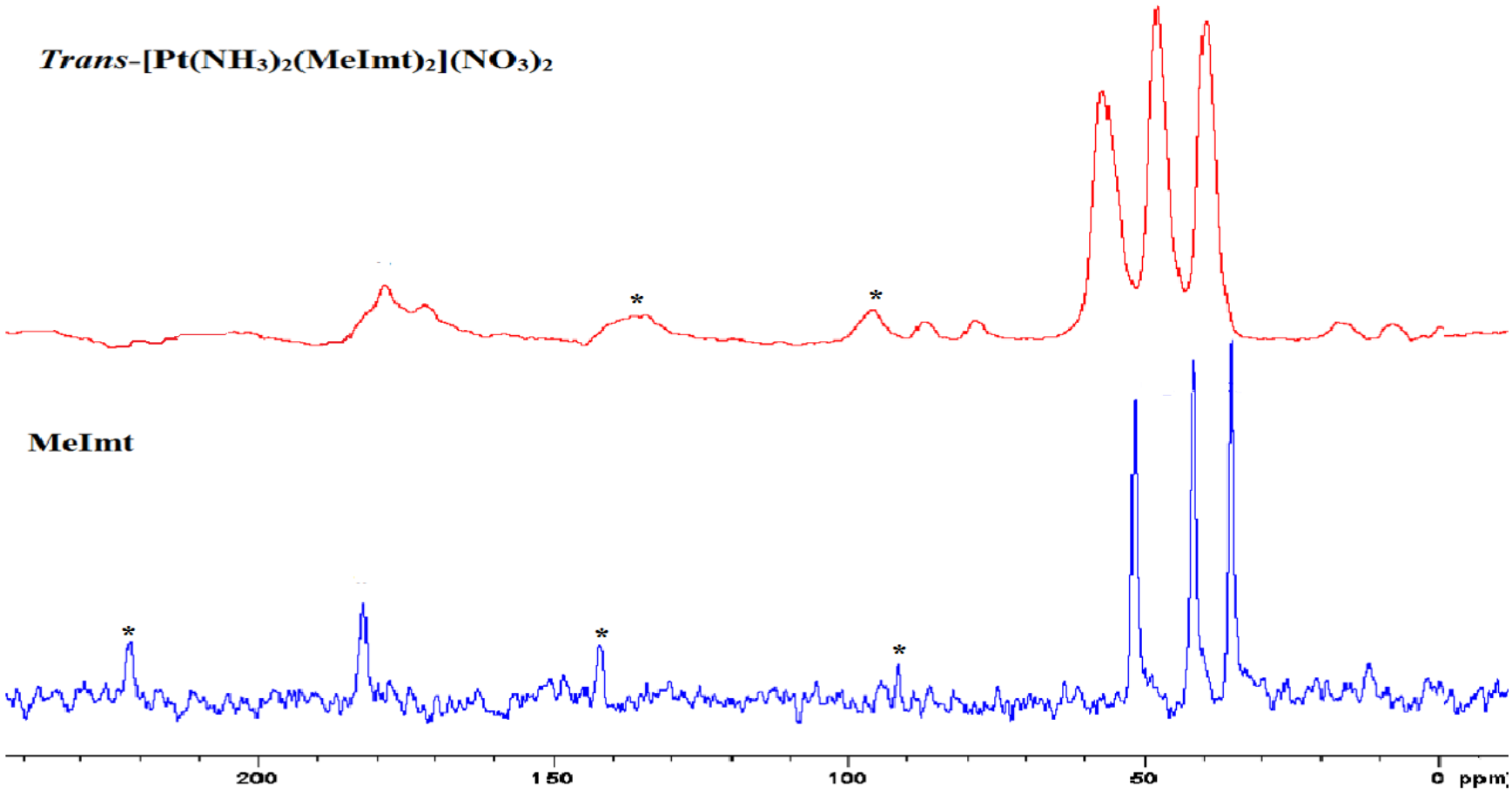
^{13}C Chemical shift (ppm) of the free EtDiaz ligand and its platinum(II) complexes in D_2O



^{13}C Chemical shift (ppm) of the free Diap ligand and its platinum(II) complexes in D_2O

