CHAPTER 1

GENERAL INTRODUCTION

1.1 Cancer

Cancer is a class of diseases characterized by the uncontrolled division of abnormal cells to form lumps or masses of tissue called tumor in a multicellular organism. Tumor may be either benign or malignant type. Benign tumors are considered as non-cancerous and rarely life threatening. These types of tumors are not able to invade the surrounding tissues and stay clustered together in a single mass. Malignant tumors however, grow rapidly and can spread to various regions of the body either using the lymphatic systems or through the bloodstream and metastasized. In this case, surgery is no more used and a pharmacological approach is the remaining treatment.[1] Roughly one person in eight of world dies in cancer.[2]

1.1.1 Types of cancer

Till date more than 200 different types of cancer have been identified according to the most affected organ or to the type of cancerous cells.[3] Scientists generally have distinguished five types of cancer on the basis of point of origin of the cancerous tumor in the body which are as follows:[3]

Carcinoma- This type of cancer begins in a tissue which covers the inner or outer surfaces of the body. It normally arises from cells originating in the endodermal or ectodermal germ layer at the time of embryogenesis. Carcinoma may be of adenocarcinoma, squamous cell carcinoma, adenosquamous carcinoma, anaplastic carcinoma, large cell carcinoma and small cell carcinoma. It has been estimated that 85% of cancers are from adenocarcinoma and squamous cell carcinoma. Adenocarcinoma is generally localized in breast, liver, kidney, ovary, thyroid, colon, stomach, lungs, salivary glands etc. while squamous cell carcinoma is localized in uterus, pancreas, gastrointestinal tract etc.

Sarcoma- Cancers that begin in connective tissues such as bone, cartilage, fat muscle, blood vessels etc. Estimation of this type of cancers is about 2-4%.

Leukaemia- Origin of this type of cancer is in blood-forming tissue like bone marrow cells. Leukaemia is mainly located in blood with an estimation of 4-6%.

Lymphoma- A group of blood cell tumors that originate from lymphatic tissues (B and T lymphocytes) are said to be lymphoma cancer. The two main categories of lymphomas are Hodgkin lymphomas and the non-Hodgkin lymphomas. These types

of cancers are mainly located in lymph nodes, spleen, skin, brain, bones, reproductive organs and lungs. 5-7% of all cancers are from lymphoma.

Myeloma- Cancer that originates in the bone marrow cells such as plasmocyte.

1.1.2 Different existing cancer treatments

In order to obtain more efficacious cancer treatment, a good analysis from the medical team must be first established. The choice of treatment is based on the cancer type, its size, stage of the disease and the general health condition of the patient. Treatment of cancer is still mainly focused on many conventional therapies such as chemotherapy, radiotherapy, hormone and surgery etc. in spite of developing some new approaches towards cancer treatments.[4]

Surgery- Surgery is the most common and oldest technique which has been widely used in the past. Surgery is often used to remove tumor, if it remains small or reasonably well defined. It was the only existing treatment against cancer and becoming more and more efficient day by day. However, surgery is effective only for benign tumors. Additional treatment with chemotherapy and radiotherapy is necessary to remove the cancer cells which are in metastatic form.

Radiotherapy- In radiotherapy, radiation such as X-rays or radiopharmaceuticals is used to destroy the cancer cells specifically. Radiotherapy is used to remove primary tumor but is more difficult to use in case of metastatic stage.

Hormone therapy- Hormone therapy is a treatment that involves the manipulation of endocrine system by administrating specific hormones particularly steroid hormones. Steroid hormones are observed to be powerful drivers of gene expression in definite cancer cells.

Immunotherapy- This cancer treatment involves the use of an antibody by inducing, enhancing or suppressing an immune response. Immunotherapy prevents cell proliferation by blocking the activity of some receptors encoded oncogenes.

Chemotherapy- Low molecular weight drugs that selectively destroy tumors or may demonstrate limited growth come under category of chemotherapeutic treatments. Originally, nitrogen mustards were used as chemotherapeutic agents. Their antileukemic properties were being identified at the time of First World War. Since then number of chemotherapeutic agents have been developed for clinical purpose and recently in 2004 near about 1000 chemotherapeutic agents were undergoing

clinical trials.[5] However, the major problem associated with chemotherapy is their severe toxicities that damage vital organs such as kidneys, liver etc. and lack of tolerability in some patients. The non-selective biodistribution throughout the body is the cause of their toxicity.

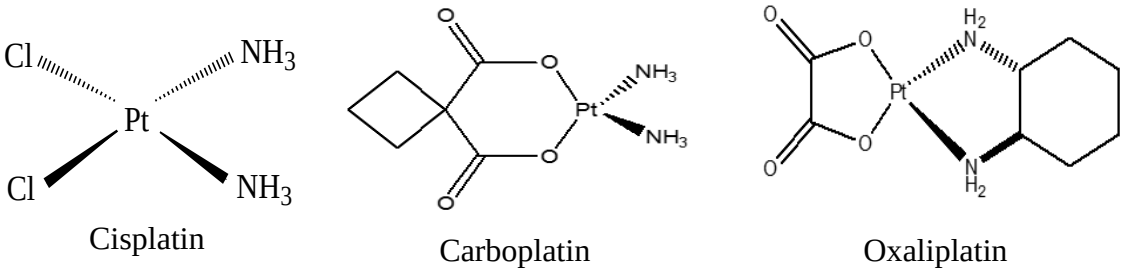
Therefore, the major goal in anticancer drug discovery process is to develop innovative therapies that exhibit a real improvement in effectiveness and tolerability.[6]

1.2 Cancer treatments by Platinum Therapies

1.2.1 Bases of modern chemotherapy

The successful development of metal-containing anticancer drugs began with the serendipitous discovery of cisplatin by Rosenberg and co-workers in 1960s.[7] Rosenbarg was studying the effect of an electric field on the growth of bacteria *E. coli*, using platinum electrodes and observed that these electrodes generated the soluble platinum complexes (*cis*-diaminedichloroplatinum(II), *cis*-diaminetetrachloroplatinum(IV)) which inhibited the cells from multiplying. This observation led to the development of *cis*-diaminedichloroplatinum(II) as an antimetastatic agent.[8] *Cis*-diaminedichloroplatinum(II) commonly known as cisplatin (Fig.1.1) was synthesized for the first time in 1845 by Peyrone[9] and the structure was elucidated in 1893 by Alfred Warner.[10] Alfred Warner was the pioneer to establish the basis of coordination chemistry and demonstrated that ammonia can coordinate to a metal ion by donating its lone pair electrons in a coordinate bond.

Cisplatin entered into clinical testing in 1971[11] and approved as chemotherapeutic agent by the American Food and Drugs Administration (FDA) in 1978. Since then cisplatin has become one of the most widely used anticancer drugs with an estimation of 70% patients receiving the compound as part of their treatment.[12] The success of cisplatin as an anticancer agent has stimulated a new field for research in bioinorganic chemistry. In the year 1993 and 2002, the two platinum based drugs: carboplatin and oxaliplatin (Fig.1.1) received worldwide approval in a routine clinical use.[13,14]



Cisplatin Carboplatin Oxaliplatin

Fig.1.1 Structures of cisplatin, carboplatin and oxaliplatin.

1.2.2 Mode of action

Cisplatin is a well-known antitumor drug and its principal target is DNA. It is accountable for the cure of more than 90% of testicular cancers[15] and also takes part in the treatment of other kinds of cancer including ovarian, bladder, head and neck, lymphomas and melanomas.[16] The mode of action of cisplatin has been extensively studied. Cisplatin is generally believed to kill cancer cells by covalently binding to the N7 position of the purine bases of DNA.[17-19] Formation of the cisplatin drug-DNA adducts interfere with the cells repair mechanism, which ultimately leads apoptosis and cell death.[20] However, cisplatin cannot react with DNA directly and before such binding can occur, the platinum drug undergoes hydrolysis leads to the substitution of chloride ligands by one or two water molecules. Antitumor activity of aquated cisplatin derives from the capability of its DNA adduct cross-links formation.[21] The highly reactive aquated cisplatin (Fig. 1.2) interact with DNA mainly at N7 of guanine, forming guanine-guanine (GG) intrastrand cross-links adducts[21-25] (Fig.1.3).[26]

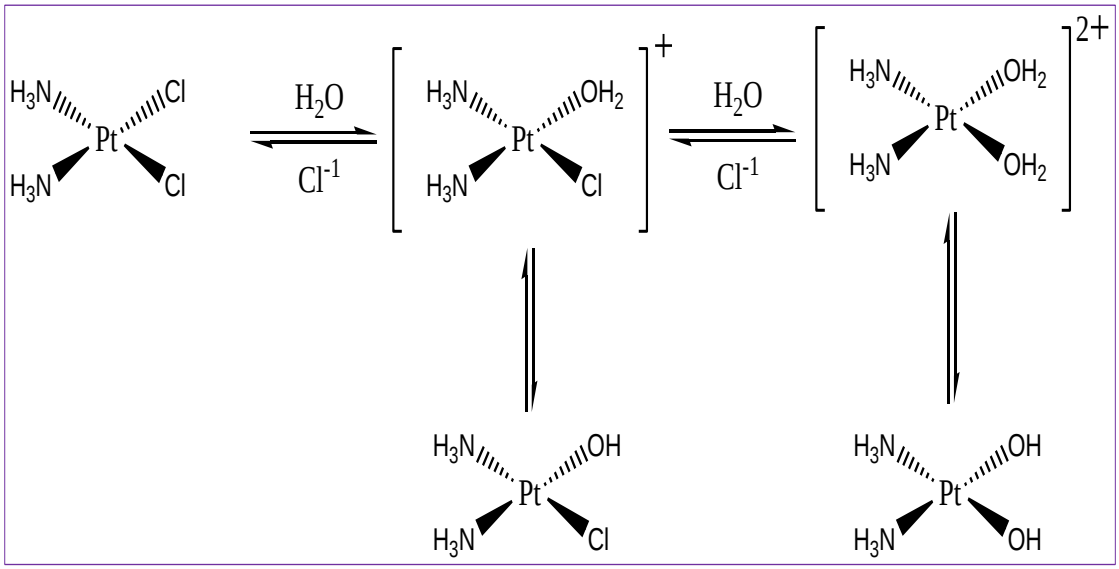


Fig.1.2 Hydrolysis of cisplatin.

