CHAPTER 1

Introduction to Ruthenium complexes as anticancer agents.

An overview about Medicinal Inorganic Chemistry is given, with special attention to the role that platinum and ruthenium play in it.

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The desire to understand how the individual molecules that make up cells organize, interact, and communicate to form living systems has led to the burgeoning field of chemical biology, an interfacial area of science that combines aspects of chemistry and biology. The defining feature of chemical biology is the use of chemical approaches and small molecules to interrogate or manipulate biology [1]. These small molecules are synthetic or naturally occurring ones that, for example, bind to DNA to affect protein expression levels, bind to proteins to inhibit their function, interact with lipids to alter membrane integrity, or become fluorescent in response to a metabolic event. Because small molecules can affect biochemical function, there is a clear link between chemical biology and pharmacology and medicine [2].

1.1 Bioinorganic chemistry: Metals in medicine

While Life was thought to be built exclusively from organic "bricks", inorganic small molecules also have had a long history in both biology and medicine. In the late 1800´s, experiments carried out with blood samples revealed the existence of iron containing compounds in this fluid [3]. The presence of metals in different enzymes was proven [4] and bioinorganic chemistry was granted the status of a separate discipline in the 1970´s [5]. Nowadays, it is known that inorganic elements play diverse biological roles, such as stabilization of structures (e.g. $CaCO_3$ stabilizes the structure of the bones; the $PO4^{3-}$ groups stabilize the DNA structure), transport of molecules (e.g. haemoglobin, an iron containing protein, which transports oxygen in the bloodstream), transfer of electrons (e.g. cytochrome *c*), redox and other enzymatic reactions (copper, iron, zinc and manganese form part of several metalloenzymes), etc. The fact that some metal ions are essential for life also suggested the possibility of incorporating metal atoms into drugs.

Ancient civilizations used gold and copper for healing purposes, and the modern era of drug discovery was ushered in when arsenic-containing salvarsan was discovered as an antisyphilis agent which was synthesized and tested in the beginning of the 20th century by Ehrlich (*Fig.1.1*) [6].

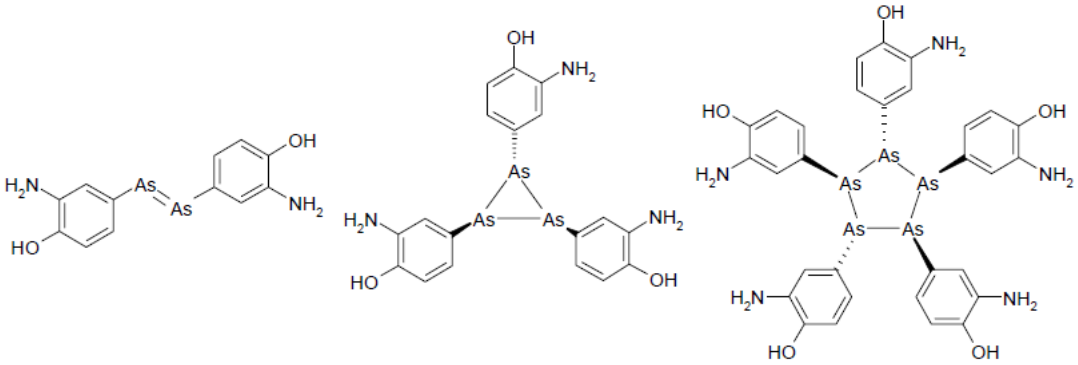

Fig.1.1: *Molecular structure of the arsenic drug salvarsan as proposed by Ehrlich (left). In 2005, salvarsan was proven to consist of a mixture of cyclic species (centre and right).*

Inorganic compounds should therefore not be overlooked in the realm of chemical biology, since their distinctive electronic, chemical, and photophysical properties render them particularly useful for a variety of applications [7-9].