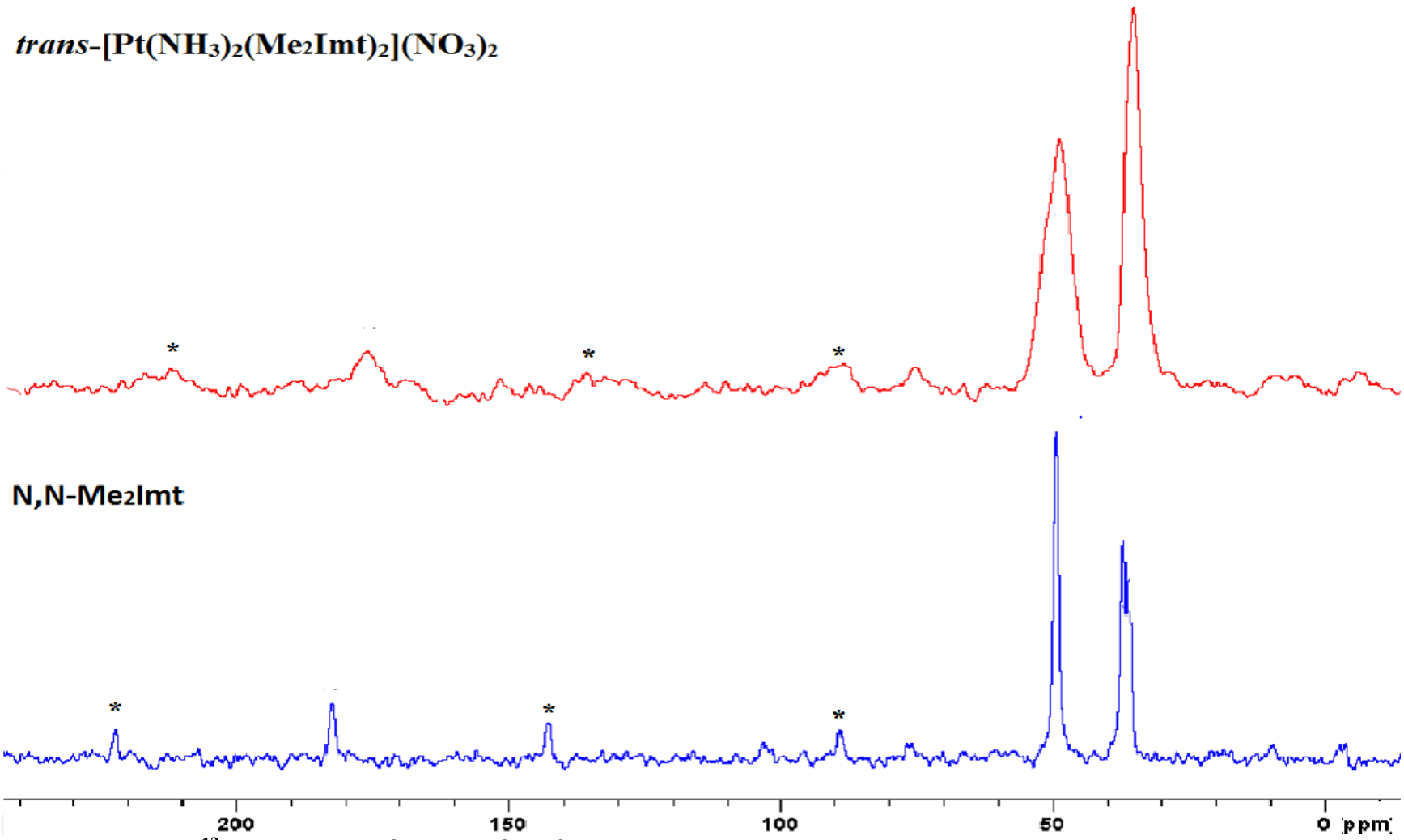
Appendix D

^{13}C solid state NMR



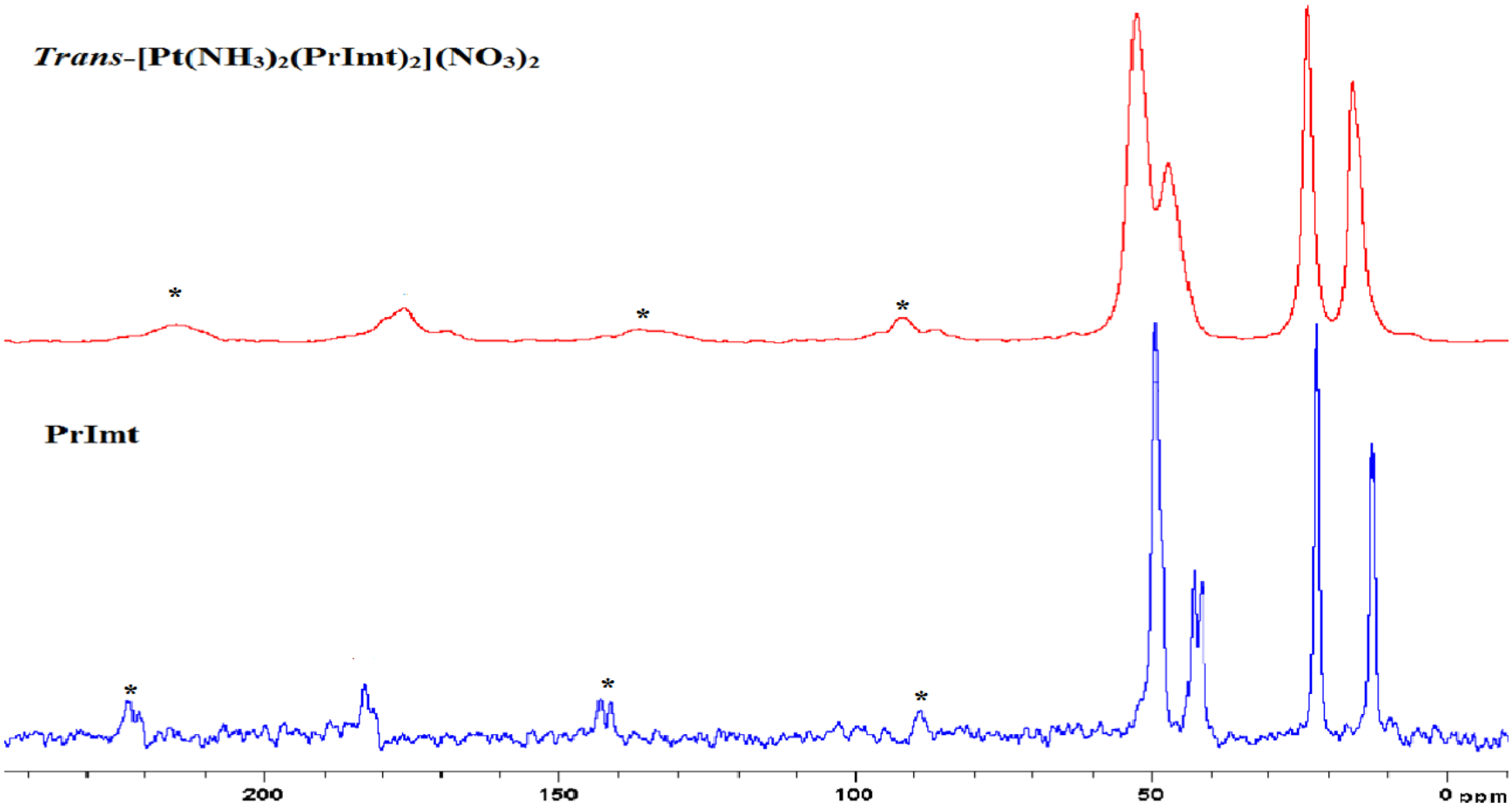
^{13}C Chemical shift (ppm) of the free N-MeImt ligand and its *trans* platinum(II) complex