The other two modes of action that have been described so far for cisplatin are interstrand interaction (between GG and GA on two different strands) and DNA-protein interaction.[27] Some other platinum derivatives such as satraplatin, iproplatin, tetraplatin[28] etc. present a covalent interaction with DNA. It has been observed that satraplatin is active against prostate cancer[29], oxaliplatin is active against colorectal tumors[30] while carboplatin displays the same activity as that of cisplatin.[31-33] Thus, DNA binding is not sufficient enough to describe the differences in activities of platinum based drugs on human tumors.

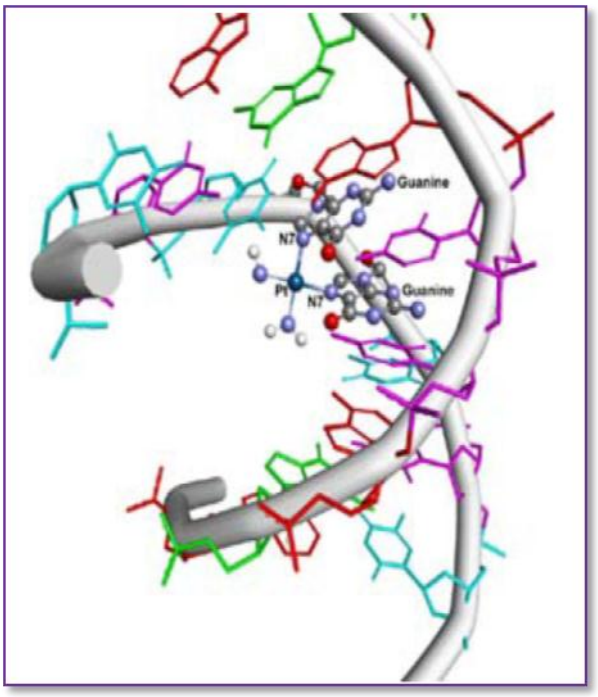


Fig. 1.3 Cisplatin form adducts with the N7 nitrogen of guanine bases (PDB code 3LPV).[26]

1.2.3 From platinum to ruthenium

In spite of the activities of these platinum metal agents against a variety of cancers, their use is associated with unpleasant side effects for the patients which include gastrointestinal symptoms (nausea, vomiting, diarrhea, abdominal pain), renal tubular injury, neuromuscular complications and often a rapid development of clinical resistance.[34] In addition, platinum is not effective for many common types of cancer.[35] The major limitation associated with the clinical application of cisplatin is the development of cisplatin resistance. In some cases, 10% of patients have displayed inherent resistance to this drug whereas 20% of patients treated with cisplatin will

eventually relapse with cisplatin resistant cancer. Studies carried out by Ozols *et. al.* have demonstrated that more than 70% of ovarian cancer patients are cured with cisplatin treatment at the beginning. However, 5 years later, the use of the same drug enables to cure only 15 to 20% of ovarian cancer[36] indicating the resistance power developed by cancer cells against this drug.[37] Expansion of tumor cell in the heterogeneous tumor cell population during initial treatment is the main cause of cisplatin resistance. Occurrence of acquired resistance to cisplatin has been explained by several mechanisms such as changes in cellular uptake, efflux on drug, inhibition of apoptosis, increased drug detoxification and increased DNA repair. Today, side effects and resistances induced by platinum complexes lead to an ongoing search to investigate new therapeutic approach with improved anticancer therapies.[19] In the search for drugs with reduced toxicity and broader spectrum of activity, other metal than platinum have been considered, such as vanadium, rhodium, ruthenium etc. These non-platinum metal complexes show different mechanism of action, biodistribution and toxicities from that of platinum-based drugs and might therefore be active against human malignancies that have either an intrinsic or an acquired resistance to them. Among these, ruthenium complexes are very promising especially from the viewpoint of overcoming cisplatin resistance with a low general toxicity.

1.3 Ruthenium anticancer complexes

The biological activity of ruthenium complexes were first recognized by Dwyer group in the 1950s.[38-40] Ruthenium complexes have found their way into the clinic and their properties are exploited for various purposes. The complexes of ruthenium have the potential to use as immunosuppressants (*cis*-$[Ru(III)(NH_3)_4(HIm)_2]^{3+}$), antimicrobials ($[Ru(II)Cl_2(chloroquine)_2]$ against malaria and others for the treatment of Chagas disease), antibiotics (the Ru(III) derivative of thiosemicarbazone against *Salmonella Typhi* and *Enterobacteria Faecalis*), nitrosyl delivery/scavenger tools (the Ru(III) polyaminocarboxylates known as AMD6245 and AMD1226 to treat stroke, septic shock, arthritis and diabetes), vasodilator/vasoconstrictor agents and as drugs for cancer chemotherapy.[41]

1.3.1 Ruthenium properties that make it suitable for biological applications

Ruthenium possesses three main properties that make it well suitable for medicinal applications: (1) multiple accessible oxidation states (2) ligand exchange kinetics and (3) the ability to mimic iron in binding to certain biomolecules.[42]

Oxidation state- Ruthenium complexes have the ability to access a range of oxidation states (II, III and IV most commonly) and all are easily accessible under physiologically relevant conditions. The energy required for inter conversion between these states is relatively low, allowing for inter conversion of oxidation state easily inside the cell.[43] In these oxidation states, ruthenium center is predominantly hexacoordinated, with essentially octahedral geometry. The coordination environment present around ruthenium plays a significant role in stabilizing the complexes in its oxidation states, hence influences the redox potential of the central metal atom.[44, 45] In biological systems the reducing agents such as glutathione, ascorbate etc. are able to reduce ruthenium(III) and ruthenium(IV)[46,47] whereas molecular oxygen and cytochrome oxidase readily oxidize ruthenium(II) in certain complexes.[48-50] The redox potential of ruthenium complexes can be exploited in order to enhance the efficacy of ruthenium based drugs in the clinical trails.[41,51] The relatively inert ruthenium(III) complexes are administered, which are activated by reduction within cancerous cells. In many cases, altered metabolism associated with cancer results in a lower oxygen concentration in tumor tissues, in comparison to healthy ones, hence promoting a reductive environment (Fig. 1.4). The reduction of ruthenium(III) to ruthenium(II) can be catalyzed by various proteins which include mitochondrial and microsomal single electron transfer proteins. The mitochondrial proteins are of particular interest in drug design as they can initiate apoptosis.[41] It is also possible for the ruthenium(II) complexes to convert back to inert ruthenium(III) by a variety of biological oxidants, if they leave the cancerous environment.

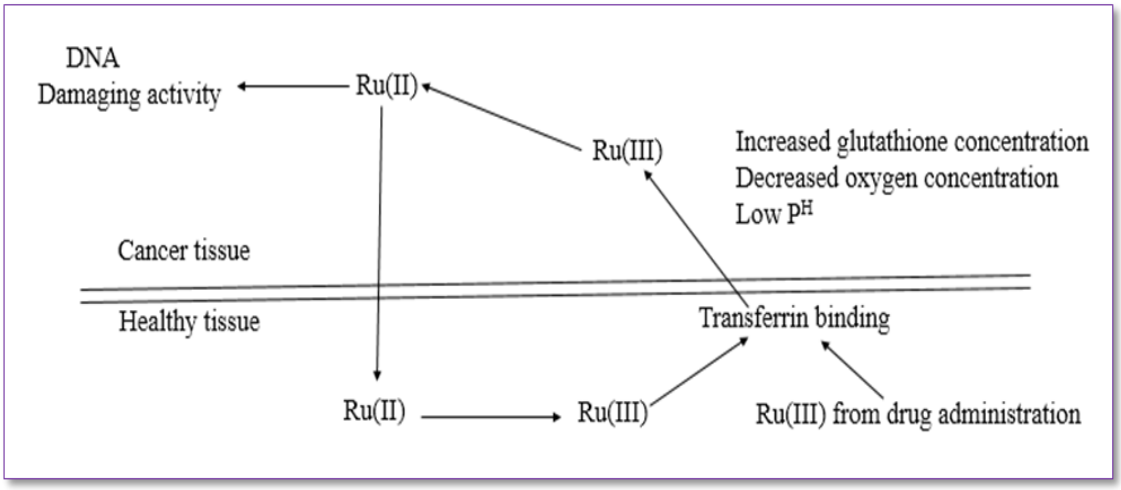


Fig. 1.4 The change of oxidation states of ruthenium in cancer and healthy cells.