What are the properties of metal ions that impart utility to biology? Because inorganic elements comprise the bulk of the periodic table, the diversity of these properties is likewise broad and has been thoroughly covered by several books in the field of bioinorganic chemistry [10-12]. A brief summary of the general chemical properties of metals is given below.

- **Charge:** Metal ions are positively charged in aqueous solution, but that charge can be manipulated depending on the coordination environment so that a metal complexed by ligands can be cationic, anionic, or neutral.
- **Interactions with ligands:** Metal ions bind to ligands via interactions that are often strong and selective. The ligands impart their own functionality and can tune properties of the overall complex that are unique from those of the individual ligand or metal. The thermodynamic and kinetic properties of metal-ligand interactions influence ligand exchange reactions.
- **Structure and bonding:** Metal-ligand complexes span a range of coordination geometries that give them unique shapes compared with organic molecules. The bond lengths, bond angles, and number of coordination sites can vary depending on the metal and its oxidation state.
- **Lewis acid character:** Metal ions with high electron affinity can significantly polarize groups that are coordinated to them, facilitating hydrolysis reactions.
- **Partially filled d-shell:** For the transition metals, the variable number of electrons in the d-shell orbitals (or f-shell for lanthanides) imparts interesting electronic and magnetic properties to transition metal complexes.
- **Redox activity:** Coupled with the variability of electrons in the d-shell is the ability for many transition metals to undergo one-electron oxidation and reduction reactions.

Biology has taken advantage of these chemical properties of metals to perform several functional roles. This is by no means an exhaustive list but rather a primer to highlight important themes. When it comes to applying inorganic compounds to biology, chemists are not restricted to the naturally bioavailable set of metals and can take advantage of the properties of biologically exotic elements, including second and third row transition elements and the lanthanide (Ln) elements. This research area has been developed and metal-based compounds have appeared from anecdotic to widely used as treatments for several diseases as stomach ulcers (bismuth) [13]; diabetes (vanadium) [14]; rheumatoid arthritis (gold) [15] and cancer (platinum) [16]. Another important set of applications of metal complexes in medicine is their

use for diagnosis purposes such as for example contrast agent in Magnetic Resonance Imaging (MRI) (gadolinium-, manganese- or iron-based complexes) [17,18] or as radio-pharmaceuticals (^{99m}technetium, ^{68}galium or ^{186}rhenium) [19,20]. A cartoon-picture of the current use of metal-based and inorganic compounds in the clinic is depicted in *Fig. 1.2* [21].

