Palladium(II) Complexes as Possible Chemotherapeutic Agents: Synthesis, Kinetics of Reaction and In Vitro Screening

Alena Corcoran

A thesis submitted to the Graduate Faculty
in partial fulfillment of the requirements for the
Masters of Science Degree in the Department of Chemistry,
Faculty of Science,
University of Prince Edward Island.

Alena Corcoran
Charlottetown, P.E.I.
March 2007

Your file Votre référence
ISBN: 978-0-494-32107-2
Our file Notre référence
ISBN: 978-0-494-32107-2

SIGNATURE PAGE

iii

REMOVED

SIGNATURE PAGE

iv

REMOVED

Acknowledgements

I wish to extend my sincere thanks to Dr. Haines, my supervisor, for his endless hours of guidance, assistance, patience, mentorship and knowledge throughout my time spent while attending the University of Prince Edward Island. I would like to thank him for instilling within me a love for medicinally related research by provoking my interest in the field.

A special thanks goes to Dr. Alastair Cribb for providing training in the methodology of *in vitro* cell line screening. Without him this project would not have been possible. I wish to extend him thanks for his patience, instruction and assistance. I would also like to thank his team of researchers at the Atlantic Veterinary College for their assistance throughout the project.

I would like to thank the remainder of the chemistry department at UPEI for their instruction and guidance throughout my masters project as well as my undergraduate degree. They have made my academic experiences enjoyable and I attribute my accomplishments to their enthusiasm and quality of instruction.

A special thanks goes to Dr Stan Brown from Queens University in Kingston, ON, for the use of his stopped-flow spectrometer. Completion of the kinetics section in this project would not have been possible without his gracious hospitality.

Finally I wish to thank my family and friends for their endless support and encouragement. Without you none of this would be possible.

Abstract

This work reports the synthesis and characterization of a series of palladium(II) analogues of cisplatin. The Pd(II) compounds are analogues composed of tridentate polypyridyl chelate ligands with one chloro ligand attached to the palladium metal. A similar binuclear compound previously synthesized was also used in the studies as a comparison for a binuclear compound *versus* a mononuclear one.

The kinetics of reaction of guanosine hydrate with $[Pd(Terpy)Cl]Cl^+$, $[Pd(B_H)Cl]Cl^+$, $[Pd(B_{Me})Cl]Cl^+$, $[Pd(B_{Et})Cl]Cl^+$ and $[Pd(B_{Bu})Cl]Cl^+$ were studied under pseudo-first-order conditions using stopped-flow spectrometry. The rate law for each compound was determined to be : Rate = $k_{obs}[Pd(ligand)Cl]^+ = (k_1 + k_2[Gu])[Pd]$. From the temperature dependence of $[Pd(Terpy)Cl]Cl^+$, $[Pd(B_H)Cl]Cl^+$ and $[Pd(B_{Bu})Cl]Cl^+$, activation parameters were calculated using the Eyring equation.

The five substituted Pd(II) terpy compounds and the binuclear Pd(II) tppz compound were subjected to reaction with calf thymus DNA indicating a positive reaction.

Octanol-water partition coefficients were calculated for each of the Pd(II) compounds indicating a trend that supports active uptake by cellular membranes based on ligand structure.

The six Pd(II) compounds were subject to *in vitro* screening against human ovarian cancer (SKOV3), human breast cancer (MCF7) and human colon cancer (HT-29) cell lines. All six compounds presented significant cytotoxic effects towards all three cell lines. The significance is an increase in toxicity for $[Pd(Terpy)Cl]Cl$ and $[Pd(B_{Bu})Cl]Cl$.

Table of Contents

List of Illustrations

Figures

Tables

1. Introduction

1.1 An Introduction to Cancer

To fully define the objective and necessity of this work, we must first examine the history and biological definitions of cancer. It is essential to obtain a knowledge of the diseased cellular growth compared to normal cellular growth to fully comprehend the complexity of the disease and its need for new novel treatments. This disease accounts for approximately 70,000 deaths each year in Canada alone. This brief introduction will give insight into the definition and characterization of this deadly disease that is currently the leading cause of premature death in Canada.[5]

1.1.I. Definition of Cancer

Cancer is an ancient disease that was known to exist as early as Egyptian times when humans first began to record their activities.[23] Today it is estimated that at least one in three people will develop cancer during their lifetime. Statistics show that one in four men and one in five women will lose their lives to this devastating disease.[11]

The history of cancer research began approximately 140 years ago when a German physiologist, Johannes Mueller, demonstrated that cancers were made up of cells. This discovery began the search for structural changes that would define the difference between a normal and a cancerous cell.[11] Today we know that cancer exhibits several distinguishing features that are specific to the disease.[2]

Primarily, cancerous cells are defined as abnormal cells that grow very rapidly and eventually develop into a detectable mass, also known as a tumor. In general, tumors that grow relatively

slowly are more likely to stay grounded to one area of the body until they have reached an advanced stage. This is one characteristic that can distinguishes a malignant tumor from a benign tumor. Secondly, the disorder of the cells' growth is unique to cancer, for cancer cells do not exhibit well-defined boundaries in their growth patterns. Thirdly, cancerous cells have the unusual ability to travel from their place of origin to other parts of the body. Once relocated, the cells will begin growing again, providing that the conditions for growth are appropriate. This unique feature of cancer that enables it to travel and grow within the body is known as metastasis.[2]

1.1.II. Understanding Cellular Growth

As mentioned above, to obtain a necessary understanding and knowledge of diseases such as cancer, we must first examine normal cellular composition and growth in depth.

Tissues found in the human body can be categorized into four groups; general supporting tissues known as mesenchyme; tissue-specific cells known as epithelia; defense cells making up what is known as the reticuloendothelial system; and the nervous system. The mesenchyme supporting tissues are composed of connective tissues, fibroblasts which are the composition of collagen fibres, and associated proteins, bone, cartilage, muscle, blood vessels, and lymphatics. The epithelial cells are specific cells of the different organs of the body. The reticuloendothelial system is comprised of a wide group of cells which are derived from precursor cells in the bone marrow which then give rise to red and white blood cells. Some of the cells, lymphocytes and macrophages, are distributed throughout the body as free or fixed components of other organs such as the liver, or as separate organs, such as the spleen and lymph. Lymphoid cells are distributed throughout the body and act as filters for cells, bacteria and other foreign materials. The nervous system is

composed of the central nervous system, the brain and spinal cord, and the peripheral nervous system of nerves that lead from these centralized structures.[2,11]

Each tissue in the body has it's own specific cells that maintain structure and function. The specific cells are grouped into a standard pattern to form organs. Different organs in the body differ only in the nature of the specific cells and the arrangement and distribution of the supporting mesenchyme, not in overall structure.[11]

Understanding normal cell and tissue composition is only the first step in understanding diseases such as cancer, we also need to examine the control of growth in normal cells. Growth is a general term that indicates an alteration in size of cell mass. Growth is the end product of several interrelated influences, such as proliferation, differentiation, cell death, cell contacts and blood supply.[23]

There is a precise mechanism in normal growth and development that allows individual organs to reach a specific size. If a tissue is injured, the surviving cells will begin to grow and multiply to replace the damaged cells. When the desired size is reached, the growth process stops again.[11]

As cellular multiplication occurs, cells accumulate errors in their DNA that result in senescence after approximately 40 doublings. This factor alone would result in all cells eventually dying, however there is a mechanism of asymmetrical division present some cells that leads to a daughter cell that retains the potential of unlimited proliferation. These cells are called stem cells.[23] Stem cells are present in most organs as special reserves which are capable of growing in response to a stimulus, such as an injury, since most adult cells cannot proliferate. The stem cells respond to the stimulus and develop into the organ specific cells that are required. [11]

Cancer cells are assumed to proliferate faster than normal cells, but this isn't exactly the case. Proliferation rates of tumor cells in relation to normal cells are not that different, what is different is the lack of stop signals in the cancer cells that maintain normal tissue stasis. Tumor cells progressing from differentiated to de-differentiated changes the circumstances and proliferation rates increase. This is seen in shorter doubling times of metastases when compared with noncancerous cells in a benign tumor.[23]

The mechanism of growth control is very important, yet only partly understood. The size of a tumor influences its chances of metastasizing. The larger the tumor, the more likely it is to spread to other areas of the body. Much research has been focused on the factors controlling stem cell growth in red and white cells and their relationship to tumor development, for we know that there is a close relationship between growth factor production and tumor growth.[2]

Overall tumor growth cannot be understood without also examining cell death as a main contributor. There are two types of cell death, necrotic and apoptotic. Necrosis is a passive process while apoptosis is a macromolecular synthesis involving programmed cell death. Tumor growth is also affected by metastasis. Cancer cells are removed from a tumor mass by migration of live cells into blood and lymphatic vessels during metastasis.[23]

1.1.III. Classification of Tumors

We generally recognize a tumor cell by the fact that the cells have shown a specific degree of abnormal growth. A tumor is composed of cells that are no longer responsive to many or all normal growth-controlling mechanisms.[11]

There are three classifications of tumors. The first class is benign tumors that arise in any

tissue, grow locally and cause damage by local pressure or obstruction. Benign tumors tend to not spread to other areas of the body. The second class is *in situ* tumors that are usually very small and develop within the epithelia. These tumors remain in the epithelial layer and do not invade the basement membrane and supporting mesenchyme. The third class is malignant tumors which have a specific capacity to invade and destroy the mesenchyme. This invasion requires nutrients that are provided through the blood stream in normal tissues. Some tumors of this class can produce proteins that stimulate the growth of blood vessels into the tumor, which in turn allows for continuous growth. The vessels are not very well formed and are easily damaged, this means that the tumor cells may penetrate these and surrounding lymphatic cells, hence allowing tumor fragments to be carried to local lymph nodes or other organs where secondary tumors can arise.[11]

1.2. An Introduction Cancer Treatment

Treatments t rely on spatial separation of tumors and normal tissue, as in the case of surgery and radiotherapy, or on minor and empirical differences in the responses of tumors and normal tissues to systemic treatments, as in the case of chemotherapy. Because of this, almost all cancer treatments cause a significant amount of damage to surrounding normal tissues.[41]

If a tumor is diagnosed before metastasis has occurred, it may often be cured by local treatment with surgery and / or radiotherapy. If metastasis has occurred, systemic treatment with cytotoxic drugs is often the only treatment that is effective at treating all the sites of metastatic disease. Alternative approaches are also administered quite frequently. Such approaches include: Hormone therapy, which can induce the remission of cancers in hormone sensitive tissues such as breast and prostate; photodynamic therapy, which is usually administered in combination with

chemotherapy; and immunotherapy, which uses antibodies to stimulate specific components of the immune system.[41]

We will examine each method of treatment briefly, however this work is focused on the chemotherapy method.

1.2.I. Surgery

Surgery is the oldest method of treating cancer, and it remains to be one of the most widely used methods of diagnosis and treatment. A tumor may be considered inoperable due to the following conditions: It may be too large, and hence technically impossible to remove; it may be of a type that is rarely cured by surgery; it may be situated anatomically so that it cannot be approached surgically; there may be evidence of distant metastasis.[40]

Surgery is used in three different respects. These are biopsy, staging and treatment. A biopsy is the removal of a piece or all of the tumor for examination and diagnosis. Staging is the examination of other tissues following a biopsy. Staging is performed so that the physician can determine the full extent and spread of the cancer. Treatment is surgery that is performed to cure the disease or to ease it, also known as palliative surgery. Palliative surgery is used to prevent or eliminate some effects of the cancer.[2]

1.2.II. Radiation Therapy

Radiation therapy is widely used to treat cancer patients. Approximately half of the cancer patients who have been treated have had radiation therapy at some stage of their treatment. Similar to surgery, radiation does have its limitations. It is useful for local treatment, but not effective for

tumors that have presented metastasis.[2]

Radiation therapy is based on the principal that when energy of radiation passes through a cell it causes disorganization of the atomic particles within the cell that are involved in cellular division.[2] Ionising radiations generate reactive oxygen species which then produce DNA strand damage that can result in cell death. Because the oxygen is taken from the air, it follows that the composition of the air influences the radiation response. The responses have been found to be near a maximum at the oxygen concentration in normal air and trials of higher oxygen levels have not shown to be beneficial.[23]

There are two types of responses to radiation that cells exhibit depending on their vulnerability. A radiosensitive cell requires only a small dose of radiation to kill it, whereas a radioresistant cell requires a tremendous amount of radiation to kill it. In radioresistant cells radiation may not kill the cell at all. The survival of normal cells is directly proportional to the amount of radiation administered.[11]

There are two reasons why radioresistance occurs. Firstly, conventional types of radiation work on cancer cells that are in areas of high oxygen concentration, as mentioned above. These cells are called highly oxygenated cells while those in low oxygen areas are called hypoxic cells. The central part of the tumor is composed of many hypoxic cells, so conventional X-rays and gamma rays are ineffective in destroying this central area. Secondly, human cells are known to repair themselves when exposed to radiation. Cancer cells have less capacity to recover from radiation damage than normal cells, however some cells develop ways of recovering before the next dose, thus escaping any permanent damage.[2]

Radiation therapy may be used as a curative measure in a small number of tumors, however

it is administered in high doses to many patients with the knowledge that the chance for a cure is very slim. Radiotherapy is generally used in combination with surgery and / or chemotherapy for curative measures, however it is also administered to many patients for the purpose of providing temporary relief of symptoms caused by advanced stages of cancer.[40]

Radiation exhibits many side effects depending on the dosage administered. General reactions include fatigue, loss of energy, loss of appetite, nausea and low blood counts. A temporary break in the treatment can alleviate many of these side effects. More serious side effects are changes in the skin which range from mild redness to irritation or burns, temporary hair loss and inflamation of the heart lining. These side effects can be temporary but some can be permanent depending on the dosage requirements.[2]

1.2. III Chemotherapy

Chemotherapy is another form of cancer treatment that is widely used in modern medicine. Chemotherapy is defined as the treatment of cancer by various chemicals. Metastasis reduces the potential for cure by surgery or radiation therefore the need for chemotherapeutic agents that can travel *via* the blood stream and lymph channels are essential.[2]

The chemicals involved are directed at disrupting the cell cycle of cancerous cells. This involves altering the DNA, RNA and / or protein molecules, hence depriving the cells of substances they require for growth and cell division. Synthetic chemotherapeutic agents are classified according to whether they interact with DNA directly, such as cisplatin, or antagonise metabolites needed for DNA synthesis, such as methotrexate.[23]

Chemotherapeutic agents are more effective against small tumors than large tumors and must

act at specific phases of the cell cycle. Such agents are only active against cells that are in the process of cell division, hence the most susceptible tumors are those with a large growth fraction. Chemotherapeutic agents also affect normal and neoplastic cells. Normal cells that divide rapidly such as hair follicle epithelia and bone marrow are usually the most sensitive. Blood component therapy with transfusions of red cells, white cells and platelets are necessary to control the problems of anemia, bleeding and infection, which are common side effects of the treatment.[40]

Combination chemotherapy is commonly used for it has been determined that the response rate and duration is improved if multiple agents are administered. Combination multidrug treatments must present the following criteria: Each drug should be active against the tumor when used alone; the drugs should have different mechanisms of action; the toxic effects of the drugs should not overlap.[40]

Before a drug can be administered for public usage, the National Cancer Institute has assigned three phases of development through which it must pass. Phase one consists of the clinical pharmacology. Within this phase, an established maximum tolerated dose and toxicity parameters are determined. This phase is the pharmacologic evaluation of the drug. Phase II consists of screening for clinical activity. This phase involves the treatment of 20-30 patients in each of a range of tumor types to evaluate tumor response rate, mechanism of action and cell cycle sensitivity. Phase III involves controlled clinical trials and combinational studies. [40]

1.2.IV Hormone Therapy

Hormone therapy is an alternative approach to the conventional cancer treatments illustrated above. Hormone therapy is used to treat cancers that are derived from hormone-sensitive tissues.

It is not curative, but useful in palliation for many with breast cancer and prostate cancer.[23]

Hormones influence cellular function and growth regulation by penetrating the cell and binding to specific hormone receptors. The hormone receptor complex is then transported to the nucleus. Within the nucleus, it attaches to the responsive cell genome, thus stimulating the genome and increasing protein synthesis. This increase in protein results in changes in cell growth, division and function. These steps in the transport and incorporation of the hormone can be blocked or altered at a number of points throughout.[40]

Hormone therapy is unique because it does not cause cell death by destruction of nuclear material, as is the case in chemotherapy or radiotherapy. Instead, hormones affect cell growth, cell division and function through physiologic mechanisms.

1.2.V. Photodynamic Therapy

Photodynamic therapy is another alternative approach to cancer treatment for skin, head and neck cancers. Photodynamic therapy is based on the principal that visible light can have devastating effects on cancer cells in the presence of light-sensitive chemicals known as porphyrins. Porphyrins are inactive until they are activated by light of 630nm. In the presence of molecular oxygen, toxic free radicals are then generated. Porphyrins can be given to a patient where they will selectively accumulate in some tumors due to the enhanced blood supply of the tumor. Laser light can then be targeted to the cancer, thereby initiating the free radical production and consequently causing cell destruction. It is hypothesized that the cytotoxic effects occur *via* membrane destruction and not DNA damage because photodynamic therapy does not generate mutations.[23]

1.2.VI. Immunotherapy

Immunotherapy is another alternative approach to cancer treatment. Immunotherapy involves manipulation of the immune response that then leads to tumor regression. Approaches to immunotherapy are described as "active" if they stimulate the immune system in the tumor bearing host and as "passive" if immune effectors, cells or antibodies, are injected into that host.[41] Most of the work involving immunotherapy has been performed with animals bearing transplantable tumors. Such tumors provide a poor model for immunotherapy of human cancer, but there is a significant possibility for future treatments to be curative. [41] Since immunotherapy is not a commonly prescribed treatment, it will not be discussed in detail.

1.3. Metallic Complexes in Chemotherapy

1.3.I. Metals in Medicine

Metals in medicine can be traced back to approximately 5000 years ago, however only in the past 30 years have metal based drugs, such as cisplatin, been used to treat various types of cancers.[13] Medicinal applications of metals has been facilitated by our knowledge of the coordination and redox properties of metal ions. It is well known that metal centers are attracted to biomolecules, which are the constituents of proteins and nucleic acids. The positively charged metal center is electrostatically attracted to the negatively charged biomolecule, hence providing a vast spectrum of drug interaction possibilities.[49]

One major disadvantage of metal complexes as drugs is accumulation of metal in the body. Detrimental side effects can be the result of metal accumulation, so biodistribution, clearance of the

complexes and pharmacological specificity need to be assessed when considering administration of such drugs. Favorable physiological responses of the drugs need to be demonstrated through ***in vitro*** testing with targeted biomolecules and tissues, as well as with xenografts and animal models before a drug can enter clinical trials.[49]

1.3.II. Platinum-Based Anticancer Agents

The use of inorganic complexes for the treatment of humans is a relatively new area of research due to prejudices held in the past. History held that most medicinal research was represented by organic synthesis departments only. It was a common belief that the use of metals in medicine to conquer diseases such as cancer would be ineffective because the heavy metals would be much too toxic to be of any therapeutic use. Discovery and success of metal based complexes has changed this notion forever.[22]

1.3.II.a. Cisplatin

cis-Diamminedichloroplatinum(II), also known as cisplatin, **(Figure 1.1)** was originally synthesized in 1844 by Michele Peyrone, however it was not until much later that its potential as a chemotherapeutic agent was discovered.[25]

In 1969 Barnett Rosenberg made the serendipitous discovery that the compound had tumor-inhibiting properties. These properties were observed while examining the inhibitory effect of an electric field on bacterial growth. The bacterium of interest was a gram negative bacillus known as *Escherichia coli*. Rosenberg had included a set of platinum electrodes, submerged in a medium solution containing ammonium chloride, in the growth chamber of the bacteria culture. Platinum

electrodes were used because platinum had been known to be unusually inert in a biological environment.[25]

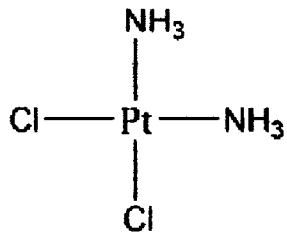


Figure 1.1 - Cisplatin

Upon later inspection of the bacterial cultures, it was found that the normal 2-5μm long bacteria had developed into long filaments up to 300 times their original length. T. Krigas made the discovery that ammonium hexachloroplatinate(IV), $Pt(NH_4)_2Cl_6$, had formed from electrolysis of the platinum electrodes present in the media. Further studies determined that the addition of this compound to similar bacterial cultures did not yield the same filamentation, however it did lead to bacteriocidal activity. It was later determined that light was needed in order to photochemically convert the $(NH_4)_2PtCl_6$ to $Pt(NH_3)_2Cl_4$. Once again, further studies determined that the addition of this compound added to similar bacterial cultures did not yield the same filamentation.[25] Based on these results, it was determined that perhaps the compound existed in two forms, the *cis* and the *trans* isomers. The *trans* isomer was the compound orginally synthesized and added to the cultures, so this left the *cis* isomer to be tested. As speculated, addition of the *cis* isomer to similar bacterial cultures did yield the filamentation that was desired. Based on these results, it was concluded that *cis* isomers were required for cytotoxic activity due to the steric constraints of DNA interactions.[25]

In addition to the Pt(IV) compound that was extracted from the media, researchers also found

a Pt(II) active agent to be present. This Pt(II) compound was cisplatin. The observation that cisplatin was able to inhibit bacterial cellular division prompted Rosenberg and his associates to test the compound in larger forms of life, such as various tumor cell lines and mouse tumors.[16] Subsequent testing yielded results that the compound was an efficient anti-tumor drug.[25]

Despite initial reservations in regards to drugs containing heavy metals, cisplatin entered clinical trials in 1971, in which excessive toxicity was at first discouraging. Despite toxicity levels, testing continued yielding promising results, such as a phase I trial of male germ-cell tumors with strong results from cisplatin. By 1978 cisplatin was approved for use in treatment of cancers and was being administered to patients with solid state tumors. Cisplatin was shown to induce durable remissions and provided cures in many patients with metastatic diseases.[16]

Despite 25 years of further testing, cisplatin's efficacy against germ-cell tumors has not been surpassed. Today it remains to be one of most frequently used treatments for solid tumors such as testicular, ovarian, bladder, breast, esophageal, cervical, lung, head and neck.[22] By 1983 cisplatin was the US's biggest selling antitumor drug, and today it represents annual sales topping $500 million US.[27]

Cisplatin is used in conjunction with other treatments to increase its efficacy. Studies have shown that doxorubicin, etoposide, cytosinarabinoside, methotrexate and 5-fluorouracil are at least additive if not synergistic to treatments with cisplatin.[22]

Despite the efficacy of cisplatin, it presents a number of drawbacks. The main disadvantage of cisplatin is its dose-limiting side effects. Patients exhibit severe signs of nephrotoxicity (kidney damage), neurotoxicity (nervous system damage) and otoxicity (hearing damage).[46] Other side effects that are not as severe include renal toxicity, elevated blood pressure, diarrhea, severe nausea

and vomiting, sensory neuropathy, emetogenesis (intestinal damage), hypomagnesemia (magnesium depletion) and myelosuppression (decreased blood cells).[14] Some of these side effects can be ameliorated by aggressive diuresis, intravenous hydration and the use of anti-emetics.[25] Administration of lower concentrations, to reduce side effects, limit the drug's effectiveness as a chemotherapeutic agent. The average dosage of cisplatin administered to a patient is 100mg/m^2/day for up to five consecutive days, every four weeks.[16]

It is hypothesized that such toxicity effects are due to the cleavage of chloro ligands from the complex followed by subsequent binding of the platinum center to intracellular DNA of non-cancerous cells or to proteins and other extracellular components.[25]

Another major disadvantage of cisplatin involves the cancer cells developing a natural resistance to the drug. Cancers that are initially responsive to cisplatin treatments aquire resistance to the drug over time in addition to the cancers that present resistance from the beginning. A cancer can acquire a resistance for four reasons: reduced cellular uptake; increased repair of metal-DNA adducts; increased tolerance of metal-DNA lesions; and deactivation of cisplatin by thiol containing proteins. Such resistances are normally the reason for treatment failure.[46]

1.3.II.b. Carboplatin

Research into new and similar chemotherapeutic drugs has been a vast area of research since the discovery of cisplatin's antitumor capabilities. More than 30 of over 3000 derivatives have reached clinical trials, however the majority of these drugs have been discarded due to their lack of advantages over cisplatin.[14]

cis-Diammine,1,1-cyclobutanedicarboxylato-platinum(II), also known as carboplatin, (**Figure**

1.2) was developed by Johnson Matthey Technology.[46] Carboplatin was synthesized on the speculation that using less labile leaving groups would reduce toxicity levels. Studies presented results that proved that substituting the chloride leaving groups with more stable ligands resulted in similar antitumor activity.[14]

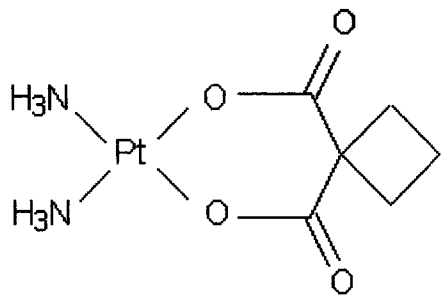


Figure 1.2 - Carboplatin

Carboplatin was found to be similar to cisplatin in that bone marrow toxicity was the dose-limiting factor in clinical trials. However, the drug was advantageous by way of exhibiting little or no nephrotoxicity or neurotoxicity, and showed milder gastrointestinal side-effects. The reduced toxicity was attributed to the slow nucleophilic substitution of the chelated dicarboxylato leaving group in addition to the low reactivity of the platinum(II) complex from its slow ring opening of the compound.[46]

The reduced toxicity of carboplatin allowed for outpatient administration without the need for diuresis. Carboplatin also has the advantage of higher dosage administration. A patient can receive a dose of 900mg/m^2/day *versus* the cisplatin dosage of 60-120mg/m^2/day. Currently carboplatin is the only drug, other than cisplatin, that has obtained worldwide clinical usage.[46]

1.3.II.c.AMD-473

cis-Dichloroammine-2-methylpyridineplatinum(II), also known as AMD-473, (**Figure 1.3**) was developed by Johnson Matthey and the Cancer Research Campaign. AMD-473 was synthesized as a sterically hindered platinum complex to be used for treatment for cisplatin resistant cancers.[46] The purpose of the sterically hindered non-leaving group is to slow associative substitution reactions on the platinum(II) center, hence allowing biological nucleophiles to bind prior to hydrolysis of the chloro ligands.[46]

AMD-473 displays an *in vitro* cytotoxicity intermediate between cisplatin and carboplatin. Although clinical trials have not been completed, it has been shown to overcome cisplatin resistance from deactivation by thiol-containing proteins, as well as reduced cellular uptake and enhanced DNA repair.[46]

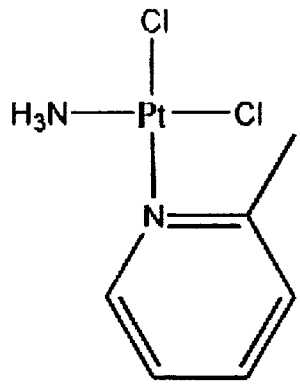


Figure 1.3 - AMD-473

1.3.II.d.Oxaliplatin

Oxaliplatin, also known as Eloxatin, was also designed to overcome the problem of cisplatin resistance. It was approved by the FDA in August 2002. It is administered as a combination treatment with 5-fluorouracil plus leucovorin. It is used to treat patients by shrinking tumors and

delaying tumor growth of colorectal cancer.[46] Colorectal cancer is a form of cancer that is untreatable by cisplatin.[16]

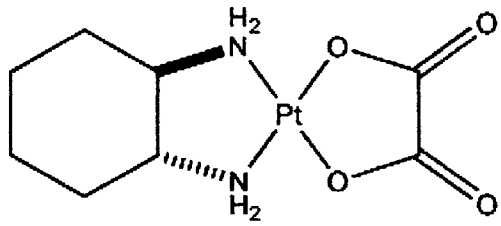


Figure 1.4 - Oxaliplatin

Many of the compounds synthesized have shown mild improvements when compared with cisplatin treatments. Only mild amelioration in toxicity levels and types of cancers that are able to be treated in this fashion have been discovered. It is for this reason that researchers continue to search for a drug that will improve the quality of treatment for this devastating disease.

1.3.III. Metal Induced Apoptosis

Cisplatin provides an excellent model to illustrate how metal ions induce apoptosis in living cellular tissues. Cisplatin forms covalent adducts with a number of different cellular macromolecules, however, it's cytotoxic properties have been linked to its ability to form bifunctional-DNA adducts.[24] The negatively charged metal center coordinates predominantly to the N7 nitrogen position of purine nucleotides, guanine and adenine. (**Figure 1.5**) The resulting coordination complex forms intrastrand or interstrand platinum-DNA cross-links. (**Figure 1.6**)

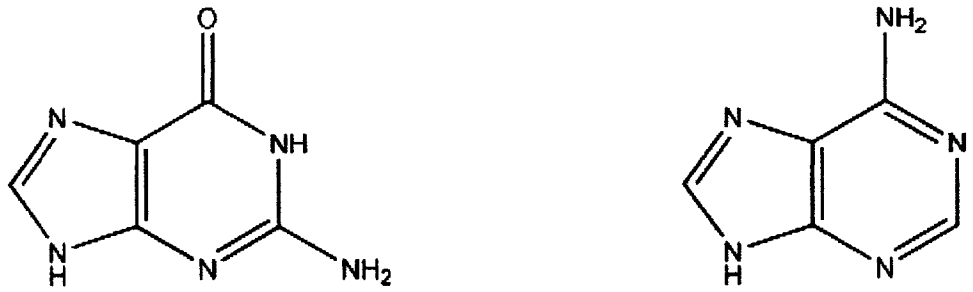


Figure 1.5 - Guanine and Adenine

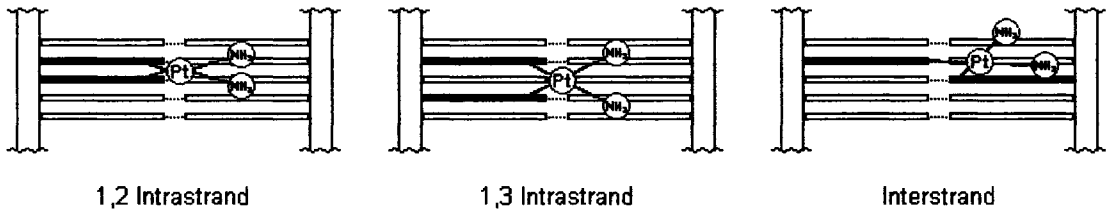


Figure 1.6 - Platinum-DNA cross-linkages (Figure taken from reference 29)

There are three possibilities of platinum-DNA covalent cross-links, 1,2 intrastrand, 1,3 intrastrand and interstrand. Studies based on the binding properties of cisplatin with DNA have shown that the drug presents a preference for the N7 nitrogens on two adjacent guanines on the same strand, resulting in 1,2 intrastrand crossing. It has also been shown that cisplatin can bind to the N7 and N1 nitrogen of adenine as well as the N3 nitrogen of cytosine. These covalent cross-links cause significant distortion of the helical structure of DNA, resulting in inhibition of DNA replication and transcription, subsequently inhibiting tumor growth and possibly eventual tumor starvation.[25]

Figure 1.7 shows a representative illustration of cisplatin bound to the specific nitrogens in DNA as mentioned above.

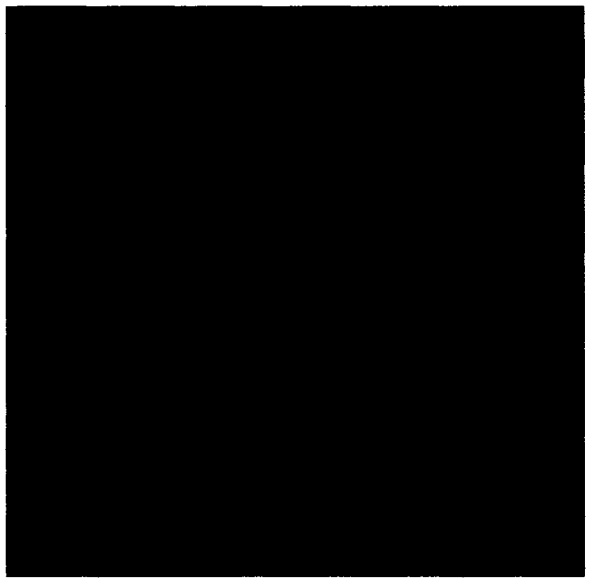


Figure 1.7 - Model of cisplatin bound to DNA (Figure taken from reference 29)

For such coordinations to occur, cisplatin must be converted to its positive, active form. However, due to the high concentration of chloride ions in the bloodstream (~100mM) cisplatin remains intact, neutral and inactive. The high concentration of water *versus* the lower concentration of chloride ions inside the cell allows the hydrolysis. It is for this reason that cisplatin is administered intravenously in a sterile saline solution. Once administered, cisplatin enters the cell by passive diffusion or active uptake by cellular transmembrane proteins.[25] Once inside the cell, the neutral form of cisplatin undergoes hydrolysis, replacing the chloride ions by water molecules. (**Figure 1.8**) The hydrolysis results in a positively charged, active compound that is able to undergo nucleophilic substitution with specific bases found in DNA strands.[24]

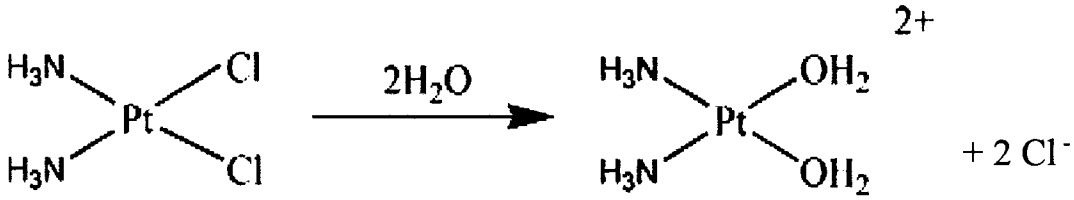


Figure 1.8 - Hydrolysis of Cisplatin

It is the nucleophilic substitution of cisplatin that is responsible for its cytotoxic effects. The substitution is initiated by the positive charge of the platinum(II) metal that is attracted to the negatively charged phosphodeoxyribose backbone of the DNA strand.[25] Cisplatin coordinates to DNA *via* specific nitrogen atoms due to the following properties. These nitrogen atoms do not form hydrogen bonds with their complementary base pairs within the double helix of the DNA complex. Since they are free from H-bonds, they are free to undergo nucleophilic substitution with the electrophilic, active form of cisplatin.[25]

1.4 Palladium Compounds in Cancer Treatment

The following section discusses a known treatment that uses palladium isotopes in radiation therapy, as well as a discussion regarding the advantages of palladium based chemotherapeutic agents *versus* well known agents such as cisplatin that use platinum metal complexation. Research using palladium metal complexes as possible chemotherapeutic agents is relatively new and there is little background information concerning the potential usage of the such complexes.