*** Spinning side band**



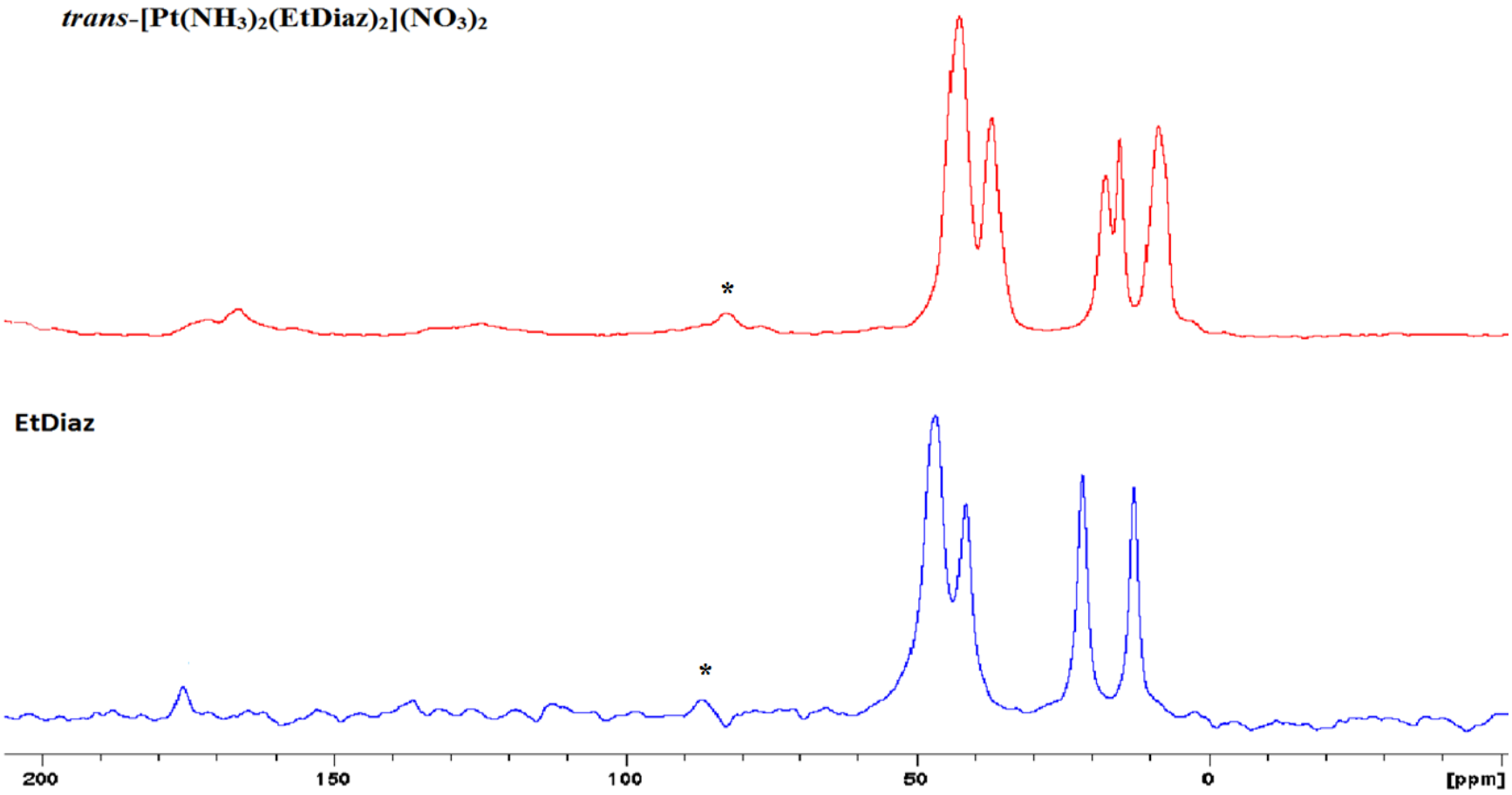
^{13}C Chemical shift (ppm) of the free N,N-Me$_2$Imt ligand and its *trans* platinum(II) complex

*** Spinning side band**



^{13}C Chemical shift (ppm) of the free N-PrImt ligand and its *trans* platinum(II) complex

*** Spinning side band**



^{13}C Chemical shift (ppm) of the free EtDiaz ligand and its *trans* platinum(II) complex

*** Spinning side band**

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PAPERS

1. A. Z. A. Mustafa, **M. Y. A. Jomaa**, M. Monim-ul-Mehboob, M. Altaf, M. Fettouhia A. A. Isaba, M. I. M. Wazeer, H. Stoeckli-Evans, G.Bhatia and V. Dhuna, Synthesis, Spectroscopic Characterization, Crystal Structure and *in vitro* Cytotoxicity of Tetrakis(thione)platinum(II) Complexes. **(Submitted)**.

2. **Mohammed Y. A. Jomaa**, M. Altaf, Anvarhusein A. Isab and Mohammed I. M. Wazeer, Synthesis, characterization and in vitro cytotoxicity studies of Bis(triethylphosphine) platinum(II) complexes with thione ligands. **(Under review)**.