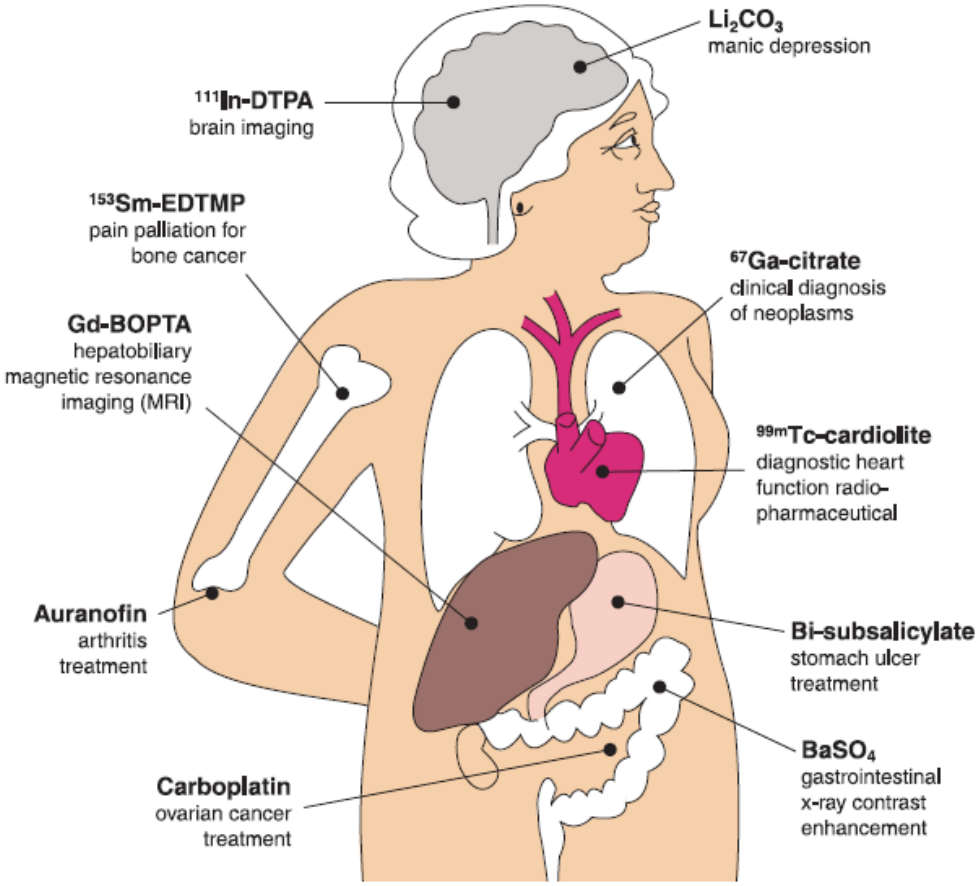

Fig. 1.2: *Overview of the use of metal-based drugs in medicine.*

❖ ***Metal Complexes for Probing DNA***

An ongoing effort toward unlocking biological information contained within DNA is the pursuit of molecules that can specifically interact with, label, or cleave oligonucleotide sequences. DNA sequences of interest can be specifically targeted by taking advantage of sequence-dependent changes in overall three-dimensional shape and surface electronic distribution. Metal complexes are especially well suited for DNA interaction because the electropositive metal center is innately attracted to the negatively charged phosphate backbone of DNA. Metal centers also serve as unique three dimensional structural scaffolds, which can be adjusted to fit into the base stacks and grooves of targeted DNA sequences.

1.2 Platinum-based anticancer drugs:

1.2.1 Pt(II) complexes:

❖ **The discovery of cisplatin as an anticancer agent:**

Medicinal inorganic chemistry as a discipline is considered to have boosted with the discovery of the anticancer properties of cisplatin [22]. Cisplatin was the first chemical compound to become the subject of a mechanistic study: its mechanism of action was investigated, as well as the way to optimize its activity.

❖ **History of cisplatin, a leading anti-cancer drug:**

cis-diamminedichloridoplatinum(II) was first described by Peyrone in 1845 [23]. Together with its *trans* analogue, this complex was used by Werner in 1893 as the first example of isomers in Coordination Chemistry. Its activity against cancer remained, however, unknown until 1964, when Rosenberg realized that the platinum electrodes used in one of his experiments affected bacterial growth [24,16]. The main species responsible for that was found to be *cis*-$Pt(NH_3)_2Cl_2$, which was formed slowly by reaction of the electrodes with the electrolyte NH_4Cl solution. The drug entered clinical trials in 1971 and by the end of 1987 it was already the most widely used anticancer medicine [25]. Unfortunately, the use of this compound did not bring a definitive end to cancer, since it only showed anticancer activity against certain types of tumours. Some tumours resist the action of cisplatin, this resistance being in some cases intrinsic, but also in some others acquired. Finally, cisplatin therapy produces severe side-effects, namely neurotoxicity, ototoxicity, nausea, vomiting, bone marrow dysfunction and nephrotoxicity, the latter being dose-limiting. Research has been focused on several fronts. Understanding the transport of the drug in the body and its cellular uptake, as well as its mechanism of action inside the cell, is crucial for the design of improved pharmaceuticals. The development of synthetic methods that rapidly yield compound libraries to be screened afterwards for anticancer activity allows for a very efficient trial-and-error strategy. Since cisplatin is indeed effective against certain tumours, studies are also being done about how to avoid its undesired side effects, while still retaining the therapeutic value of the drug.