1.4.1. Current Palladium Cancer Treatment Method: Brachytherapy

A localized form of radiation therapy, known as brachytherapy, uses radioactive isotopes of

palladium to treat prostate and breast cancer. This treatment was first explored in 1987 primarily for the treatment of prostate cancer, which is the second leading cause of male cancer death in America today. The first effective treatment of breast cancer using this methodology was administered in September of 2005.[39]

Using ultrasound as a guide, physicians inject seeds of the palladium-103 isotope, which are small rice sized radioative portions, into the site of the tumor using hollow needles. Once injected, the seeds release varying amounts of radiation to the infected cells (high radiation within the prostate, low radiation for breast cancer). The radioactive release from the seeds occurs over a period of two months before the palladium-103 becomes inactive. Since the seeds become inactive over time they do not have to be removed from the system.[3, 39]

It should be noted that brachytherapy is also performed using radioactive iodine isotopes in addition to the palladium mentioned above, however it has been shown that the palladium-103 isotope presents the most effective treatment results. [3] Researchers estimate that approximately 20% of breast cancers may be treated using this method. In addition, 90% of long-term follow up care of prostate cancer patients present a disease-free survival rate.[39]

1.4.II Advantages of Palladium Based Chemotherapeutic Agents

Coordination processes and complex formation processes of palladium(II) are very similar to those of platinum(II), hence the scientific community has recently shown a great interest in palladium(II) ions used to mimic the binding properties of platinum(II) complexes.[33] Palladium(II) analogues of platinum(II) complexes are of great interest as potential chemotherapeutic agents in addition to treatments currently used, for they are isostructural.

Palladium(II) derivatives have similar coordination geometry and hence form square-planar compounds similar to platinum(II). Palladium(II) compounds do have the potential advantage that they are known to attain equilibrium much more quickly than platinum(II) systems.[1] Palladium(II) systems have been shown to exhibit ~ 10^6 times faster kinetics than their platium(II) analogues.[17] Based on this, one could predict that the palladium(II) complexes would react faster in a biological system as well, thus allowing for possible reduction in exposure time required to effectively terminate a tumor.

1.5 Kinetic Studies

Chemical reaction mechanisms are determined using chemical kinetics. The rate law can be derived on the basis of changes in rate as a function of reactant concentration. The rate law then provides insight into the stoichiometry of the reactants in the rate determining step and the characteristic rate of the reaction.[20]

Activation entropy ($\Delta S^{\neq}$) and enthalpy ($\Delta H^{\neq}$) parameters can be estimated by altering the temperature of reaction. The Eyring equation, derived using Transition State Theory, (**equation 1.1**) provides numeric values for these parameters. A plot of ln (k / T) versus 1 / T provides values for the activation enthalpy, $\Delta H^{\neq}$, and the activation entropy, $\Delta S^{\neq}$, from the slope and intercept of the Eyring plot, respectively. [20]

Equation 1.1 $$\ln (k / T) = [\ln (\kappa / h) + \Delta S^{\neq} / R] - \Delta H^{\neq} / (RT)$$

k = rate constant, T = absolute temperature

κ = Boltzmann's constant, h = Plank's constant,

R = ideal gas constant

The activation enthalpy parameter lends insight into the bond making and bond breaking processes that a reaction exhibits. The activation entropy parameter provides an explanation of degrees of disorder on going from the initial state to the transition state of the reaction of interest.[20]

1.5.I. Guanine

The kinetics of reaction of metallic complexes with guanine, a representative nitrogenous base contained in DNA, can be measured. Studies have shown that positively charged metal centers undergo substitution with nitrogenous bases found in DNA, most commonly guanine and adenine. Cisplatin is the most extensively studied compound of this nature.[24]

Since palladium(II) and platinum(II) compounds are congeners, both having low spin d^8 systems and exhibiting square planar geometry, it is expected that they will react *via* similar mechanisms. They are expected to undergo nucleophilic substitution reactions with guanine *via* an associative mechanism. A dissociative pathway has also been known to exist with such compounds when bulky ligands are present to slow the substitution rates due to sterics of interaction.[18]

1.5.II. DNA

Metal based chemotherapeutic drugs are known to interact with DNA *via* nonconvalent bonds with nucleic acids. These interactions can be by classical intercalation, groove binding, and/or external electrostatic interactions.[10] Different binding scenarios result from a decrease in the free energy of the system. This decrease can be due to electrostatic interactions, hydrogen bonding, London dispersion forces and entropy increases for hydrophobic interactions.[19]

The specific type of interaction of drugs with the double helix of DNA depend on factors

such as planarity and aromaticity.[9] Intercalation is an interaction that involves π-stacking of a ligand between adjacent base pairs of DNA. For such an interaction to occur, the ligand must be flat, such as in the case for aromatic systems annulated with heterocyclic rings.[19] DNA binding ability and base sequence specificity can be modified by changing the structure of the ligand present in the drug, hence altering drug effectiveness.[13]

Calf-Thymus DNA contains a specific number of substitution sites and gives insight as to the different forms of interaction and stoichiometry of drugs in relation to the DNA strands.

1.6 Octanol-Water Partition Coefficients

Octanol-water partition coefficients (K_{OW}) are simple thermodynamic measurements that are extremely useful in estimating bioaccumulation. Using a liquid model membrane with aqueous and lipid barriers (ie, octanol-water) gives insight to the physico-chemical properties of drugs on their permeability coefficients for biological membranes and on the maximum flux of drugs into the body.[34]

The K_{OW} values describe the hydrophobicity or hydrophilicity of a compound. The value of K_{OW} is derived from the Nernst distribution law (**equation 1.2**) and is defined as the ratio of the concentration of the complex in octanol *versus* the concentration of the complex in water. The resulting value correlates to the potential uptake of the complex through the lipid bilayer of cells resulting in overall bioaccumulation of the complex in the body.[34]

Equation 1.2 $$K_{OW} = C^{O} / C^{W}$$

1.7 In Vitro Screening

Viability becomes fundamental once a cell is extracted from its normal *in vivo* environment. Experimental manipulations are carried out *in vitro* for the purpose of determining potential cytotoxicity of compounds being studied for use as chemotherapeutic agents. Once the cytotoxicity has been established *in vitro*, *in vivo* testing is administered. *In vitro* testing is performed first for economic and humane reasons.[12]

Converting *in vitro* results to an *in vivo* system is a difficult task. There are many aspects not present in *in vitro* experiments that need to be acknowledged in *in vivo* systems. *In vitro* experiments simplify a biological system to a great extent. It does not take into account the differences in exposure time once it reaches the are in question, the chances in concentration of drugs while passing through the system, the rate of change of drug metabolism, tissue penetration, clearance and excretion. There is also the fact that many non-toxic substances become toxic after being metabolized by the liver. There is also a possibility that toxic *in vitro* compounds may become detoxified by the liver enzymes. Despite these drawbacks, *in vitro* screening is the best method of testing new potential chemotherapeutic agents today.[12]

Toxicity is a very complex event *in vivo*. In a living system, there may be direct cellular damage, physiological effects such as transport in the kidney or neurotoxicity in the brain, inflammatory effects and other systemic effects. It is very difficult to monitor such systemic and physiological effects *in vitro*, so most assays determine effects only at a cellular level. These cellular level effects are more commonly known as cytotoxicity. Cytotoxicity can vary depending on the nature of the study and whether cells are killed or only have their metabolism altered.[12]

1.7.I. In Vitro Screening Assays

Assays oversimplify the events that they measure and are employed because they are cheap, easily quantified and reproducible. Choosing an assay depends on the agent under study, the nature of the response, and the particular target cell. Assays are divided into five major classes: 1- Viability which measures immediate or short-term responses. 2- Survival which measures the long-term retention of self-renewal capacity. 3- Metabolic, usually microtitration based, which measures metabolic responses at the time of exposure or measures the same parameter two or three population doublings after exposure. 4- Transformation which measures survival in an altered state. Transformation assays used *in vitro* include the following; ancorage independence; reduced density limitation of cell proliferation; evidence of mutagenesis. Mutagenesis is measured by sister chromatid exchange. 5- Irritancy which measures a response that is analogous to inflammation, allergy or irritation *in vivo*. Irritancy assays are not commonly used assays for it is difficult to model such responses *in vitro* so the assay monitors cytokine release in organotypic cultures as a predictive measure. This area is in early stages of development but shows promise for future testing.[12]

1.7.I.a. Viability Assays

Viability assays are used to measure the proportion of viable cells after a traumatic procedure such as cell freezing and thawing. Viability assays rely on a breakdown in the membrane integrity that is determined by the uptake of a dye that is normally taken up and retained by viable cells. The drawbacks of viability assays are that the effects are immediate and do not always predict survival, and that dye exclusion is known to overestimate viability.[12]

1.7.I.b Survival Assays

Survival assays are long-term tests that demonstrate survival rather than short-term toxicity which may be reversible. Short-term tests are convenient and easy to perform, however they reveal only cells that are dead at the time of the assay. Cells that have been subjected to toxic influences often show effects several hours or days later. Survival is defined as the retention of regenerative capacity. Survival is measured by plating efficiency, thus demonstrating proliferative capacity for several cell generations present. The clonogenic assay presents cells with high enough efficiency that the colonies can be considered representative of the cell population as a whole. An efficiency greater than 10% is acceptable because such assays present such variation. Variations are due to the following components of the assay: concentration of the agent; invariate agent concentrations, duration of exposure to the agent; time of exposure to the agent; cell density during exposure; cell density during cloning; colony size; and solvents used.[12]

1.7.I.c Metabolic Assays

Metabolic assays are used to reduce time and labor involved in survival assays. Metabolic assays were designed to combat the problem of poor plating efficiencies found in survival assays. They do not measure survival directly, instead they measure the net increase in the number of cells, the increase in the total amount of protein or DNA, or the residual ability to synthesize proteins or DNA. Based on these measurements, survival is determined as the retention of metabolic or proliferative ability by the cell after the toxic agent has been removed. The drawback of this assay is that it cannot determine the difference between a reduction in metabolic or proliferative activity and a reduced number of cells, hence survival assays such as the clonogenic assay are to be

administered as confirmation for any significant observations.[12]

The microtitration assay is one of the most common form of metabolic assays. This involves the use of multiwell plates to approach replicate sampling in tissue culture. The plates are easy to use, economic, useful for automated handling and of good optical quality. The 96-well plate is the most popular, each well has the capacity to hold up to 10^5 cells. Each individual well has 28-32mm^2 of growth area and 0.1-0.2 ml medium capacity. The microtitration method allows for replicate sampling of viability measurements of a large number of samples simultaneously.[12]

Two commonly used microtitration assays are the MTT based and the SRB based (sulforhodamine B).[21] MTT is a yellow water-soluble tetrazolium dye that is reduced by live cells to a purple, insoluble, formazan product,[12] whereas SRB is a purple protein binding dye.[38] Both the MTT and SRB methods provide similar dose/response curves, however the SRB method appears to be more sensitive. The MTT method provides higher IC-50 values whereas the SRB method provides better linearity with cell number and a higher reproducibility ratio.[21]

The MTT assay is a tetrazolium assay that involves a timed step where cells are incubated with tetrazolium. Once incubated, the number of viable cells are estimated based on the amount of formazan produced. This method relies on the ability of viable cells to reduce tetrazoliums to formazans.[35] The MTT assay is commonly performed, however to save time and to increase efficiency, the National Cancer Institute has adopted the SRB assay as a main source of chemotherapeutic testing.[38] The SRB assay provides a chemical fixation of the cells in the culture at the end of the assay. This allows for simultaneous staining and processing without time critical steps. The SRB assay measures whole culture protein content, thus eliminating the constraint of the cells ability of reducing one chemical to another. The SRB growth viability is based on the

assumption that dead cells either lyse and are removed from the culture, or otherwise simply do not contribute to the end point result.[35]

Other advantages of the SRB assay include its colorimetric end point that is nondestructive, indefinitely stable and visible to the naked eye.[38] The SRB assay also provides a low variation between cell-lines. The underlying advantage of the SRB assay is that it does not depend on enzymatical activity but instead on protein content.[21]

1.8 Project Objectives

The goal of this project is to synthesize and characterize a series of palladium analogues of cisplatin. There are six compounds in total, all synthesized based on tridentate polypyridyl chelating ligands and one chloro ligand attached to the palladium(II) center. The desired compounds include one formed from the tppz ligand, and five derivatives based on substituted terpyridyl ligands. (See **Table 2.1**) The compounds are to be characterized using FTIR, ^{1}H NMR, ^{13}C NMR, ESMS and CHN elemental analysis. The goal of the different syntheses is to examine the effect of steric bulk and lipophilicity of the ligands on the anti-cancer properties of the compounds.

The compounds are to be reacted with guanosine hydrate, to determine the reaction mechanism nucleophilic subsitution, and calf thymus DNA to determine the reaction of the complexes on a macromolecular level. These kinetic studies will determine the ability of the complexes to coordinate to DNA base pairs. The compounds are to be subjected to octanol-water partition coefficients to estimate their bioaccumulation in cells.

The compounds are to be screened *via in vitro* methods against human ovarian, breast, and colon cancer cells to determine cellular cytotoxicity.

2. Experimental

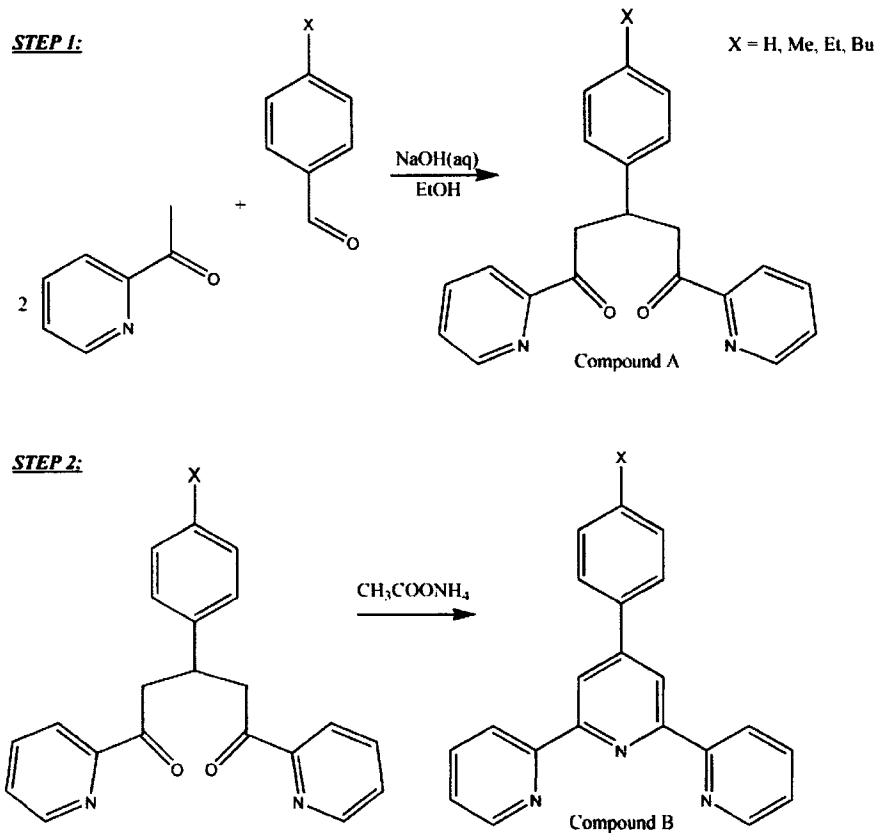
2.1 Materials

A listing of the solvents and chemicals used in the experimental procedures are tabulated in **Appendix A.** All materials were used as received unless otherwise stated.

2.2 Ligand Synthesis

2,2',6',2"-Terpyridyl and 2,3,5,6-tetrakis(2-pyridyl)pyrazine were purchased directly from Aldrich. Synthesis of a series of substituted terpyridines and their palladium(II) complexes was based on modification of literature methods.[6,7,8,15]

The reaction scheme that was followed to synthesize the terpyridyl ligands in this study is given as **Scheme 2.1.**



Scheme 2.1 - Ligand Synthesis Reaction Scheme

The compounds will be abbreviated as in **Table 2.1** for the remainder of the text, including the results and discussion section.

Table 2.1 - Compound Abbreviations

Compound	*Abbreviation*
1,5-*bis*(2-pyridyl)-3-(phenyl)pentane-1,5-dione	A_H
1,5-*bis*(2-pyridyl)-3-(p-methylphenyl)pentane-1,5-dione	A_{Me}
1,5-*bis*(2-pyridyl)-3-(p-ethylphenyl)pentane-1,5-dione	A_{Et}
1,5-*bis*(2-pyridyl)-3-(p-butylphenyl)pentane-1,5-dione	A_{Bu}
2,3,5,6-tetrakis(2-pyridyl)pyrazine	Tppz

2,2',6,2"-terpyridyl	Terpy
4'-phenyl-2,2',6,2"-terpyridyl	B_H
4'-(p-methylphenyl)-2,2',6,2"-terpyridyl	B_{Me}
4'-(p-ethylphenyl)-2,2',6,2"-terpyridyl	B_{Et}
4'-(p-butylphenyl)-2,2',6,2"-terpyridyl	B_{Bu}
dichloro(2,3,5,6-tetrakis(2-pyridyl)pyrazine)dipalladium(II) dichloride	$[Pd_2(Tppz)Cl_2]Cl_2$
chloro(2,2',6,2"-terpyridyl)palladium(II) chloride	[Pd(Terpy)Cl]Cl
chloro(4'-phenyl-2,2',6,2"-terpyridyl)palladium(II) chloride	$[Pd(B_H)Cl]Cl$
chloro(4'-p-methylphenyl-2,2',6,2"-terpyridyl)palladium(II) chloride	$[Pd(B_{Me})Cl]Cl$
chloro(4'-p-ethylphenyl-2,2',6,2"-terpyridyl)palladium(II) chloride	$[Pd(B_{Et})Cl]Cl$
chloro(4'-p-butylphenyl-2,2',6,2"-terpyridyl)palladium(II) chloride	$[Pd(B_{Bu})Cl]Cl$

2.2.I. Intermediate Ligand Synthesis

Similar procedures were followed for the synthesis of the intermediate ligands. Each synthesis is provided in detail due to subtle differences in procedure and observations. Analytical details on all complexes are reported in **section 3.1**.

2.2.I.a. 1,5-Bis(2-pyridyl)-3-(phenyl)pentane-1,5-dione (A_H)

Sodium hydroxide, 3.10g (77.5mmol), was dissolved in 36.0 mL of deionized water. Ethanol, 51.0 mL, was added to the solution followed by the dropwise addition of 12.6 mL (112 mmol) 2-acetylpyridine and 4.80 mL (47.2 mmol) benzaldehyde. The resulting yellow solution was stirred at room temperature for one hour allowing a bright orange solution to form with a small amount of precipitate.

Cold deionized water, 45.0 mL, was added to the solution resulting in immediate

precipitation of a viscous pink solid. The substance was isolated by vacuum filtration, washed with cold ethanol, and placed in a vacuum desiccator for 24 hours. The compound was recrystallized from hot ethanol on a steam bath, yielding 11.2g (72.0%) of white powder A_H.

2.2.I.b. 1,5-Bis(2-pyridyl)-3-(p-methylphenyl)pentane-1,5-dione (A_{Me})

Sodium hydroxide, 3.01g (75.4 mmol), was dissolved in 36.0 mL of deionized water. Ethanol, 51.0 mL, was added to the solution followed by the dropwise addition of 12.6 mL (112 mmol) 2-acetylpyridine and 3.60 mL (46.3 mmol) p-tolualdehyde. The resulting orange solution was stirred at room temperature for one hour at which time a deep orange solution formed together with a small amount of precipitate.

Cold deionized water, 45.0 mL, was added to the solution resulting in immediate precipitation of a viscous pink solid. The solution was left at room temperature for 24 hours to allow the precipitate to separate and settle to the bottom of the liquid. The precipitate, which formed a solid cake, was crushed, vacuum filtered, washed with cold ethanol, and dried in a vacuum desiccator for 24 hours. The compound was recrystallized from hot ethanol on a steam bath, yielding 12.9g (80.7%) of white powder A_{Me}.

2.2.I.c. 1,5-Bis(2-pyridyl)-3-(p-ethylphenyl)pentane-1,5-dione (A_{Et})

Sodium hydroxide, 3.09g (77.3 mmol), was dissolved in 36.0 mL of deionized water. Ethanol, 51.0 mL, was added to the solution followed by the dropwise addition of 12.6 mL (112 mmol) 2-acetylpyridine and 4.20 mL (48.2 mmol) 4-ethylbenzaldehyde. The resulting yellow solution was stirred at room temperature for one hour. A red solution formed, and a small amount

of precipitate was produced.

Cold deionized water, 45.0 mL, was added to the solution resulting in immediate precipitation of a viscous pink solid. The solution was left at room temperature for 24 hours to allow the precipitate to separate and settle to the bottom of the liquid. The precipitate, which formed a solid cake, was crushed, vacuum filtered, washed with cold ethanol, and dried in a vacuum desiccator for 24 hours. The compound was recrystallized from hot ethanol on a steam bath, yielding 12.0g (69.6%) of white powder A_{Et}.

2.2.I.d. 1,5-Bis(2-pyridyl)-3-(p-butylphenyl)pentane-1,5-dione (A_{Bu})

Sodium hydroxide, 0.750g (18.8 mmol), was dissolved in 9.00 mL of deionized water. Ethanol, 12.8 mL, was added to the solution followed by the dropwise addition of 3.15 mL (28.1 mmol) 2-acetylpyridine and 2.00 mL (12.0 mmol) 4-n-butylbenzaldehyde. The resulting pink solution was stirred at room temperature for one hour, forming a deep red solution containing a small amount of precipitate.

Cold deionized water, 15.0 mL, was added to the solution resulting in immediate precipitation of a viscous pink solid. The solution was left at room temperature for 24 hours to allow the precipitate to separate and settle to the bottom of the liquid. The substance was crushed and isolated by vacuum filtration, washed with cold ethanol, and placed in a vacuum desiccator for 24 hours. The product was recrystallized by addition of petroleum ether to a hot isopropanol solution of the compound, yielding 2.87g (62.1%) of white powder A_{Bu}.

2.2.II. Ligand Synthesis

Similar procedures were followed for the synthesis of the final ligands. Each synthesis is provided in detail due to subtle differences in procedure and observations.

2.2.II.a. 4'-Phenyl-2,2',6,2"-terpyridyl (B_H)

1,5-Bis(2-pyridyl)-3-(phenyl)pentane-1,5-dione, A_H, 5.01g (16.2 mmol), was combined with ammonium acetate, 62.5g (811 mmol), in 625 mL ethanol. The solution was allowed to reflux for two hours yielding a deep yellow solution which was cooled and filtered by vacuum filtration to extract any unreacted compound. A large excess of deionized water was added producing an orange solution with a yellow precipitate. The solution was held at 5°C for 12 hours. The bright yellow precipitated product was isolated by vacuum filtration, washed with ice cold ethanol and placed in a vacuum desiccator for 24 hours. The compound was recrystallized from hot ethanol on a steam bath, yielding 3.03g (64.6%) of compound B_H.

2.2.II.b. 4'-(p-Methylphenyl)-2,2',6,2"-terpyridyl (B_{Me})

1,5-*Bis*(2-pyridyl)-3-(p-methylphenyl)pentane-1,5-dione, A_{Me}, 4.50g (13.1 mmol), was combined with ammonium acetate, 25.3g (328 mmol), in 200 mL glacial acetic acid. The solution was allowed to reflux for two and a half hours yielding a deep maroon solution. The solution was then neutralized using aqueous sodium carbonate, yielding an orange solution with a small amount of precipitate. Dichloromethane (310 mL) was added to extract the desired product. The green organic layer was evaporated to dryness yielding a green oily substance. Deionized water (250mL) was added and the mixture was allowed to sit at 25°C for 36 hours, producing a solid precipitate in

place of the oily substance. The yellow precipitate was scraped off the flask, isolated by vacuum filtration, washed with deionized water, and dried in a vacuum desiccator for 24 hours. The compound was recrystallized from hot ethanol on a steam bath, yielding 3.20g (75.0%) of compound B_{Me}.

2.2.II.b. 4'-(p-Ethylphenyl)-2,2',6,2"-terpyridyl (B_{Et})

1,5-*Bis*(2-pyridyl)-3-(p-ethylphenyl)pentane-1,5-dione, A_{Et}, 4.00g (11.3 mmol), was combined with ammonium acetate, 25.1g (325 mmol), in 200 mL glacial acetic acid. The solution was refluxed for two and a half hours yielding a deep maroon solution. The solution was then neutralized using aqueous sodium carbonate, yielding an orange solution with some precipitate. Dichloromethane (700mL) was added to extract the desired product. The red organic layer was evaporated to dryness yielding a deep red oily substance. Deionized water (250mL) was added and allowed to sit at 25°C for 48 hours, producing a mixture of precipitate and oil. The water was decanted and methanol was added to dissolve the substance. Deionized water was added to precipitate the desired product. The flask was placed on a freeze dryer for 72 hours. The deep yellow precipitate was scraped off the flask, isolated by vacuum filtration, washed with deionized water, and dried in a vacuum desiccator for 24 hours. The compound was recrystallized from hot ethanol on a steam bath, yielding 2.08g (54.8%) of compound B_{Et}.

2.2.II.b. 4'-(p-Butylphenyl)-2,2',6,2"-terpyridyl (B_{Bu})

1,5-*Bis*(2-pyridyl)-3-(p-butylphenyl)pentane-1,5-dione, A_{Bu}, 0.400g (1.04mmol), was combined with ammonium acetate, 2.55g (33.1mmol), in 200 mL glacial acetic acid. The solution

was refluxed for two and a half hours yielding a deep maroon solution. The solution was then neutralized using aqueous sodium carbonate, yielding an orange solution with some precipitate. Dichloromethane (310mL) was added to extract the desired product. The orange organic layer was evaporated to dryness yielding a green oily substance. Deionized water (250mL) was added and the mixture was allowed to sit at 25°C for 48 hours, producing a mixture of precipitate and oil. The water was decanted and methanol was added to dissolve the substance. Deionized water was added to precipitate the desired product and the flask was placed on the freeze dryer for 5 days. The compound B_{Bu} remained as an oil and was unable to be isolated. The compound was used in oil form. To ensure that the terpyridyl complex was formed, a sample of it was added to ferrous ammonium sulfate, yielding a deep purple solution, which is characteristic of terpyridyl compounds.[36]

2.3. Metal Complexation

Similar procedures were followed for the metal complexation of the compounds. Each synthesis is provided in detail due to subtle differences in procedure and observations.

2.3.I. Chloro(2,2',6,2"-terpyridyl)palladium(II) chloride {[Pd(terpy)Cl]Cl}

Sodium tetrachloropalladate(II), 0.630g (2.14 mmol), 80.0 mL deionized water and 24.0 mL concentrated hydrochloric acid were refluxed for one hour to yield a bronze solution. The solution was subjected to hot vacuum filtration. 2,2',6,2"-Terpyridyl, 0.501g (2.14 mmol), in methanol was added in a dropwise fashion to the warm solution. Upon this addition a light pink precipitate formed immediately. The mixture was left to stir at 25°C for 24 hours. The pink precipitate was isolated

by vacuum filtration, washed sequentially with deionized water, methanol, chloroform, dichloromethane, acetone and ethanol to ensure removal of any impurities, and dried in a vacuum desiccator for 24 hours, yielding 0.866g (98.5%) of compound [Pd(terpy)Cl]Cl. **(Figure 2.1)**

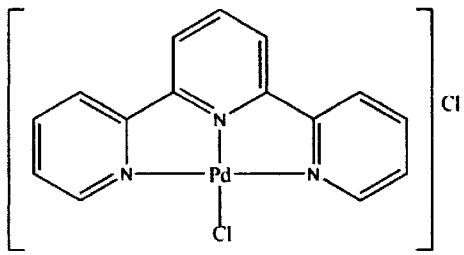


Figure 2.1: [Pd(terpy)Cl]Cl

*2.3.II. Chloro(4'-phenyl-2,2',6,2"-terpyridyl)palladium(II) chloride {**[Pd(B_H)Cl]Cl**}*

Sodium tetrachloropalladate(II), 0.477g (1.62 mmol), 80.0 mL deionized water and 24.0 mL concentrated hydrochloric acid were refluxed for one hour to yield a bronze solution. The solution was subjected to hot vacuum filtration. 4'-Phenyl-2,2',6,2"-terpyridyl, B_H, 0.504g (1.63 mmol), in chloroform was added in a dropwise fashion to the warm solution. The mixture was left to stir vigorously at 25 °C for 24 hours. The pale pink precipitate was isolated by vacuum filtration, washed sequentially with deionized water, methanol, chloroform, dichloromethane, acetone and ethanol to ensure removal of any impurities, and dried in a vacuum desiccator for 24 hours, yielding 0.780g (99.0%) of compound [Pd(B_H)Cl]Cl. **(Figure 2.2)**

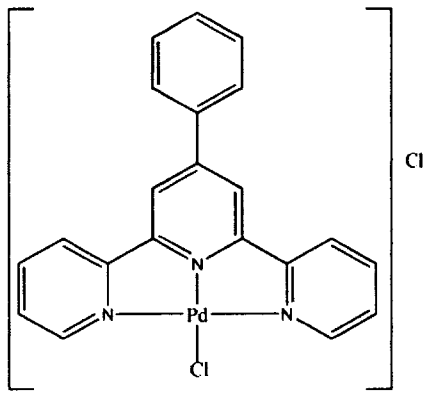


Figure 2.2: $[Pd(B_H)Cl]Cl$

2.3.III. Chloro(4'-p-methylphenyl-2,2',6,2"-terpyridyl)palladium(II) chloride {$[Pd(B_{Me})Cl]Cl$}

Sodium tetrachloropalladate(II), 0.455g (1.55 mmol), 80.0 mL deionized water and 24.0 mL concentrated hydrochloric acid were refluxed for one hour to yield a bronze solution. The solution was subjected to hot vacuum filtration. 4'-(p-Methylphenyl)-2,2',6,2"-terpyridyl, B_{Me}, 0.505g (1.56 mmol), in chloroform was added in a dropwise fashion to the warm solution. The mixture was left to stir vigorously at 25°C for 48 hours. The peach precipitate was isolated by vacuum filtration, washed sequentially with deionized water, methanol, chloroform, dichloromethane, acetone and ethanol to ensure removal of any impurities, and dried in a vacuum desiccator for 24 hours, yielding 0.586g (75.7%) of compound $[Pd(B_{Me})Cl]Cl$. **(Figure 2.3)**

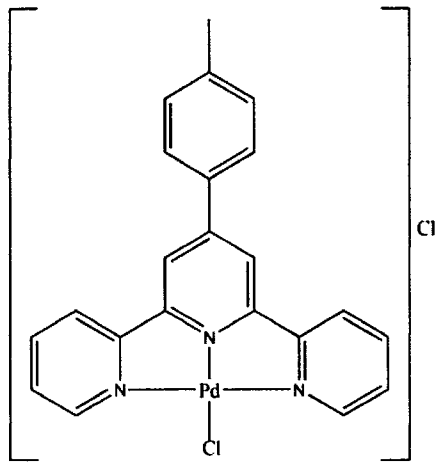


Figure 2.3: [Pd(B_{Me})Cl]Cl

2.3.IV. Chloro(4'-p-ethylphenyl-2,2',6,2"-terpyridyl)palladium(II) chloride {[Pd(B_{Et})Cl]Cl}

Sodium tetrachloropalladate(II), 0.442g (1.50 mmol), 80.0 mL deionized water and 24.0 mL concentrated hydrochloric acid were refluxed for one hour to yield a bronze solution. The solution was subjected to hot vacuum filtration. 4'-(p-Ethylphenyl)-2,2',6,2"-terpyridyl, B_{Et}, 0.506g (1.50 mmol), in chloroform was added in a dropwise fashion to the warm solution. The mixture was left to stir vigorously at 25 °C for 96 hours. The pale beige precipitate was isolated by vacuum filtration, washed sequentially with deionized water, methanol, chloroform, dichloromethane, acetone and ethanol to ensure removal of any impurities, and dried in a vacuum desiccator for 24 hours, yielding 0.401g (51.9%) of compound [Pd(B_{Et})Cl]Cl. **(Figure 2.4)**

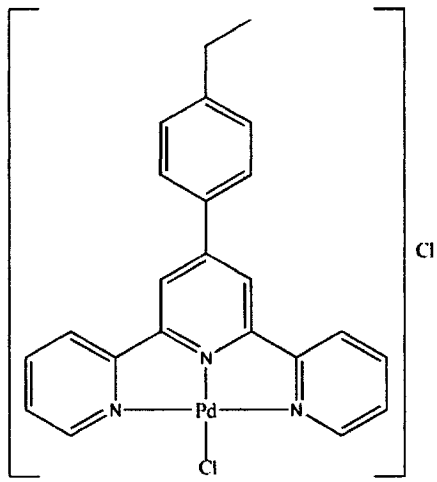


Figure 2.4: $[Pd(B_{Et})Cl]Cl$

*2.3.V. Chloro(4'-p-butylphenyl-2,2',6,2"-terpyridyl)palladium(II) chloride {**[Pd(B$_{Bu}$)Cl]Cl**}*

Sodium tetrachloropalladate(II), 0.301g (1.02 mmol), 120mL methanol and 12.0mL concentrated hydrochloric acid were refluxed for one hour to yield a bronze solution. The solution was subjected to hot vacuum filtration. A dichloromethane solution of 4'-(p-butylphenyl)-2,2',6,2"-Terpyridyl, B_{Bu}, was added dropwise to the warm solution of Na_2PdCl_4 resulting in the immediate formation of a beige precipitate. The mixture was left to stir vigorously at 25°C for 48 hours. The pale beige precipitate was isolated by vacuum filtration, washed sequentially with deionized water, methanol, chloroform, dichloromethane, acetone and ethanol to ensure removal of any impurities, and dried in a vacuum desiccator for 24 hours, yielding 0.300g (54.0%) of compound $[Pd(B_{Bu})Cl]Cl$. **(Figure 2.5)**

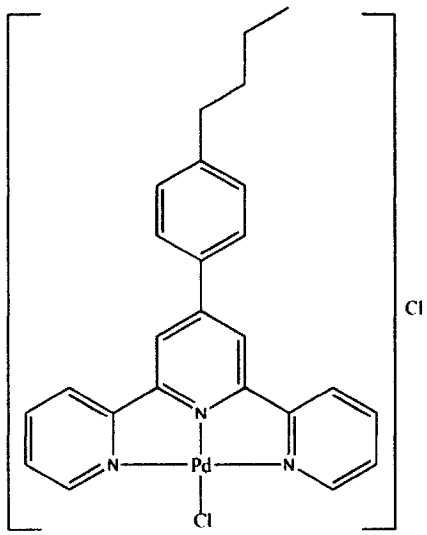


Figure 2.5: $[Pd(B_{Bu})Cl]Cl$

2.3.VI. Dichloro(2,3,5,6-tetrakis(2-pyridyl)pyrazine)dipalladium(II) dichloride

{[Pd_2(Tppz)Cl_2]Cl_2}

A sample from previous work was used in the following experiments. For synthesis details refer to reference 7.