Ligand exchange kinetics- Despite the flexibility in oxidation states, ruthenium complexes have displayed relatively slow ligand exchange rates in water in comparison with other transition metal complexes. The range of these exchange rates is around 10^{-2} to 10^{-4} s^{-1} which is on the timescale of an average cell's lifetime, thereby giving ruthenium high kinetic stability and preventing rapid equilibriation reactions.[52]

Iron mimicking- Ruthenium occupies in the same chemical group with iron, thereby can mimic iron in binding to serum transferrin and albumin.[53] These proteins (transferrin and albumin) transport iron ions in the blood plasma for metabolic purposes.[54] There are mainly two sites of transferrin at which it binds iron ion reversibly. Due to the similarity between iron(III) and ruthenium(III), ruthenium ion can capable of binding transferrin receptor at these particular sites. Since, rapidly dividing cells have a greater demand for iron, they increase the number of transferrin receptors on their surfaces, resulting in sequestration of more circulating iron-loaded transferrin. This implies the increase in concentration of ruthenium ion in the cancerous cells compared to healthy cells.[55] In this way, selectivity of the ruthenium drugs increases with reduced toxicity (Fig. 1.5).

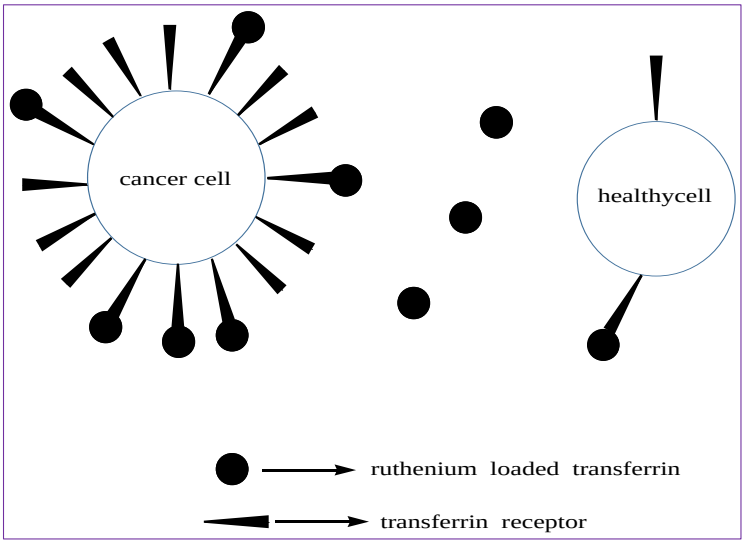
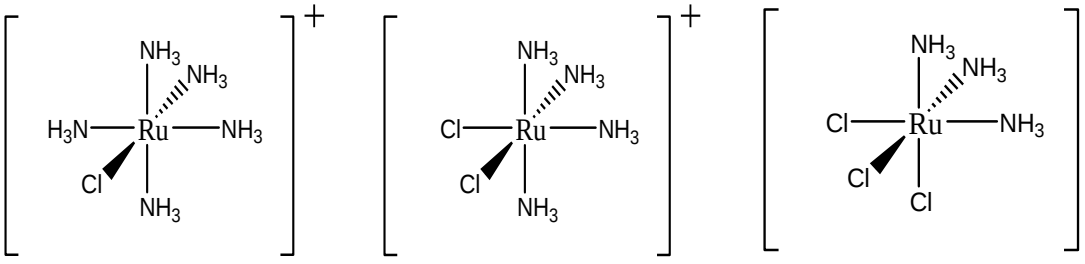


Fig.1.5 Schematic representation of selective uptake of transferrin by cancer cells.

1.3.2 Classification of ruthenium complexes with anticancer properties

1.3.2.1 Ammine-chlorido derivatives

Clarke and co-workers proposed the anticancer properties of chloride-ammine ruthenium(II) and ruthenium(III) complexes with general formula $[Ru(NH_3)_{6-x}Cl_x]^{Y+}$ in 1980s.[56] Ruthenium with +2 oxidation state are expected to bind with DNA similar to cisplatin and indeed the first experiment performed with the complexes $[Ru(II)(NH_3)_5Cl]^+$ (Fig.1.6) and $[Ru(II)(NH_3)_5(H2O)]^{2+}$ fulfilled this expectation.[57-59] The complexes are also tested for cytotoxicity in cancer cell lines but yielded disappointing results. Interestingly, the ruthenium complexes like *cis*-$[Ru(III)(NH_3)_4Cl_2]^+$ and *fac*-$[Ru(III)(NH_3)_3Cl_3]$ have displayed a comparable antitumour activity to that of cisplatin in a few selected cell lines.[42,60] One of the main issues with biological applications of these complexes is their poor water solubility.



$[Ru(II)(NH_3)_5Cl]^+$ *cis*-$Ru(III)(NH_3)_4Cl_2]^+$ *fac*-$[Ru(III)(NH_3)_3Cl_3]$

Fig. 1.6 Ammine-chlorido derivatives.

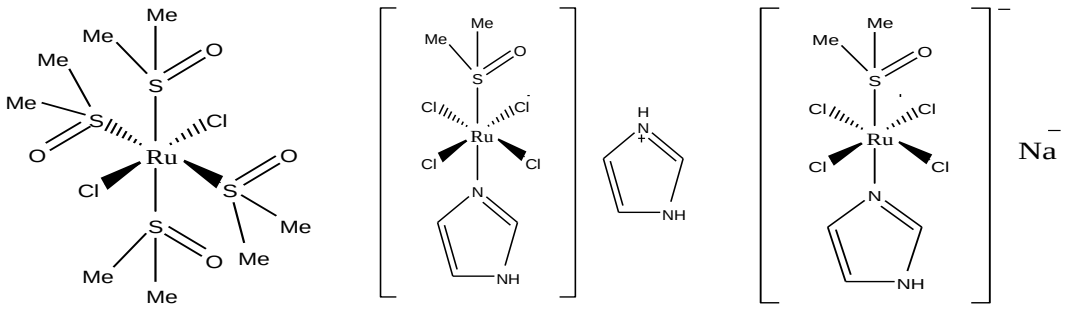
1.3.2.2 Dimethyl sulfoxide complexes

The next major class of compounds studied by Alessio, Sava and co-workers were highly water soluble Ru(II)chlorido-DMSO complexes where ammine ligands are substituted by DMSO molecules yield compounds with improved solubility.[61,62] Both *cis*- and *trans*- [Ru(II)Cl_2(DMSO)$_4$] (Fig.1.7) are able to coordinate to guanine residues of DNA but the *trans*-[Ru(II)Cl_2(DMSO)$_4$] complex is found to be much more cytotoxic than its *cis* counterpart.[63] The *trans*-[Ru(II)Cl_2(DMSO)$_4$] complex also seemed to overcome cisplatin resistance, as seen in the case of the P388 leukaemia cell line, indicating that this isomer shows a good antimetastatic activity.[64]

Based on the above mentioned promising compounds, a series of dimethyl sulfoxide-ruthenium complexes have been designed and among them the two most significant compounds are Na[*trans*-Ru(III)Cl_4(DMSO)(Him)], (Him = imidazole), nicknamed NAMI, and the more stable (Im)[*trans*-Ru(III)Cl_4(DMSO)(Him)], known as NAMI-A (Fig.1.7). NAMI-A is the first ruthenium complex to reach clinical testing for anticancer activity and has recently completed phase-I studies. It has been observed that two chlorido ligands of NAMI-A are substituted by aqua ligands. This hydrated species become more reactive to bind to several biomolecules, including DNA, proteins etc.[65,66] However, mechanism of action of both NAMI and NAMI-A is thought not to be directly related to binding to DNA, because *in vitro* studies have shown that NAMI-A can only bind weakly to DNA.[67]

1.3.2.3 NAMI-A type of complexes

In the search for novel ruthenium(III) complexes with better pharmacological profile, Bergamo *et. al*. synthesized a series of compounds analogues to NAMI-A. These compounds have the similar chemical structure like NAMI-A, but differ from their nature of coordinated nitrogen ligand such as pyrazole, thiazole and pyrazine.



trans-[Ru(II)Cl_2(DMSO)$_4$] Im[*trans*-Ru(III)Cl_4(DMSO)(Him)] Na[*trans*-Ru(III)Cl_4(DMSO)(Him)]

Fig.1.7 Structural formulas of dimethylsulfoxide complexes.

This modification leads to the better stability of NAMI-A in aqueous solution compared to the parent compound (Fig.1.8).[68] On the other hand, Groessl *et. al*. have recently reported that the NAMI-A analogues with the corresponding azole heterocycle ligands, such as triazole, 4-amino-1,2,4-triazole, etc. (Fig. 1.8), provide interesting pharmacological properties as well as display higher antiproliferative activity towards some human tumor cell lines *in vitro*.[69] In addition, the triazole analogue shares many similar characteristics with NAMI-A, for example, they all undergo hydrolysis when the compounds are dissolved within a physiological buffer at pH 7.4 and have a stronger binding affinity for proteins than corresponding platinum-based tumor inhibitors.[69,70] Webb *et. al*. synthesized a series of pyridine based derivatives of NAMI-A along with their sodium ion compensated analogues. These complexes show very high reduction potential demonstrated by electrochemical studies (Fig. 1.9).[71] Because of the similar structures and properties of these analogous with NAMI-A, they may become new potential members of NAMI-A-type antimetastatic agents.