❖ **Cisplatin: mechanism of action:**

Concerning the mechanisms of action, cisplatin remains in its intact dichlorido form in the blood stream due to the high extracellular chloride concentration (100 mM). Once it enters the cells, hydrolysis of one or both chlorido ligands to afford aqua complexes

$[Pt(NH_3)_2Cl(OH_2)]^+$ or $[Pt(NH_3)_2(OH_2)_2]^{2+}$ will occur due to the lower chloride intracellular concentration (4-50 mM). These aqua species will then react with the biological targets [26]. DNA is recognized to be the main intracellular target of platinum-based drugs and Pt-nucleic acids interactions have been abundantly reviewed [26-29]. Platinum(II) compounds interact with DNA *via* the coordination of the *cis*-$[Pt(NH_3)_2]$ fragment to the N7 atom of purine bases (guanine and adenine) by displacing the aqua ligands to form mainly 1,2- *cis*(GG) and *cis*(AG), 1,3-intrastrand *cis*(GNG) crosslinks or interstrand crosslinks [26] (*Fig. 1.3*)

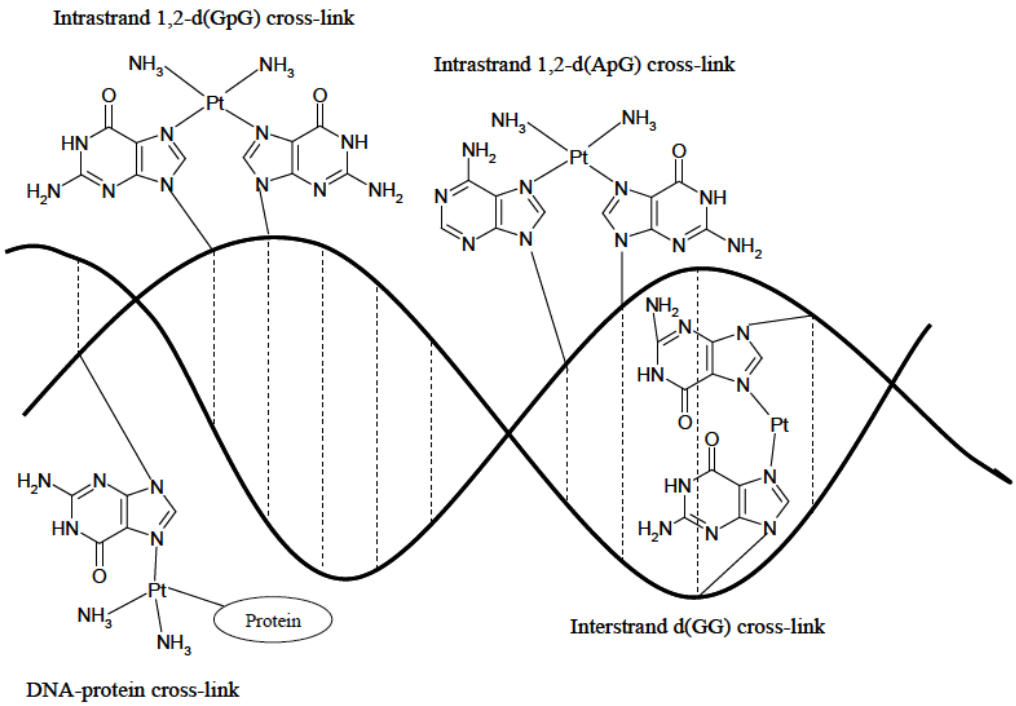

Fig.1.3: *Schematic view of a double-stranded DNA, depicting some of the most commonly occurring Pt-DNA adducts.*

Such crosslinks distort the structure of DNA duplex, notably by bending it significantly toward the major groove modifying the binding site of proteins. This bending can interfere with several transduction pathways including the p53 pathway, a protein responsible of induction of cell-cycle arrest or apoptosis in response to cellular stress and mutated in the majority of human tumors. Moreover, it has been shown that cisplatin can induce G2-arrest leading to cell death [26,27].