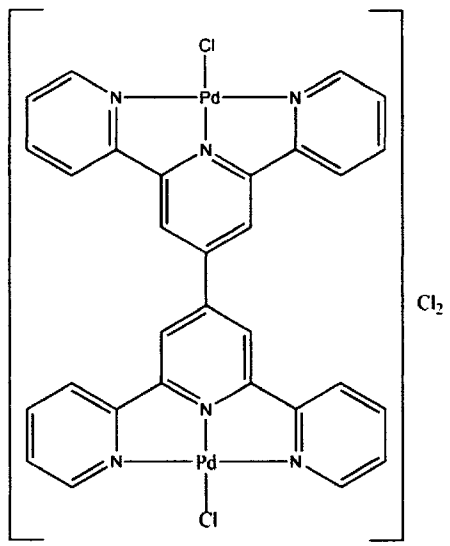


Figure 2.6: $[Pd_2(Tppz)Cl_2]Cl_2$

2.4 Methodology

^{13}C and ^{1}H NMR spectra of the complexes were obtained on a Bruker Avance 300MHz NMR spectrometer. The FTIR spectra were measured using a Perkin Elmer 1600 Series FTIR Infrared Spectrometer. Elemental analysis (C,H,N) were performed by Guelph Chemical Laboratories Inc., Ontario. Mass spectra were measured at Queens University, Ontario, on a Micromass/VG Quatro Triple Quadrupole Instrument using an electrospray ionization source in the positive ion mode.

UV/Vis spectra were measured on a Hewlett Packard 8453 Diode Array spectrometer attached to a Pentium 75MHz computer running the Hewlett-Packard's Kinetics software package. Stopped-flow kinetics for [Pd(Terpy)Cl]Cl and [Pd(B_{H})Cl]Cl were performed using a Hi-Tech SF-61 DX2 double mixing stopped-flow spectrometer, thermostated to ± 0.1 °C using a Lauda model RM6 refrigerated recirculating water bath.. The kinetic data were fitted using KinetAsyst software. The stopped-flow kinetics for [Pd(B_{Me})Cl]Cl, [Pd(B_{Et})Cl]Cl and [Pd(B_{Bu})Cl]Cl were performed using a DX-17MV Applied Photophysics stopped-flow spectrometer, thermostated to ± 0.1 °C using a Lauda model RM6 refrigerated recirculating water bath using a PolyScience Digital temperature controller. The kinetic data were fitted using Pro-Data SX software. Data were plotted using Microsoft Excel.

Cytotoxic activity was calculated using absorbance measurements. The cells were agitated on a Plate Shaker 20 from National Labnet Co. The absorbance measurements were performed on a Molecular Devices SpecMax 340 Diode Array Spectrometer running the SoftMax Pro software package. Statistical analysis were performed using the software package Prism 3.03 by Novell.

2.5 Characterization

2.5.I. NMR Spectroscopy

Samples of the intermediate compounds (designated compound A in **Scheme 2.1**), the final ligands (designated compound B in **Scheme 2.1**) and the subsequent palladium(II) complexes were measured in deuterated DMSO.

2.5.II. FTIR Spectroscopy

Spectra of the compounds A_H, A_{Me}, and A_{Et} were measured using a chloroform cast method, whereas compound A_{Bu} was characterized using a KBr pellet method. The final ligands (designated compound B in **Scheme 2.1**) and the subsequent palladium(II) complexes were characterized using a KBr pellet method.

2.6 Kinetics

Kinetics of reaction of guanosine hydrate (**Figure 2.7**) with the above series of substituted palladium(II) compounds were studied under pseudo-first-order conditions using stopped-flow spectrometry. Guanosine hydrate was used in place of guanine due to the insolubility of guanine in aqueous solutions.

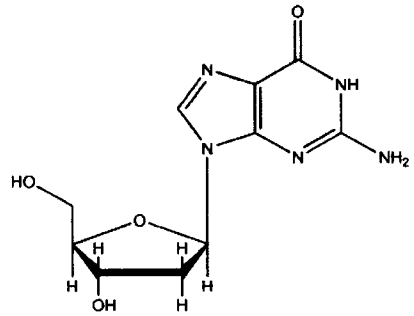


Figure 2.7 - Guanosine Hydrate

Pseudo-first-order conditions are achieved by having one of the two reactants present in at least a ten-fold excess over the other reactant. The metal complexes, which were sparingly soluble, were kept at a constant concentration of 50% saturation, giving a concentration of approximately 1 x 10^{-6}M. The concentration of guanosine hydrate was varied from 5 x 10^{-4}M to 1.25 x 10^{-4}M. Each of the solutions were subjected to a further two fold dilution once combined in the stopped-flow spectrometer. The temperature values were varied from 15°C to 35°C.

The value of k_{obs} was reported for each set of conditions. This value was reported as the mean of repetitive measurements that produced results consistent within 1%.

The kinetics of reaction of $[Pd_2(Tppz)Cl_2]Cl_2$ were performed using UV/Vis spectrometry and can be found in reference 7. In addition, the kinetics of reaction of [Pd(Terpy)Cl]Cl and $[Pd(B_H)Cl]Cl$ were performed previously using stopped-flow spectrometry and can be found in reference 8.

2.7 Calf Thymus DNA Experiment

A common method for determining substrate interaction with nucleic DNA is spectrophotometric titrations with the complex in question and calf thymus DNA.[45]

The palladium solutions were fixed at a concentration of 5.0×10^{-5}M. The solutions contained 0.5% DMSO with buffer solvent. 0.5% DMSO was used as solvent to to obtain significance when comparing to the control used in the *in vitro* cell line screening results where the same concentrations were used. The buffer solvent was composed of 10mM Tris-HCL/50mM NaCl (pH 7.2).[45]

A stock solution of DNA was added in increments to provide a maximum ratio of 9:1, Pd:DNA. The concentration per nucleotide was determined by absorption spectroscopy using the known molar extinction coefficient value of $6600 M^{-1}cm^{-1}$ at 260nm.[45]

A buffer blank (containing the same amount of DNA and buffer used in the experimental vial, plus 0.5% DMSO) was used in each measurement. The Pd/DNA solutions were allowed to incubate at room temperature for 10 minutes before experimental measurement was taken *via* UV/Vis spectroscopy. The absorbance changes were measured at ~240 nm for each complex.

2.8 Octanol-Water Partition Coefficients

Octanol-water partition coefficients were measured for each substituted Pd(II) complex using the slow-stir method.[34] Saturated aqueous solutions of the Pd(II) complexes were added to an equal amount of purified octanol and allowed to stir slowly at room temperature for 15 days.

Absorbance measurements were taken using UV/Vis spectroscopy. For each measurement a control of water / octanol (stirred with no Pd(II) compounds) was used. The octanol-water partition coefficient is the ratio of the final concentration the complex in octanol *versus* water. The results reported are the mean value of triplicate experimentals providing an error within 1%.

2.9 In-Vitro cell line screening

The commonly used concentration unit in bioassays is micromolarity, μM. Hence, this unit will be used throughout the *in-vitro* cell line screening sections in this text.

2.9.I. Cell Line Preparation

Three human cancer cell lines were used for *in vitro* screening experiments. The three cell lines were ovarian cancer (SKOV3), breast cancer (MCF7) and colon cancer (HT-29), supplied by the Atlantic Veterinary College.

The individual cell lines were rapidly thawed from a liquid nitrogen holding tank. The cells were mixed with 14 mL of a sterile cell medium (composed of 50mL fetal bovine serum, 20mL L-glutamine and 1L of RPMI-1640 medium) using a sterile pipette. The resulting mixtures were then transferred to a sterile straight-necked vented flask in a sterile vented hood. The flasks containing the medium/cell mixture were incubated at 37°C under an atmosphere of 5.0% CO_2 to obtain optimum growth.

Once the cells had settled (24 hrs) the medium was removed by vacuum suction and the cells were rinsed with 10mL of sterile phosphate buffer solution (PBS). The PBS solution was composed of sodium chloride, potassium chloride, sodium phosphate and potassium phosphate. The PBS was removed by vacuum suction and 14 mL of fresh media solution was added to the cells. The cells were then incubated as above. The procedure of rinsing and changing media was repeated every three days for the remainder of the experimental time period.

Once the cells began to multiply, it was required to subculture the cells into smaller aliquots to keep a minimum number of cells per flask for optimal growth and health. Cellular subculturing

involved the removal of the medium and rinsing with PBS as above. Once the rinsing procedure was complete, 1mL of trypsin was added to the cells. The cells were then incubated as above for a period of 5-10 minutes. The incubation allowed the cells to be released from fixation to the flask so that they visibly floated in the trypsin. Medium was then added to the trypsinized cells and mixed throughly. Once the cells were free floating in the medium/trypsin solution, they were transferred to new sterile flasks in appropriate aliquots. Subculturing was performed in ratios of 1:2, 1:3, 1:4, 1:5, 1:6 depending on the number of cells initially present before subculturing and the amount of cells desired in the new flasks.

2.9.II. Sulforhodamine B Assay

The SRB assay was followed using reference procedures.[31] To each well of the 96-well plate(100μL) 25μL of cold 50% (w/v) trichloroacetic acid (TCA) was added to reach a final concentration of 10% TCA. The well plate was then incubated at 4°C for one hour to allow the cells to fix in place. The supernatant was then discarded by vacuum suction. The well plate was then washed five times with deionized water and left to air dry at room temperature. At this point the well plates were stable and able to be stored until use.

The SRB solution was composed of 0.4% w/v Sulforhodamine B in 1% acetic acid. 100μL was added to each well in the well plate. The well plate was then incubated at room temperature for ten minutes. In order to remove any unbound SRB the plate was washed five times with 1% acetic acid. After the plates were left to air dry, 100μL of Tris buffer was added to each well. The well plate was agitated for five minutes to solubilize any bound SRB stain. The optical densities were then read on an automated spectrophotometric plate at 515nm.

2.9.III. Calibration Curve

Calibration curves were required in order to determine the concentration of each specific cell line that fell within a linear range of the SRB assay. The calibration curve determines the concentration of cells per well to be used during the drug screening process.

After trypsonizing the cells as described earlier and combining them with fresh medium, the cells were counted using a hemacytometer to achieve a result of 80 000 cells per mL of medium. Once this concentration of cells was acquired, serial dilutions were made using the aliquot. There was a range of nine concentrations plus a control with zero cells to be read as a blank measurement. The solutions were added to a 96-well plate, leaving the outside wells containing medium only, for they tend to dry out and produce invalid results.

The well plate was incubated at 37°C under an atmosphere of 5.0% CO_2 for 24 hours to allow the cells to adhere to the surface again. For each cell line there were four 96-well plates plated with the above cell concentrations. The first was fixed at time equals zero (i.e. after the 24 hour adherence time allowance). The other three plates were fixed at subsequent 24, 48, and 72 hour time intervals. Fixing the cells was as described above and the SRB assay was then performed to determine the calibration curve.

A linear dependence was found for each time trial. The ovarian cancer cells were subsequently plotted at 20 000 cells per mL medium, the breast cancer cells at 10 000 cells per mL medium, and the colon cancer cells at 20 000 cells per mL medium.

2.9.IV. Drug Screening

For each trial (three trials in total), serial dilutions were initially prepared in DMSO. The

solutions were kept frozen throughout the trial period to prevent decomposition. Each day, 25μL of each solution was diluted in 5mL fresh medium to reach a 0.5% DMSO ratio of the desired concentrations; 50μM, 25μM, 10μM, 1μM, 0.1μM and 0.01μM for cisplatin (used as a comparative control substance on each plate), 50μM, 10μM, 1μM, 0.1μM for [Pd(Terpy)Cl]Cl, [Pd(B_{Et})Cl]Cl and [Pd(B_{Bu})Cl]Cl, 25μM, 10μM, 1μM, 0.1μM for [Pd(B_{H})Cl]Cl and [Pd(B_{Me})Cl]Cl, and 10μM, 1μM, 0.1μM, 0.01μM for [Pd_2(Tppz)Cl_2]Cl_2.

The cells were plated according to the above concentrations for each cell line. After a 24 hour incubation period at 37°C under an atmosphere pressure of 5.0% CO_2, the medium was removed by vacuum suction and fresh medium containing the desired drugs were added to the appropriate wells. The control plate was fixed at this time using the SRB assay. (See **Appendix B** for a sample well plate)

The cells were rinsed and fresh medium and drugs were added every 24 hours throughout the experiment followed by incubation as mentioned as above. Appropriate plates containing ranges of concentrations of each drug were fixed at time periods of 24, 48 and 72 hours. The plates were subjected to the SRB assay. The experiments provided a full dose response curve for each drug in question (four concentrations, three time periods).

2.9.VI. Statistical Analysis

Triplicate analysis of each concentration of experimental drug and control drug were performed for each cell line of interest.

In total there were three distinct measurements: Control optical density (C) was a measure of absorbance of cells with only media present. Test optical density (T) was a measure of cell death.

Optical density at time zero (T_0) was a measure of absorbance of cells with only media present at time equals zero on the control plate. DMSO / Initial drug effects were determined to be zero.[31]

Cell toxicity and growth inhibition values were calculated using the following equations. The cell kill equation is used when $T < T_0$(equation = $100 \times [(T - T_0)/T_0]$). The growth inhibition equation is used when $T > T_0$(equation = $100 \times [(T - T_0)/(C - T_0)]$). Cell kill values are negative, this means that x% of the cells were killed in the solution. Growth inhibition values are positive, x% of the cells are growing as normal, and 100-x% of the cells are experiencing growth inhibition.[31]

Statistical analysis were performed using two-way ANOVA methods. A two-way ANOVA allows for the study of population means when populations are classified according to two factors simultaneously. It also allows for the examination of interactions between these factors.[32]

Three statistical values were calculated; interaction value, concentration value, and drug value. The interaction value states whether or not the interaction is statistically significant, meaning that there is a difference in the overall shapes of the dose response curves between the experimental and control drug. If the interaction is significant, the results for concentration and drug may be irrelevant. Simply stating a significant interaction is a strong enough statement to prove that the drugs are statistically different. The concentration value states whether or not the concentration is statistically significant, meaning that there is a difference in dose effects of the two drugs in question. The drug value states whether or not the drug is statistically significant, meaning that there is a difference in results of the two drugs in question. If the drug value was determined to be statistically significant Bonferroni post-tests were used to determine which concentrations effects were statistically different. The Bonferroni method was used because it makes correction for multiple comparisons and guarantees that the probability of any false rejection among all comparisons made is no greater than 0.05.[32]

3. Results and Discussion

The compounds will be abbreviated as in **Table 2.1**.

3.1 Characterization

In the following section results of spectroscopic characterization of the dione intermediate ligands, substituted terpyridyl ligands and consequent palladium(II) complexes are tabulated. These results show successful synthesis of the desired compounds.

It should be noted that a sample of $[Pd_2(Tppz)Cl_2]Cl_2$ from previous work[7] was used in the experimental procedures.

3.1.I. NMR Spectroscopy

3.1.I.a. ^1H NMR

Table 3.1 shows the significant peaks for the ^1H NMR spectra for the dione intermediate compounds, designated compound A in **Scheme 2.1**.

Table 3.1 - ^1H NMR (compound A)

Compound	*Chemical Shift (ppm)*	*Multiplicity*	*Number of H*	*Assignment*
A_H	8.29	d d	2	pyridine H
	7.71	m	4	pyridine H
	7.27	m	2	pyridine H
	7.00	m	5	phenyl H
	4.01	m	1	CH
	2.00	d	2	CH_2

	1.75	d	2	CH_2
A_{Me}	8.29	d d	2	pyridine H
	7.71	m	4	pyridine H
	7.27	m	2	pyridine H
	6.93	m	4	phenyl H
	4.01	m	1	CH
	2.00	d	2	CH_2
	1.75	d	2	CH_2
	2.06	s	3	Methyl CH_3
A_{Et}	8.29	d d	2	pyridine H
	7.71	m	4	pyridine H
	7.27	m	2	pyridine H
	6.97	m	4	phenyl H
	4.01	m	1	CH
	2.00	d	2	CH_2
	1.74	d	2	CH_2
	2.45	m	2	Ethyl CH_2
	1.06	t	3	Ethyl CH_3
A_{Bu}	8.67	d d	2	pyridine H
	7.89	m	4	pyridine H
	7.63	m	2	pyridine H
	7.13	m	4	phenyl H
	3.92	m	1	CH
	3.50	d	4	CH_2
	2.45	m	2	Butyl CH_2
	1.47	m	2	Butyl CH_2

	1.23	m	2	Butyl CH_2
	0.85	t	3	Butyl CH_3

Table 3.2 shows the significant peaks for the ^1H NMR spectra for the final ligands, designated compound B in **Scheme 2.1**.

Table 3.2 - ^1H NMR (compound B)

Compound	*Chemical Shift (ppm)*	*Multiplicity*	*Number of H*
$\mathbf{B_H}$	8.72	m	6
	8.05	t	2
	7.93	d	2
	7.81	m	5
$\mathbf{B_{Me}}$	8.71	m	6
	8.04	t	2
	7.83	d	2
	7.45	m	4
	2.41	s	3
$\mathbf{B_{Et}}$	8.71	m	6
	8.04	t	2
	7.87	d	2
	7.43	m	4
	2.71	q	2
	1.28	t	3
$\mathbf{B_{Bu}}$	Since the compound could not be isolated in pure form, NMR was unable to be performed. (See experimental)		

Since palladium(II) is a d^8, low spin, diamagnetic metal ion, it is possible to measure the NMR spectra of the complexes of the palladium(II) metal with the above ligands. **Table 3.3** shows the significant peaks for the ^1H NMR spectra for the palladium(II) complexes.

Table 3.3 - ^1H NMR (palladium complexes)

Compound	*Chemical Shift (ppm)*	*Multiplicity*	*Number of H*
[Pd(Terpy)Cl]Cl	8.65	m	7
	8.47	d t	2
	7.88	d t	2
[Pd(B_H)Cl]Cl	9.02	s	2
	8.90	d	2
	8.70	d d	2
	8.49	d t	2
	8.22	m	2
	7.88	d t	2
	7.69	m	3
[Pd(B_{Me})Cl]Cl	9.03	d	1
	8.91	m	4
	8.69	d	1
	8.45	m	2
	8.14	d	1
	8.00	d	1
	7.86	m	2
	7.45	m	2
	2.43	s	3

[Pd(B_{Et})Cl]Cl	8.95	s	2
	8.87	d	2
	8.63	d	2
	8.45	t	2
	8.15	d	2
	7.84	t	2
	7.49	d	2
	2.74	q	2
	1.27	t	3
[Pd(B_{Bu})Cl]Cl	8.99	s	2
	8.88	m	2
	8.75	d	2
	8.50	t	2
	8.15	d	1
	7.93	m	1
	7.47	m	4
	2.69	m	2
	1.62	m	2
	1.33	m	2
	0.933	t	3

3.1.I.b. ^{13}C NMR

Table 3.4 shows the significant peaks for the ^{13}C NMR spectra for the dione intermediate compounds, designated compound A in **Scheme 2.1**.

Table 3.4 - ^{13}C NMR (compound A)	
Compound	*Chemical Shift (ppm)*
$\mathbf{A_H}$	201.4, 153.9, 149.1, 147.7, 136.5, 135.7, 127.7, 127.5, 126.1, 119.3, 45.9, 38.0
$\mathbf{A_{Me}}$	201.4, 153.9, 148.2, 147.7, 136.9, 134.9, 128.7, 127.5, 126.1, 119.3, 45.9, 37.6, 20.5
$\mathbf{A_{Et}}$	201.5, 154.0, 148.2, 147.9, 136.3, 135.7, 127.5, 126.1, 124.8, 119.0, 46.1, 37.9, 27.6, 15.3
$\mathbf{A_{Bu}}$	200.0, 152.8, 149.1, 145.6, 137.6, 134.9, 128.1, 127.4, 126.5, 121.3, 43.6, 36.5, 34.4, 33.1, 22.6, 13.8

Table 3.5 shows the significant peaks for the ^{13}C NMR spectra for the final ligands, designated compound B in **Scheme 2.1**.

Table 3.5 - ^{13}C NMR (compound B)	
Compound	*Chemical Shift (ppm)*
$\mathbf{B_H}$	155.7, 155.0, 149.6, 149.4, 137.6, 137.5, 129.5, 129.4, 126.9, 124.6, 121.0, 118.0
$\mathbf{B_{Me}}$	155.7, 155.0, 150.0, 149.4, 139.3, 137.5, 134.6, 130.0, 126.7, 124.5, 120.9, 117.7, 20.8
$\mathbf{B_{Et}}$	155.7, 155.0, 151.0, 149.4, 139.5, 137.5, 134.7, 128.8, 126.9, 124.2, 120.9, 118.1, 30.1, 15.4
$\mathbf{B_{Bu}}$	Since the compound could not be extracted in powder form, NMR was unable to be performed. (See experimental)

Due to solubility limitations, the ^{13}C NMR was unable to be performed for the palladium complexes.

3.1.II. FTIR Spectroscopy

Table 3.6 shows the significant peaks for the FTIR spectra for the dione intermediate compounds, designated compound A in **Scheme 2.1**.

Table 3.6 - FTIR (compound A)

Compound	*Peak (cm^{-1})*	*Strength*	*Assignment*
A_H	1588.9	s	C = N
	1570.9, 1466.5	m	Aromatic C = C
	3057.7	m	C - H stretch
	752.6	s	C - H o.o.p. bend
	1693.1	s	C = O
A_{Me}	1589.2	s	C = N
	1571.2, 1466.9	m	Aromatic C = C
	3055.7	m	Aromatic C-H stretch
	749.4	s	Aromatic C-H o.o.p. bend
	1692.4	s	C = O
	1435.4, 1346.7	m	Alkane C-H bend
	2919.8	s	Alkane C-H stretch
A_{Et}	1588.8	s	C = N
	1571.0, 1466.3	m	Aromatic C = C
	3054.6	m	Aromatic C-H stretch
	749.9	s	Aromatic C-H o.o.p. bend
	1692.4	s	C = O
	1435.2, 1346.8	m	Alkane C-H bend
	2916.6	s	Alkane C-H stretch

A_{Bu}	1583.5	s	C = N
	1568.4, 1462.6	m	Aromatic C = C
	3050.0	m	Aromatic C-H stretch
	765.9	s	Aromatic C-H o.o.p. bend
	1701.4	s	C = O
	1435.9, 1368.6	m	Alkane C-H bend
	2926.8	s	Alkane C-H stretch

Table 3.7 shows the significant peaks for the FTIR spectra for the final ligands, designated compound B in **Scheme 2.1**.

Table 3.7 - FTIR (compound B)

Compound	*Peak (cm^{-1})*	*Strength*	*Assignment*
B_H	1583.7	s	C = N
	1566.0, 11467.1	s	Aromatic C = C
	3049.3	w	C - H stretch
	760.3	s	C - H o.o.p. bend
B_{Me}	1584.2	s	C = N
	1586.2, 1467.4	s	Aromatic C = C
	3055.6	m	Aromatic C-H stretch
	788.0	s	Aromatic C-H o.o.p. bend
	1440.9, 1389.0	s	Alkane C-H bend
	2911.1	w	Alkane C-H stretch
B_{Et}	1584.3	s	C = N
	1586.6, 1466.1	s	Aromatic C = C

	3055.4	w	Aromatic C-H stretch
	703.4	s	Aromatic C-H o.o.p. bend
	1433.8, 1388.0	m	Alkane C-H bend
	2931.9	w	Alkane C-H stretch
B_{Bu}	Since the compound could not be extracted in powder form, NMR was unable to be performed. (See experimental)		

Table 3.8 shows the significant peaks for the FTIR spectra for palladium(II) complexes.

Table 3.8 - FTIR (palladium complexes)

Compound	*Peak (cm^{-1})*	*Strength*	*Assignment*
[Pd(terpy)Cl]Cl	1585.6	s	C = N
	1570.5, 1471.7	s	Aromatic C = C
	3089.2	w	C - H stretch
	767.7	s	C - H o.o.p. bend
[Pd(A_H)Cl]Cl	1597.0	s	C = N
	1533.5, 1416.4	s	Aromatic C = C
	3086.1	w	C - H stretch
	769.4	s	C - H o.o.p. bend
[Pd(A_{Me})Cl]Cl	1598.2	s	C = N
	1533.0, 1425.7	m	Aromatic C = C
	3090.5	m	Aromatic C-H stretch
	785.8	s	Aromatic C-H o.o.p. bend
	1401.9, 1356.1	w	Alkane C-H bend
	3010.2	w	Alkane C-H stretch

[Pd(A_{Et})Cl]Cl	1598.0	s	C = N
	1534.0, 1462.0	m	Aromatic C = C
	3061.0	m	Aromatic C-H stretch
	786.0	s	Aromatic C-H o.o.p. bend
	1403.1, 1357.0	m	Alkane C-H bend
	3009.5	s	Alkane C-H stretch
[Pd(A_{Bu})Cl]Cl	1596.6	s	C = N
	1529.0, 1429.8	m	Aromatic C = C
	3057.2	m	Aromatic C-H stretch
	783.9	s	Aromatic C-H o.o.p. bend
	1402.6, 1342.9	m	Alkane C-H bend
	3005.1	m	Alkane C-H stretch

The FTIR spectra of the palladium(II) complexes are similar to those of the free ligand differing only slightly in wavelengths. The shift in wavelength corresponds to successful metal complexation of the ligands.[48]

3.1.III. ESMS

Table 3.9 shows the theoretical and experimental parent ion peaks for the compounds A, B and the corresponding palladium(II) complexes.

Table 3.9 - ESMS

Compound	*Theoretical (g/mol)*	*Experimental (g/mol)*
$[A_H H]^+$	331.14	331.01
$[A_{Me} H]^+$	345.16	345.08

$[A_{Et}\ H]^+$	359.18	358.95
$[A_{Bu}\ H]^+$	310.13	386.49
$[B_H\ H]^+$	310.13	310.30
$[B_{Me}\ H]^+$	324.15	324.13
$[B_{Et}\ H]^+$	338.16	338.09
$[Pd(terpy)Cl]^+$	375.97	375.88
$[Pd(B_H)Cl]^+$	452.00	451.86
$[Pd(B_{Me})Cl]^+$	466.02	465.89
$[Pd(B_{Et})Cl]^+$	480.03	479.86
$[Pd(B_{Bu})Cl]^+$	543.00	542.78

3.1.IV. C,H,N Analysis

Table 3.10 shows the theoretical and experimental C,H,N percentages of the compounds A, B and the corresponding palladium(II) complexes.

Table 3.10 - C,H,N Analysis

Compound	*Experimental*			*Theoretical*		
	% C	*% H*	*% N*	*% C*	*% H*	*% N*
A_H	76.6	5.4	9.0	76.3	5.5	8.5
A_{Me}	76.8	6.1	8.6	76.7	5.9	8.1
A_{Et}	77.0	6.2	8.3	77.1	6.2	7.8
A_{Bu}	77.7	6.7	7.1	77.7	6.8	7.3
B_H	81.2	4.4	13.2	81.5	4.9	13.6
B_{Me}	81.5	5.4	12.5	81.7	5.3	13.0
B_{Et}	81.5	5.6	12.4	81.9	5.7	12.3
[Pd(terpy)Cl]Cl	43.5	2.5	10.3	43.9	2.7	10.2

[Pd(B_H)Cl]Cl	51.6	2.8	8.1	51.8	3.1	8.6
[Pd(B_{Me})Cl]Cl	52.4	3.5	8.2	52.8	3.4	8.4
[Pd(B_{Et})Cl]Cl	53.5	3.5	7.9	53.7	3.7	8.2
[Pd(B_{Bu})Cl]Cl	59.2	4.4	7.8	59.2	4.6	8.3

3.2 Kinetics

The kinetics of reaction of $[Pd_2(Tppz)Cl_2]Cl_2$ with guanine were performed using UV/Vis spectrometry and can be found in reference 7. The results will not be discussed in this section for the purpose of these kinetic studies was to determine the effects of a carbon chain extension on the molecules in regards to the rate of reaction.

3.2.I. UV Vis Spectrometry

Previous UV/Vis spectrometry studies have shown an absorbance change upon reaction of 5 x 10^{-5}M guanosine hydrate with 50% saturation, 1 x 10^{-6}M, [Pd(Terpy)Cl]Cl and [Pd(B_H)Cl]Cl.[36] **(Figure 3.1, Figure 3.2)** This absorbance change indicated that a chemical reaction between the two compounds had occurred. Similar studies were performed with [Pd(B_{Me})Cl]Cl, [Pd(B_{Et})Cl]Cl and [Pd(B_{Bu})Cl]Cl indicating a similar reaction for the compounds.**(Figure 3.3, Figure 3.4, Figure 3.5)** These preliminary results graphically represent the similarity in substitution reaction with the Pd compounds and guanosine hydrate. The spectral change occurred during the time of mixing in the 1 cm cuvette of the diode array spectrometer, so it was necessary to use stopped-flow spectrometry to measure the kinetics of the reaction.

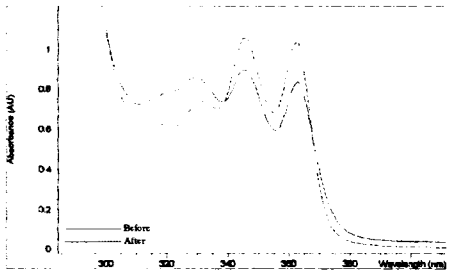


Figure 3.1 - Absorbance *versus* Time for $[Pd(terpy)Cl]^+ + Gu$
(Figure taken from reference 8)

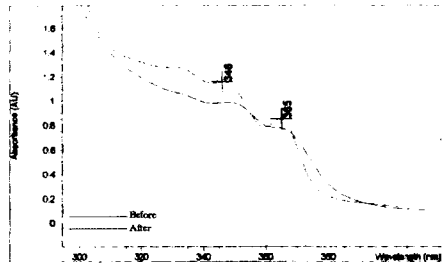


Figure 3.2 - Absorbance *versus* Time for $[Pd(B_H)Cl]^+ + Gu$
(Figure taken from reference 8)

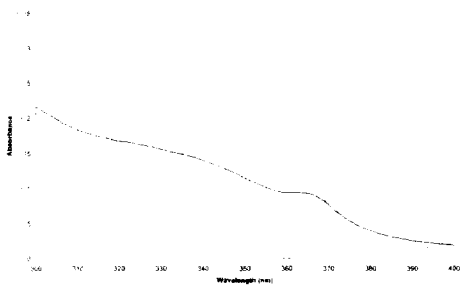


Figure 3.3 - Absorbance *versus* Time for $[Pd(B_{Me})Cl]^+ + Gu$

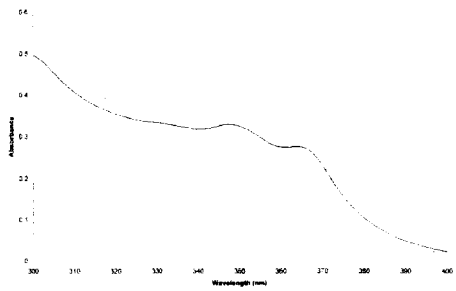


Figure 3.4 - Absorbance *versus* Time for $[Pd(B_{Et})Cl]^+ + Gu$

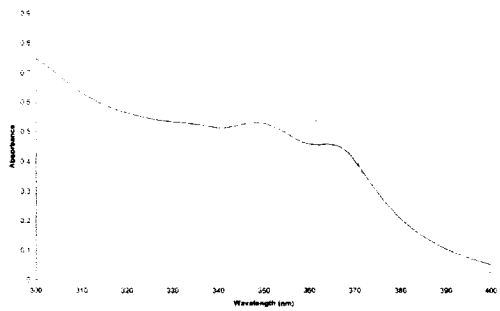


Figure 3.5 - Absorbance *versus* Time for $[Pd(B_{Bu})Cl]^+ + Gu$

3.2.II. Kinetics of Reaction with Guanosine Hydrate

Absorbance *versus* time changes were measured at a wavelength of 340-350 nm. The specific wavelength was chosen independently for each compound depending on the maximum peak height observed in the above UV/Vis spectra. It should also be noted that in the presence of physiological saline solution (9% w/v NaCl) the reaction was completely supressed, hence counter ion concentration dependence was not measured.

The change in absorbance as a function of time was exponential, indicating the reaction to be first order with respect to the palladium complex concentration. For each of the five Pd compounds, similar kinetics of reactions were observed. This provides us with a rate law as follows: Rate = k_{obs}[Pd(ligand)Cl]$^+$, where the value of k_{obs}, the observed first order rate constant, was determined from the absorbance *versus* time data using a non-linear least-squares fit.

Data were collected as a function of guanosine concentration at five temperatures and concentrations. The results for [Pd(TerpyCl]Cl and [Pd(B_H)Cl]Cl were reported previously[8], however will be revisited in this discussion for comparison clarification. The results for [Pd(B_{Bu})Cl]Cl will be used as a comparison trend, and represent the results for [Pd(B_{Me})Cl]Cl and [Pd(B_{Et})Cl]Cl for temperature dependence studies.

A plot of k_{obs} *versus* [Gu] illustrates the dependene of k_{obs} on [Gu] over the experimental temperature range for each compound. From this linear dependence, the y-intercept and slope are determined. The dependence shows that the Rate = k_{obs}[Pd(ligand)Cl]$^+$ = (k_1 +k_2[Gu])[Pd] where k_1 is the S_N1 or dissociative pathway (**Figure 3.6**) and k_2 is the S_N2 or associative pathway (**Figure 3.7**).

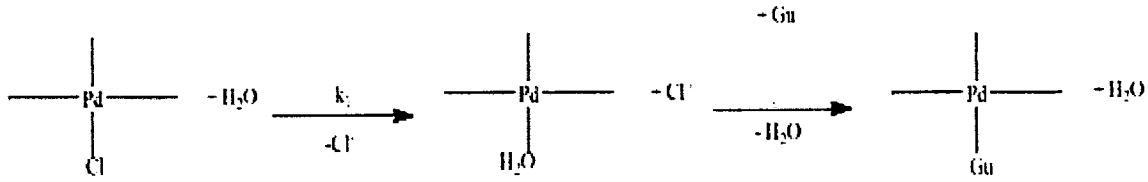


Figure 3.6 - Dissociative Reaction Mechanism

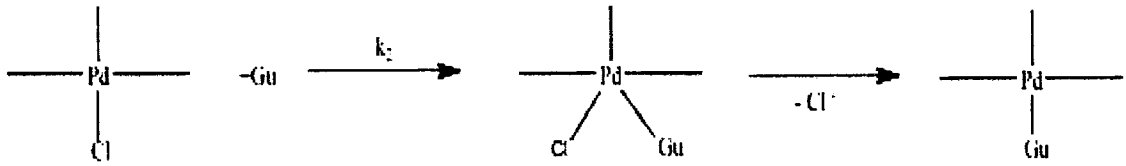


Figure 3.7 - Associative Reaction Mechanism

Appendix C show the data collected for [Pd(Terpy)Cl]Cl and [Pd(B_H)Cl]Cl.[8] **Table 3.11** and **Figure 3.8** show the data collected for [Pd(B_{Bu})Cl]Cl.