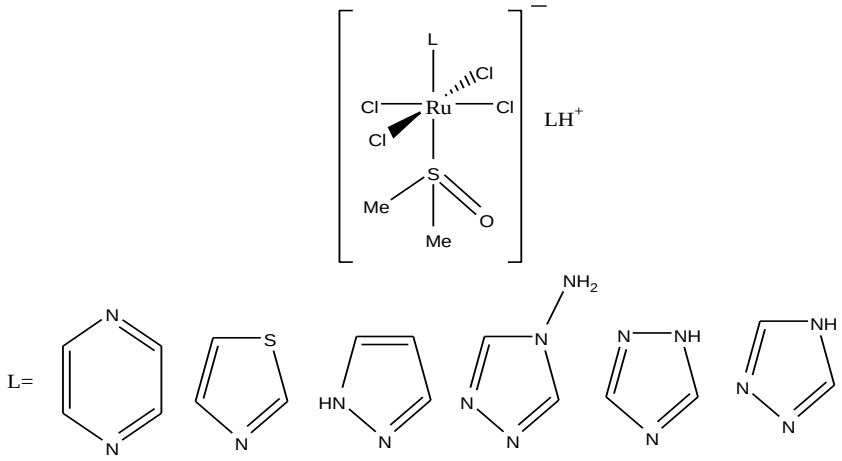


Fig.1.8 Chemical structure of NAMI-A derivatives.

1.3.2.4 Complexes with other heterocyclic ligands

Keppler and co-workers prepared a group of complexes which are called as "Keppler-type" compounds. The formula of Keppler type compound is *trans*-$[RuCl_4(L)_2]^-$, where L is imidazole (KP418) or indazole (KP1019 and KP1339), and the counterion $(LH)^+$ or Na^+ (Fig.1.10). KP1019 and KP1339 are reported to be inhibited platinum resistant colorectal carcinomas in rats.[72] Mechanism of action of these complexes are thought to differ considerably from that of cisplatin. "Activation by reduction"

process and the transferrin-mediated transport into the cells seem to play a significant role in determining the efficiency of the "Keppler-type" complexes.[73]

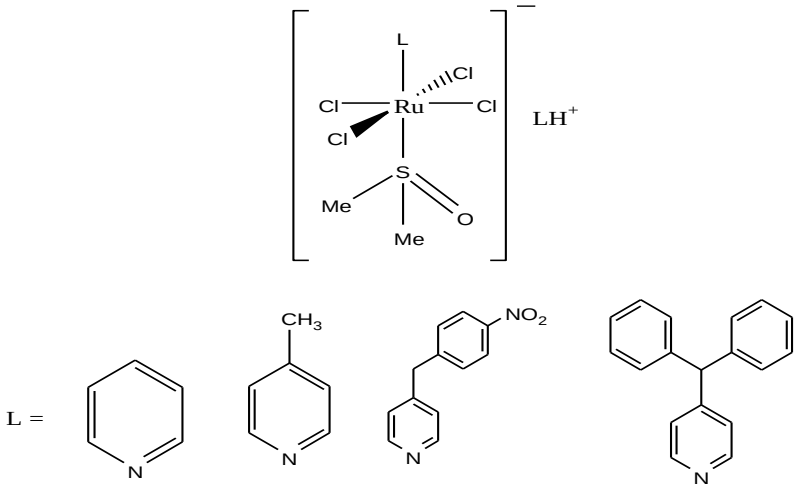
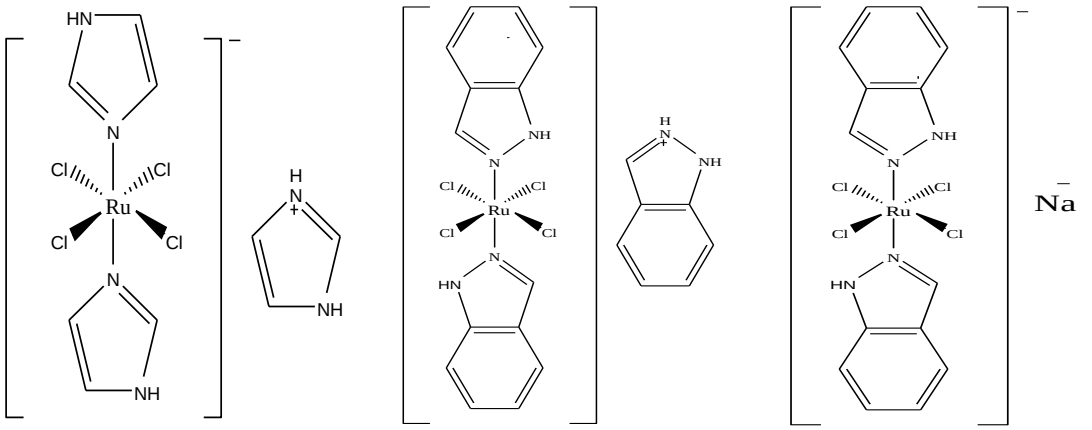


Fig.1.9 pyridine analogue of NAMI-A complex.

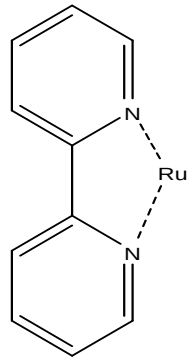


Im[*trans*-$RuCl_4(Him)_2$]) Ind[*trans*-$RuCl_4(Hind)_2$ Na[*trans*-$RuCl_4(Hind)_2$])

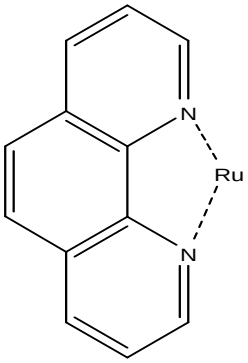
Fig.1.10 Structural formulas of KP418(Im[*trans*-$RuCl_4(Him)_2$]), KP1019(Ind[*trans*-$RuCl_4(Hind)_2$]), KP1339 (Na[*trans*-RuCl4$(Hind)_2$]).

1.3.2.5 Ruthenium polypyridyl complexes

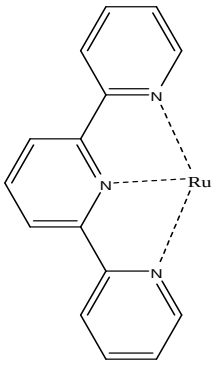
Ruthenium polypyridyl complexes have been extensively studied due to their ease of synthesis, stability, photoluminescence properties and the ability to intercalate DNA. Typical polypyridyl ligands which are commercially available and readily form stable complexes with ruthenium included 2,2'-bypyridine (bpy), 1,10-phenanthroline (phen) and 2,2':6'2"-terpyridine (terpy) (Fig.1.11).



2,2'-bypyridine



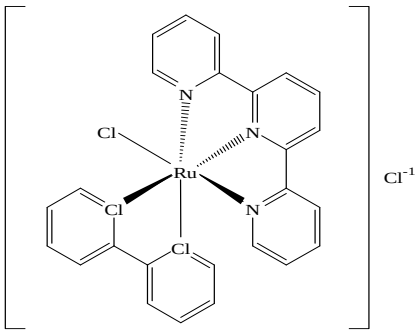
1,10-phenanthroline



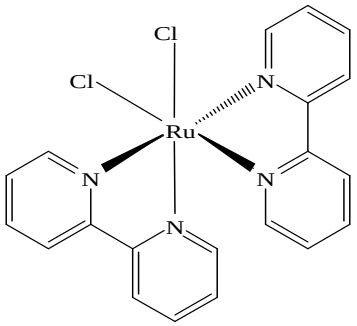
2,2':6'2"-terpyridine

Fig.1.11 Polypyridyl ligands.

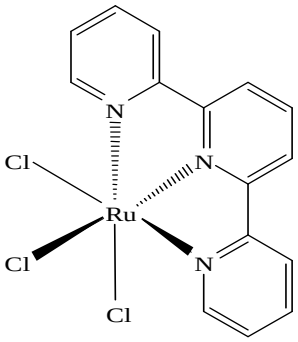
Δ and Λ enantiomers of *cis*-[Ru(bpy)$_2$Cl$_2$], *mer*-[Ru(terpy)Cl$_3$] are some of the earliest ruthenium polypyridyl complexes studied for potential anticancer activities. Several ruthenium polypyridyl complexes are synthesized and investigated for their DNA binding and antitumor activities against murine L1210 leukaemia and human cervix carcinoma HeLa cancer cell lines. Among them, only *mer*-[Ru(III)(tpy)Cl$_3$] complex is reported to be antitumor active (Fig.1.12).[74] It has been able to form interstrand DNA cross-links,[75,76] but poor water solubility hampered their further progress into the clinical trials.