❖ **Development of new platinum anticancer agents:**

Thousands of platinum compounds have been synthesized in an attempt to overcome the problems of cisplatin. Surprisingly none of these has been able to substitute cisplatin

in routine chemotherapy treatments. The observation of the first platinum complexes synthesized and their efficacies as antitumour agents led to what was called the "structure-activity relationships" (SAR´s) [25]. This was a list of structural characteristics that a platinum complex was thought to require in order to show an antitumour activity. Subsequently every new compound was designed according to these rules. The most successful of the second-generation platinum compounds is *cis*-diammine-1,1-cyclobutane-dicarboxylatoplatinum(II), also known as carboplatin (*Fig.1.4*). Since its introduction in 1986 it has been preferred to cisplatin in the treatment of many platinum-sensitive malignancies. Carboplatin has less severe side effects than cisplatin, but it is cross-resistant with it. Its activity is equivalent to cisplatin in the treatment of ovarian cancers, however in the treatment of testicular, head and neck cancers cisplatin is superior [30,31]. Two other second- and third-generation compounds have been approved for clinical use. *cis*-diammine(glycolato)platinum(II) (nedaplatin) [32] (*Fig.1.4*) was approved in 1995 by the Health and Welfare Ministry in Japan [33] and various studies of combined therapies of the platinum complex with other drugs are undergoing clinical trials for the treatment of urothelial, uterine, lung, esophageal or testicular cancer, amongst others [34-39]. (1R,2R-diaminocyclohexane)oxalatoplatinum(II) (oxaliplatin) [40] (*Fig.1.4*) was approved in France and in a few other European countries mainly for the treatment of metastatic colorectal cancer. Clinical studies pointed out that the myelosuppression and nephrotoxicity caused by oxaliplatin are less intense in comparison with cisplatin treatment, however neuropathy occurs more frequently in case of the patients treated with this third-generation compound [33].

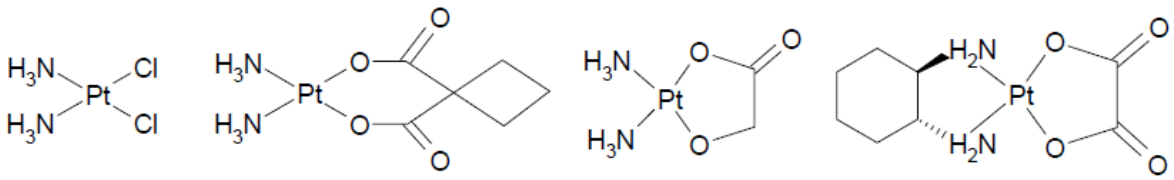

Fig.1.4: *Molecular structure of a few selected platinum drugs. From left to right: cisplatin, carboplatin, nedaplatin and oxaliplatin.*

Since it became evident that mere analogues of cisplatin or carboplatin would probably not offer any substantial clinical advantages over the existing drugs, as complexes of this kind can be expected to have similar biological consequences to cisplatin, some platinum complexes were synthesised which contradicted the SAR´s.

1.2.2 Pt(IV) complexes:

Pt(IV) compounds present an octahedral geometry introducing two extra binding sites compared to Pt(II) complexes. Moreover, the kinetic inertness of Pt(IV) compounds toward reduction or ligand substitution makes them unlikely to react with biological nucleophiles. This increases their lifetime in blood and thus their chance to reach the tumor and enter the cells intact. It is hypothesized that the activity of Pt(IV) compounds follows the reduction of Pt(IV) to Pt(II) and the subsequent release of the two axial ligands leading to the biologically active Pt(II) species.