Table 3.11 - [Pd(B_{Bu})Cl]Cl Kinetic Data					
Temperature	*292.7K*	*298.0K*	*301.8K*	*305.6K*	*309.8K*
[Gu] (M)	k_{obs} (s^{-1})	k_{obs} (s^{-1})	k_{obs} (s^{-1})	k_{obs} (s^{-1})	k_{obs} (s^{-1})
6.30×10^{-5}	31.6	14.7	17.7	24.8	29.7
1.25×10^{-4}	22.9	24.4	30.1	39.8	48.2
1.88×10^{-4}	17.7	34.8	41.4	48.5	66.9
2.50×10^{-4}	9.86	44.8	56.0	69.7	82.3
k_1 (s^{-1})	2.89	4.56	4.78	9.36	12.6
k_2 ($M^{-1}s^{-1}$)	1.13×10^5	1.61×10^5	2.02×10^5	2.30×10^5	2.83×10^5

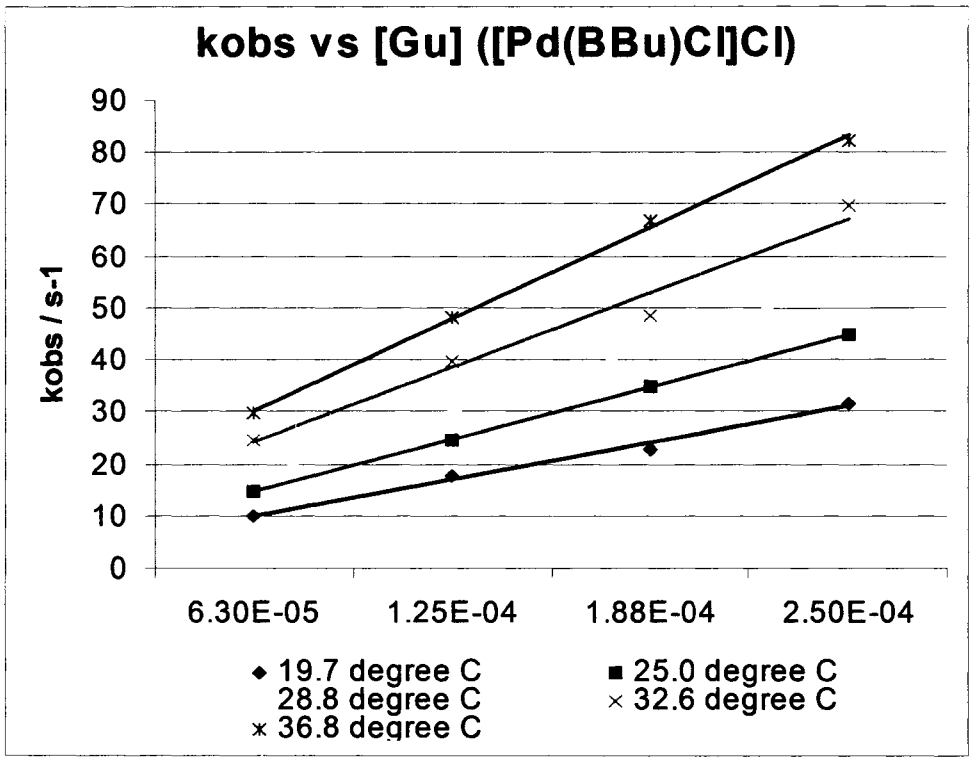


Figure 3.8 - k_{obs} *versus* [Gu] for [Pd(B_{Bu})Cl]Cl

The striking similarities of the kinetics of reaction with [Gu] for the three Pd compounds indicate that the effect of an additional phenyl group and carbon chain do not affect the rate of reaction. The differences between the three Pd compounds are at the opposite end of the molecule to the site of nucleophilic attack, hence presenting very negligible effects on the incoming nucleophile. To confirm that this trend is equally represented for [Pd(BMe)Cl]Cl and [Pd(BEt)Cl]Cl, the kinetics of reaction were performed at 25 °C. The data are represented in **Table 3.12** and illustrated in **Figure 3.9**.

Table 3.12 - Kinetic Data Comparison (25 °C)					
Compound	*[Pd(Terpy)Cl]Cl*	*[Pd(B_H)Cl]Cl*	*[Pd(B_{Me})Cl]Cl*	*[Pd(B_{Et})Cl]Cl*	*[Pd(B_{Bu})Cl]Cl*
[Gu] (M)	k_{obs} (s^{-1})	k_{obs} (s^{-1})	k_{obs} (s^{-1})	k_{obs} (s^{-1})	k_{obs} (s^{-1})
6.30×10^{-5}	30.0	17.1	15.6	15.19	14.7
1.25×10^{-4}	45.5	24.5	27.5	24.27	24.4
1.88×10^{-4}	63.0	30.0	32.8	36.2	34.8
2.50×10^{-4}	77.5	37.2	40.4	47.14	44.8
k_1 (s^{-1})	13.1	10.8	9.15	3.76	4.56
k_2 ($M^{-1}s^{-1}$)	2.56×10^5	1.05×10^5	1.28×10^5	1.73×10^5	1.61×10^5

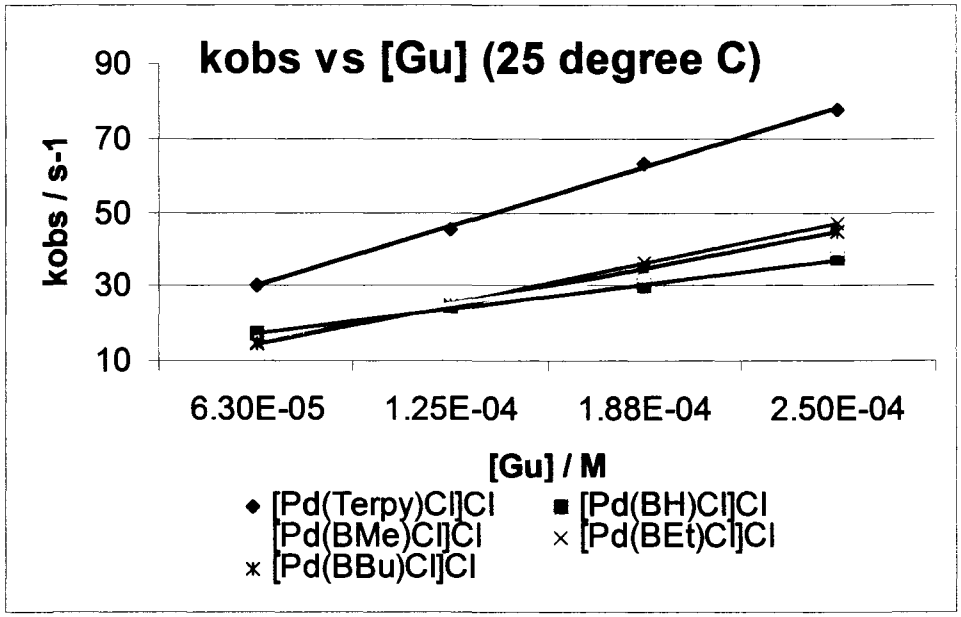


Figure 3.9 - k_{obs} *versus* [Gu] at 25 °C

Within experimental error, all five Pd compounds present the same rate of reaction. The k_2 values for the associate mechanism, for all compounds are within a factor of 2 of each other. However the k_1 values, for the dissociative mechanism, are slightly higher for [Pd(Terpy)Cl]Cl

versus its substituted analogues. The presence of a phenyl group can stabilize a structure through resonance. This stabilization leads to a π system that is electron withdrawing, hence creating a stronger Pd-Cl bond. This is known as a negative inductive effect.[4] Since the remaining compounds do not provide any additional resonance, their k_1 values are effectively equal to one another.

The proposed reaction mechanism correlates to literature results of $[Pt(Terpy)Cl]^+$ interaction with L-cysteine.[43] The proposed mechanism also parallels other Pd based nucleophilic substitution reactions, which also exhibit similar rate values.[30]

To complete the kinetic study, entropy and enthalpy activation parameters were calculated from the temperature dependence using the Eyring equation. (**Equation 1.1**) A plot of ln(k/T) *vs* (1/T) is used to calculate values for ΔS^* and ΔH^*. ΔS^* = [Intercept - ln(k/h)]R and ΔH^* = - (R x Slope). The data values for [Pd(Terpy)Cl]Cl and $[Pd(B_H)Cl]Cl$ can be found in **Appendix C**. **Table 3.13** contains the data values for $[Pd(B_{Bu})Cl]Cl$.

Table 3.13 - $[Pd(B_{Bu})Cl]Cl$ - ln(k/T) *versus* 1/T values

T (K)	*1/T (K^{-1})*	*k_1 (s^{-1})*	*ln (k_1/T)*	*k_2 ($M^{-1}s^{-1}$)*	*ln (k_2/T)*
292.7	3.42×10^{-3}	2.89	-4.62	1.13×10^5	5.96
298.0	3.36×10^{-3}	4.56	-4.18	1.61×10^5	6.29
301.8	3.31×10^{-3}	4.78	-4.15	2.02×10^5	6.51
305.6	3.27×10^{-3}	9.36	-3.49	2.30×10^5	6.62
309.8	3.23×10^{-3}	12.6	-3.20	2.83×10^5	6.82
Y-int		21.3		21.4	
Slope		-7.59×10^3		-4.50×10^3	

Table 3.14 lists a comparison of the entropy and enthalpy activation parameters.

Table 3.14 - Activation Entropy and Enthalpy Comparison

	Dissociative Pathway		*Associative Pathway*	
	ΔS_1^* *(J/Kmol)*	ΔH_1^* *(kJ/mol)*	ΔS_2^* *(J/Kmol)*	ΔH_2^* *(kJ/mol)*
[Pd(Terpy)Cl]Cl	-88.6	39.8	-42.9	29.6
[Pd(B_H)Cl]Cl	-87.8	41.0	-50.4	29.4
[Pd(B_{Bu})Cl]Cl	-20.5	63.1	-19.6	37.4

A negative ΔS_1^* is explained by the electrostriction due to charge creation.[42] When the Pd(II) compound exchanges its Cl^- for a neutral H_2O there is an overall increase in charge. The separation of charges of $[Pd(ligand)H_2O]^+$ and Cl^- cause an increase in ordering of the solvent, resulting in a negative entropy charge. A negative ΔS_2^* is explained by an decrease in the overall number of molecules on going from the initial state to the transition state; $[Pd(ligand)Cl]^+$ and Gu join together to become one, $[Pd(ligand)Cl\ Gu]^+$.

ΔH_1^* is found to be slightly larger than ΔH_2^* since breaking a bond in the dissociative pathway requires more energy than does creating a bond in the associative pathway.

These values correlate to literature values found for similar Pd(II) complexes,[44] however they differ slightly when compared to similar Pt(II) complexes.[43] The kinetics of reaction with [Pt(Terpy)Cl]Cl have been previously measured, and show similar reaction mechanisms, but differ in entropy and enthalpy activation parameter values. Pt(II) is a softer metal center, therefore it is speculated that it may be more sensitive to electronic communication with the aromatic rings of the terpy ligand than Pd(II). Pd(II) and Pt(II) terpy complexes have been shown to exhibit similar structural and reactivity properties, hence the differences in activation parameters must be due to the

properties of the transition states. Less extensive orbital overlap in the five-coordinate transition state of Pd(II) may result in weaker and longer metal-to-ligand bonds. This may in turn lead to a less compact structure when compared to Pt(II), hence explaining the subtle differences in activation parameters.

3.3 Calf Thymus DNA Experiment

A common method for determining the interaction of metal based compounds with nucleic DNA is spectrophotometric titration with the compound in question and calf thymus DNA. Successful experimentation requires that the absorption band of the metallic complex is different than that of the calf thymus DNA.[45]

Experimentation requires varying concentrations of calf thymus DNA in excess over the metallic complexes. Six Pd(II) compounds ([Pd(Terpy)Cl]Cl, [Pd(B_H)Cl]Cl, [Pd[B_{Me})Cl]Cl, [Pd(B_{Et})Cl]Cl, [Pd(B_{Bu})Cl]Cl and [Pd_2(Tppz)Cl_2]Cl_2) were measured in a series of five sets of replicates with calf thymus DNA in ranges of concentrations that reached an excess ratio of 9:1 complex to DNA. Absorption changes in the spectra are indicative of successful binding. However the results were unquantifiable due to band overlaps, as explained below.

The solubilities of the substituted Pd(II) compounds were exceedingly low, thus giving very small absorbances in the 1cm pathlength cell of the UV/Vis spectrometer. In contrast the absorbance of the calf thymus DNA was extremely high due to its required large excess over Pd(II). The absorption bands of the Pd(II) complexes were masked by the absorption band of calf thymus DNA. The DNA provided a maximum peak at 260nm while the Pd(II) compounds absorbed at approximately 240nm, which was underlying the sides of the DNA peak. Statistically reproducible

spectral changes were unobtainable due to the absorption overlap. The changes observed in the spectra were consistent, however subtracting a small spectral change in Pd(II) complex from a large spectral absorbance of DNA resulted in unquantifiable results.

3.4. Octanol-Water Partition Coefficients

Octanol-Water partition coefficients (K_{OW}) are thermodynamic measurements that are useful in estimating bioaccumulation.[34] **Table 3.15** shows the results for the octanol-water partition coefficients.

Table 3.15 - K_{OW} Values

Compound	*K_{OW} Values*	*Percent Error*
[Pd(Terpy)Cl]Cl	0.0208	5%
[Pd(B_H)Cl]Cl	0.958	4%
[Pd(B_{Me})Cl]Cl	0.804	5%
[Pd(B_{Et})Cl]Cl	0.459	6%
[Pd(B_{Bu})Cl]Cl	2.57	3%
[Pd_2(Tppz)Cl_2]Cl_2	0.277	4%

Extending the length of the substituted carbon chain present on the ligand is expected to alter the hydrophobicity of the compound. It is expect that addition of carbons to the chain would increase the hydrophobicity of the complexes, thus making them more able to permeate to cellular membranes.

For [Pd(Terpy)Cl]Cl the value of K_{OW} is very small indicating that the compound is quite hydrophilic and so would not be expected to easily penetrate the hydrophobic lipid bilayer present

in cells. However upon examination of the *in vitro* cell line screening data (discussed in the next section) we observe that this complex is one of the more reactive complexes studied. It is speculated that despite its lower bioaccumulation, its cytotoxic effects once inside the cell are enhanced due to the rigidity of the compound and its ability to intercalate into the grooves of DNA, in addition to the nucleophilic substitution method of attack described in the kinetics section.

The addition of a phenyl ring to the [Pd(Terpy)Cl]Cl complex is expected to increase the hydrophobicity of the compound. This expected trend supported the observation of a dramatic increase in the K_{OW} value. The fact that the [Pd(B_H)Cl]Cl complex is non-planar may also be a contributing factor to the increased solubility of the complex in the non-aqueous layer. The interesting trend to make note of when comparing the two compounds is that the K_{OW} values indicate an increase in lipophilicity, however the *in vitro* cytotoxicity results exhibit an opposite correlation between the two.

With the introduction of longer carbon chains to the base compound [Pd(B_H)Cl]Cl a significant increase in hydrophobicity is expected. Upon experimentation, including error analysis, we observe the unexpected trend that the K_{OW} values for [Pd(B_H)Cl]Cl, [Pd(B_{Me})Cl]Cl and [Pd(B_{Et})Cl]Cl are very close suggesting very little difference in hydrophobicity and hence very little difference in bioaccumulation of the compounds. This trend also corresponds to the measured similar cytotoxicities of the three compounds. The striking feature is that they are all significantly more hydrophobic than the parent [Pd(Terpy)Cl]Cl complex.

The most interesting trend observed is the dramatic increase in the K_{OW} value for the [Pd(B_{Bu})Cl]Cl complex. We expect the compound to be more hydrophobic due to the extended carbon chain. This trend is clearly shown, exhibiting a drastic increase in hydrophobicity over and

above its H, Me, and Et congeners. Once again this correlates to the cytotoxicity of the complexes.

The $[Pd_2(Tppz)Cl_2]Cl_2$ complex is less soluble in aqueous solutions than its [Pd(Terpy)Cl]Cl counterpart. The fact that the K_{OW} value is increased by an order of magnitude in comparison is not unexpected given the extremely hydrophobic nature of the tppz ligand. Again, when comparing the octanol-water partition coefficients to the cytotoxicity of the compounds, the values correlate to the toxicity results acquired.

3.5. In Vitro Screening Results

The following section will discuss the results for the *in vitro* cell line screening of the series of six substituted Pd(II) compounds. Previous work (reference 8) presents preliminary screening experiments of [Pd(Terpy)Cl]Cl and $[Pd(B_H)Cl]Cl$. The results suggest that such compounds may be efficient as cytotoxic treatments for solid state tumors such as ovarian. Similar complexes of Au metal centers were previously reported to have similar results.[37]

This work provides a thorough analysis of all six compounds against solid state tumors that are effectively treated with similar drugs such as cisplatin. The following section will discuss the results for SKOV3 (human ovarian) and MCF7 (human breast) cancer cell lines. It should also be noted that HT-29 (human colon) cancer cells were also screened, however due to a cell line contamination there are unquantifiable results. Upon visual observation of the testing, the drugs exhibited similar cytotoxicity to this cell line in all cases.

For space conservation, only selected results will be presented in the following text. A full listing of the data, including statistical analysis, can be found in **Appendix D** for the ovarian cancer cell line and **Appendix F** for the breast cancer cell line. A complete graphical representation of the

results can be found in **Appendix E** for the ovarian cancer cell line and **Appendix G** for the breast cancer cell line. The positive values present on the graph correspond to % normal growth whereas the negative numbers correspond to % cell kill. (Note: The stars present on the graphical illustrations represent the P values for the Bonferroni post-tests. This test is used if the experimental drug and the control drug are determined to be statistically different. The Bonferroni method determines which concentrations of the drugs are statistically different and to what extent. One star is equivalent to a $P < 0.05$, two stars are equivalent to $P < 0.01$ and three stars are equivalent to $P < 0.001$.)

3.5.I. $[Pd_2(Tppz)Cl_2]Cl_2$

Although the $[Pd_2(Tppz)Cl_2]Cl_2$ compound is not a substituted terpyridyl complex, it was included in this work because it has been previously shown to interact with guanine by nucleophilic substitution and the inner-sphere coordination about the Pd ion is very similar having three aromatic N donors plus one Cl ligand.[7] This nucleophilic substitution is similar to cisplatin whereas the electrophilic metal center is electrostatically attracted to the N7 nitrogen of the guanine molecule. Based on this finding, it is speculated that $[Pd_2(Tppz)Cl_2]Cl_2$ may have similar potential to cisplatin as an anti-cancer drug. Furthermore, the complex does have two metal centers that parallel that present in the [Pd(Terpy)Cl]Cl and analogue complexes.

3.5.I.a. Ovarian Cancer Cell Line

Figure 3.10 and **Figure 3.11** represent the results for *in vitro* screening for time periods of 24 hours and 72 hours respectively.

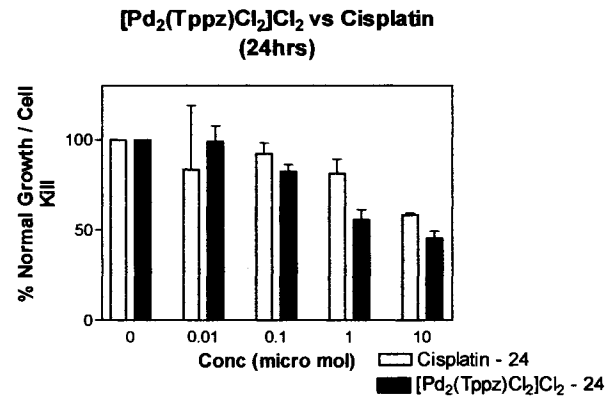


Figure 3.10 - $[Pd_2(Tppz)Cl_2]Cl_2$ *versus* cisplatin (24 hrs)

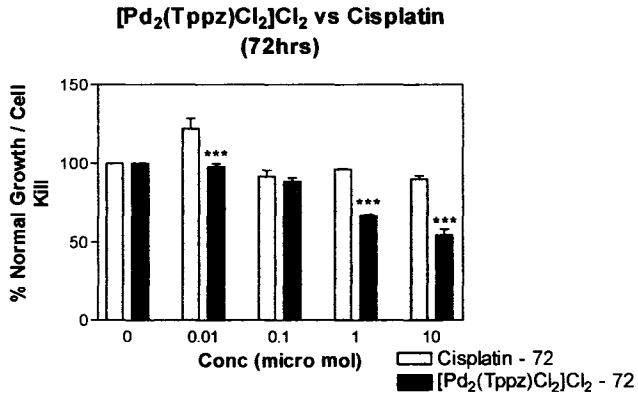


Figure 3.11 - $[Pd_2(Tppz)Cl_2]Cl_2$ *versus* cisplatin (72 hrs)

As illustrated, the $[Pd_2(Tppz)Cl_2]Cl_2$ complex is more reactive than the control cisplatin at both time trials and all concentrations. Both compounds are cytotoxic as growth inhibitors of the cancer cells. As stated above, we expect a similar mechanism of reaction to cisplatin, however the differences in reactivity could be explained by the structural differences of the two compounds. $[Pd_2(Tppz)Cl_2]Cl_2$ is a ditopic compound hence there is a possibility of two separate bonding sites whereas cisplatin has only one site of attack. A second possibility for the increase in reactivity is the planarity and rigidity of $[Pd_2(Tppz)Cl_2]Cl_2$. A possible intercalation mechanism could also be

speculated for the $[Pd_2(Tppz)Cl_2]Cl_2$ whereas cisplatin does not exhibit traits indicative of intercalation.

3.5.I.b. Breast Cancer Cell Line

Figure 3.12 and **Figure 3.13** represent the results for *in vitro* screening for time periods of 24 hours and 72 hours respectively.

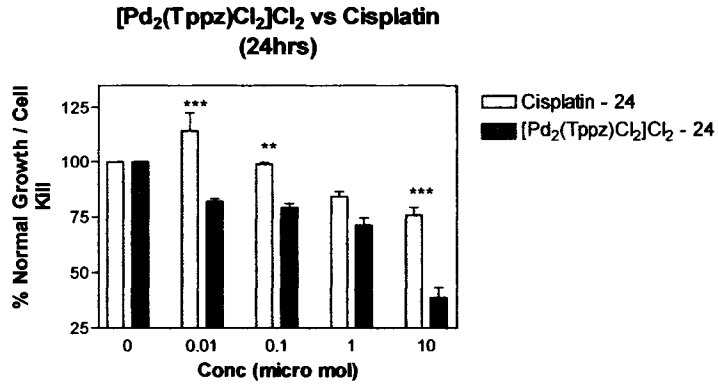


Figure 3.12 - $[Pd_2(Tppz)Cl_2]Cl_2$ *versus* cisplatin (24 hrs)

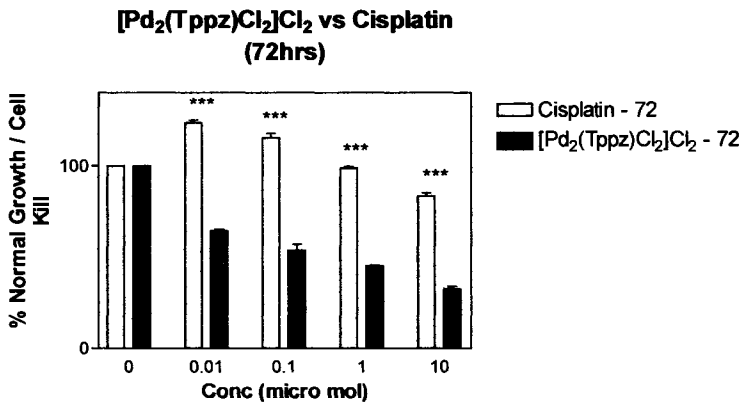


Figure 3.13 - $[Pd_2(Tppz)Cl_2]Cl_2$ *versus* cisplatin (72 hrs)

Similarly to the ovarian cancer cell line, $[Pd_2(Tppz)Cl_2]Cl_2$ is more reactive than the control cisplatin. It may be postulated that the $[Pd_2(Tppz)Cl_2]Cl_2$ complex, like the [Pd(Terpy)Cl]Cl complex, has a substituted site to coordinate to DNA base pairs, as well as a planar structure that enables intercalation, as described earlier. It should be noted that both compounds, $[Pd_2(Tppz)Cl_2]Cl_2$ and cisplatin, are slightly less reactive towards the breast cancer cell lines when compared to the ovarian cancer cell line. This is merely an observation and our chemical experiments do not provide an explanation.

3.5.II. [Pd(Terpy)Cl]Cl

[Pd(Terpy)Cl]Cl is the base compound from which the remaining five complexes were derived. Previous preliminary *in vitro* screening has shown [Pd(Terpy)Cl]Cl to be effective as a cytotoxic drug.[8] A complete analysis is provided for ovarian and breast cancer cells to represent a full dose response of the compound.

Previous work has also shown that [Pd(Terpy)Cl]Cl interacts with guanosine hydrate by nucleophilic substitution.[8] Similarly to $[Pd_2(Tppz)Cl_2]Cl_2$, [Pd(Terpy)Cl]Cl undergoes subsitution with guanine *via* a mechanism that is exhibited also by cisplatin, where the electrophilic metal center is electrostatically attracted to the N7 nitrogen of the guanine molecule. Based on this finding, it is speculated that [Pd(Terpy)Cl]Cl may have similar potential to cisplatin as an anti-cancer drug.

3.5.II.a. Ovarian Cancer Cell Line

Figure 3.14 and **Figure 3.15** represent the results for *in vitro* screening for time periods of 24 hours and 72 hours respectively.

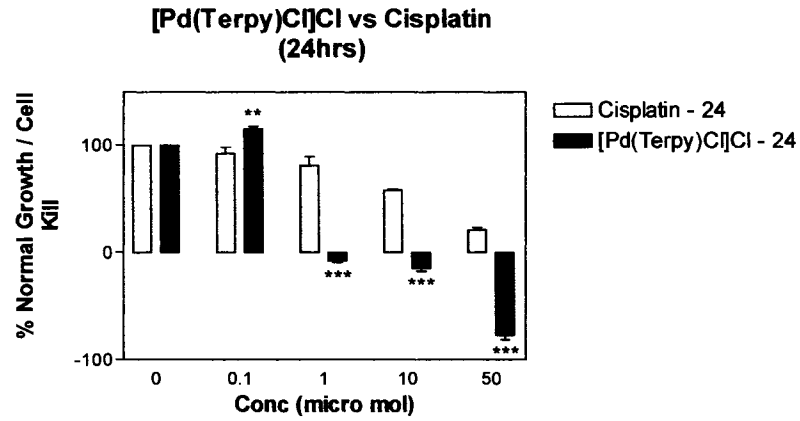


Figure 3.14 - [Pd(Terpy)Cl]Cl *versus* cisplatin (24 hrs)

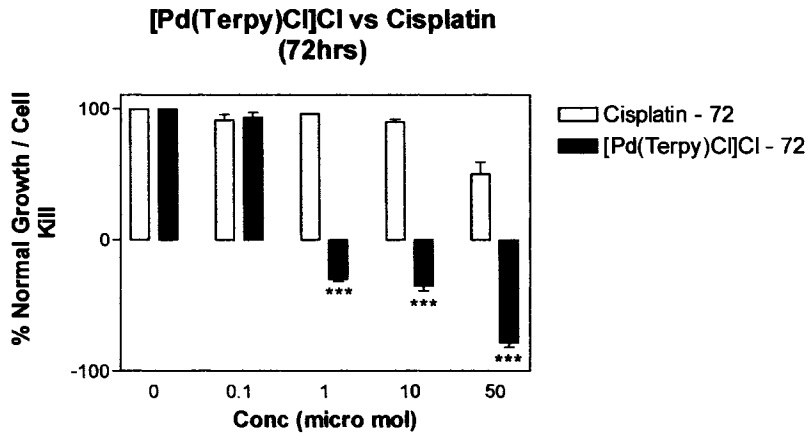


Figure 3.15 - [Pd(Terpy)Cl]Cl *versus* cisplatin (72 hrs)

The results for the screening of [Pd(Terpy)Cl]Cl are both surprising and promising. When compared to cisplatin, [Pd(Terpy)Cl]Cl is far more active as a cytotoxic agent. Cisplatin presents its activity based on growth inhibition, whereas [Pd(Terpy)Cl]Cl presents actual cell kill. Even at a time trial of 24 hours and a concentration of 0.1 micromolar, [Pd(Terpy)Cl]Cl presents approximately 50% cell kill whereas cisplatin allows for almost 100% normal growth of the cells.

As previously stated, a Pd based compound is expected to react at a faster rate than its Pt

based analogue due to its ability to attain equilibrium at a much faster rate. Palladium is more labile than platinum, and although the two metals form complexes with similar geometries, palladium(II) systems have been shown to exhibit ~ 10^6 times faster kinetics than their platinum (II) analogues.[17] This phenomenon is cleary supported by the screening data.

Both compounds have only one site for nucleophilic attack, so the drastic change in toxicity cannot be explained as it was in the case of $[Pd_2(Tppz)Cl_2]Cl_2$. In addition to this, [Pd(Terpy)Cl]Cl is planar and rigid, hence it is capable of an intercalation mechanism in addition to the anticipated nucleophilic attack. An intercalation mechanism would also explain the compound's high reactivity when comparing it to its low bioaccumulation (K_{OW}) value.

3.5.II.b. Breast Cancer Cell Line

Figure 3.16 and **Figure 3.17** represent the results for *in vitro* screening for time periods of 24 hours and 72 hours respectively.

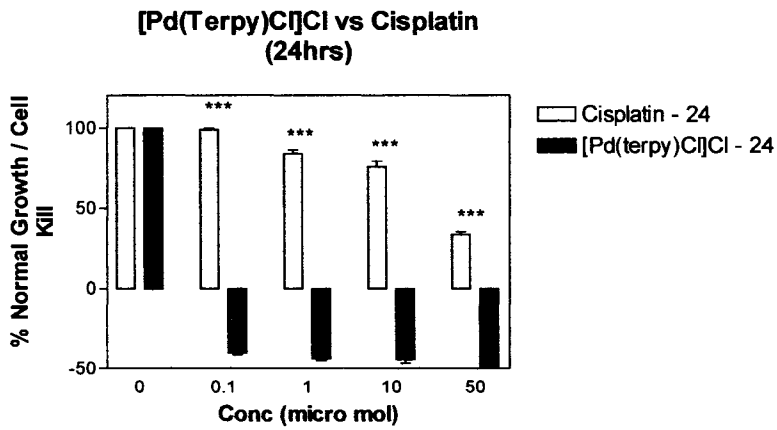


Figure 3.16 - [Pd(Terpy)Cl]Cl *versus* cisplatin (24 hrs)

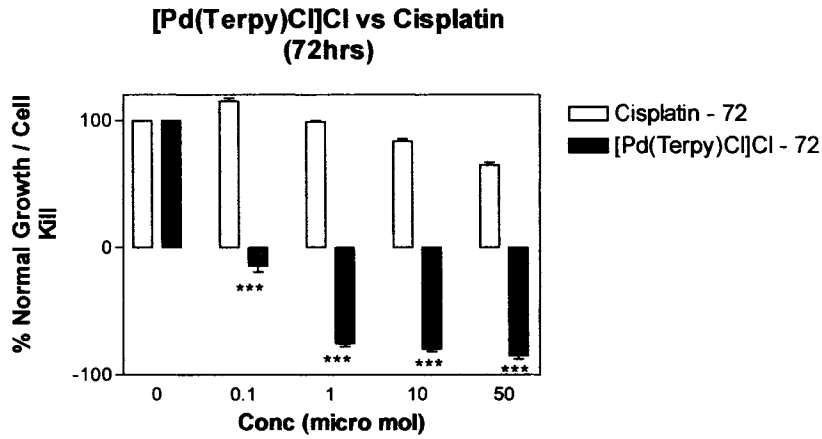


Figure 3.17 - [Pd(Terpy)Cl]Cl *versus* cisplatin (72 hrs)

[Pd(Terpy)Cl]Cl is more reactive than the control cisplatin. The discussion is very similar to the ovarian cancer cell line, however it should be noted that at low concentrations [Pd(Terpy)Cl]Cl is slightly less reactive towards the breast cancer cell lines when compared to the ovarian cancer cell line. However as you increase the concentration to fifty micro molar the reactivity is much more pronounced in the breast cancer cell line at a time trial of 24 hours.

3.5.III. [Pd(B_H)Cl]Cl

[Pd(B_H)Cl]Cl is the first compound in the series of substituted terpyridyl compounds synthesized for this work. [Pd(B_H)Cl]Cl and [Pd(Terpy)Cl]Cl differ by a single substituted phenyl ring thus eliminating the planarity of the compound and increasing its bioavailability. Previous preliminary *in vitro* screening has shown [Pd(B_H)Cl]Cl to be cytotixic against human ovarian breast cancer cells.[8] A complete analysis is provided for ovarian and breast cancer cells to represent a full dose response of the compound.

The preliminary screening results indicate that [Pd(B_H)Cl]Cl interacts with guanosine hydrate by nucleophilic substitution.[8] [Pd(B_H)Cl]Cl exhibits subsitution to guanosine through a mechanism that is expressed also by cisplatin, whereby the electrophilic metal center is electrostatically attracted to the N7 nitrogen of the guanosine molecule. Based on this finding, it is speculated that [Pd(B_H)Cl]Cl may have similar potential to cisplatin as an anti-cancer drug.

3.5.III.a. Ovarian Cancer Cell Line

Figure 3.18 and **Figure 3.19** represent the results for *in vitro* screening for time periods of 24 hours and 72 hours respectively.