[Ru(II)(bpy)(tpy)Cl]Cl



cis-[Ru(II)(bpy)$_2$Cl$_2$



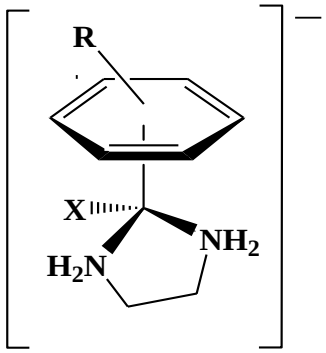
mer-[Ru(III)(tpy)Cl$_3$]

Fig. 1.12 Structural formula of ruthenium Polypyridyl complexes.

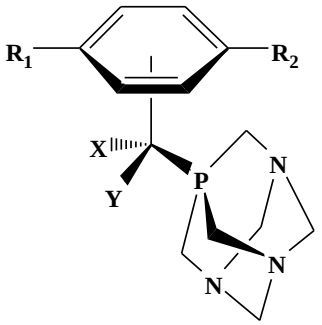
1.3.2.6 Organoruthenium complexes

A novel group of arene ruthenium(II) diammine complexes of the type [(η6-arene)Ru(II)(en)X] [PF6], where en is ethylenediamine and X is chloride or iodide (Fig. 1.13) developed[77] are believed to exert a strong antitumor activity against cancer

cells *in vitro* and are associated with DNA interaction.[78.79] The chlorido or iodido ligands are readily lost to yield the more reactive aqua complex.[80] DNA appears to be a target for these compounds, which bind preferentially to the guanine residues and also interact "non-covalently" via both arene intercalation and minor groove binding.[81,82] RAPTA-T ([(η6-toluene)Ru(II)(pta)Cl_2], where pta is 1,3,5-triaza-7-phosphaadamantane), one of the lead compounds, reduces the growth of lung metastases in mice bearing a mammary carcinoma and with only mild effects on the primary tumors (Fig.1.13).[83] RAPTA-T is also found to inhibit some steps of the metastatic process such as detachment from the primary tumor cell mass, migration/invasion and re-adhesion to a new growth substrate in breast cancer cell line. The mechanism of action of the RAPTA compounds is yet to be investigated.[84]



[(η6-arene)Ru(II)(en)X]



[(η6-arene)Ru(II)(pta)XY]

Fig.1.13 $[(\eta6\text{-arene})Ru(II)(en)X]^+$ (where arene= benzene, p-cymene, biphenyl, 5,8,9,10-tetrahydroanthracene or 9,10-dihydroanthracene, X is Cl or I) [(η6-arene)Ru(II)(pta)XY] (where R1, R2 = alkyl groups; X and Y = Cl/ different μ-dicarboxylate ligands.

1.3.3 Ruthenium complexes undergoing clinical trials

1.3.3.1 NAMI-A

NAMI-A (Fig.1.7) developed by Sava and co-workers, is the first ruthenium based drugs to undergo human clinical trials.

Preclinical studies

NAMI-A is capable of preventing the metastases formation as well as inhibiting their growth once established.[88] It has shown selective activity toward lung metastases formation, as observed from preclinical animal studies[89,90] and found to be active against various solid tumors.[91] Stability of NAMI-A can be improved by dissolving in

physiological concentration of sodium chloride because the complex is very unstable in physiological conditions like (pH 7.4, [Cl]=0.1 M, 37 °C).[92] NAMI-A is reported to be 1053 times less cytotoxic than cisplatin but have the same binding capacity to calf thymus DNA and can induced the numbers of GG and AG intrastrand adducts.[93] Extensive studies on structure, chemical properties[94] and effects on cellular level[95] of NAMI-A have been performed, but its exact mode of activity still remains unexplored. The proposed mechanisms of action of NAMI-A include: (1) blocking the cell cycle progression in the G2/M phase[96,97], (2) Preventing matrix metalloproteinases[98] (3) increase of extracellular matrix around tumor vasculature, thereby preventing neoplastic cells from invading adjacent tissues and blood vessels[97] and (4) binding to nucleic acids, therefore, resulting in direct effect on tumor cell DNA.[93]

In preclinical studies, administration of NAMI-A with a frequent smaller dosages shows more prominent antimetastatic effects.[99] It is seem to be independent of the type of primary tumor or the stage of growth of metastases but reduces the weight of lung metastases more than their number.[100]

Clinical trial

A phase I clinical trial and pharmacokinetic study of this antimetastatic agent began in 1999 and was reported in 2004.[101] In this study, 24 patients displaying various metastatic solid tumors including colorectal, lung, melanoma, ovarian and pancreatic cancers etc. refractory to conventional therapies are treated according to a dose escalation protocol. Patients are administrated with NAMI-A as a 3-h intravenous infusion daily for 5 days every 3 weeks. Twelve dose levels are administered to two groups of patients in the range of 2.4 mg/m^2/day (12 mg/m^2/cycle) - 500 mg/m^2/day (2500 mg/m^2/cycle). At a dose of 400 mg/m^2/day, patients suffer from transient painful blisters on the hands and feet. This painful blister formation is considered a dose-limiting toxicity and the recommended dose of NAMI-A for phase II studies is determined to be 300 mg/m^2/day.[101] 20 out of 24 patients in this study (83%) are evaluated for tumor responses. Only one heavily pretreated patient having progressive metastatic non-small cell lung cancer shows disease stabilization up to 21 weeks. Phase II clinical trials of NAMI-A has recently been conducted.[102, 103]

1.3.3.2 KP1019

Contemporary with the development of NAMI-A by Sava and co-workers, Keppler and co-workers discovered KP1019, [InH][*trans*-$RuCl_4(In)_2$] (In = indazole), a stable ruthenium(III) complex containing two indazole ligand coordinated to the metal center via nitrogen atoms (Fig.1.10).

Preclinical studies

It shows remarkable cytotoxic activity by inducing apoptosis in a number of cancer cell lines as well as in a primary cisplatin resistant colorectal tumors.[104, 105] It induces apoptosis in colorectal cell lines mainly through the intrinsic mitochondria pathway.[106, 107] The antitumor activity of these type of complexes are tested with different cancer cells including P388 and 1210 leukaemia, B16 melanoma and AMNN colon carcinoma and display positive response with favorable toxicity. Hydrolysis of KP1019 is also investigated by Keppler *et. al*. under different conditions[108,109] and appears to be more stable toward aquation and is readily taken up by the cells than NAMI-A. KP1019 also shows activity towards primary explants of human tumors that are found to be resistant to a variety of standard chemotherapeutic agents.[110]

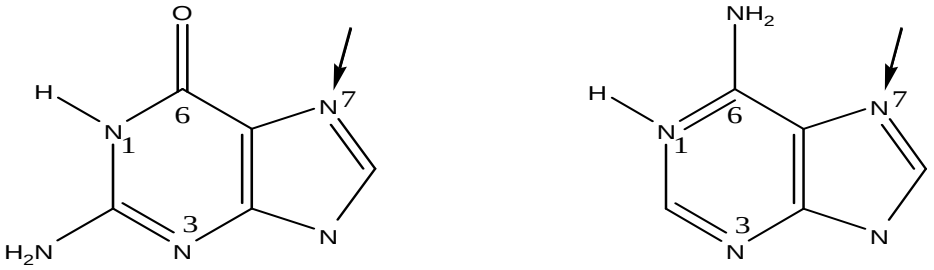
Clinical trails

KP1019 is the second ruthenium based agents that reached human clinical trials. A phase-I clinical trial and pharmacokinetic study has recently been reported.[111,112] In this study, 8 patients with advanced and refractory solid tumors (including colorectal, endometrial, melanoma, and bladder carcinomas) are administered with KP1019 with doses ranging from 25 to 600 mg twice weekly for 3 weeks and administered at a rate of 10ml/min. It has been observed that two of the eight patients dropped out of the study after only one treatment cycle and of the remaining six evaluable patients five experiences disease stabilization for 8–10 weeks. No significant dose-limiting toxic side effects could be observed. Its Phase II trials for the patients who are suffering from advanced colorectal cancer is currently being planned.[113]

1.3.4 Biological target of ruthenium drug

It is generally accepted that most drugs exert their action by binding to DNA.[114,115] Increasing evidence in the literatures reveal that anticancer activity of ruthenium complexes is based on their capability to coordinatively bind with DNA nucleobases.[116-119] Further, many experimental evidence suggest that the N7 site of

guanine bases of DNA (Fig. 1.14) is mainly the preferred binding target of ruthenium complexes, although binding to adenine and cytosine may also occur.[116-121]



Guanine Adenine

Fig. 1.14 Schematic diagram of guanine and adenine.

The mechanism of DNA binding has been explored and found that ruthenium complexes like KP1019 is capable of forming crosslinks between DNA strands but differs from the DNA intrastrand cross-links favored by cisplatin.[122] Binding to DNA via an additional mode was achieved when an intercalating polypyridyl ligand was added to the ruthenium system. On the other hand, organometallic arene ruthenium complexes are appear to coordinate DNA nucleobases through H-bonding and π stacking interactions.[123] But NAMI-A, the most successful ruthenium based anticancer drug developed till date, displays a unique behaviour. DNA damage is not the reason of its *in vivo* ability to reduce metastases weight.[124] NAMI-A appears to bind strongly to serum proteins, including the iron transporter transferrin and it induces cell arrest in the premitotic G(2)-M phase.[68]

Therefore, a deeper understanding of the interaction of ruthenium complexes with DNA and protein are very significant to reveal the mode of action of ruthenium-based drugs.