Within the various families of experimental Pt(IV) compounds developed so far, different drug design strategies were applied including: i) tuning the rate of reduction of the Pt(IV) center and the compound's lipophilicity to optimize the release of Pt(II) drug into the cell [41] and ii) making use of the two axial positions to couple Pt(II) moieties to bioactive ligands to allow "bifunctional" and targeted therapy [42]. Satraplatin, a *trans*-diacetato-*cis*-dichlorido Pt(IV) compounds with a cyclohexylamine ligand (*Fig. 1.5*) has been shown to induce cell death in cisplatin-resistant cell lines including human ovarian, lung and prostate cancer cell lines [43,41]. Satraplatin passed Phase I clinical trials in which myelo suppression and nausea appeared as dose limiting toxicities (DLTs). It reached Phase II clinical trial for the treatment of small-cells lung cancer and hormone refractory prostate cancer. It also gave interesting results in Phase III clinical trials in combination with prednisone on patients with refractory cancer [Satraplatin and prednisone against refractory cancer (SPARC trial)] reducing by 40 % the risk of cancer progression. However, it was rejected by the FDA due to lack of benefits in terms of overall survival. It is currently under Phase I, II and III clinical trials in combination with different drugs.

Some groups took advantage of the extracellular stability of the Pt(IV) metal center to build up targeted cisplatin pro-drugs and "bifunctional" compounds bearing organic drugs. The main limitation of this approach is the intrinsic inertness of Pt(IV) complexes which renders the exchange of the axial ligands impossible and limits the scope of ligands that can be introduced in these positions to the ones that can be incorporated during the oxidation step. Following this methodology, some *trans*-dihydroxido and *trans*-dichorido complexes were synthesized by oxidation with hydrogen peroxide or chlorine gas respectively [44,45]. Keppler *et al.* reacted the dihydroxido complex with succinic anhydride to obtain complexes with free carboxylate groups available for further coupling with alcohols or amines [46]. Dyson *et al.* developed a cisplatin-based Pt(IV) complex incorporating two equivalents of ethacrynic acid (ethacraplatin, *Fig. 1.5*), a known inhibitor of the enzyme Glutathione-S-transferase which is supposed to be involved in the

mechanism of resistance to cisplatin. Indeed, ethacraplatin showed a faster toxicity against all tested cisplatin-resistant cell lines compared to cisplatin alone [47].