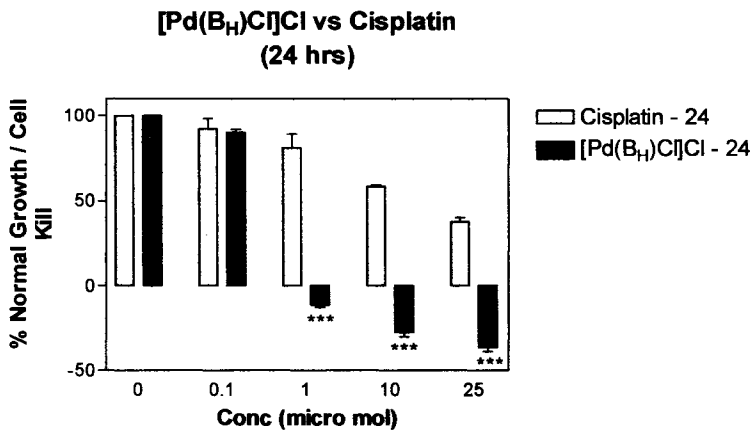


Figure 3.18 - [Pd(B_H)Cl]Cl *versus* cisplatin (24 hrs)

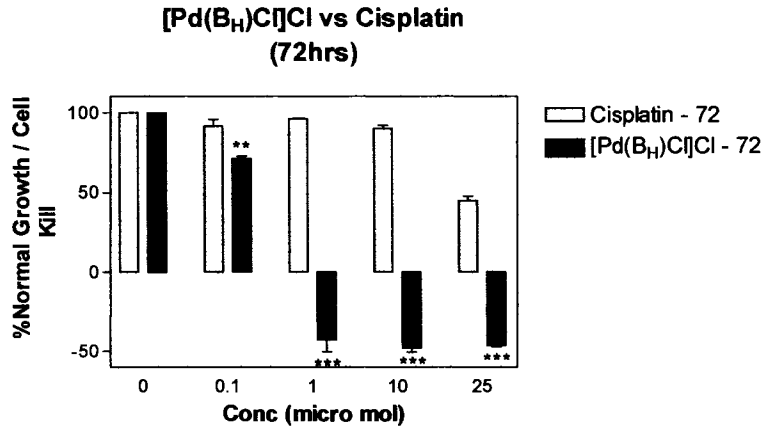


Figure 3.19 - [Pd(B_H)Cl]Cl *versus* cisplatin (72 hrs)

[Pd(B_H)Cl]Cl is much more effective as a cytotoxic agent than cisplatin as in the previous two compounds. Similarly to [Pd(Terpy)Cl]Cl, [Pd(B_H)Cl]Cl exhibits cell kill rather than simple growth inhibition as with cisplatin.

Based on the K_{OW} values, it is expected that [Pd(B_H)Cl]Cl would be a more effective cytotoxic agent because its bioavailability is much higher, however we see an opposite trend in the results. [Pd(B_H)Cl]Cl is less active than its [Pd(Terpy)Cl]Cl counterpart. It is speculated that the reason for the decrease in activity is due to the reduction of the planarity of the compound, hence lessening the intercalation reaction pathway.

3.5.III.b. Breast Cancer Cell Line

Figure 3.20 and **Figure 3.21** represent the results for *in vitro* screening for time periods of 24 hours and 72 hours respectively.

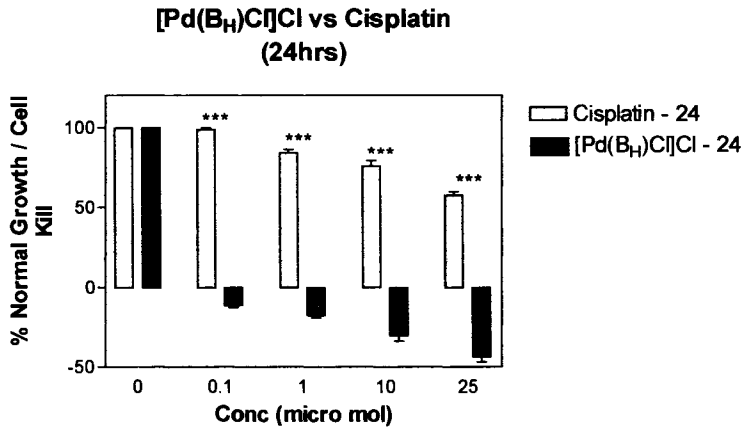


Figure 3.20 - [Pd(B_H)Cl]Cl *versus* cisplatin (24 hrs)

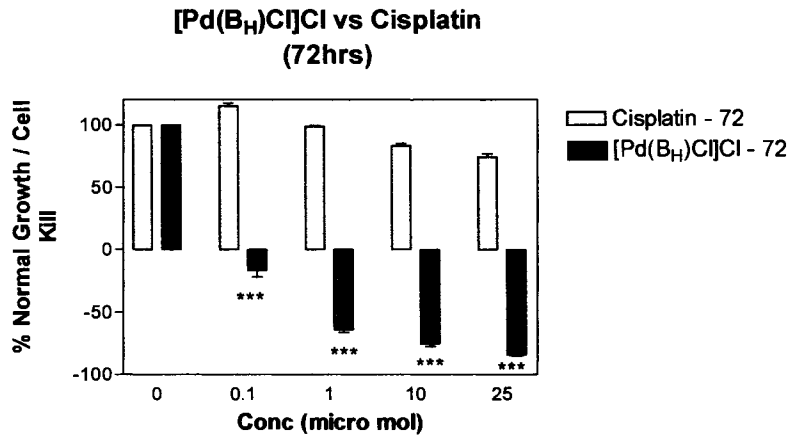


Figure 3.21 - [Pd(B_H)Cl]Cl *versus* cisplatin (72 hrs)

Similarly to the ovarian cancer cell line, [Pd(B_H)Cl]Cl is more reactive than the control cisplatin. This may be explained as it was for the previous complexes, however it should be noted that at concentrations of 0.1μM [Pd(B_H)Cl]Cl is slightly less reactive towards the breast cancer cell lines when compared to the ovarian cancer cell line. This trend is very similar to that expressed by the [Pd(Terpy)Cl]Cl complex.

3.5.IV. [Pd(B_{Me})Cl]Cl

[Pd(B_{Me})Cl]Cl is the second compound in the series of substituted terpyridyl compounds synthesized for this work. [Pd(B_H)Cl]Cl and [Pd(B_{Me})Cl]Cl differ by a single substituted methyl group in the para position on the phenyl ring. This extra methyl group was expected to increase the bioavailability, however the K_{OW} values for both compounds are very similar, hence are expected to exhibit similar cytotoxicities.

The kinetics performed in this work indicate that [Pd(B_{Me})Cl]Cl interacts with guanosine hydrate by nucleophilic substitution. [Pd(B_{Me})Cl]Cl exhibits subsitution to guanosine through a mechanism that is exhibited also by cisplatin, whereby the electrophilic metal center is electrostatically attracted to the N7 nitrogen of the guanosine molecule. As with the other complexes discussed above it is speculated that [Pd(B_{Me})Cl]Cl may have similar potential to cisplatin as an anti-cancer drug. A complete analysis is provided for ovarian and breast cancer cells to represent a full dose response of the compound.

3.5.IV.a. Ovarian Cancer Cell Line

Figure 3.22 and **Figure 3.23** represent the results for *in vitro* screening for time periods of 24 hours and 72 hours respectively.

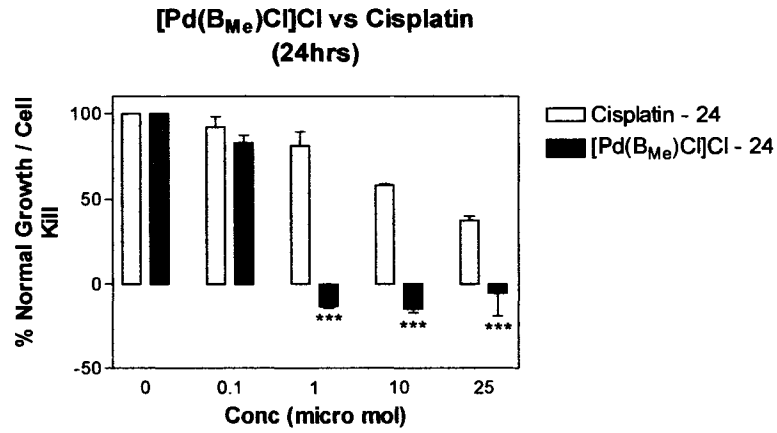


Figure 3.22 - [Pd(B_{Me})Cl]Cl *versus* cisplatin (24 hrs)

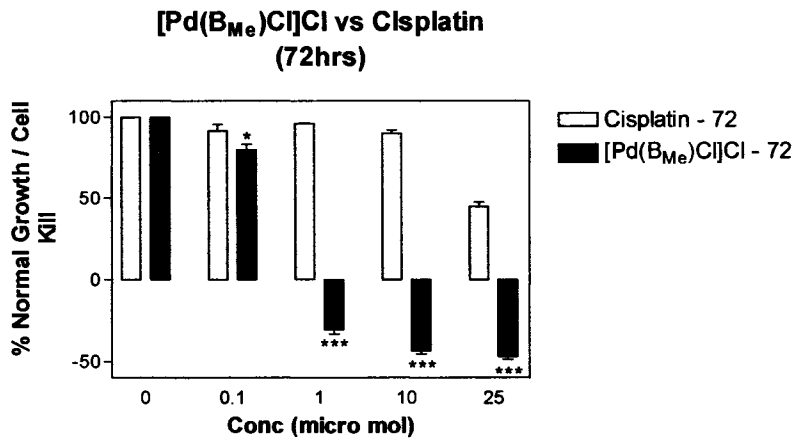


Figure 3.23 - [Pd(B_{Me})Cl]Cl *versus* cisplatin (72 hrs)

Interestingly, the activity for [Pd(B_{Me})Cl]Cl is slightly less than its [Pd(B_H)Cl]Cl congener. Since the site of reaction is virtually identical in the congeners, it is speculated that the methyl group adds added sterics, hence reducing its activity slightly when compared to [Pd(B_H)Cl]Cl.

At 24 hours [Pd(B_{Me})Cl]Cl reacts similarly to cisplatin, exhibiting activity based on growth inhibition. Increasing the time period to 72 hours results in actual cell kill. Although effective on a shorter time scale, the activity increases as the time of exposure increases.

3.5.IV.b. Breast Cancer Cell Line

Figure 3.24 and **Figure 3.25** represent the results for *in vitro* screening for time periods of 24 hours and 72 hours respectively.

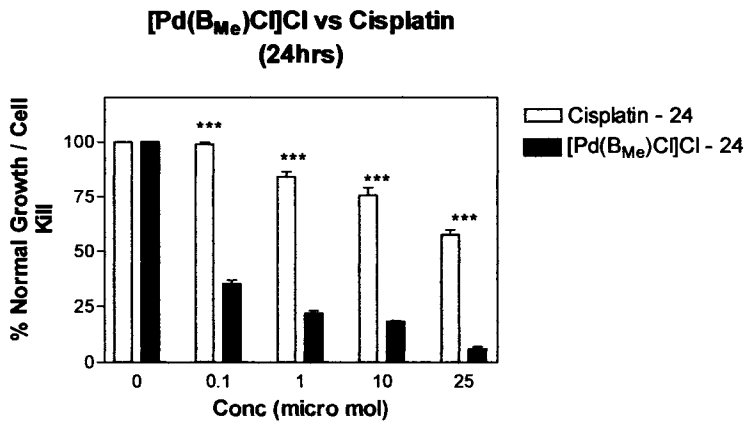


Figure 3.24 - [Pd(B_{Me})Cl]Cl *versus* cisplatin (24 hrs)

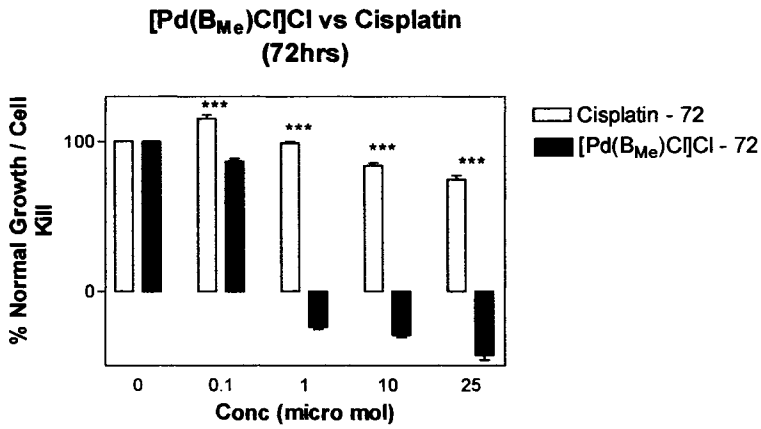


Figure 3.25 - [Pd(B_{Me})Cl]Cl *versus* cisplatin (72 hrs)

Once again, [Pd(B_{Me})Cl]Cl is more reactive than the control cisplatin. This can be explained as was with the ovarian cancer cell line discussed above. This compound has the distinguishing feature that it is more reactive towards breast cancer cells than it is towards ovarian cancer cells. The

previous compounds discussed exhibited the opposite trend in reactivity. At all time trials there exists cell kill with the breast cancer cell line whereas for 24 and 48 hours there proved to be only growth inhibition exhibited with the ovarian cancer cell line.

3.5.V. [Pd(B_{Et})Cl]Cl

[Pd(B_{Et})Cl]Cl is the third substituted terpyridyl compound in the series synthesized for this work. The introduction of an ethyl group was expected to increase the bioavailability, relative to the methyl substituted congener. However the K_{OW} values for [Pd(B_H)Cl]Cl, [Pd(B_{Me})Cl]Cl and [Pd(B_{Et})Cl]Cl are very similar, therefore they are expected to exhibit similar cytotoxicities.

Similarly to [Pd(B_{Me})Cl]Cl, the kinetics indicate that [Pd(B_{Et})Cl]Cl interacts with guanosine hydrate by nucleophilic substitution. [Pd(B_{Et})Cl]Cl undergoes nucleophilic subsitution to guanosine similarly to cisplatin, whereby the electrophilic metal center is electrostatically attracted to the N7 nitrogen of the guanosine molecule. As with the other complexes discussed above it is speculated that [Pd(B_{Et})Cl]Cl may have potential as an anti-cancer drug. A complete analysis is provided for ovarian and breast cancer cells to represent a full dose response of the compound.

3.5.V.a. Ovarian Cancer Cell Line

Figure 3.26 and **Figure 3.27** represent the results for *in vitro* screening for time periods of 24 hours and 72 hours respectively.

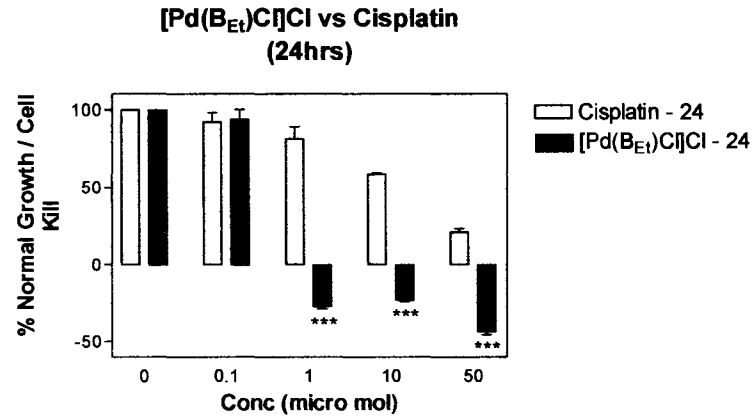


Figure 3.26 - [Pd(B$_{Et}$)Cl]Cl *versus* cisplatin (24 hrs)

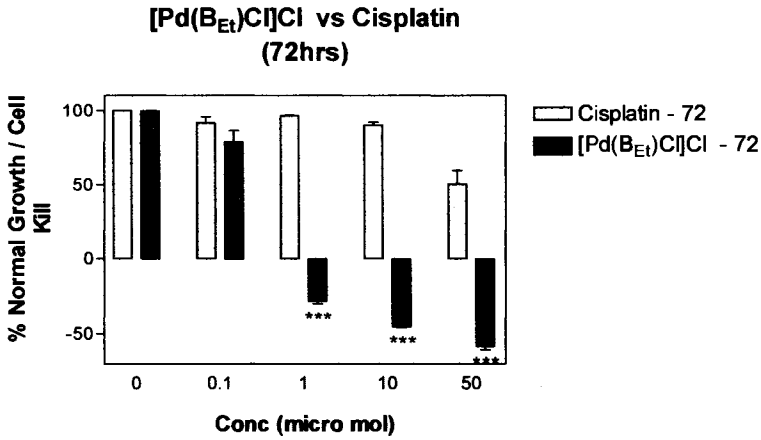


Figure 3.27 - [Pd(B$_{Et}$)Cl]Cl *versus* cisplatin (72 hrs)

The activity for [Pd(B$_{Et}$)Cl]Cl is very similar to its [Pd(B$_{Me}$)Cl]Cl congener. It is apparent that the presence of an ethyl group has very little effect towards the cytotoxicity of the compound.

At 24 hours [Pd(B$_{Et}$)Cl]Cl reacts similarly to cisplatin, exhibiting activity based on growth inhibition. Increasing the time period to 72 hours results in actual cell kill. Although effective on a short time scale, the activity increases proportionally to the time of exposure of the drug to the cells.

3.5.V.b. Breast Cancer Cell Line

Figure 3.28 and **Figure 3.29** represent the results for *in vitro* screening for time periods of 24 hours and 72 hours respectively.

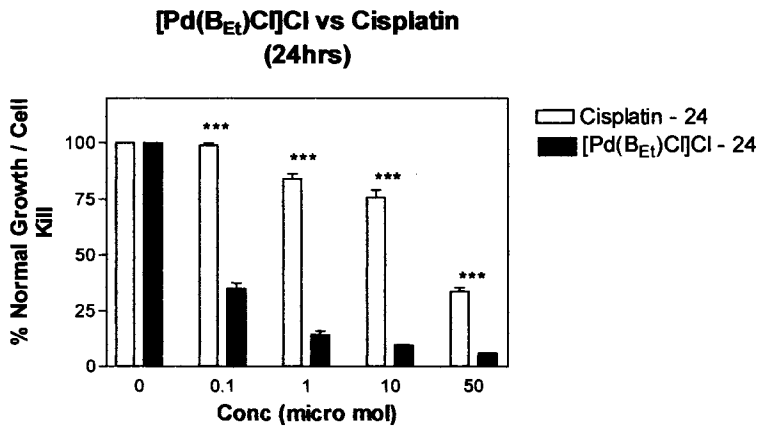


Figure 3.28 - [Pd(B_{Et})Cl]Cl *versus* cisplatin (24 hrs)

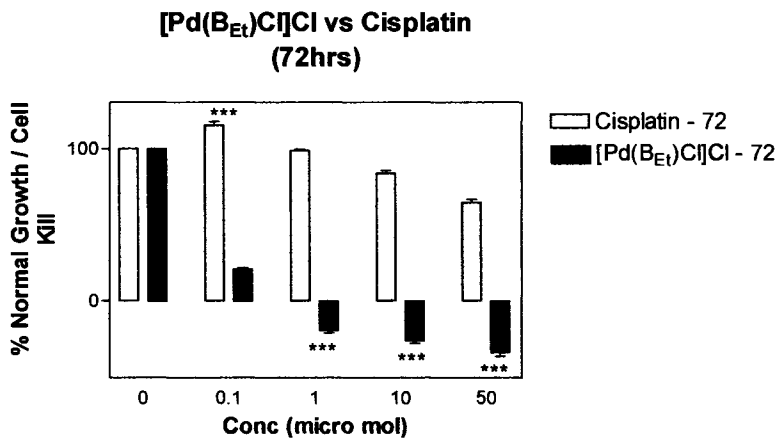


Figure 3.29 - [Pd(B_{Et})Cl]Cl *versus* cisplatin (72 hrs)

[Pd(B_{Et})Cl]Cl is more reactive than the control cisplatin for the breast cancer cell line. The discussion is similar to the ovarian cancer cell line, as above. This compound is similar to [Pd(B_{Me})Cl]Cl in that it is more reactive towards breast cancer cells than it is towards ovarian cancer

cells. The previous compounds discussed, with the exception of [Pd(B_{Me})Cl]Cl, exhibited the opposite trend in reactivity. For [Pd(B_{Et})Cl]Cl, at all time trials there exists cell kill with the breast cancer cell line whereas for 24 and 48 hours proved only to exhibit only growth inhibition with the ovarian cancer cell line.

3.5.VI. [Pd(B_{Bu})Cl]Cl

[Pd(B_{Bu})Cl]Cl is our final substituted terpyridyl compound in the series synthesized for this work. The extended carbon chain has been shown to greatly increase the compound's bioavailability, clearly expressed by the drastic increase in the K_{OW} value. Based on its estimated bioavailability, the compound is expected to exhibit a very high cytotoxicity within cells.

The kinetics of interaction performed in this work indicate that [Pd(B_{Bu})Cl]Cl interacts with guanosine hydrate by nucleophilic substitution. [Pd(B_{Bu})Cl]Cl exhibits a mechanistic subsitution reaction to guanosine similar to cisplatin, whereby the electrophilic metal center is electrostatically attracted to the N7 nitrogen of the guanosine molecule. As with the other complexes discussed above it is speculated that [Pd(B_{Bu})Cl]Cl may have potential as an anti-cancer drug. A complete analysis is provided for ovarian and breast cancer cells to represent a full dose response of the compound.

3.5.VI.a. Ovarian Cancer Cell Line

Figure 3.30 and **Figure 3.31** represent the results for *in vitro* screening for time periods of 24 hours and 72 hours respectively.

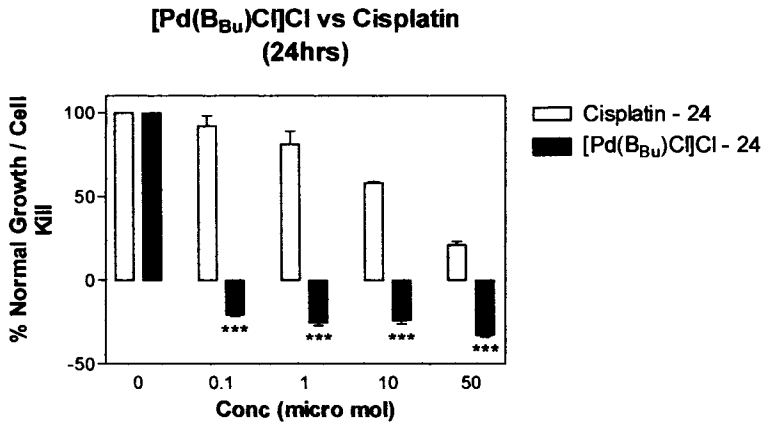


Figure 3.30 - [Pd(B_{Bu})Cl]Cl *versus* cisplatin (24 hrs)

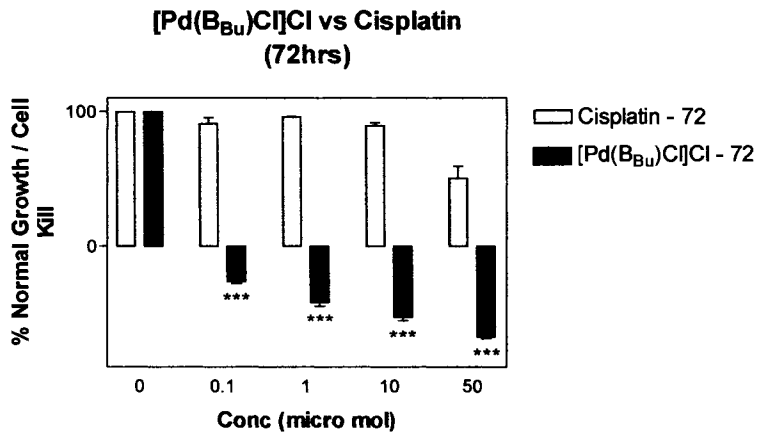


Figure 3.31 - [Pd(B_{Bu})Cl]Cl *versus* cisplatin (72 hrs)

The cytotoxicity of [Pd(B_{Bu})Cl]Cl is very similar to that of [Pd(Terpy)Cl]Cl. The high bioavailability of [Pd(B_{Bu})Cl]Cl seems to compensate for the lack of an intercalation mechanism exhibited by [Pd(Terpy)Cl]Cl.

This compound is the striking feature of the entirety of this work. It supports our hypothesis that increasing the carbon chain on the ligand increases the compounds bioavailability and hence increasing the cytotoxicity of the complex.

3.5.VI.b. Breast Cancer Cell Line

Figure 3.32 and **Figure 3.33** represent the results for *in vitro* screening for time periods of 24 hours and 72 hours respectively.

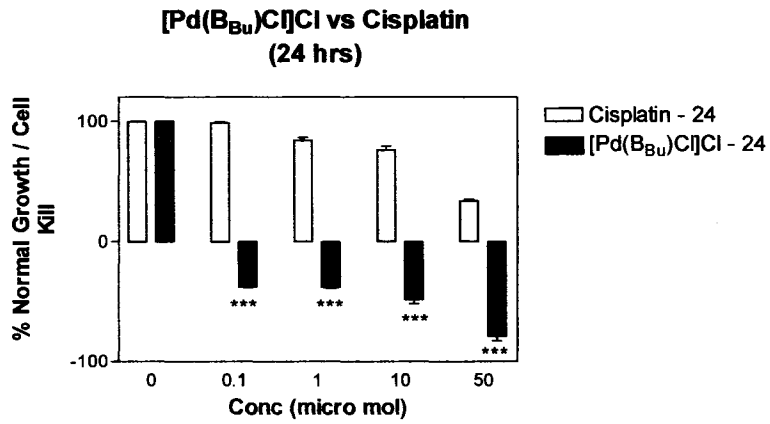


Figure 3.32 - [Pd(B_{Bu})Cl]Cl *versus* cisplatin (24 hrs)

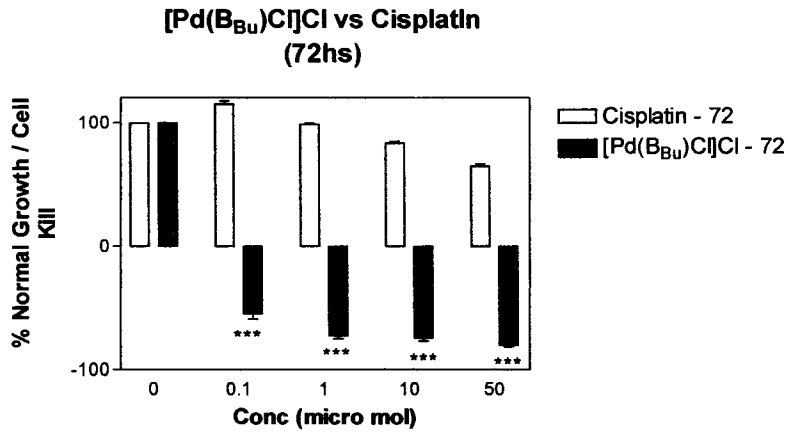


Figure 3.33 - [Pd(B_{Bu})Cl]Cl *versus* cisplatin (72 hrs)

Once again, the results are similar to those of [Pd(Terpy)Cl]Cl, however [Pd(B_{Bu})Cl]Cl exhibits a greater cytotoxicity towards the breast cancer cell line at lower concentrations. The ability to reduce the required concentration to provide similar reactivity may be the most important feature that this compound exhibits.

3.6. Future Work

The six Pd(II) compounds used in this project all exhibit promising potential as possible chemotherapeutic agents. Although this work is only the beginning, the palladium(II) complexes have shown to be successful at inhibiting / killing cancer. Future work would involve an expansion of this family of drugs including a more defined family of binuclear Pd(II) compounds to examine the effects of dual reaction sites in similar compounds.

Experiments to quantify the amount of drug actively being taken into the cells is desirable. A possible method would be to lyse the cells after the desired incubation period and measure the lysate for Pd content using methods such as atomic adsorption.

A more in depth study of *in vitro* screening against various types of cancer would also be desired. A larger series of cell lines and time trials would determine the range of cancers that these drugs could possibly treat and give us a greater insight to their efficacy.

In vitro testing against normal human cells would also be beneficial to determine whether these drugs are targeted towards cancer cells or simply kill cells in general. After extensive *in vitro* testing, clinical trials would begin by using *in vivo* models (rats, mice, pigs, etc) would model the compounds for human application.

Complexation with cyclodextrins is also worth investigation. A cyclodextrin forms a host/guest complex with lipophilic compounds making them more soluble in aqueous solutions. The cyclodextrins can act as drug carriers until the compounds reach the plasma membrane of the target cell. Once at the cell membrane, the drug enters the cell leaving the cyclodextrin behind in intracellular fluid which will be excreted at a later time. The cyclodextrins do not increase drug activity, but instead aid in delivery.

4. Conclusions

In conclusion, a series of Pd(II) analogues of cisplatin have been successfully synthesized and characterized using ^{1}H NMR, ^{13}C NMR, FTIR, ESMS, and C,H,N analysis. The Pd(II) compounds are composed of tridentate polypyridyl chelate ligands with one chloro ligand attached to the palladium metal. A related binuclear compound previously synthesized was also used in the studies as a comparison for a binuclear *versus* mononuclear compound.

The kinetics of reaction of guanosine hydrate with $[Pd(Terpy)Cl]Cl^{+}$, $[Pd(B_{H})Cl]Cl^{+}$, $[Pd(B_{Me})Cl]Cl^{+}$, $[Pd(B_{Et})Cl]Cl^{+}$ and $[Pd(B_{Bu})Cl]Cl^{+}$ complex cations were studied under pseudo-first-order conditions using stopped-flow spectrometry. The studies provided a proposed mechanism of a parallel associative and dissociative pathways for all five compounds. The rate law for each compound was determined to be ; Rate = $k_{obs}[Pd(ligand)Cl]^{+} = (k_1 + k_2[Gu])[Pd]$. The differences in ligand structure for each compound were determined to be insignificant when describing the rate of reaction or mechanism of nucleophilic substitution. From the temperature dependence of $[Pd(Terpy)Cl]Cl^{+}$, $[Pd(B_{H})Cl]Cl^{+}$ and $[Pd(B_{Bu})Cl]Cl^{+}$, activation parameters were calculated using the Eyring equation. These values supported the proposed two pathway mechanism of interaction.

The five substituted Pd(II) terpy compounds and the binuclear Pd(II) tppz compound were subjected to reaction with calf thymus DNA. Although there absorbance change was unquantifiable, spectral changes indicated a similar reaction with each Pd(II) compound.

Octanol-water partition coefficients were calculated for each of the Pd(II) compounds.The base terpy compound presented the lowest value at 0.02, whereas the B_{H}, B_{Me}, and B_{Et} compounds were very similar. The striking result was the drastic increase with the B_{Bu} ligand to 2.57 indicating that the increase in length of the chain drastically increased the hydrophobicity.

The six Pd(II) compounds were subject to *in vitro* screening against human ovarian cancer, human breast cancer and human colon cancer. All six compounds presented significant cytotoxic effects towards all three cell lines. The significant increase in cytotoxicity by [Pd(Terpy)Cl]Cl, relative to the other palladium(II) complexes, is explained by a secondary mechanism of intercalation due its rigid structure. The similar high toxicity of [Pd(B_{Bu})Cl]Cl is explained by an increased uptake by the cells as predicted by the water-octanol partition coefficients.

5. References

1- Ahu, S.; Matilla, A.; Tercero, J.M.; Vijayaragavan, V.; and Walmsley, J.A. *Inorg. Chim. Acta,* **2004**, *357*, 411-420

2- Anku, V. *What to Know About the Treatment of Cancer*; Madrona, Seattle, **1984**

3- Blasko, J.C.; and Grimm, P.D. *Int. J. Rad. Oncol. Biol. Phys.* **2000**, *46*, 839-850

4- Bruice, P.Y. *Organic Chemistry*, 3rd ed; Prentice Hall, New Jersey, **2001**

5- Canadian Cancer Association, January **2005**, www.cancer.ca

6- Constable, E.C.; Harverson, P.; Smith, D.R.; and Whall, L.A. *Tetrahedron.* **1994**, *50*(26), 7799-7806

7- Corcoran, A.; and Haines, R.I. *Inorg. React. Mech.* **2006**, *6*, 83-9

8- Corcoran, A. Honors dissertation, UPEI, **2004**

9- Cusumano, M; DiPietro, M. L.; Giannetto, A.; and Romano, F. *Inorg. Chem.* **2000**, *39*, 50-55

10- Cusumano, M.; DiPietro, M.L; and Giannetto A. *Inorg. Chem.* **1999**, *38*, 1754-1758

11- Franks, L.M.; and Teich, N.M. *Introduction to the Cellular and Molecular Biology of Cancer*. Oxford University Press, New York, **1997**

12- Freshney, R.I. *Culture of Animal cells - A Manual of Basic Technique* 4 ed. Wiley-Liss, New York, **2000**

13- Gao, J.; Reibenspies, J.H.; and Martell, A.E. *J. Inorg. Biochem.* **2003**, *94*, 272-278

14- Giandomenico, C.M.; and Wong, E. *Chem. Rev.* **1999**, *99*, 2451-2466

15- Goodall, W.; Wild, K.; Arm, K.J.; and Williams, G. *J. Chem. Soc.* **2002**, *2*, 1669-1681

16- Guminski, A.D., Harnett, P.R.; and deFazio, A. *Lancet Oncol.* **2002**, *3*, 312-318

17- Hay, R.W.; *Reaction Mechanism of Inorganic and Organometallic Systems*. Oxford Press, New York, **1991**

18- Hay, R.W. *Reaction Mechanisms of Metal Complexes*. Horwood Publishing Ltd, England, **2000**

19- Jing, B.; Zhang, M.; and Shen. T. *Spectrochim. Acta Part A*. **2004**, *60*, 2635-2641

20- Jordan, R.B. *Reaction Mechanism of Inorganic and Organometallic Systems*. Oxford Press, New York, **1991**

21- Keepers, Y.P,; Pizao, P.E.; Peters, G.J.; Van Ark-Otte, J.; Winograd, B.; and Pinedo, H.M. *Eur. J. Cancer*. **1991**, *27*, 897-900

22- Keppler, B.K. ed. *Metal Complexes in Cancer Chemotherapy*. VCH Publishers, New York, **1993**

23- King, R.J.B. *Cancer Biology*. Wesley Longman, England, **1996**

24- Lippard, S.J. Jamieson, E.R. *Chem. Rev.* **1999**, *9*, 2467-2495

25- Lippert, B. Ed. *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*. Verlag Hervetica Chimica Acta, Toronto, **1999**

26- Liu S.M. Website, 1991, http://www2.mrc-lmb.cam.ac.uk/personal/sl/Html/Mechanism.html #pt-DNA

27- McGowan, M.A.D.; and McGowan, P.C. *Inorg. Chem. Comm.* **2000**, 337

28- McGuire, W.L. *Cancer Chemotherapy 1*. Martinus Nijhoff, London, **1983**

29- Miller J.P. Website, http://www.jonathanpmiller.com/intercalation/

30- Moawad, M. M.; Anis, S. S.; and Eskander, M. L. *Transition Met. Chem.* **2001**, *26*, 50-56

31- Monks, A.; Scudiero, D.; Skehan, P.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Viagro-Wolff, A.; Gra-Goodrich, M.; Campbell, H.; Mayo, J.; and Boyd, M. *J. Nat. Canc. Inst.* **1991**, *83*, 757-766

32- Moore, D.S.; McCabe G.P. *Introduction to the Practice of Statistics, 4th ed.* W.H. Freeman and Company, United States of America, **2003**

33- Nagy, Z.; Fabian,I.; Benyei, A.; and Sovago,I. *J. Inorg. Biochem.* **2003**, *94*, 291-299

34- Ropel,L; Belveze, L.S.; Aki, S.M.V.K.; Stadtherr, M.A.; and Brennecke, J.F. *Green Chem*, **2005**, 7(Advance Article)

35- Rubinstein, L.V.; Shoemaker, R.H.; Paull, K.D.; Simon, R.M.; Tosini, S.; Skehan, P.; Scudiero, D.A.; Monks, A.; Boyd, M.R. *J. Nat. Can. Inst.* **1990**, *82*, 1113-1118

36- Schilt, A.A.; Smith, F.G.; and Heimbuch, *A. Anal. Chem.* **1956**, *28*, 809

37- Shi, P.; Jiang,Q.; Zhao,Y.; Yangmiao, Z.; Lin, J.; Lin, L; Ding, J.; and Guo, Z. *J. Biol. Inorg. Chem.* **2006**, *11*, 745-752

38- Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J.T.; Bokesch, H.; Kenney, S.; and Boyd, M.R. *J. Nat. Canc. Inst.* **1990**, *82*, 1107-112

39- Stillwater Palladium, 2004, http://wwwstillwaterpalladium.com

40- Stryker, J.A. *Radiation Oncology*. Medical Examination, New York, **1985**

41- Tannock, I.F.; and Hill, R.P., *The Basic Science of Oncology 2 ed.* McGraw-Hill, New York, **1992**

42- Tobe, M.L.; and Burgess, J. *Inorg. React. Mech.* Addison Wesley Longman Ltd, England, **1999**

43- Van Eldik, R.; Bugarcic, Z. C.; and Liehr, G. *J. Chem. Soc.* **2002**, 2825-2830

44- Van Eldik, R.; and Kotowski, M.. *Inorg. Chem.* **1986**, *25*, 3896-3899

45- Wang, X.; Chao, H.; Li, H.; Hong, X.; Liu, Y.; Tan, L.; and Ji, L. *J. Inorg. Biochem.* **2004**, *98*, 1143-1150

46- Wheate, N.J.; and Collins, J.G. *Coord. Chem. Rev.* **2003**, *241*, 133-145

48- Yasuda, T.; Yamaguchi, I.; and Yamamoto, T. *Adv. Mat.* **2006**, *14(4)*, 293-296

49- Zhang, C.X.; and Lippard, S.J. *Curr. Opin. Chem. Bio.* **2003**, *7*, 481-489

Appendix A - *Solvents and Chemicals*

Chemical	*Supplier*
2-Acetylpyridine	Aldrich
2,2',6',2"-Terpyridyl	Aldrich
4-Ethylbenzaldehyde	Aldrich
4-n-butylbenzaldehyde	Caledon
Acetic Acid	Fisher
Acetone	Caledon
Ammonium Acetate	BDH
Benzaldehyde	BDH
Calf Thymus DNA	Sigma
Celite	Fisher
Chloroform	Commercial Alcohols INC.
cis-Diamminedichloroplatinum(II)	Aldrich
Deuterated Chloroform	CDN Isotopes
Deuterated Dimethyl Sulphoxide	CDN Isotopes
Dichloromethane	Caledon
DMSO (biological grade)	Caledon
Ethanol	Commercial Alcohols INC.
Fetal Bovine Serum	CanSera
Glacial Acetic Acid	Fisher
Guanosine Hydrate	Aldrich
Hydrochloric Acid	Caledon
Isopropanol	Caledon
L-Glutamine	Sigma
Methanol	Caledon

Octanol	Sigma
p-Tolualdehyde	Aldrich
Petroleum Ether	Caledon
Potassium Chloride	Fisher
Potassium Phosphate (K_2HPO_4)	Fisher
RPMI-1640 Medium	Sigma
Sodium Hydroxide	BDH
Sodium Chloride	Sigma
Sodium tetrachloropalladate(II)	Aldrich
Sodium Phosphate (Na_2HPO_4)	Fisher
Sulforhodamine B	Sigma
Tris Buffer	Fisher
Trypsin	Sigma

Appendix B - *Sample Well-Plate Scheme*

Control Plate: Measures initial drug effects at time = 0.