1.4 Computational approach

When an adduct formation occurs between biomolecules (DNA/proteins) and ruthenium complex, the thermodynamic stability and the functional properties of biomolecule will change. Understanding that how the adduct formation affects the structural or mechanical properties of biomolecules is the most significant step towards elucidating the functional mechanism of binding. In this regards, development of computational modeling tools as well as *ab initio* quantum mechanical calculations for investigating their binding properties are very

interesting.[125-127] One of the most popular computational approach is the density functional theory (DFT) method. In recent years, DFT has gained increasing interest from both in its conceptual and computational aspects.[128-131] Computational DFT basically deals with predicting molecular properties of a system with better quality/cost ratio while series of reactivity descriptors including global hardness, global softness, electronegativity, chemical potential, electrophilicity index, fukui function etc. have been introduced within the context of conceptual DFT.[132] DFT acts as a close connector between theory and experiment and often leads to important clues regarding the geometric, electronic and spectroscopic properties of the systems being studied.

In the past decades, computational studies made valuable contributions to compute electronic structure on drug-nucleoside complexes.[133-136] In a recent DFT study, the hydrolysis of the two ruthenium(III) antitumor complexes has been intensively studied at a wide range of different levels of theory.[124,137,138] Thermodynamics of binding of antitumor ammine, amine and immine complexes of ruthenium(II) and ruthenium(III) to DNA and peptides have been examined computationally[139] Further, studies on structural and energetic properties of organometallic ruthenium(II) diamine anticancer compounds and their interaction with nucleobases are explored using the DFT (BP86) and MP2 calculations together with *Car-Pari-nello* molecular dynamic calculations.[140] The theoretical predictions correlate very well with experimental findings.

1.5 Scope of the work

In conclusion, ruthenium complexes are particularly important in the clinic because of their low toxicity and shows promising anticancer activity in cells, animals and humans. In this chapter, we presented a brief introduction to ruthenium complexes and their anticancer activities in order to gain more insight about the way they function and subsequently, how they can be improved. It has been observed that, till date, two ruthenium complexes (NAMI-A and KP1019) are being evaluated for phase II clinical trials. Polypyridyl ruthenium(II) complexes have also been the focal point of present research works due to their DNA cleaving properties. However, the major limitation associated with these complexes is their unknown mechanism of action. Therefore, the investigation of the reaction pathway and binding properties of

ruthenium(III) and polypyridyl ruthenium(II) complexes toward biomolecules are very important for determining the reactive nature of these molecules. Further, need for DFT studies to analyze the structure and reactivity of these complexes as well as their interaction with biomolecules at molecular level are stressed.

- **Chapter 2** presents the overview about the basic theoretical and computational aspects used for this study.
- Structure and reactivity of some selected NAMI-A type of complexes are analyzed in **chapter 3.** A complete description is given regarding the geometries and usefulness of DFT based descriptors including global hardness, electrophilicity, chemical potential local philicity etc. in analyzing the reactivity of these complexes.
- In **chapter 4** hydrolysis mechanism of two NAMI-A type of complexes have been investigated in order to understand their mechanism of action at the molecular level. Our calculations provide a picture of hydrolysis of complexes with stepwise loss of chloride and DMSO ligands up to second aquation.
- In order to examines the stability and binding affinity of NAMI-A and its type, [4-amino-1,2,4-triazolium][*trans*-RuCl4 (4-amino-1,2,4-triazole) (DMSO-S)], inside the protein environment, we have studied their interaction with human serum albumin (**HSA**) by molecular docking and two layer QM/MM hybrid methods, which is included in **chapter 5A**. Interaction mechanism of ruthenium(III) complex, [Hind] [*trans*-RuCl4(2H-indazole) (DMSO-S)] with histidine and cysteine has been dealt in **chapter 5B**. This theoretical study provides detailed structural properties and energetics for the ruthenium(III) complexes. To establish the nitric oxide scavenging ability of NAMI-A as well as for understanding its antimetastatic activity, an interaction mechanism of NAMI-A with nitric oxide is carried out and is presented in **Chapter 5C.**
- In **chapter 6**, we describe the interaction of ruthenium(III) complexes with normal and mismatch base pair.
- **Chapter 7** deals with interaction of ruthenium(II) polypyridyl complexes with DNA sequences. Here, we have evaluated the information regarding the intercalative binding mode of the complexes with DNA receptors.
- In **Chapter 8**, the significant conclusions derived in this thesis have been summarized.

References

1. National Cancer Institute, www.cancer.gov.
2. World Health Organization: http://www.who.int/. (Accessed on June 13th 2009: http://www.who.int/entity/healthinfo/global_burden_disease/GBD_report_2004update_part2. Pdf.
3. "Defining Cancer". *National Cancer Institute*.
4. B. Bastien, PhD Thesis, University of strasbourg, 2012.
5. K. Sikira, O. Timbs, Exp. *Rev. Anticancer Ther*., 2004, **4**, s11-s12.
6. A. R. Sharma, D. M. Gangrade, S. D. Bakshi, J. S. John, *Int. J. Chem. Tech. Res*., 2014, **6**, 828-837.
7. B. Rosenberg, L. V. Van Camp, T. Krigas, *Nature (London)*, 1965, **205**, 698-699.
8. V. H. Mansour, B. Rosenbarg, L. Van Camp, J. E. Trosco, *Nature*, 1969, **222**, 385-386.
9. M. Peyrone, *Justus Liebigs Ann. Chem*, 1844, **51**, 1-29.
10. A. Z. Warner, *Anorg. Allg. Chem*., 1893, **3**, 267-330.
11. D. Higby, H. Wallace, D. Albert, J. Holland, *Cancer*, 1974, **33**, 1219-1225.
12. A. Dorcier, W. H. Ang, S. Bolano, L. Gonsalvi, L. J.-Jeannerat, G. Laurenczy, M. Peruzzini, A. D. Phillips, F. Zanobini, P. J. Dyson, *Organometallics*, 2006, **25**, 4090-4096.
13. M. D. Hall, T. W. Hamble, *Coord. Chem. Rev*., 2002, **232**, 49 –67.
14. N. J. Wheate, S. Walker, G. E. Craig, R. Oun, *Dalton Transactions*, 2010, **39**, 8113–8127.
15. F. Calabrò, P. Albers, C. Bokemeyer, C. Martin, L. H. Einhorn, A. Horwich, S. Krege, H. J. Schmoll, C. N. Sternberg, G. Daugaard, *Eur. Urol.*, 2012, **61**, 1212-1221.
16. E. R. Jamieson, S. J. Lippard, *Chem. Rev*., 1999, **99**, 2467-2498.
17. D. Wang, S. J. Lippard, *Nat.Rev.Drug Disc*., 2005, **4**, 307-320.
18. I. Kostova, *Recent Patents Anti-Cancer Drug Disc*., 2006, **1**, 1-22.
19. I. Ott, R. Gust, *Arch. Pharm*., 2007, **340**, 117–126.
20. C. A. Rabik, M. E. Dolan, *Cancer Treat. Rev.*, 2007, **33**, 9-23.

21. J. P. Caradonna, S. J. Lippard, M. J. Gait, M. Singh, *J. Am. Chem. Soc*., 1982, **104**, 5793-5795.

22. A. M. J. Fichtinger-Schepman, A. T. Van Oosterom, P. H. M. Lohman, F. Berends, *Cancer Res.*, 1987, **47**, 3000-3004.

23. A. M. J. Fichtinger-Schepman, J. L. Van der Veer, J. H. J. Den Hartog, P. H. M. Lohman, J. Reedijk, *Biochemistry*, 1985, **24**, 707-713.

24. J. Filipski, K. W. Kohn, W. M. Bonner, *Chemico-Biological Interactions*, 1980, **32**, 321-330.

25. A. Eastman, *Biochemistry*, 1986, **25**, 3912-3915.

26. J. C. G.-Ramos, R. G.-Murillo, F. C.-Guzman, L. R.-Azuara, *J. Mex. Chem. Soc*., 2013, **57**, 245-259.

27. K. Chvalova, V. Brabec, J. kasparkova, *Nucleic acids res*., 2007, **35**, 1812-1821.

28. D. Lebwohl, R Canetta, *Eur. J. Cancer*, 1998, **34**, 1522-1534.

29. G. Sonpavde, C. N. Sternbarg, *Future Oncol*, 2009, **5**, 931-940.

30. M. F. Morelli, A. Santomaggio, E. Ricevuto, K. Cannita, F. De Galitiis, M. Tudini, G. Bruera, M. Mancini, M. Pelliccione, F. Calista, F. Guglielmi, F. Martella, P. Lanfiuti baldi, G. Porzio, A. Russo, N. Gebbia, S. Iacobelli, P. Marchetti, C. Ficorella, *Oncol. Rep*., 2010, **23**, 1635-1640.

31. P. J. Souquet, M. Krzakowki, R. Ramlau, X. S. Sun, G. Lopez-Vivanco, C. Puozzo, J. C. Pouget, M. C. Pinel, R. Rosell, *Clin. Lung Cancer*, 2010, **11**, 105-113.

32. H. S. kim, G. W. Lee, J. H. Kim, H. Y. Kim, J. H. Kwon, H. H. Song, H. J. Kim, J. Y. Jung, G. Jang, G. R. Choi, S. M. Park, T. R. Shin, H. S. Lee, D. Y. Jang, *Lung Cancer*, 2010, **70**, 71-76.