	1	2	3	4	5	6	7	8	9	10	11	12
A	M	M	M	M	M	M	M	M	M	M	M	M
B	M	50a	50a	25b	25b	25c	25c	50d	50d	50e	50e	M
C	M	50a	50a	25b	25b	25c	25c	50d	50d	50e	50e	M
D	M	a-	a-	b-	b-	c-	c-	d-	d-	e-	e-	M
E	M	a-	a-	b-	b-	c-	c-	d-	d-	e-	e-	M
F	M	md+	md+	m+	m+	cis	cis	cis-	cis-	10f	f-	M
G	M	md+	md+	m+	m+	cis	cis	cis-	cis-	10f	f-	M
H	M	M	M	M	M	M	M	M	M	M	M	M

Legend:

M = Media Only

md+ = Media, 0.5% DMSO, cells

50a = 50 μL drug A (in appropriate Media/DMSO solution), cells

25b = 25 μL drug B (in appropriate Media/DMSO solution), cells

25c = 25 μL drug C (in appropriate Media/DMSO solution), cells

50d = 50 μL drug D (in appropriate Media/DMSO solution), cells

50e = 50 μL drug E (in appropriate Media/DMSO solution), cells

10f = 10 μL drug F (in appropriate Media/DMSO solution), cells

cis = 50 μL cisplatin (in appropriate Media/DMSO solution), cells

m+ = Media, cells

a- = 50 μL drug A (in appropriate Media/DMSO solution), no cells

b- = 25 μL drug B (in appropriate Media/DMSO solution), no cells

c- = 25 μL drug C (in appropriate Media/DMSO solution), no cells

d- = 50 μL drug D (in appropriate Media/DMSO solution), no cells

e- = 50 μL drug E (in appropriate Media/DMSO solution), no cells

f- = 10 μL drug F (in appropriate Media/DMSO solution), no cells

cis- = 50 μL cisplatin (in appropriate Media/DMSO solution), no cells

Sample Test Plate: 24 hour exposure time.

	1	2	3	4	5	6	7	8	9	10	11	12
A	M	M	M	M	M	M	M	M	M	M	M	M
B	M	m-	m-	50a	50a	10a	10a	1a	1a	0.1a	0.1a	M
C	M	m-	m-	50a	50a	10a	10a	1a	1a	0.1a	0.1a	M
D	M	m+	m+	50b	50b	10b	10b	1b	1b	0.1b	0.1b	M
E	M	m+	m+	50b	50b	10b	10b	1b	1b	0.1b	0.1b	M
F	M	md+	md+	50c	50c	10c	10c	1c	1c	0.1c	0.1c	M
G	M	md+	md+	50c	50c	10c	10c	1c	1c	0.1c	0.1c	M
H	M	M	M	M	M	M	M	M	M	M	M	M

Legend:

M = Media only

m- = media, no cells

m+ = media, cells

md+ = media, 0.5% DMSO, cells

50= 50 μL drug A (in appropriate Media/DMSO solution), cells

50= 50 μL drug B (in appropriate Media/DMSO solution), cells

50 = 50 μL cisplatin (in appropriate Media/DMSO solution), cells

10a = 10 μL drug A (in appropriate Media/DMSO solution), cells

10b = 10 μL drug B (in appropriate Media/DMSO solution), cells

10c = 10 μL cisplatin (in appropriate Media/DMSO solution), cells

1a = 1 μL drug A (in appropriate Media/DMSO solution), cells

1b = 1 μL drug B (in appropriate Media/DMSO solution), cells

1c = 1 μL cisplatin (in appropriate Media/DMSO solution), cells

0.1a = 0.1 μL drug A (in appropriate Media/DMSO solution), cells

0.1b = 0.1 μL drug B (in appropriate Media/DMSO solution), cells

0.1c = 0.1 μL cisplatin (in appropriate Media/DMSO solution), cells

Appendix C - Kinetic Data

Table C1 - [Pd(Terpy)Cl]Cl Kinetic Data (Taken from reference 8)					
Temperature	*287.9 °K*	*291.5 °K*	*298.0 °K*	*302.4 °K*	*307.2 °K*
[Gu] (M)	k_{obs} (s^{-1})	k_{obs} (s^{-1})	k_{obs} (s^{-1})	k_{obs} (s^{-1})	k_{obs} (s^{-1})
6.30×10^{-5}	17.5	21.5	30.0	37.0	47.5
1.25×10^{-4}	26.0	32.5	45.5	53.0	67.0
1.88×10^{-4}	35.0	43.0	63.0	72.0	87.5
2.50×10^{-4}	44.5	55.0	77.5	90.0	110
k_1 (s^{-1})	8.25	10.3	13.9	18.5	25.8
k_2 ($M^{-1}s^{-1}$)	1.44×10^5	1.78×10^5	2.56×10^5	2.85×10^5	3.33×10^5

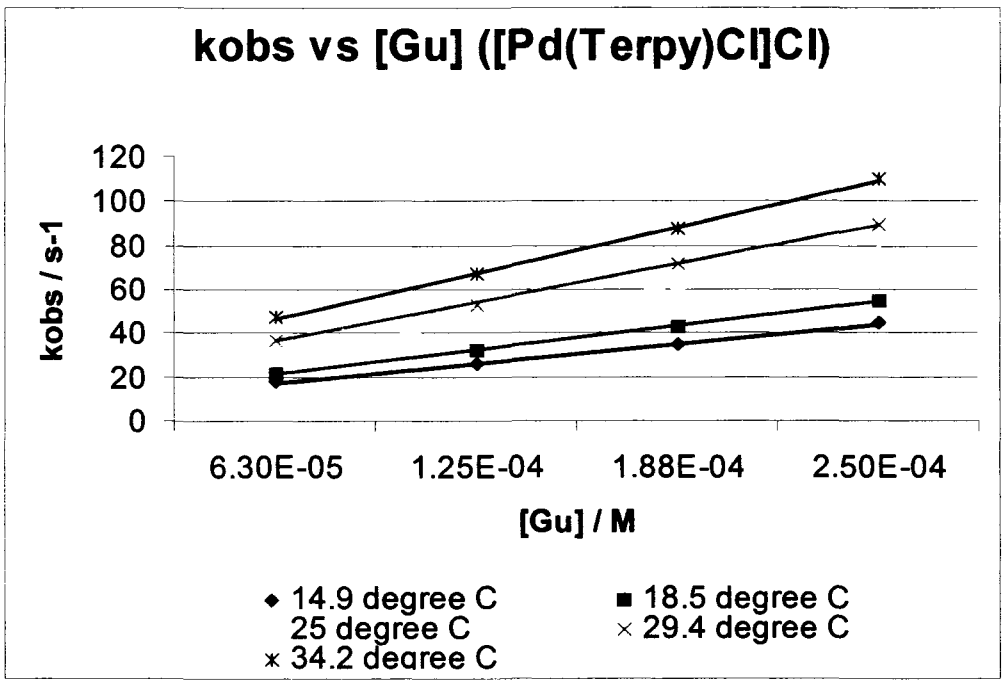


Figure C1 - Plot of k_{obs} *versus* [Gu] for [Pd(Terpy)Cl]Cl (Taken from reference 8)

Table C2 - [Pd(B_H)Cl]Cl Kinetic Data (Taken from reference 8)					
Temperature	*287.2 °K*	*292.0 °K*	*298.0 °K*	*302.6 °K*	*307.2 °K*
[Gu] (M)	k_{obs} (s^{-1})	k_{obs} (s^{-1})	k_{obs} (s^{-1})	k_{obs} (s^{-1})	k_{obs} (s^{-1})
6.30×10^{-5}	9.25	12.4	17.1	21.1	27.4
1.25×10^{-4}	13.2	18.6	24.5	30.0	35.2
1.88×10^{-4}	16.6	23.2	30.0	39.1	46.1
2.50×10^{-4}	20.5	29.8	37.2	46.0	54.4
k_1 (s^{-1})	5.57	6.76	10.8	13.2	17.8
k_2 ($M^{-1}s^{-1}$)	5.95×10^4	9.10×10^4	1.05×10^5	1.33×10^5	1.47×10^5

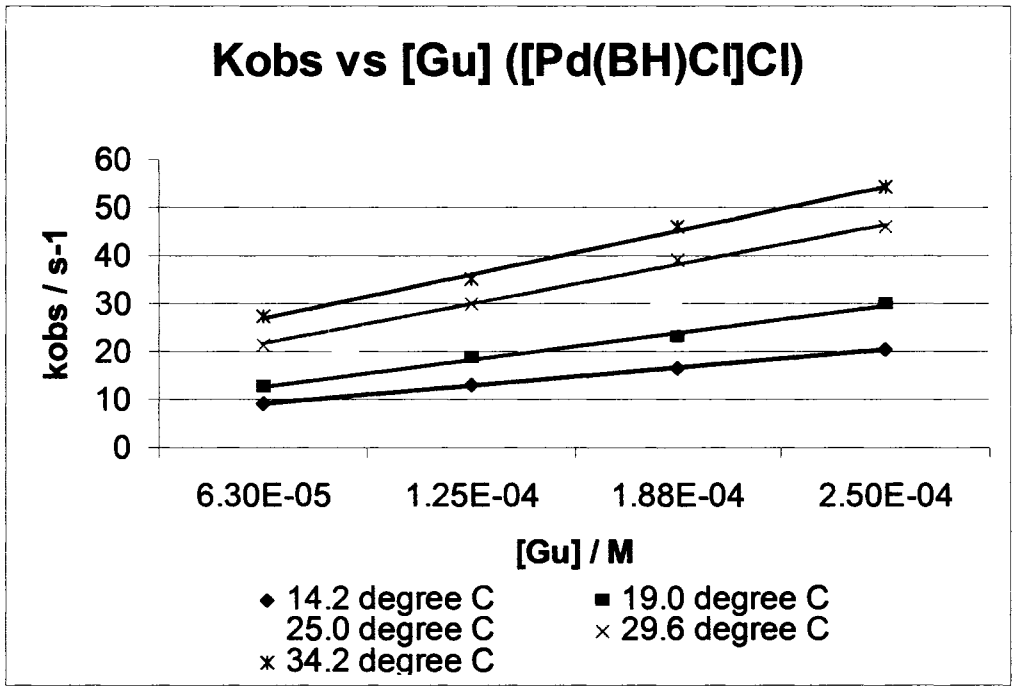


Figure C2 - Plot of k_{obs} *versus* [Gu] for [Pd(B_H)Cl]Cl (Taken from reference 8)

Table C3 - [Pd(Terpy)Cl]Cl - ln(k/T) *versus* 1/T values (Taken from reference 8)					
T (°K)	*1/T (K^{-1})*	*k_1 (s^{-1})*	*ln (k_1/T)*	*k_2 ($M^{-1}s^{-1}$)*	*ln (k_2/T)*
287.9	3.47 x 10^{-3}	8.25	-3.55	1.44 x 10^5	6.22
291.5	3.43 x 10^{-3}	10.3	-3.34	1.78 x 10^5	6.41
298.0	3.36 x 10^{-3}	13.9	-3.07	2.56 x 10^5	6.76
302.4	3.31 x 10^{-3}	18.5	-2.80	2.85 x 10^5	6.85
307.2	3.26 x 10^{-3}	25.8	-2.48	3.33 x 10^5	6.99
Y-int		13.1		18.6	
Slope		-4.79 x 10^3		-3.56 x 10^3	

Table C4 - [Pd(B_H)Cl]Cl - ln(k/T) *versus* 1/T values (Taken from reference 8)					
T (°K)	*1/T (K^{-1})*	*k_1 (s^{-1})*	*ln (k_1/T)*	*k_2 ($M^{-1}s^{-1}$)*	*ln (k_2/T)*
287.2	3.48 x 10^{-3}	5.57	-3.94	5.95 x 10^4	5.33
292.0	3.42 x 10^{-3}	6.76	-3.77	9.10 x 10^4	5.74
298.0	3.36 x 10^{-3}	10.8	-3.32	1.05 x 10^5	5.87
302.6	3.30 x 10^{-3}	13.2	-3.13	1.33 x 10^5	6.09
307.2	3.26 x 10^{-3}	17.8	-2.85	1.47 x 10^5	6.17
Y-int		13.2		17.7	
Slope		-4.93 x 10^3		-3.54 x 10^3	

Appendix D - *Human Ovarian Cancer (SKOV3) Cell Line Screening Data*

D.I. $[Pd_2(Tppz)Cl_2]Cl_2$ Screening Results

Table D1 - $[Pd_2(Tppz)Cl_2]Cl_2$ – 24 hrs Data

Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.150 (SD=0.019)			T_0 = 0.285 (SD=0.056)			T_0 = 0.166 (SD=0.065)		
	Ctrl = 0.205 (SD=0.035)			Ctrl = 0.386 (SD=0.013)			Ctrl = 0.257 (SD=0.041)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
10.0	0.174	0.001	43.6	0.326	0.028	40.6	0.214	0.008	52.7
1.00	0.183	0.007	60.0	0.330	0.029	44.6	0.223	0.021	62.6
0.100	0.199	0.016	89.1	0.368	0.022	82.2	0.235	0.016	75.8
0.0100	0.214	0.018	116	0.379	0.016	93.1	0.246	0.031	87.9

Table D2 - $[Pd_2(Tppz)Cl_2]Cl_2$ – 48 hrs Data

Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.150 (SD=0.019)			T_0 = 0.285 (SD=0.056)			T_0 = 0.166 (SD=0.065)		
	Ctrl = 0.662 (SD=0.079)			Ctrl = 0.635 (SD=0.048)			Ctrl = 0.610 (SD=0.122)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
10.0	0.326	0.008	34.4	0.412	0.010	36.3	0.318	0.016	34.2
1.00	0.474	0.004	63.3	0.492	0.029	59.1	0.460	0.005	66.2
0.100	0.479	0.005	64.3	0.515	0.008	65.7	0.444	0.015	62.6
0.0100	0.527	0.017	73.6	0.585	0.010	85.7	0.587	0.026	94.8

Table D3 - $[Pd_2(Tppz)Cl_2]Cl_2$ – 72 hrs Data

Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.150 (SD=0.019)			T_0 = 0.285 (SD=0.056)			T_0 = 0.166 (SD=0.065)		
	Ctrl = 0.724 (SD=0.055)			Ctrl = 0.912 (SD=0.034)			Ctrl = 0.865 (SD=0.095)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*

10.0	0.421	0.015	47.2	0.654	0.020	58.9	0.568	0.005	57.5
1.00	0.526	0.015	65.5	0.699	0.006	66.0	0.641	0.008	68.0
0.100	0.684	0.010	93.0	0.825	0.014	86.1	0.768	0.014	86.1
0.0100	0.726	0.024	100	0.906	0.025	99.0	0.823	0.019	94.0

Table D4 - $[Pd_2(Tppz)Cl_2]Cl_2$ – Data Summary

conc (μmol)	*% Growth Ave*	*SD (error)*	*Bonferroni*
Time = 24 hrs			
10.0	45.7	6.32	P > 0.050
1.00	55.7	9.77	P > 0.050
0.100	82.4	6.63	P > 0.050
0.0100	99.1	15.1	P > 0.050
Time = 48 hrs			
10.0	27.9	1.15	P < 0.010
1.00	48.2	3.55	P < 0.050
0.100	47.3	1.55	P < 0.050
0.0100	61.2	10.6	P > 0.050
Time = 72 hrs			
10.0	54.5	6.37	P < 0.001
1.00	66.5	1.29	P < 0.001
0.100	88.4	4.00	P > 0.050
0.0100	97.8	3.36	P < 0.001

Table D5 - $[Pd_2(Tppz)Cl_2]Cl_2$ – Statistical Data

	24 hrs			*48 hrs*			*72 hrs*		
	%Var (Tot)	*P val*	*Sig*	*%Var (Tot)*	*P val*	*Sig*	*%Var (Tot)*	*P val*	*Sig*
Interaction	7.160	0.5005	N	7.650	0.0391	Y	15.46	<0.0001	Y

Concentration	45.24	0.0272	Y	67.6	<0.0001	Y	53.30	<0.0001	Y
Drug	1.620	0.3880	N	15.78	0.0002	Y	26.04	<0.0001	Y

D.II. [Pd(Terpy)Cl]Cl Screening Results

Table D6 - [Pd(Terpy)Cl]Cl – 24 hrs Data

Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.150 (SD=0.019)			T_0 = 0.285 (SD=0.056)			T_0 = 0.166 (SD=0.065)		
	Ctrl = 0.275 (SD=0.114)			Ctrl = 0.385 (SD=0.029)			Ctrl = 0.228 (SD=0.057)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
50.0	0.039	0.004	-74.0	0.043	0.001	-84.9	0.047	0.007	-71.7
10.0	0.130	0.016	-13.3	0.229	0.027	-19.6	0.147	0.008	-11.4
1.00	0.142	0.011	-5.30	0.266	0.010	-6.70	0.148	0.023	-10.8
0.100	0.295	0.011	116.0	0.397	0.065	112.0	0.240	0.011	119.4

Table D7 - [Pd(Terpy)Cl]Cl – 48 hrs Data

Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.150 (SD=0.019)			T_0 = 0.285 (SD=0.056)			T_0 = 0.166 (SD=0.065)		
	Ctrl = 0.620 (SD=0.131)			Ctrl = 0.692 (SD=0.024)			Ctrl = 0.562 (SD=0.064)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
50.0	0.040	0.005	-73.3	0.046	0.003	-83.9	0.043	0.004	-74.1
10.0	0.120	0.014	-20.0	0.205	0.026	-28.1	0.124	0.009	-25.3
1.00	0.137	0.017	-8.70	0.257	0.030	-9.80	0.146	0.037	-12.0
0.100	0.593	0.028	94.3	0.733	0.062	110.1	0.477	0.040	78.5

Table D8 - [Pd(Terpy)Cl]Cl – 72 hrs Data									
Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.150 (SD=0.019)			T_0 = 0.285 (SD=0.056)			T_0 = 0.166 (SD=0.065)		
	Ctrl = 0.780 (SD=0.023)			Ctrl = 0.990 (SD=0.010)			Ctrl = 0.847 (SD=0.084)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
50.0	0.041	0.001	-72.7	0.043	0.006	-84.9	0.037	0.002	-77.7
10.0	0.104	0.006	-30.7	0.163	0.012	-42.8	0.112	0.010	-32.5
1.00	0.109	0.020	-27.3	0.196	0.011	-31.2	0.112	0.013	-32.5
0.100	0.771	0.016	98.6	0.892	0.044	86.1	0.817	0.036	95.6

Table D9 - [Pd(Terpy)Cl]Cl – Results Summary			
conc (μmol)	*% Growth Ave*	*SD (error)*	*Bonferroni*
Time = 24 hrs			
50.0	-76.9	7.06	$P < 0.001$
10.0	-14.8	4.30	$P < 0.001$
1.00	-7.6	2.87	$P < 0.001$
0.100	115	3.68	$P < 0.010$
Time = 48 hrs			
50.0	-77.1	5.87	$P < 0.001$
10.0	-24.5	4.10	$P < 0.001$
1.00	-10.2	1.72	$P < 0.001$
0.100	94.3	15.7	$P > 0.050$
Time = 72 hrs			
50.0	-78.4	6.15	$P < 0.001$
10.0	-35.3	6.54	$P < 0.001$
1.00	-30.4	2.70	$P < 0.001$
0.100	93.4	6.51	$P > 0.050$

Table D10 - [Pd(Terpy)Cl]Cl – Statistial Data									
	24 hrs			*48 hrs*			*72 hrs*		
	%Var (Tot)	*P val*	*Sig*	*%Var (Tot)*	*P val*	*Sig*	*%Var (Tot)*	*P val*	*Sig*
Interaction	16.63	<0.0001	Y	17.90	<0.0001	Y	22.89	<0.0001	Y
Concentration	67.40	<0.0001	Y	57.13	<0.0001	Y	42.88	<0.0001	Y
Drug	15.23	<0.0001	Y	23.75	<0.0001	Y	33.53	<0.0001	Y

D.III. [Pd(B_H)Cl]Cl Screening Results

Table D11 - [Pd(B_H)Cl]Cl – 24 hrs Data									
Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.150 (SD=0.019)			T_0 = 0.285 (SD=0.056)			T_0 = 0.166 (SD=0.065)		
	Ctrl = 0.275 (SD=0.114)			Ctrl = 0.385 (SD=0.029)			Ctrl = 0.228 (SD=0.057)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
25.0	0.101	0.018	-32.7	0.169	0.019	-40.7	0.106	0.021	-36.1
10.0	0.109	0.015	-27.3	0.193	0.018	-32.3	0.128	0.006	-22.9
1.00	0.135	0.021	-10.0	0.245	0.038	-14.0	0.148	0.034	-10.8
0.100	0.267	0.007	93.6	0.375	0.057	90	0.220	0.043	87.1

Table D12 - [Pd(B_H)Cl]Cl – 48 hrs Data									
Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.150 (SD=0.019)			T_0 = 0.285 (SD=0.056)			T_0 = 0.166 (SD=0.065)		
	Ctrl = 0.620 (SD=0.131)			Ctrl = 0.692 (SD=0.024)			Ctrl = 0.562 (SD=0.064)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
25.0	0.088	0.006	-41.3	0.153	0.033	-46.3	0.103	0.012	-38.0
10.0	0.079	0.007	-47.3	0.147	0.018	-48.4	0.101	0.016	-39.2

1.00	0.104	0.027	-30.7	0.224	0.04	-21.4	0.121	0.017	-27.1
0.100	0.539	0.063	82.8	0.685	0.046	98.3	0.541	0.026	94.7

Table D13 - [Pd(B_H)Cl]Cl – 72 hrs Data

Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.150 (SD=0.019)			T_0 = 0.285 (SD=0.056)			T_0 = 0.166 (SD=0.065)		
	Ctrl = 0.780 (SD=0.023)			Ctrl = 0.990 (SD=0.010)			Ctrl = 0.847 (SD=0.084)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
25.0	0.082	0.008	-45.3	0.156	0.007	-45.3	0.086	0.015	-48.2
10.0	0.071	0.013	-52.7	0.149	0.009	-47.7	0.094	0.003	-43.4
1.00	0.065	0.007	-56.7	0.169	0.012	-40.1	0.114	0.008	-31.3
0.100	0.608	0.059	72.7	0.799	0.067	72.9	0.634	0.090	68.7

Table D14 - [Pd(B_H)Cl]Cl - Results Summary

conc (μmol)	*% Growth Ave*	*SD (error)*	*Bonferroni*
Time = 24 hrs			
25.0	-36.5	4.03	P < 0.001
10.0	-27.5	4.70	P < 0.001
1.00	-11.6	2.13	P < 0.001
0.100	90.2	3.26	P > 0.050
Time = 48 hrs			
25.0	-41.9	4.21	P < 0.001
10.0	-45.0	5.06	P < 0.001
1.00	-26.4	4.67	P < 0.001
0.100	91.9	8.12	P > 0.050
Time = 72 hrs			
25.0	-46.3	1.37	P < 0.001

10.0	-47.9	4.65	$P < 0.001$
1.00	-42.9	12.8	$P < 0.001$
0.100	71.4	2.36	$P < 0.010$

Table D15 - [Pd(B_H)Cl]Cl – Statistical Data

	24 hrs			*48 hrs*			*72 hrs*		
	%Var (Tot)	*P val*	*Sig*	*%Var (Tot)*	*P val*	*Sig*	*%Var (Tot)*	*P val*	*Sig*
Interaction	15.68	<0.0001	Y	19.65	<0.0001	Y	22.02	<0.0001	Y
Concentration	59.39	<0.0001	Y	52.79	<0.0001	Y	38.23	<0.0001	Y
Drug	24.00	<0.0001	Y	26.66	<0.0001	Y	39.27	<0.0001	Y

D.IV. [Pd(B_{Me})Cl]Cl Screening Results

Table D16 - [Pd(B_{Me})Cl]Cl – 24 hrs Data

Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.150 (SD=0.019)			T_0 = 0.285 (SD=0.056)			T_0 = 0.166 (SD=0.065)		
	Ctrl = 0.234 (SD=0.052)			Ctrl = 0.420 (SD=0.025)			Ctrl = 0.270 (SD=0.043)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
25.0	0.118	0.019	-21.3	0.243	0.031	-14.7	0.129	0.002	-22.3
10.0	0.121	0.026	-19.3	0.248	0.030	-13.0	0.146	0.020	-12.0
1.00	0.129	0.011	-14.0	0.254	0.021	-10.9	0.143	0.016	-13.9
0.100	0.227	0.034	91.7	0.390	0.031	77.8	0.249	0.013	79.8

Table D17 - [Pd(B_{Me})Cl]Cl – 48 hrs Data

Conc (μmol)	*Trial 1*	*Trial 2*	*Trial 3*
	T_0 = 0.150 (SD=0.019)	T_0 = 0.285 (SD=0.056)	T_0 = 0.166 (SD=0.065)
	Ctrl = 0.576 (SD=0.036)	Ctrl = 0.703 (SD=0.024)	Ctrl = 0.604 (SD=0.078)

	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
25.0	0.088	0.007	-41.3	0.173	0.038	-39.3	0.101	0.024	-39.2
10.0	0.078	0.017	-48.0	0.141	0.011	-50.5	0.092	0.017	-44.6
1.00	0.090	0.011	-40.0	0.152	0.017	-46.7	0.083	0.006	-50.0
0.100	0.518	0.192	86.4	0.603	0.042	76.1	0.515	0.059	79.7

Table D18 - [Pd(B_{Me})Cl]Cl – 72 hrs Data

Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.150 (SD=0.019)			T_0 = 0.285 (SD=0.056)			T_0 = 0.166 (SD=0.065)		
	Ctrl = 0.715 (SD=0.077)			Ctrl = 0.922 (SD=0.030)			Ctrl = 0.918 (SD=0.072)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
25.0	0.081	0.007	-46.0	0.142	0.009	-50.2	0.092	0.005	-44.6
10.0	0.09	0.011	-40.0	0.161	0.005	-43.5	0.088	0.004	-47.0
1.00	0.104	0.015	-60.7	0.186	0.029	-34.7	0.124	0.013	-25.3
0.100	0.597	0.057	79.1	0.835	0.068	86.3	0.720	0.052	73.7

Table D19 - [Pd(B_{Me})Cl]Cl - Results Summary

conc (μmol)	*% Growth Ave*	*SD (error)*	*Bonferroni*
Time = 24 hrs			
25.0	-19.5	4.11	$P < 0.001$
10.0	-14.8	3.96	$P < 0.001$
1.00	-12.9	1.76	$P < 0.001$
0.100	83.1	7.50	$P > 0.050$
Time = 48 hrs			
25.0	-39.9	1.22	$P < 0.001$
10.0	-47.7	3.00	$P < 0.001$
1.00	-45.6	5.09	$P < 0.001$

0.100	80.7	5.23	P > 0.050
Time = 72 hrs			
25.0	-46.9	2.91	P < 0.001
10.0	-43.5	3.49	P < 0.001
1.00	-30.2	4.73	P < 0.001
0.100	79.7	6.36	P < 0.050

Table D20 - [Pd(B_{Me})Cl]Cl – Statistical Data

	24 hrs			*48 hrs*			*72 hrs*		
	%Var (Tot)	*P val*	*Sig*	*%Var (Tot)*	*P val*	*Sig*	*%Var (Tot)*	*P val*	*Sig*
Interaction	15.63	<0.0001	Y	19.90	<0.0001	Y	22.11	<0.0001	Y
Concentration	58.25	<0.0001	Y	48.89	<0.0001	Y	40.84	<0.0001	Y
Drug	23.11	<0.0001	Y	30.49	<0.0001	Y	36.74	<0.0001	Y

D.V. [Pd(B_{Et})Cl]Cl Screening Results

Table D21 - [Pd(B_{Et})Cl]Cl – 24 hrs Data

Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.150 (SD=0.019)			T_0 = 0.285 (SD=0.056)			T_0 = 0.166 (SD=0.065)		
	Ctrl = 0.234 (SD=0.052)			Ctrl = 0.420 (SD=0.025)			Ctrl = 0.270 (SD=0.043)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
50.0	0.085	0.018	-43.3	0.171	0.026	-40.0	0.088	0.009	-47.0
10.0	0.114	0.048	-24.0	0.224	0.025	-21.4	0.126	0.023	-24.1
1.00	0.109	0.030	-27.3	0.216	0.063	-24.2	0.116	0.006	-30.1
0.100	0.239	0.090	106.0	0.409	0.046	91.9	0.253	0.008	83.7

Table D22 - [Pd(B_{Et})Cl]Cl – 48 hrs Data

Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.150 (SD=0.019)			T_0 = 0.285 (SD=0.056)			T_0 = 0.166 (SD=0.065)		
	Ctrl = 0.576 (SD=0.036)			Ctrl = 0.703 (SD=0.024)			Ctrl = 0.604 (SD=0.078)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
50.0	0.069	0.007	-54.0	0.122	0.008	-57.2	0.081	0.007	-51.2
10.0	0.103	0.017	-31.3	0.190	0.013	-33.3	0.108	0.016	-34.9
1.00	0.120	0.018	-20.0	0.220	0.020	-22.8	0.121	0.012	-27.1
0.100	0.534	0.145	90.1	0.702	0.014	99.8	0.488	0.035	73.5

Table D23 - [Pd(B_{Et})Cl]Cl – 72 hrs Data

Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.150 (SD=0.019)			T_0 = 0.285 (SD=0.056)			T_0 = 0.166 (SD=0.065)		
	Ctrl = 0.715 (SD=0.077)			Ctrl = 0.922 (SD=0.030)			Ctrl = 0.918 (SD=0.072)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
50.0	0.068	0.004	-54.7	0.119	0.002	-58.2	0.063	0.008	-62.0
10.0	0.083	0.007	-44.7	0.153	0.013	-46.3	0.092	0.003	-44.6
1.00	0.111	0.029	-26.0	0.195	0.029	-31.6	0.120	0.020	-27.7
0.100	0.678	0.068	93.5	0.752	0.004	73.3	0.694	0.028	70.2

Table D24 - [Pd(B_{Et})Cl]Cl - Results Summary

conc (μmol)	*% Growth Ave*	*SD (error)*	*Bonferroni*
Time = 24 hrs			
50.0	-43.4	3.50	$P < 0.001$
10.0	-23.2	1.53	$P < 0.001$
1.00	-27.2	2.96	$P < 0.001$
0.100	93.8	11.3	$P > 0.050$

Time = 48 hrs			
50.0	-54.1	3.00	P < 0.001
10.0	-33.2	1.81	P < 0.001
1.00	-23.3	3.58	P < 0.001
0.100	87.8	13.3	P > 0.050
Time = 72 hrs			
50.0	-58.3	3.69	P < 0.001
10.0	-45.2	0.99	P < 0.001
1.00	-28.4	2.86	P < 0.001
0.100	79.0	12.6	P > 0.050

Table D25- [Pd(B_{Et})Cl]Cl – Statistical Data

	24 hrs			*48 hrs*			*72 hrs*		
	%Var (Tot)	*P val*	*Sig*	*%Var (Tot)*	*P val*	*Sig*	*%Var (Tot)*	*P val*	*Sig*
Interaction	15.94	<0.0001	Y	17.83	<0.0001	Y	21.22	<0.0001	Y
Concentration	62.18	<0.0001	Y	54.28	<0.0001	Y	39.69	<0.0001	Y
Drug	20.87	<0.0001	Y	26.77	<0.0001	Y	37.73	<0.0001	Y