33. Y. Fan, N. M. Lin, S. L. Ma, L. H. Luo, L. Fang, Z. Y. Huang, H. F. Yu, F. Q. Wu, *Acta Pharmacol. Sin*., 2010, **31**, 746-752.

34. M. Markman, *Expert. Opin. Drug. Saf.*, 2003, **6**, 597-607.

35. V. Brabec, J. Kasparkova, *Drug Resist. Updat*., 2005, **8**, 131–146.

36. R. Ozols, R. Young, *Semin. Oncol.*, 1991, **18**, 222-232.

37. R. Ozols, *Curr. Probl. Cancer*, 1992, **16**, 61–126.

38. F. P. Dwyer, E. C. Gyarfas, W. P. Rogers, J. H. Koch, *Nature*, 1952, **170**, 190–191.

39. F. P. Dwyer, E. Mayhew, E. M. F. Roe, A. Shulman, *Br. J. Cancer*, 1965, **19**, 195–199.

40. E. Meggers, *Curr. Opin. Chem. Biol*., 2007, **11**, 287–292.

41. C. S. Allardyce, P. J. Dyson, *Platinum Metals Rev.*, 2001, **45**, 62-69

42. M. J. Clarke, F. Zhu, D. R. Frasca, *Chem. Rev*., 1999, **99**, 2511–2534.

43. S. Page, "*Ruthenium compounds as anticancer agents". Education in Chemistry*, 2012.

44. J. Chakravarty, B. Bhattacharya, *Polyhedron*, 1996, **15**, 1047–1055.

45. S. Baitalik, B. Adhikary, *Polyhedron*, 1997, **16**, 4073–4080.

46. M. J. Clarke, S. Bitler, D. Rennert, M. Buchbinder, A. D. Kelman, *J. Inorg. Biochem.*, 1980, **12**, 79-87.

47. G. Sava, I. Capozzi, K. Clerici, G. Gagliardi, E. Alessio, G. Mestroni, *Clin. Exp. Metastasis*, 1998, **16**, 371–379.

48. D. M. Stanbury, O. Haas, H. Taube, *Inorg. Chem*., 1980, **19**, 518-524.

49. D. M. Stanbury, W. A. Mulac, J. C. Sullivan, H. Taube, *Inorg. Chem*., 1980, **19**, 3735-3740.

50. D. M. Stanbury, D. Gaswick, G. M. Brown, H. Taube, *Inorg. Chem*., 1983, **22**, 1975-1982.

51. O. Lentzen, C. Moucheron, A. K.-D. Mesmaeker, Metallotherapeutic *drugs & metal-based diagnostic agents. John Wiley & Sons, Ltd: West Sussex*, 2005, pp 359-378.

52. E. S. Antonarakis, A. Emadi, *Cancer Chemother. Pharmacol*., 2010, **66**, 1–9.

53. F. Kratz, L. Messori, *J. Inorg. Biochem*., 1993, **49**, 79–82.

54. A. Bergamo, G. Sava, *Dalton Trans*., 2011, **40**, 7817–7823.

55. G. Sava, A. Bergamo, *Int. J. Oncol*., 2000, **17**, 353-365.

56. M. J. Clarke, *Met. Ions Biol. Syst*., 1980, **11**, 231–283.

57. M. J. Clarke, M. Buchbinder, A. D. Kelman, *Inorg. Chim. Acta*, 1978, **27**, 87-88.

58. M. J. Clarke. B. Jansen, K. A. Marx, R. Kruger, *Inorg. Chim. Acta*, 1986, **124**, 13-28.

59. V. M. Rodriguez-Bailey, K. J. La Chance-Galang, P. E. Doan, M. J. Clarke, *Inorg. Chem*., 1997, **36**, 1873-1883.

60. J. R. Durig, J. Danneman, W. D. Behnke, E. E. Mercer, *Chem.Biol. Interact*., 1976, **13**, 287-294.

61. E. Alessio, G. Mestroni, G. Nardin, W. M. Attia, M. Calligaris, G. Sava, S. Zorzet, *Inorg. Chem.*, 1988, **27**, 4099–4106.

62. I. Bratsos, A. Bergamo, G. Sava, T. Gianferrara, E. Zangrando, E. Alessio, *J. Inorg. Biochem*., 2008, **102**, 606–617.

63. E. Alessio, G. Mestroni, G. Nardin, W. M. Attia, M. Calligaris, G. Sava, S. Zorzet, *Inorg. Chem.*, 1988, **27**, 4099-4106.

64. M. Coluccia, G. Sava, F. Loseto, A. Nassi, A. Boccarelli, D. Giordano, E. Alessio, G. Mestroni, *Eur. J. Cancer*, 1993, **29A**, 1873-1879.

65. E. Gallori, C. Vettori, E. Alessio, F. González-Vílchez, R. Vilaplana, P. Orioli, A. Casini, L. Messori, *Arch. Biochem. Biophys*., 2000, **376**, 156-162.

66. L. Messori, P. Orioli, D Vullo, E. Alessio, E. Iengo, *Eur. J. Biochem*., 2000, **267**, 1206-1213.

67. A. V. Vargiu, A. Robertazzi, A. Magistrato, P. Ruggerone, P. Carloni, *J. Phys. Chem. B*, 2008, **112**, 4401-4409.

68. A. Bergamo, B. Gava, E. Alessio, G. Mestroni, B. Serli, M. Cocchietto, S. Zorzet, G. Sava, *Int. J. Oncol*., 2002, **21**, 1331-1338.

69. M. Groessl, E. Reisner, C. G. Hartinger, R. Eichinger, O. Semenova, A. R. Timerbaev, M. A. Jakupec, V. B. Arion, B. K. Keppler, *J. Med. Chem*., 2007, **50**, 2185-2193.

70. E. Reisner, V. B.Arion, M. F. C. Guedes da Silva, R. Lichtenecker, A. Eichinger, B. K. Keppler, V. Y. Kukushkin, A. J. L. Pombeiro, *Inorg. Chem*., 2004, **43**, 7083-7093.

71. M. I. Webb , R. A. Chard, Y. M. Al-Jobory , M. R. Jones , E. W. Y. Wong, C. J. Walsby, *Inorg. Chem*., 2012, **51**, 954−966.

72. S. Kapitza, M. Pongratz, M. A. Jakupec, P. Heffeter, W. Berger, L. Lackinger, B. K. Keppler, B. Marian, *J. Cancer Res. Clin. Oncol*., 2005, **131**, 101-110.

73. C. G. Hartinger, S. Zorbas-Seifried, M. A. Jakupec, B. Kynast, H. Zorbas, B. K. Keppler, *J. Inorg. Biochem*., 2006, **100**, 891-904.

74. O. Novakova, J. Kasparkova, O. Vrana, P. M. Vanvliet, J. Reedijk, V. Brabec, *Biochemistry*, 1995, **34**, 12369–12378.

75. P. M. van Vliet, J. G. Haasnoot, J. Reedijk, *Inorg. Chem*., 1994, **33**, 1934-1939.

76. P. M. van Vliet, S. M. S. Toekimin, J. G. Haasnoot, J. Reedijk, O. Novakova, O. Vrana, V. Brabec, *Inorg. Chim. Acta*, 1995, **231**, 57-64.

77. R. E. Morris, R. E. Aird, P. S. del Murdoch, H. Chen, J. Cummings, N. D. Hughes, S. Parsons, A. Parkin ,G. Boyd, D. I. Jodrell, P. J. Sadler *J. Med. Chem*., 2001, **44**, 3616–3621.

78. H. Chen, J. A. Parkinson, S. Parsons, R. A. Coxall, R. O. Gould, P. J. Sadler, *J. Am. Chem. Soc*., 2002, **124**, 3064–3082.

79. H. Chen, J. A. Parkinson, O. Novakova, J. Bella, F. Wang, A. Dawson, R. Gould, S. Parsons, V. Brabec, R. E. Morris, P. J. Sadler, *J. Am.Chem. Soc*., 2003, **125,** 173–186.

80. F. Wang, H. M. Chen, S. Parsons, L. D. H. Oswald, J. E. Davidson, P. J. Sadler, *Chem.Eur. J.*, 2003, **9**, 5810-5820.

81. H. M. Chen, J. A. Parkinson, S. Parsons, R. A. Coxall, R. O. Gould, P. J. Sadler, *J. Am. Chem. Soc.*, 2002, **124**, 3064-3082.

82. F. Y. Wang, J. Bella, J. A. Parkinson, P. J. Sadler, *J. Biol. Inorg. Chem*., 2005, **10**, 147-155.

83. A. Bergamo, A. Masi, P. J. Dyson, G. Sava, *Int. J. Oncol*., 2008, **33**, 1281–1289.

84. P. J. Dyson, G. Sava, *Dalton Trans*., 2006, 1929-1933.

85. O. Lentzen, C. Moucheron, A. Kirsch-De Mesmaeker, *Metallotherapeutic drugs & metal-based diagnostic agents. John Wiley & Sons, Ltd: West Sussex*, 2005; pp 359-378.

86. B. Armitage, *Chem. Rev*., 1998, **98**, 1171-1200.

87. M. Pauly, I. Kayser, M. Schmitz, M. Dicato, A. Del Guerzo, I. Kolber, C. Moucheron, A. Kirsch-De Mesmaeker, *Chem. Commun*., 2002, 1086-1087.

88. G. Sava, I. Capozzi, K. Clerici, G. Gagliardi, E. Alessio, G. Mestroni, *Clin. Exp. Metastasis*. 1998, **16**, 371–379.

89. G. Sava, R. Gagliardi, A. Bergamo, E. Alessio, G. Mestroni, *Anticancer Res*. 1999, **19**, 969–972.

90. G. Sava, K. Clerici, I. Capozzi, M. Cocchietto, R. Gagliardi, E. Alessio, G. Mestroni, A. Perbellini, *Anticancer Drugs*, 1999, **10**, 129–138.

91. G. Mestroni, E. Alessio, G. Sava, WO1998000431 A1, 1998.

92. M. Groessl, C. G. Hartinger, P. J. Dyson, B. K. Keppler, *J. Inorg. Biochem*., 2008, **102**, 1060−1065.

93. D. Pluim, R. C. A. M. van Waardenburg, J. H. Beijnen, J. H. M. Schellens, *Cancer Chemother Pharm*., 2004, **54**, 71-78.

94. M. Bouma, B. Nuijen, M. T. Jansen, G. Sava, A. Flaibani, A. Bult, J. H. Beijen, *Int. J. Pharm*., 2012, **248**, 239-246.

95. M. Bacac, A. C. G. Hotze, K .Van Der Schilden, J. G. Haasnoot, S. Pacor, E. Alessio, G. Sava, J. Reedijk, *J. Inorg. Biochem*., 2004, **98**, 402-412.

96. A. Bergamo, R. Gagliardi, V. Scarcia, A. Furlani, E. Alessio, G. Mestroni, G. Sava, *J. Pharmacol. Exp. Ther*., 1999, **289**, 559–564.

97. S. Zorzet, A. Bergamo, M. Cocchietto, A. Sorc, B. Gava, E. Alessio, E. Iengo, G. Sava, *J. Pharmacol. Exp. Ther*, 2000, **295**, 927–933.

98. A. Vacca, M. Bruno, A. Boccarelli, M. Coluccia, D. Ribatti, A. Bergamo, S. Garbisa, L. Sartor, G. Sava, *Br. J. Cancer*, 2002, **86**, 993–998.

99. R. Gagliardi, G. Sava, S. Pacor, G. Mestroni, E. Alessio, *Clin. Exp. Metastasis*., 1994, **12**, 93–100.

100. G. Sava, R. Gagliardi, A. Bergamo, E. Alessio, G. Mestroni, *Anticancer Res*., 1999, **19**, 969–972.

101. J. M. Ramdemaker-Lakhai, D. Van den Bongard, D. Pluim, J. H. Beijnen, J. H. Schnellenes, *Clin. Cancer Res*., 2004, **10**, 3717-3727.

102. M. Brindell, I. Stawoska, J. Supel, A. Skoczowski, G. Stochel, R. van Eldik, *J. Biol. Inorg. Chem*., 2008, **13**, 909-918.

103. E. Reisner, V. B. Arion, B. K. Keppler, A. J. L. Pombeiro, *Inorg. Chim. Acta*., 2008, **361**, 1569-1583.

104. M. Galanski, V. B. Arion, M. A. Jakupec, B. K. Keppler, *Curr. Pharm. Des*., 2003, **9**, 2078–2089.

105. S. Kapitza, M. Pongratz, M. A. Jakupec, P. Heffeter, W. Berger, L. Lackinger, B. K. Keppler, B. Marian, *J. Cancer. Res. Clin Oncol*., 2005, **131**, 101–110.

106. C. G. Hartinger, S. Zorbas-Seifried, M. A. Jakupec, B. Kynast, H. Zorbas, B. K. Keppler, *J. Inorg. Biochem*., 2006, **100**, 891-904.

107. J. Malina, O. Novakova, B. K. Keppler, E. Alessio, V. Brabec, *J. Biol. Inorg. Chem*., 2001, **6**, 435-445.

108. J. Chatlas, R. Van Eldik, B. K. Keppler, *Inorg. Chim. Acta*, 1995, **233**, 59-63.

109. O. M. Ni Dhubhghaill, W. R. Hagen, B. K. Keppler, K. G. Lipponer, P. J. Sadler, *J. Chem. Soc., Dalton Trans.*,1994, 3305-3310.

110. C. G. Hartinger, S. Zorbas-Seifried, M. A. Jakupec, B. Kynast, H. Zorbas, B. K. Keppler, *J. Inorg. Biochem.*, 2006, **100**, 891–904.

111. F. Lentz, A. Drescher, A. Lindauer, M. Henke, R. A. Hilger, C. G. Hartinger, M. E. Scheulen, C. Dittrich, B. K. Keppler, U. F. Jaehde., *Anticancer Drugs*, 2009, **20**, 97–103.

112. M. M. Henke, H. Richly, A. Drescher, M. Grubert, D. Alex, D. Thyssen, U. Jaehde, M. E. Scheulen, *Int. J. Clin. Pharmacol. Ther*., 2009, **47**, 58–60.

113. C. G. Hartinger, M. A. Jakupec, S. Zorbas- Seifried, M. Groessl, A. Egger, W. Berger, H. Zorbas, P. Dyson, J. Paul, B. K. Keppler, *Chem. Biodive.*, 2008, **5**, 2140–2155.

114. M. J. Clarke, *Coord. Chem. Rev*., 2002, **232**, 69-93.

115. A. H. Velders, A. C. G. Hotze, G. A. van Albada, J. G. Haasnoot, J. Reedijk, *J. Inorg. Chem.*, 2000, **39**, 4073-4080.

116. V. Brabec, O. Novakova, *Drug Resistance Update*, 2006, **9**, 111-122.

117. C. C. Kuehn, H. Taube, *J. Am. Chem. Soc*., 1976, **98**, 689-702.

118. S. Fruhauf, W. J. Zeller, *Cancer Res*., 1991, **51**, 2943-2948.

119. E. Gallori, C. Vettori, E. Alessio, F. G. Vilchez, R. Vilaplana, P. Orioli, A. Casini, L. Messori, *Arch. Biochem. Biophys*., 2000, **376**, 156-162.

120. M. J. Clarke, B. Jansen, K. A. Marx, R. Kruger, *Inorg. Chim. Acta*, 1986, **124**, 13-28.

121. M. A. McNamara, M. J. Clarke, *Inorg. Chim. Acta*, 1992, **195**, 175-185.

122. S. Kapitza, M. A. Jakupec, M. Uhl, B. K. Keppler, B. Marian, *Cancer lett.*, 2005, **226**, 115-121.

123. R. Fernandez, M. Melchart, A. Habtemariam, S. Parsons, P. J. Sadler, *Chem. Eur. J.*, 2004, **10**, 5173-5179.

124. A. V. Vargiu, A. Robertazzi, A. Magistrato, P. Ruggerone, P. Carloni, *J. Phys. Chem. B* , 2008, **112**, 4401-4409.

125. W. Rzeski, J. Matysiak, M. Kandefer-Szerszen, *Bioorg. Med. Chem*., 2007, **15**, 3201–3207.

126. O. A. Santos-Filho, R. B. de Alencastro, J. D. Figueroa-Villar, *Biophys. Chem*., 2001, **91**, 305–317.

127. C. H. T. P. Silva, C. A. Taft, *Biophys. Chem*., 2005, **117**, 73–77.

128. R. G. Parr, W. Yang, *Density-Functional Theory of Atoms and Molecules*; Oxford University Press: New York, 1989.

129. R. G. Parr, W. Yang, *Annu. Rev. Phys. Chem*., 1995, **46**, 701-728.

130. P. Geerlings, F. De Proft, W. Langenaeker, *Adv. Quantum. Chem*., 1999, **33**, 303-328.

131. H. Chermette, *J. Comput. Chem.,* 1999, **20**, 129-154.

132. P. Sarmah, R. C. Deka, *Int. J. Quant. Chem*., 2008, **108**, 1400-1409.

133. J. K. C.Lau, D. V. Deubel, *Chem. Eur. J*., 2005, **11**, 2849-2855.

134. M. H. Baik, R. Friesner, S. J. Lippard, *J. Am. Chem. Soc*., 2003, **125**, 14082-14092.

135. T. Zimmermann, M. Zeizinger, J. V. Burda, *J. Inorg. Biochem*., 2005, **99**, 2184-2196.

136. J. Raber, C. B. Zhu, L. A. Eriksson, *J. Phys. Chem. B*, 2005, **109**, 11006-11015.

137. J. C. Chen, L. M. Chen, S. Y. Liao, K. C. Zheng, L. N. Ji, *Dalton Trans*., 2007, **32**, 3507-3515.

138. J. C. Chen, L. M. Chen, S. Y. Liao, K. C. Zheng, L. N. Ji, *J. Phys. Chem. B*, 2007, **111**, 7862-7869.

139. N. Besker, C. Coletti, A. Marrone, N. Re, *J. Phys. Chem. B*, 2007, **111**, 9955-9964.

140. C. Gossens, I. Tavernelli, U. Rothlisberger, *J. Chem. Theory. Comput.,* 2007, **3**, 1212-1222.