D.VI. [Pd(B_{Bu})Cl]Cl Screening Results

Table D26 - [Pd(B_{Bu})Cl]Cl – 24 hrs Data

Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.150 (SD=0.019)			T_0 = 0.285 (SD=0.056)			T_0 = 0.166 (SD=0.065)		
	Ctrl = 0.205 (SD=0.035)			Ctrl = 0.386 (SD=0.013)			Ctrl = 0.257 (SD=0.041)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
50.0	0.101	0.001	-32.7	0.199	0.028	-30.2	0.109	0.008	-34.3

10.0	0.121	0.007	-19.3	0.215	0.029	-24.6	0.121	0.021	-27.1
1.00	0.114	0.016	-24.0	0.219	0.022	-23.2	0.119	0.016	-28.3
0.100	0.120	0.018	-20.0	0.231	0.016	-18.9	0.130	0.031	-21.7

Table D27 - [Pd(B_{Bu})Cl]Cl – 48 hrs Data

Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.150 (SD=0.019)			T_0 = 0.285 (SD=0.056)			T_0 = 0.166 (SD=0.065)		
	Ctrl = 0.662 (SD=0.079)			Ctrl = 0.635 (SD=0.048)			Ctrl = 0.610 (SD=0.122)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
50.0	0.070	0.008	-53.3	0.115	0.010	-59.6	0.060	0.016	-63.9
10.0	0.086	0.004	-42.7	0.123	0.019	-56.8	0.080	0.005	-51.8
1.00	0.082	0.005	-45.3	0.129	0.008	-54.7	0.090	0.015	-45.8
0.100	0.104	0.017	-30.7	0.173	0.010	-39.3	0.102	0.026	-38.6

Table D28 - [Pd(B_{Bu})Cl]Cl – 72 hrs Data

Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.150 (SD=0.019)			T_0 = 0.285 (SD=0.056)			T_0 = 0.166 (SD=0.065)		
	Ctrl = 0.724 (SD=0.055)			Ctrl = 0.912 (SD=0.034)			Ctrl = 0.865 (SD=0.095)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
50.0	0.050	0.015	-66.7	0.086	0.020	-69.8	0.056	0.005	-66.3
10.0	0.064	0.015	-57.3	0.113	0.006	-53.3	0.086	0.008	-48.2
1.00	0.092	0.010	-38.7	0.150	0.014	-47.4	0.101	0.014	-39.2
0.100	0.107	0.024	-28.7	0.208	0.025	-27.0	0.126	0.019	-24.1

Table D29 - [Pd(B_{Bu})Cl]Cl - Results Summary

conc (μmol)	*% Growth Ave*	*SD (error)*	*Bonferroni*
Time = 24 hrs			
50.0	-32.4	2.09	$P < 0.001$

10.0	-23.7	3.96	P < 0.001
1.00	-25.2	2.77	P < 0.001
0.100	-20.2	1.38	P < 0.001
Time = 48 hrs			
50.0	-58.9	5.30	P < 0.001
10.0	-50.4	7.19	P < 0.001
1.00	-48.6	5.30	P < 0.001
0.100	-36.2	4.78	P < 0.001
Time = 72 hrs			
50.0	-67.6	1.95	P < 0.001
10.0	-53.0	4.58	P < 0.001
1.00	-41.7	4.89	P < 0.001
0.100	-29.6	2.31	P < 0.001

Table D30 - [Pd(B_{Bu})Cl]Cl – Statistical Data

	24 hrs			*48 hrs*			*72 hrs*		
	%Var (Tot)	*P val*	*Sig*	*%Var (Tot)*	*P val*	*Sig*	*%Var (Tot)*	*P val*	*Sig*
Interaction	14.32	<0.0001	Y	13.76	<0.0001	Y	14.90	<0.0001	Y
Concentration	42.25	<0.0001	Y	34.21	<0.0001	Y	27.38	<0.0001	Y
Drug	42.65	<0.0001	Y	51.28	<0.0001	Y	57.19	<0.0001	Y

Appendix E - *Illustration of Human Ovarian Cancer (SKOV3) Cell Line Screening Data*

E.I. $[Pd_2(Tppz)Cl_2]Cl_2$ versus Cisplatin

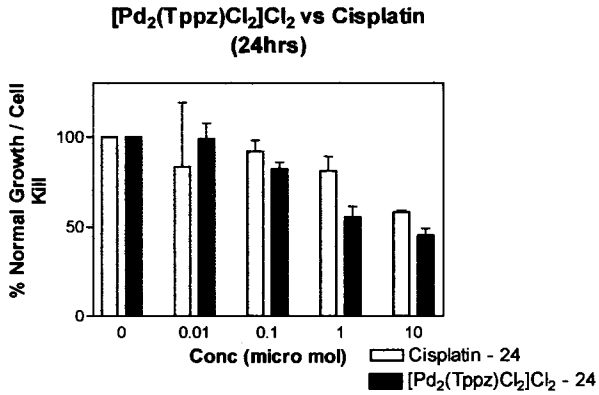


Figure E1 - $[Pd_2(Tppz)Cl_2]Cl_2$ *versus* cisplatin (24 hrs)

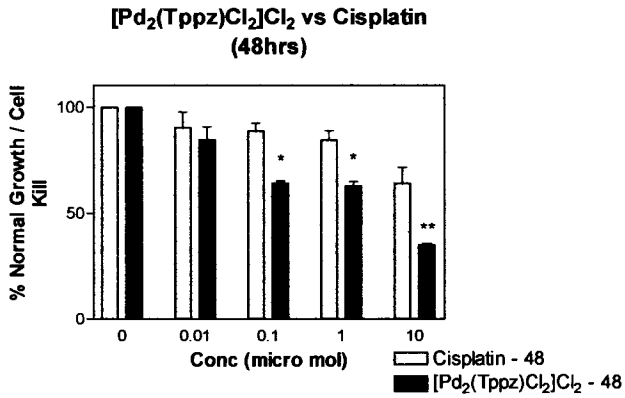


Figure E2 - $[Pd_2(Tppz)Cl_2]Cl_2$ *versus* cisplatin (48 hrs)

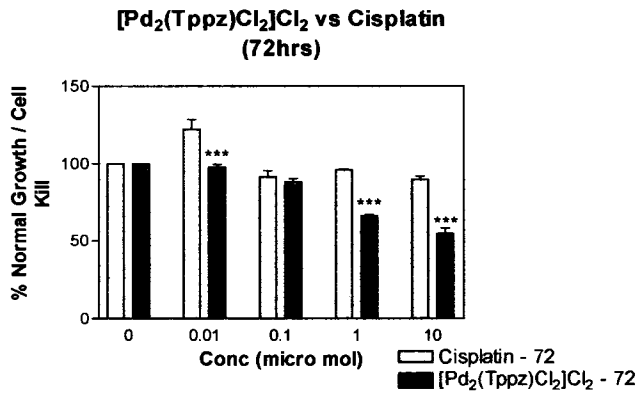


Figure E3 - $[Pd_2(Tppz)Cl_2]Cl_2$ *versus* cisplatin (72 hrs)

E.II. [Pd(Terpy)Cl]Cl versus Cisplatin

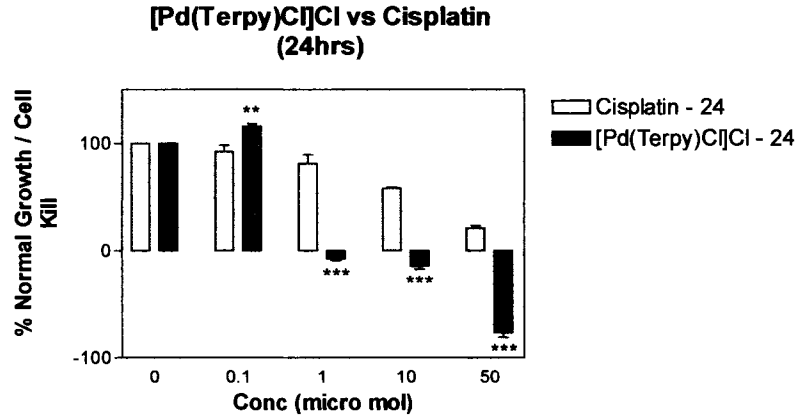


Figure E4 - [Pd(Terpy)Cl]Cl *versus* cisplatin (24 hrs)

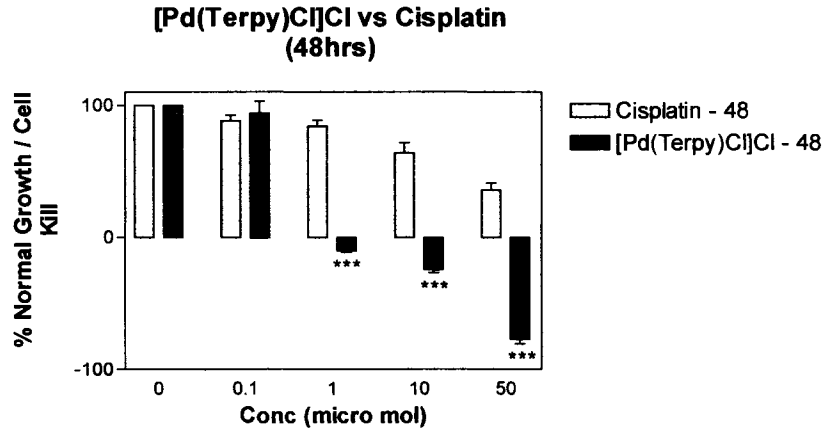


Figure E5 - [Pd(Terpy)Cl]Cl *versus* cisplatin (48 hrs)

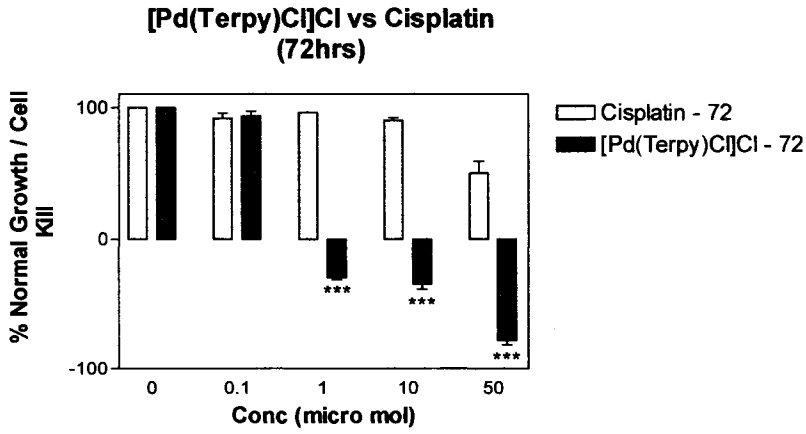


Figure E6 - [Pd(Terpy)Cl]Cl *versus* cisplatin (72 hrs)

E.III. [Pd(B$_H$)Cl]Cl versus Cisplatin

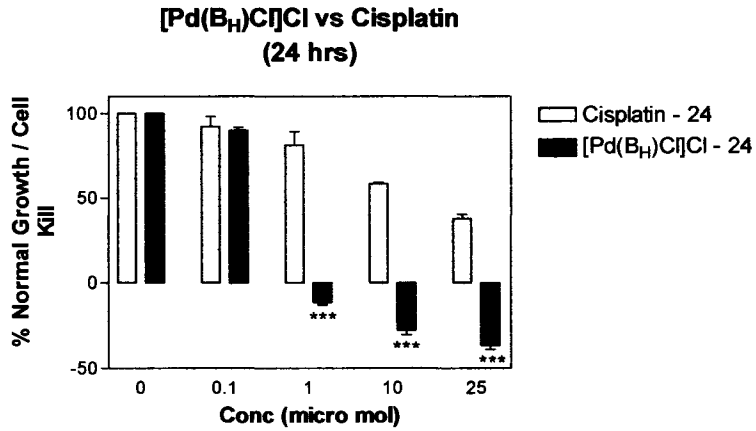


Figure E7 - [Pd(B$_H$)Cl]Cl *versus* cisplatin (24 hrs)

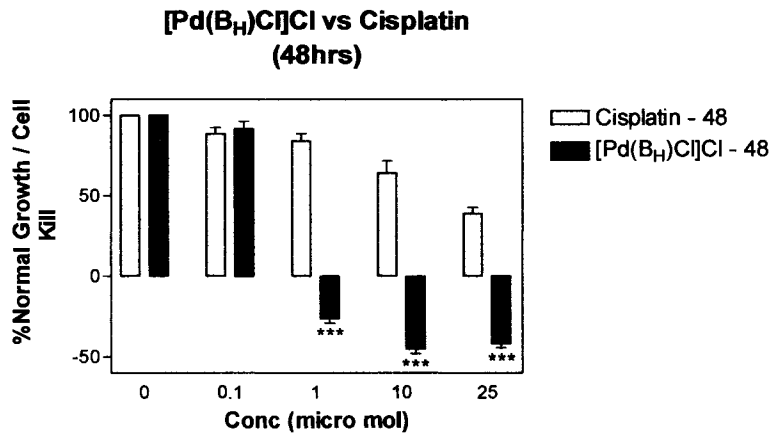


Figure E8 - [Pd(B$_H$)Cl]Cl *versus* cisplatin (48 hrs)

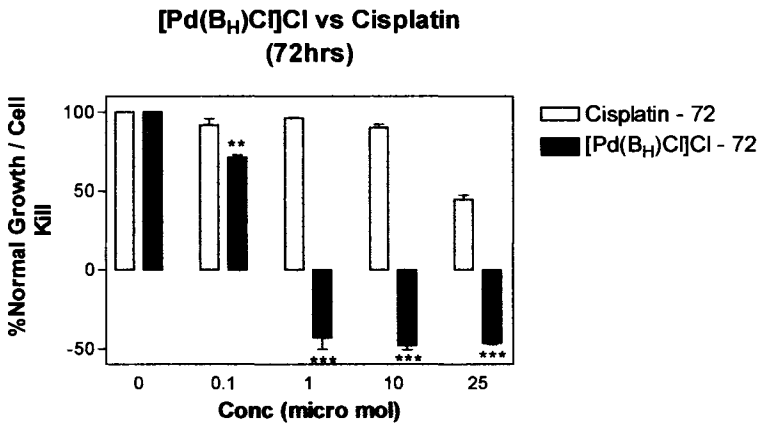


Figure E9 - [Pd(B$_H$)Cl]Cl *versus* cisplatin (72 hrs)

E.IV. [Pd(B_{Me})Cl]Cl versus Cisplatin

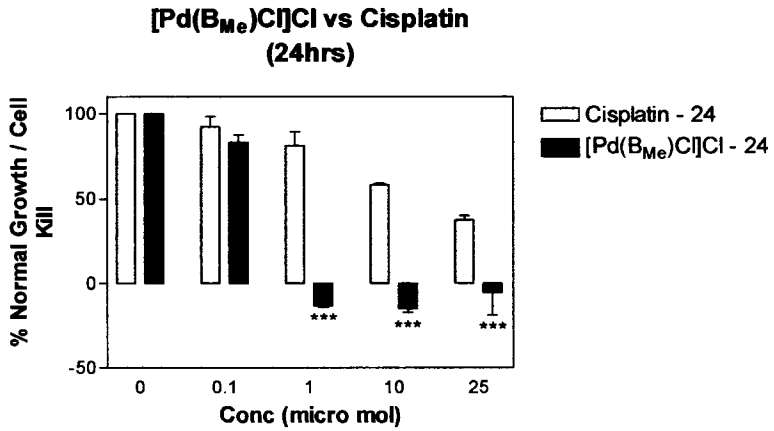


Figure E10 - [Pd(B_{Me})Cl]Cl *versus* cisplatin (24 hrs)

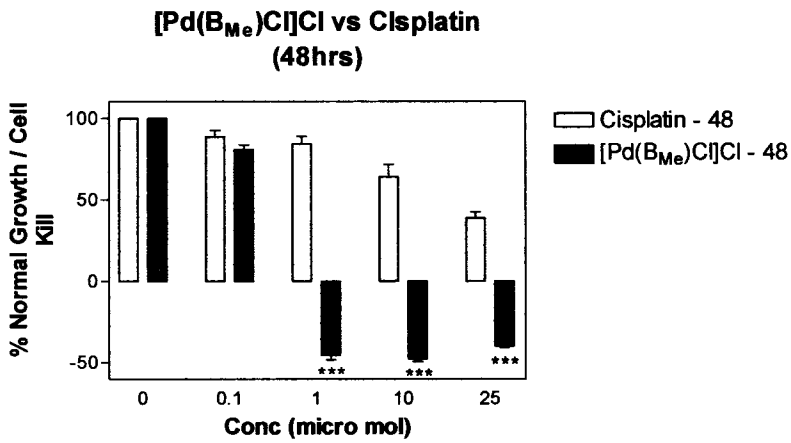


Figure E11 - [Pd(B_{Me})Cl]Cl *versus* cisplatin (48 hrs)

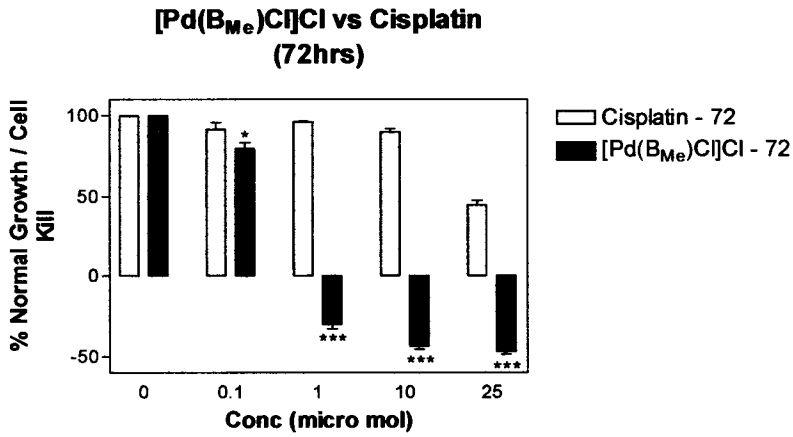


Figure E12 - [Pd(B_{Me})Cl]Cl *versus* cisplatin (72 hrs)

E.V. [Pd(B_{Et})Cl]Cl versus Cisplatin

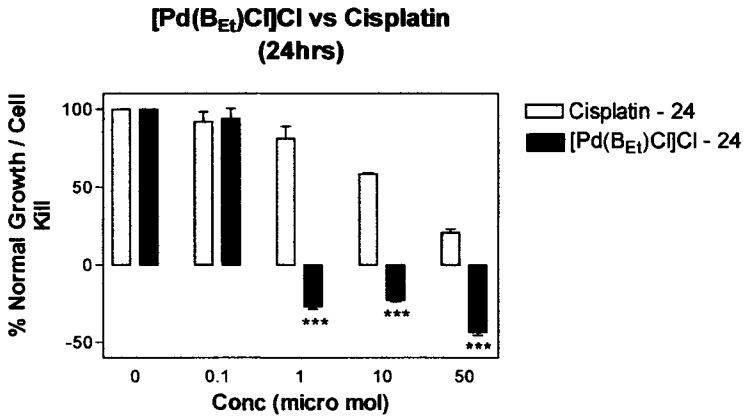


Figure E13 - [Pd(B_{Et})Cl]Cl *versus* cisplatin (24 hrs)

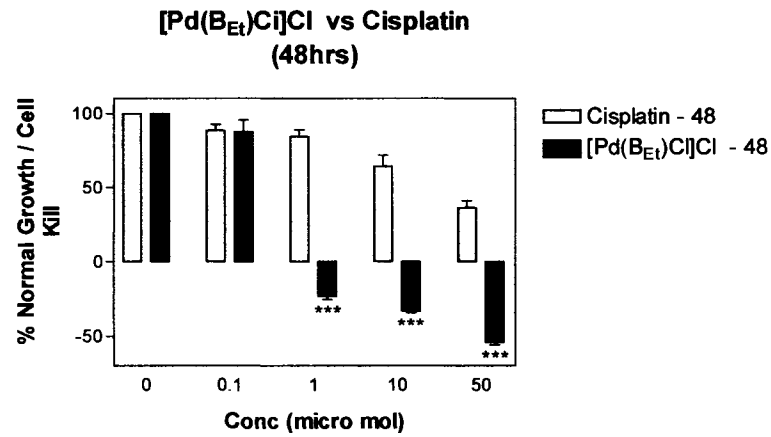


Figure E14 - [Pd(B_{Et})Cl]Cl *versus* cisplatin (48 hrs)

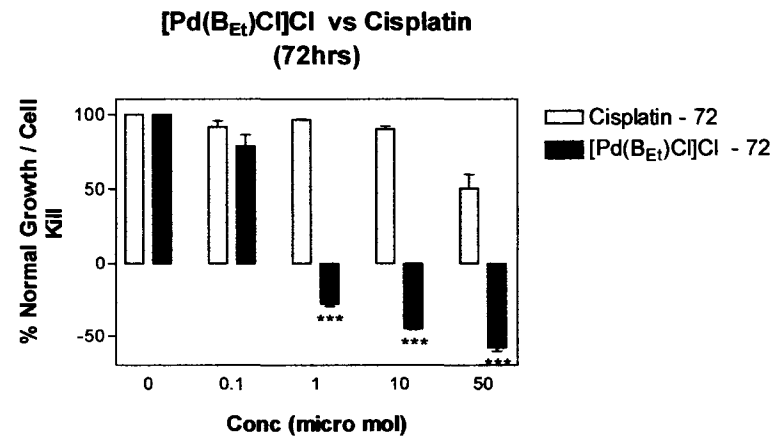


Figure E15 - [Pd(B_{Et})Cl]Cl *versus* cisplatin (72 hrs)

E.VI. [Pd(B_{Bu})Cl]Cl versus Cisplatin

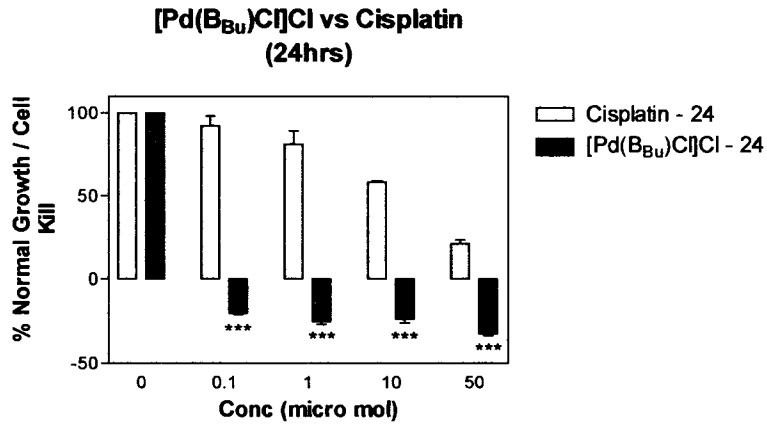


Figure E16 - [Pd(B_{Bu})Cl]Cl *versus* cisplatin (24 hrs)

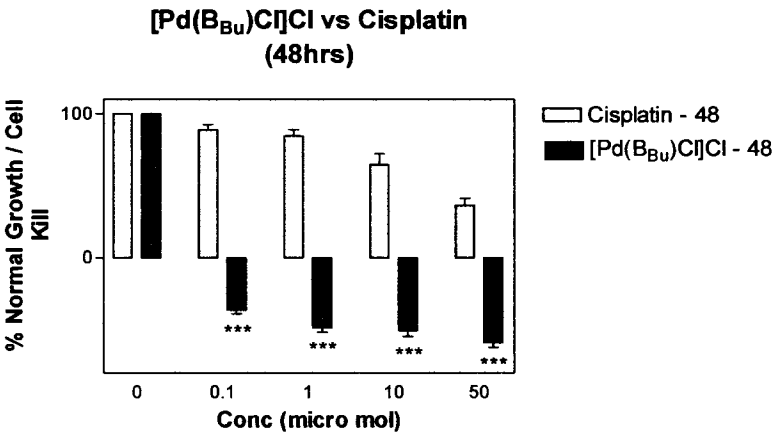


Figure E17 - [Pd(B_{Bu})Cl]Cl *versus* cisplatin (48 hrs)

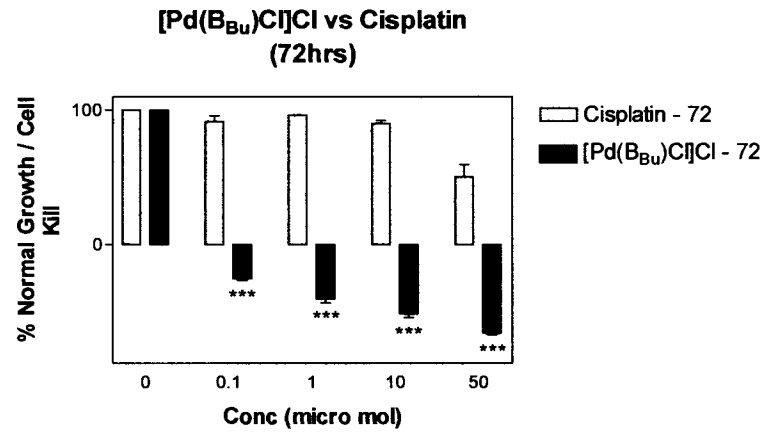


Figure E18 - [Pd(B_{Bu})Cl]Cl *versus* cisplatin (72 hrs)

Appendix F - *Human Breast Cancer (MCF7) Cell Line Screening Data*

F.I. $[Pd_2(Tppz)Cl_2]Cl_2$ Screening Results

Table F1 - $[Pd_2(Tppz)Cl_2]Cl_2$ – 24 hrs Data

Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.243 (SD=0.038)			T_0 = 0.195 (SD=0.030)			T_0 = 0.200 (SD=0.040)		
	Ctrl = 0.143 (SD=0.027)			Ctrl = 0.268 (SD=0.098)			Ctrl = 0.244 (SD=0.020)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
10.0	0.213	0.060	30.0	0.225	0.065	41.1	0.217	0.020	44.9
1.00	0.165	0.027	78.0	0.245	0.055	68.5	0.228	0.054	67.3
0.100	0.160	0.080	83.0	0.251	0.043	76.7	0.233	0.102	77.6
0.0100	0.156	0.011	84.0	0.255	0.164	82.2	0.234	0.142	79.6

Table F2 - $[Pd_2(Tppz)Cl_2]Cl_2$ – 48 hrs Data

Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.243 (SD=0.038)			T_0 = 0.195 (SD=0.030)			T_0 = 0.200 (SD=0.040)		
	Ctrl = 0.441 (SD=0.061)			Ctrl = 0.599 (SD=0.151)			Ctrl = 0.489 (SD=0.085)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
10.0	0.310	0.038	33.8	0.361	0.046	41.1	0.329	0.086	45.6
1.00	0.378	0.041	68.8	0.378	0.060	45.3	0.337	0.094	48.3
0.100	0.322	0.080	39.9	0.399	0.021	50.5	0.351	0.055	53.1
0.0100	0.310	0.076	33.8	0.469	0.038	67.8	0.371	0.123	59.9

Table F3 - $[Pd_2(Tppz)Cl_2]Cl_2$ – 72 hrs Data

Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.243 (SD=0.038)			T_0 = 0.195 (SD=0.030)			T_0 = 0.200 (SD=0.040)		
	Ctrl = 0.192 (SD=0.049)			Ctrl = 0.588 (SD=0.054)			Ctrl = 0.497 (SD=0.074)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*

10.0	0.226	0.044	33.3	0.312	0.158	29.8	0.300	0.024	34.8
1.00	0.220	0.052	45.1	0.372	0.054	45.0	0.335	0.067	46.4
0.100	0.218	0.120	49.0	0.402	0.102	52.7	0.377	0.052	60.3
0.0100	0.210	0.037	64.7	0.455	0.087	66.2	0.385	0.069	62.9

Table F4 - $[Pd_2(Tppz)Cl_2]Cl_2$ – Data Summary

conc (μmol)	*% Growth Ave*	*SD (error)*	*Bonferroni*
Time = 24 hrs			
10.0	38.7	7.74	P < 0.001
1.00	71.3	5.84	P > 0.050
0.100	79.1	3.41	P < 0.010
0.0100	81.9	2.22	P < 0.001
Time = 48 hrs			
10.0	40.2	5.92	P < 0.001
1.00	53.9	12.4	P < 0.001
0.100	47.8	6.98	P < 0.010
0.0100	53.8	17.8	P < 0.001
Time = 72 hrs			
10.0	32.6	2.57	P < 0.001
1.00	45.5	0.745	P < 0.001
0.100	54.0	5.74	P < 0.001
0.0100	64.6	1.62	P < 0.001

Table F5 - $[Pd_2(Tppz)Cl_2]Cl_2$ – Statistical Data

	24 hrs			*48 hrs*			*72 hrs*		
	%Var (Tot)	*P val*	*Sig*	*%Var (Tot)*	*P val*	*Sig*	*%Var (Tot)*	*P val*	*Sig*
Interaction	10.68	0.0016	Y	15.90	0.0004	Y	14.93	<0.0001	Y

Concentration	58.60	<0.0001	Y	25.91	<0.0001	Y	26.34	<0.0001	Y
Drug	24.88	<0.0001	Y	52.22	<0.0001	Y	58.15	<0.0001	Y

F.II. [Pd(Terpy)Cl]Cl Screening Results

Table F6 - [Pd(Terpy)Cl]Cl – 24 hrs Data

Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.243 (SD=0.038)			T_0 = 0.195 (SD=0.030)			T_0 = 0.200 (SD=0.040)		
	Ctrl = 0.334 (SD=0.020)			Ctrl = 0.254 (SD=0.014)			Ctrl = 0.289 (SD=0.020)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
50.0	0.114	0.005	-53.1	0.105	0.030	-46.2	0.105	0.002	-47.5
10.0	0.135	0.031	-44.4	0.101	0.080	-48.2	0.119	0.006	-40.5
1.00	0.131	0.008	-46.1	0.111	0.125	-43.1	0.115	0.010	-42.5
0.100	0.140	0.006	-42.4	0.120	0.142	-38.5	0.120	0.056	-40.0

Table F7 - [Pd(Terpy)Cl]Cl – 48 hrs Data

Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.243 (SD=0.038)			T_0 = 0.195 (SD=0.030)			T_0 = 0.200 (SD=0.040)		
	Ctrl = 0.455 (SD=0.045)			Ctrl = 0.405 (SD=0.211)			Ctrl = 0.593 (SD=0.068)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
50.0	0.046	0.009	-81.1	0.041	0.008	-79.0	0.049	0.085	-75.5
10.0	0.086	0.030	-64.6	0.052	0.016	-73.3	0.063	0.031	-68.5
1.00	0.097	0.003	-60.1	0.069	0.026	-64.6	0.081	0.003	-59.5
0.100	0.124	0.033	-49.0	0.089	0.068	-54.4	0.095	0.021	-52.5

Table F8 - [Pd(Terpy)Cl]Cl – 72 hrs Data									
Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.243 (SD=0.038)			T_0 = 0.195 (SD=0.030)			T_0 = 0.200 (SD=0.040)		
	Ctrl = 0.509 (SD=0.119)			Ctrl = 0.689 (SD=0.079)			Ctrl = 0.752 (SD=0.059)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
50.0	0.038	0.004	-84.4	0.020	0.005	-89.7	0.039	0.056	-80.5
10.0	0.042	0.022	-82.7	0.039	0.016	-80.0	0.049	0.024	-75.6
1.00	0.057	0.006	-76.5	0.042	0.902	-78.5	0.059	0.028	-70.5
0.100	0.228	0.097	-6.17	0.165	0.241	-15.4	0.157	0.221	-21.5

Table F9 - [Pd(Terpy)Cl]Cl – Results Summary			
conc (μmol)	*% Growth Ave*	*SD (error)*	*Bonferroni*
Time = 24 hrs			
50.0	-48.9	3.68	P < 0.001
10.0	-44.4	3.85	P < 0.001
1.00	-43.9	1.93	P < 0.001
0.100	-40.3	1.98	P < 0.001
Time = 48 hrs			
50.0	-78.5	2.81	P < 0.001
10.0	-68.8	4.37	P < 0.001
1.00	-61.4	2.80	P < 0.001
0.100	-51.9	2.74	P < 0.001
Time = 72 hrs			
50.0	-84.9	4.64	P < 0.001
10.0	-79.4	4.62	P < 0.001
1.00	-75.2	4.16	P < 0.001
0.100	-14.4	7.72	P < 0.001

Table 10 - [Pd(Terpy)Cl]Cl – Statistial Data									
	24 hrs			*48 hrs*			*72 hrs*		
	%Var (Tot)	*P val*	*Sig*	*%Var (Tot)*	*P val*	*Sig*	*%Var (Tot)*	*P val*	*Sig*
Interaction	15.38	<0.0001	Y	14.97	<0.0001	Y	15.65	<0.0001	Y
Concentration	31.59	<0.0001	Y	25.85	<0.0001	Y	25.00	<0.0001	Y
Drug	52.88	<0.0001	Y	59.09	<0.0001	Y	59.19	<0.0001	Y

F.III. [Pd(B_H)Cl]Cl Screening Results

Table F11 - [Pd(B_H)Cl]Cl – 24 hrs Data									
Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.243 (SD=0.038)			T_0 = 0.195 (SD=0.030)			T_0 = 0.200 (SD=0.040)		
	Ctrl = 0.275 (SD=0.114)			Ctrl = 0.385 (SD=0.029)			Ctrl = 0.228 (SD=0.057)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
25.0	0.153	0.010	-37.0	0.102	0.054	-47.7	0.109	0.021	-45.5
10.0	0.185	0.018	-23.9	0.135	0.047	-30.8	0.128	0.086	-36.0
1.00	0.204	0.031	-16.0	0.155	0.030	-20.5	0.168	0.005	-16.0
0.100	0.209	0.039	-14.0	0.176	0.069	-9.74	0.179	0.010	-10.5

Table F12 - [Pd(B_H)Cl]Cl – 48 hrs Data									
Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.243 (SD=0.038)			T_0 = 0.195 (SD=0.030)			T_0 = 0.200 (SD=0.040)		
	Ctrl = 0.455 (SD=0.045)			Ctrl = 0.405 (SD=0.211)			Ctrl = 0.593 (SD=0.068)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
25.0	0.101	0.042	-58.4	0.085	0.113	-56.4	0.091	0.008	-54.5
10.0	0.104	0.015	-57.2	0.090	0.022	-53.8	0.099	0.004	-50.5

1.00	0.129	0.013	-46.9	0.102	0.058	-47.7	0.103	0.024	-48.5
0.100	0.136	0.048	-44.0	0.105	0.095	-46.2	0.109	0.056	-45.5

Table F13 - [Pd(B_H)Cl]Cl – 72 hrs Data

Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.243 (SD=0.038)			T_0 = 0.195 (SD=0.030)			T_0 = 0.200 (SD=0.040)		
	Ctrl = 0.509 (SD=0.119)			Ctrl = 0.689 (SD=0.079)			Ctrl = 0.752 (SD=0.059)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
25.0	0.042	0.046	-82.7	0.028	0.045	-85.6	0.032	0.004	-84.0
10.0	0.050	0.017	-79.4	0.054	0.135	-72.3	0.053	0.013	-73.5
1.00	0.082	0.003	-66.3	0.079	0.054	-59.5	0.068	0.022	-66.0
0.100	0.227	0.087	-6.6	0.153	0.219	-21.5	0.156	0.099	-22.0

Table F14 - [Pd(B_H)Cl]Cl - Results Summary

conc (μmol)	*% Growth Ave*	*SD (error)*	*Bonferroni*
Time = 24 hrs			
25.0	-43.4	5.63	P < 0.001
10.0	-30.2	6.08	P < 0.001
1.00	-17.5	2.59	P < 0.001
0.100	-11.4	2.27	P < 0.001
Time = 48 hrs			
25.0	-56.4	1.97	P < 0.001
10.0	-53.8	3.35	P < 0.001
1.00	-47.7	0.793	P < 0.001
0.100	-45.2	1.09	P < 0.001
Time = 72 hrs			
25.0	-84.1	1.47	P < 0.001

10.0	-75.1	3.81	P < 0.001
1.00	-63.9	3.83	P < 0.001
0.100	-16.7	8.77	P < 0.010

Table F15 - [Pd(B_H)Cl]Cl – Statistical Data

	24 hrs			*48 hrs*			*72 hrs*		
	%Var (Tot)	*P val*	*Sig*	*%Var (Tot)*	*P val*	*Sig*	*%Var (Tot)*	*P val*	*Sig*
Interaction	13.76	<0.0001	Y	15.44	<0.0001	Y	15.66	<0.0001	Y
Concentration	31.26	<0.0001	Y	22.91	<0.0001	Y	23.48	<0.0001	Y
Drug	54.68	<0.0001	Y	61.56	<0.0001	Y	60.70	<0.0001	Y

F.IV. [Pd(B_{Me})Cl]Cl Screening Results

Table F16 - [Pd(B_{Me})Cl]Cl – 24 hrs Data

Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.243 (SD=0.038)			T_0 = 0.195 (SD=0.030)			T_0 = 0.200 (SD=0.040)		
	Ctrl = 0.538 (SD=0.167)			Ctrl = 0.360 (SD=0.148)			Ctrl = 0.451 (SD=0.024)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
25.0	0.261	0.104	6.10	0.202	0.115	4.24	0.220	0.082	7.98
10.0	0.299	0.084	19.0	0.224	0.087	17.6	0.245	0.015	17.9
1.00	0.305	0.024	21.0	0.235	0.055	24.2	0.251	0.101	20.3
0.100	0.338	0.133	32.2	0.258	0.153	38.2	0.287	0.085	34.7

Table F17 - [Pd(B_{Me})Cl]Cl – 48 hrs Data

Conc (μmol)	*Trial 1*	*Trial 2*	*Trial 3*
	T_0 = 0.243 (SD=0.038)	T_0 = 0.195 (SD=0.030)	T_0 = 0.200 (SD=0.040)
	Ctrl = 0.862 (SD=0.178)	Ctrl = 0.572 (SD=0.160)	Ctrl = 0.751 (SD=0.024)

	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
25.0	0.255	0.185	1.93	0.210	0.107	3.98	0.211	0.053	2.00
10.0	0.325	0.052	13.2	0.251	0.054	14.9	0.243	0.120	1.80
1.00	0.412	0.054	27.3	0.305	0.072	29.2	0.357	0.185	28.5
0.100	0.501	0.215	41.7	0.354	0.052	42.2	0.413	0.098	38.7

Table F18 - [Pd(B_{Me})Cl]Cl – 72 hrs Data

Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.243 (SD=0.038)			T_0 = 0.195 (SD=0.030)			T_0 = 0.200 (SD=0.040)		
	Ctrl = 0.901 (SD=0.127)			Ctrl = 0.922 (SD=0.248)			Ctrl = 0.866 (SD=0.158)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
25.0	0.141	0.036	-42.0	0.122	0.025	-37.4	0.102	0.024	-49.0
10.0	0.178	0.026	-26.7	0.139	0.012	-28.7	0.135	0.129	-32.5
1.00	0.191	0.030	-21.4	0.143	0.034	-26.7	0.153	0.051	-24.0
0.100	0.821	0.144	87.8	0.843	0.083	89.1	0.753	0.052	83.0

Table F19 - [Pd(B_{Me})Cl]Cl - Results Summary

conc (μmol)	*% Growth Ave*	*SD (error)*	*Bonferroni*
Time = 24 hrs			
25.0	6.10	1.86	$P < 0.001$
10.0	18.2	0.732	$P < 0.001$
1.00	21.9	2.09	$P < 0.001$
0.100	35.0	3.00	$P < 0.001$
Time = 48 hrs			
25.0	2.64	1.16	$P < 0.001$
10.0	12.0	3.69	$P < 0.001$
1.00	28.3	0.949	$P < 0.001$

0.100	40.8	1.90	P < 0.001
Time = 72 hrs			
25.0	-42.8	5.83	P < 0.001
10.0	-29.3	2.92	P < 0.001
1.00	-24.0	2.63	P < 0.001
0.100	86.7	3.21	P < 0.001

Table F20 - [Pd(B_{Me})Cl]Cl – Statistical Data

	24 hrs			*48 hrs*			*72 hrs*		
	%Var (Tot)	*P val*	*Sig*	*%Var (Tot)*	*P val*	*Sig*	*%Var (Tot)*	*P val*	*Sig*
Interaction	11.60	<0.0001	Y	13.85	<0.0001	Y	19.11	<0.0001	Y
Concentration	43.02	<0.0001	Y	33.95	<0.0001	Y	38.79	<0.0001	Y
Drug	44.93	<0.0001	Y	51.85	<0.0001	Y	41.91	<0.0001	Y

F.V. [Pd(B_{Et})Cl]Cl Screening Results

Table F21 - [Pd(B_{Et})Cl]Cl – 24 hrs Data

Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.243 (SD=0.038)			T_0 = 0.195 (SD=0.030)			T_0 = 0.200 (SD=0.040)		
	Ctrl = 0.538 (SD=0.167)			Ctrl = 0.360 (SD=0.148)			Ctrl = 0.451 (SD=0.024)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
50.0	0.261	0.115	6.10	0.205	0.026	6.06	0.245	0.054	5.98
10.0	0.270	0.146	9.15	0.211	0.020	9.70	0.225	0.121	9.96
1.00	0.278	0.063	11.8	0.224	0.080	17.6	0.234	0.086	13.5
0.100	0.340	0.088	32.9	0.248	0.047	32.1	0.301	0.084	40.2

Table F22 - [Pd(B_{Et})Cl]Cl – 48 hrs Data

Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.243 (SD=0.038)			T_0 = 0.195 (SD=0.030)			T_0 = 0.200 (SD=0.040)		
	Ctrl = 0.862 (SD=0.178)			Ctrl = 0.572 (SD=0.160)			Ctrl = 0.751 (SD=0.024)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
50.0	0.259	0.146	2.58	0.204	0.052	2.39	0.209	0.055	3.59
10.0	0.265	0.107	3.55	0.229	0.028	9.02	0.222	0.043	8.76
1.00	0.294	0.028	8.24	0.231	0.123	9.55	0.234	0.074	13.5
0.100	0.341	0.065	15.8	0.248	0.045	14.1	0.251	0.105	20.3

Table F23 - [Pd(B_{Et})Cl]Cl – 72 hrs Data

Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.243 (SD=0.038)			T_0 = 0.195 (SD=0.030)			T_0 = 0.200 (SD=0.040)		
	Ctrl = 0.901 (SD=0.127)			Ctrl = 0.922 (SD=0.248)			Ctrl = 0.866 (SD=0.158)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
50.0	0.150	0.001	-38.3	0.134	0.104	-31.3	0.139	0.089	-30.5
10.0	0.175	0.002	-28.0	0.142	0.026	-27.2	0.155	0.053	-22.5
1.00	0.189	0.089	-22.2	0.159	0.067	-18.5	0.167	0.075	-16.5
0.100	0.388	0.153	22.0	0.345	0.050	18.8	0.344	0.045	21.6

Table F24 - [Pd(B_{Et})Cl]Cl - Results Summary

conc (μmol)	*% Growth Ave*	*SD (error)*	*Bonferroni*
Time = 24 hrs			
50.0	6.05	0.06	$P < 0.001$
10.0	9.60	0.41	$P < 0.001$
1.00	14.3	2.94	$P < 0.001$
0.100	35.1	4.48	$P < 0.001$

Time = 48 hrs			
50.0	2.85	0.64	P < 0.001
10.0	7.11	3.08	P < 0.001
1.00	10.4	1.76	P < 0.001
0.100	16.7	3.23	P < 0.001
Time = 72 hrs			
50.0	-33.4	4.28	P < 0.001
10.0	-25.9	2.96	P < 0.001
1.00	-19.1	2.91	P < 0.001
0.100	20.8	1.75	P < 0.001

Table F25- [Pd(B_{Et})Cl]Cl – Statistical Data

	24 hrs			*48 hrs*			*72 hrs*		
	%Var (Tot)	*P val*	*Sig*	*%Var (Tot)*	*P val*	*Sig*	*%Var (Tot)*	*P val*	*Sig*
Interaction	13.13	<0.0001	Y	14.20	<0.0001	Y	14.61	<0.0001	Y
Concentration	50.17	<0.0001	Y	35.14	<0.0001	Y	28.98	<0.0001	Y
Drug	36.28	<0.0001	Y	50.39	<0.0001	Y	56.24	<0.0001	Y

F.VI. [Pd(B_{Bu})Cl]Cl Screening Results

Table F26 - [Pd(B_{Bu})Cl]Cl – 24 hrs Data

Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.243 (SD=0.038)			T_0 = 0.195 (SD=0.030)			T_0 = 0.200 (SD=0.040)		
	Ctrl = 0.143 (SD=0.027)			Ctrl = 0.268 (SD=0.098)			Ctrl = 0.244 (SD=0.020)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
50.0	0.035	0.001	-85.6	0.052	0.016	-73.3	0.045	0.054	-77.5

10.0	0.122	0.036	-49.8	0.091	0.091	-53.3	0.118	0.120	-41.0
1.00	0.147	0.015	-39.5	0.121	0.068	-37.9	0.126	0.086	-37.0
0.100	0.154	0.046	-36.6	0.122	0.140	-37.4	0.122	0.037	-39.0

Table F27 - [Pd(B_{Bu})Cl]Cl – 48 hrs Data

Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.243 (SD=0.038)			T_0 = 0.195 (SD=0.030)			T_0 = 0.200 (SD=0.040)		
	Ctrl = 0.441 (SD=0.061)			Ctrl = 0.599 (SD=0.151)			Ctrl = 0.489 (SD=0.085)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
50.0	0.042	0.010	-82.7	0.026	0.044	-86.7	0.035	0.052	-82.5
10.0	0.135	0.028	-44.4	0.099	0.032	-49.2	0.102	0.015	-49.0
1.00	0.156	0.018	-35.8	0.137	0.067	-29.7	0.127	0.075	-36.5
0.100	0.198	0.041	-18.5	0.149	0.090	-23.6	0.144	0.152	-28.0

Table F28 - [Pd(B_{Bu})Cl]Cl – 72 hrs Data

Conc (μmol)	*Trial 1*			*Trial 2*			*Trial 3*		
	T_0 = 0.243 (SD=0.038)			T_0 = 0.195 (SD=0.030)			T_0 = 0.200 (SD=0.040)		
	Ctrl = 0.192 (SD=0.049)			Ctrl = 0.588 (SD=0.054)			Ctrl = 0.497 (SD=0.074)		
	Mean	*SD*	*% G*	*Mean*	*SD*	*% G*	*Mean*	*SD*	*% G*
50.0	0.044	0.009	-81.9	0.037	0.010	-81.0	0.044	0.158	-78.0
10.0	0.058	0.024	-76.1	0.044	0.068	-77.4	0.059	0.068	-70.5
1.00	0.059	0.012	-75.7	0.050	0.026	-74.4	0.064	0.092	-68.0
0.100	0.119	0.069	-51.0	0.098	0.048	-49.7	0.072	0.083	-64.0

Table F29 - [Pd(B_{Bu})Cl]Cl - Results Summary

conc (μmol)	*% Growth Ave*	*SD (error)*	*Bonferroni*
Time = 24 hrs			
50.0	-78.8	6.23	$P < 0.001$

10.0	-48.0	6.35	P < 0.001
1.00	-38.2	1.27	P < 0.001
0.100	-37.7	1.21	P < 0.001
Time = 48 hrs			
50.0	-84.0	2.35	P < 0.001
10.0	-47.6	2.70	P < 0.001
1.00	-34.0	3.72	P < 0.001
0.100	-23.4	4.74	P < 0.001
Time = 72 hrs			
50.0	-80.3	2.04	P < 0.001
10.0	-74.7	3.69	P < 0.001
1.00	-72.7	4.12	P < 0.001
0.100	-54.9	7.89	P < 0.001

Table F30 - [Pd(B_{Bu})Cl]Cl – Statistical Data

	24 hrs			*48 hrs*			*72 hrs*		
	%Var (Tot)	*P val*	*Sig*	*%Var (Tot)*	*P val*	*Sig*	*%Var (Tot)*	*P val*	*Sig*
Interaction	13.41	<0.0001	Y	14.17	<0.0001	Y	15.91	<0.0001	Y
Concentration	34.00	<0.0001	Y	29.98	<0.0001	Y	21.67	<0.0001	Y
Drug	52.39	<0.0001	Y	55.73	<0.0001	Y	62.28	<0.0001	Y

Appendix G - *Illustration of Human Breast Cancer (MCF7) Cell Line Screening Data*

G.I. $[Pd_2(Tppz)Cl_2]Cl_2$ versus Cisplatin

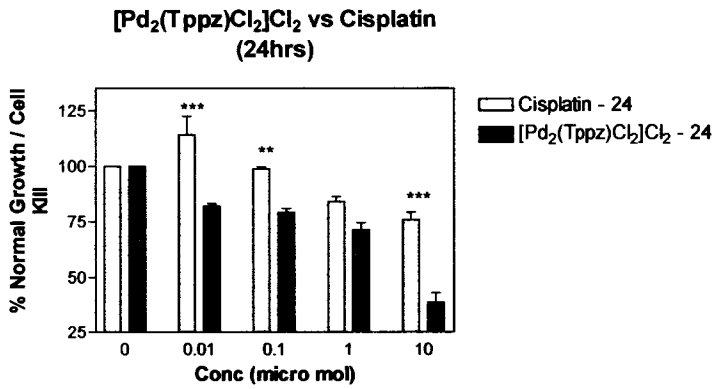


Figure G1 - $[Pd_2(Tppz)Cl_2]Cl_2$ *versus* cisplatin (24 hrs)

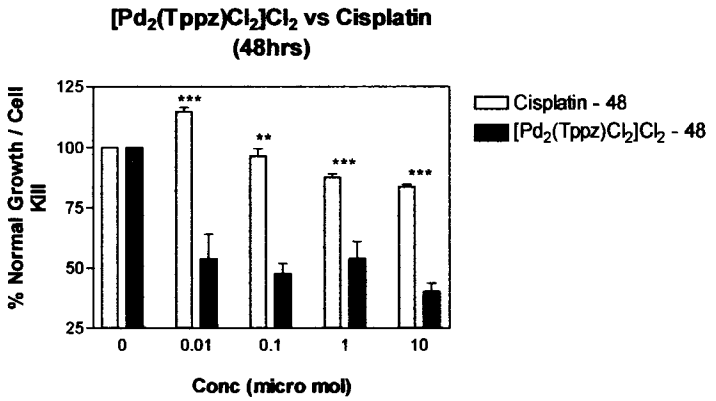


Figure G2 - $[Pd_2(Tppz)Cl_2]Cl_2$ *versus* cisplatin (48 hrs)

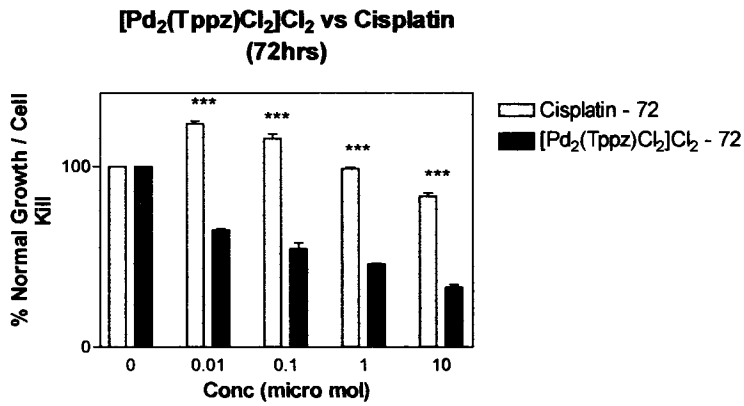


Figure G3 - $[Pd_2(Tppz)Cl_2]Cl_2$ *versus* cisplatin (72 hrs)

G.II. [Pd(Terpy)Cl]Cl versus Cisplatin

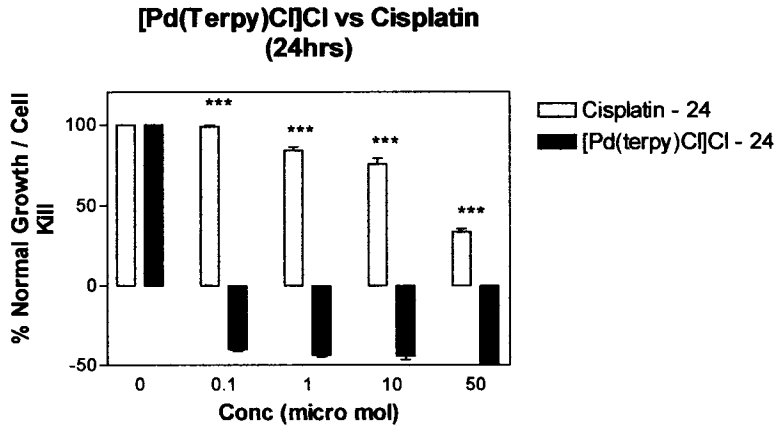


Figure G4 - [Pd(Terpy)Cl]Cl *versus* cisplatin (24 hrs)

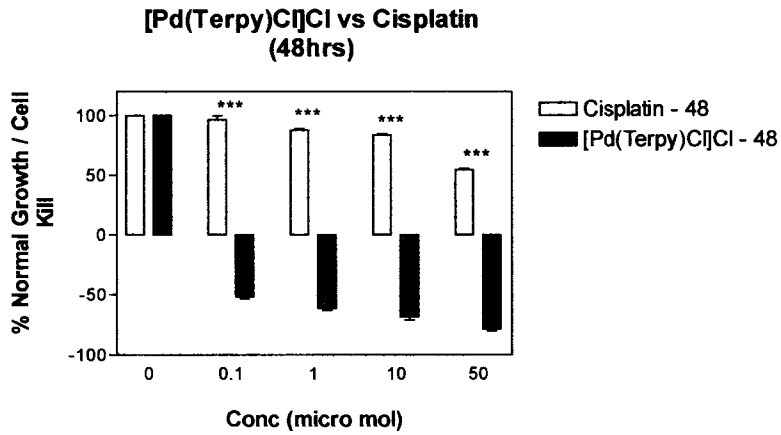


Figure G5 - [Pd(Terpy)Cl]Cl *versus* cisplatin (48 hrs)

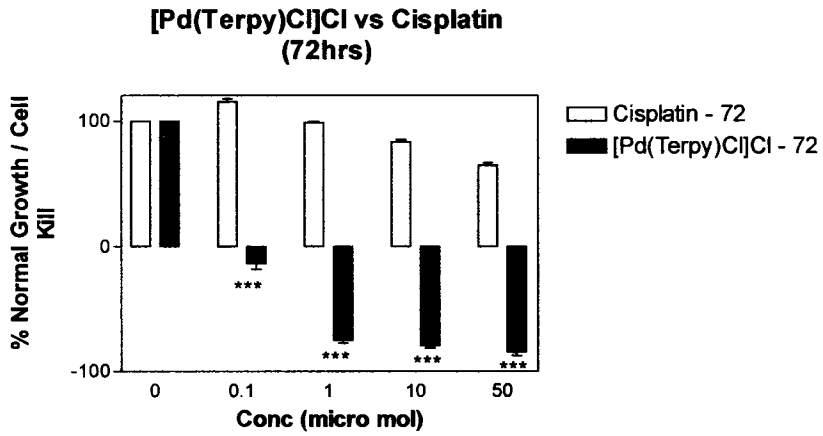


Figure G6 - [Pd(Terpy)Cl]Cl *versus* cisplatin (72 hrs)

G.III. [Pd(B$_H$)Cl]Cl versus Cisplatin

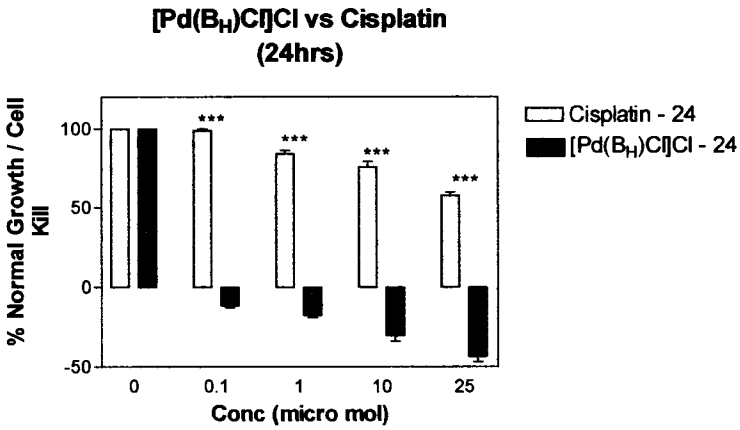


Figure G7 - [Pd(B$_H$)Cl]Cl *versus* cisplatin (24 hrs)

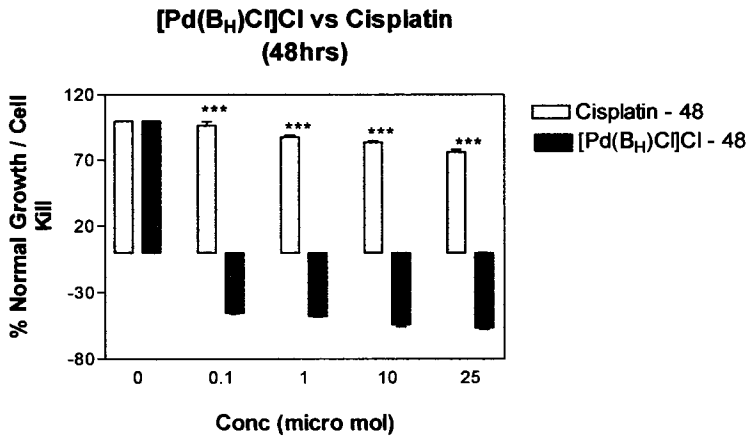


Figure G8 - [Pd(B$_H$)Cl]Cl *versus* cisplatin (48 hrs)

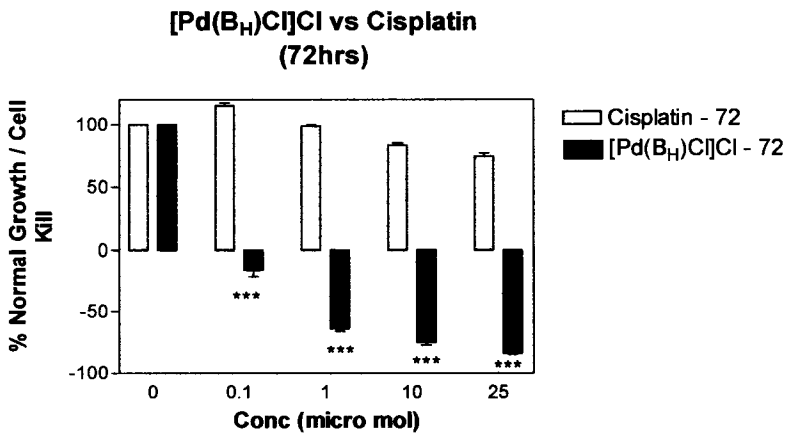


Figure G9 - [Pd(B$_H$)Cl]Cl *versus* cisplatin (72 hrs)

G.IV. [Pd(B$_{Me}$)Cl]Cl versus Cisplatin

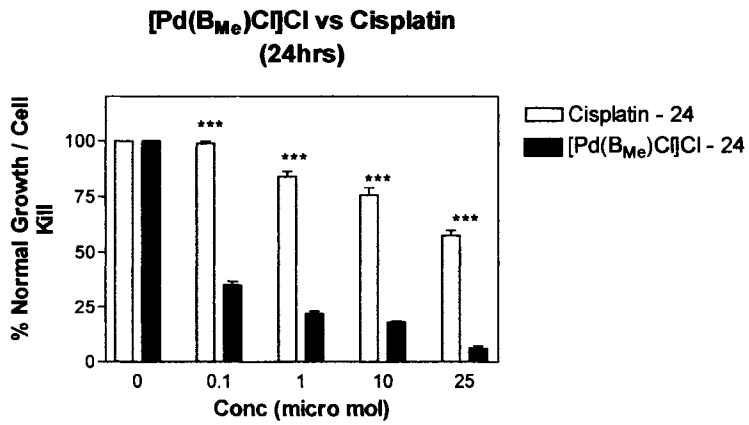


Figure G10 - [Pd(B$_{Me}$)Cl]Cl *versus* cisplatin (24 hrs)

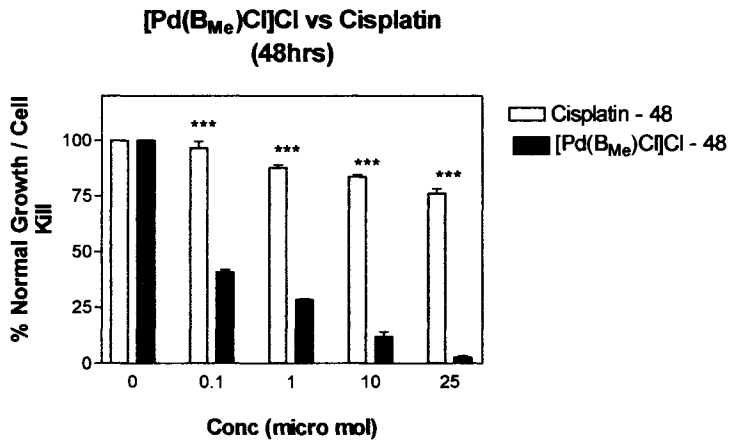


Figure G11 - [Pd(B$_{Me}$)Cl]Cl *versus* cisplatin (48 hrs)

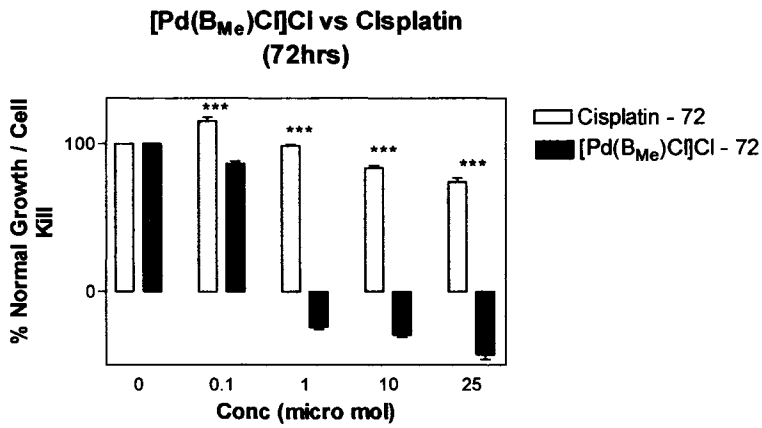


Figure G12 - [Pd(B$_{Me}$)Cl]Cl *versus* cisplatin (72 hrs)

G.V. [Pd(B_{Et})Cl]Cl versus Cisplatin

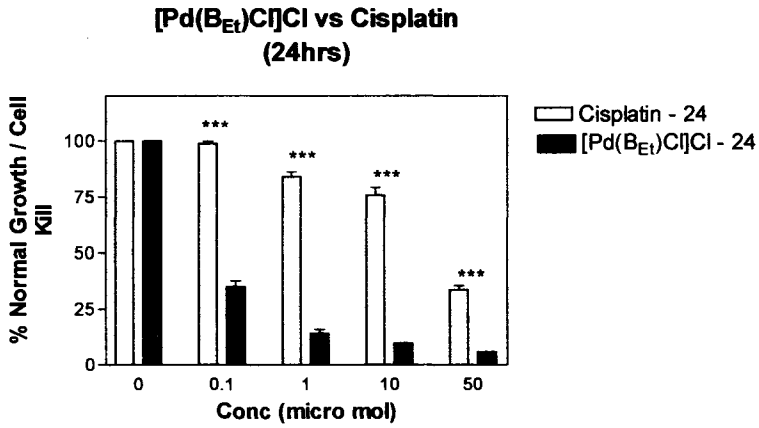


Figure G13 - [Pd(B_{Et})Cl]Cl *versus* cisplatin (24 hrs)

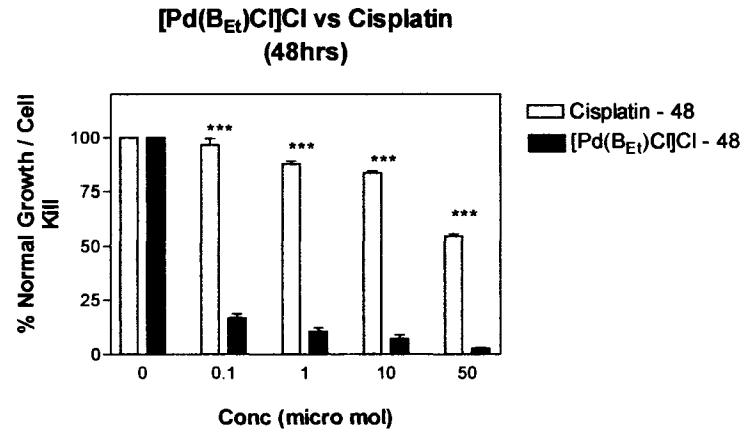


Figure G14 - [Pd(B_{Et})Cl]Cl *versus* cisplatin (48 hrs)

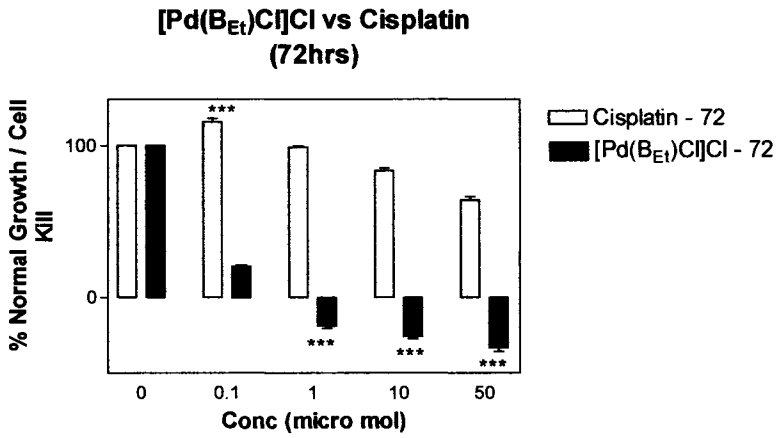


Figure G15 - [Pd(B_{Et})Cl]Cl *versus* cisplatin (72 hrs)

G.VI. [Pd(B_{Bu})Cl]Cl versus Cisplatin

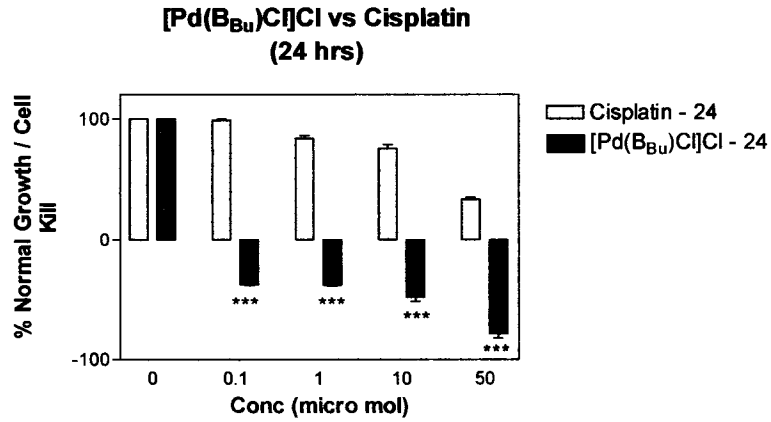


Figure G16 - [Pd(B_{Bu})Cl]Cl *versus* cisplatin (24 hrs)

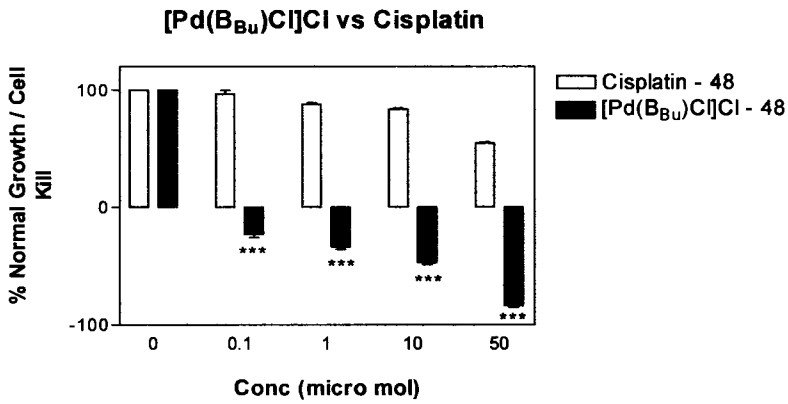


Figure G17 - [Pd(B_{Bu})Cl]Cl *versus* cisplatin (48 hrs)

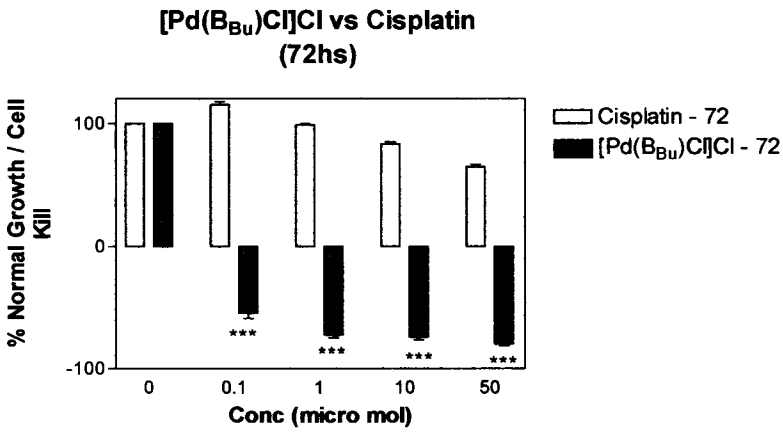


Figure G18 - [Pd(B_{Bu})Cl]Cl *versus* cisplatin (72 hrs)