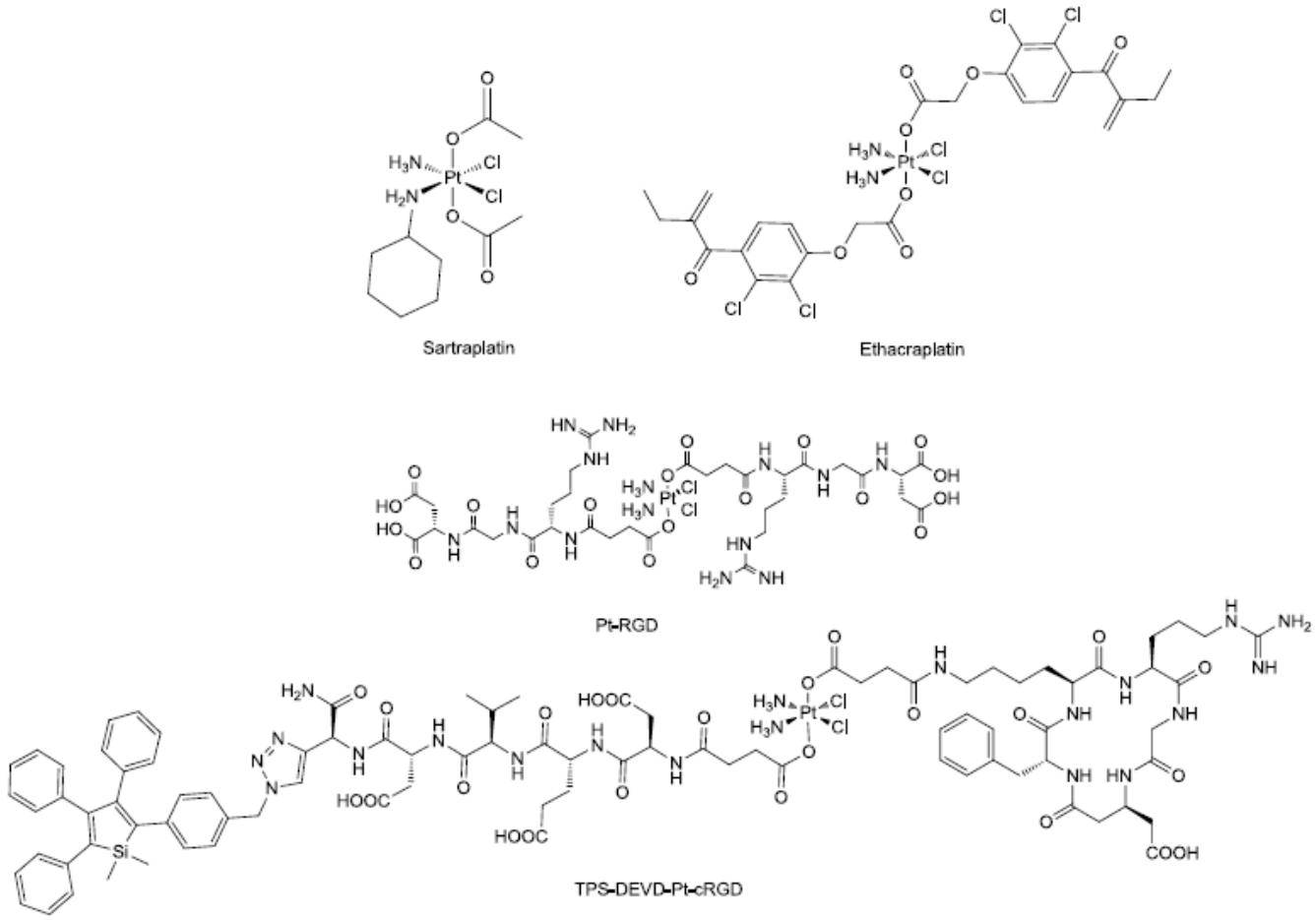

Fig. 1.5: *Examples of Pt(IV) complexes under investigation.*

Lippard *et al.* developed a series of Pt(IV) complexes presenting different peptides for targeting $\alpha_V\beta_3/\alpha_V\beta_5$ integrins overexpressed in endothelial tumor cells [47]. An example (Pt- RGD) is presented in *Fig 1.5*. A recent work from Liu *et al.* reports the development of a fluorescent Pt(IV)-based targeted agent (TPS-DEVD-Pt-cRGD, *Fig 1.5*) [48]. In this compound cRGD moiety enables it to target cancer cells overexpressing integrin $\alpha_V\beta_3$ receptors. Moreover, the drug-induced apoptosis by *in cellulo* generated cisplatin was detected monitoring the fluorescence of the tetraphenylsilole moiety (TPS) activated by caspase-3.

Understanding the cellular mechanism of cisplatin-induced effects is thus a major goal in medicinal inorganic chemistry in order to rationally design new compounds that overcome resistance mechanisms and present decreased side effects.

1.3 A possible alternative to platinum therapy: ruthenium complexes

In the search for drugs with improved clinical effectiveness, reduced toxicity and a broader spectrum of activity, other metals than platinum have been considered, such as rhodium and ruthenium. Non-platinum active compounds are likely to have different mechanisms of action, biodistribution and toxicities than platinum-based drugs and might therefore be active against human malignancies that have either an intrinsic or an acquired resistance to them. Ruthenium complexes are very promising; especially from the viewpoint of overcoming cisplatin resistance with a low general toxicity. Ruthenium has found its way into the clinic, where its properties are exploited for very miscellaneous uses. The radiophysical properties of ^{97}Ru can be applied to radiodiagnostic imaging [49,50]. Other ruthenium compounds have potential as immunosuppressants (*cis*-$[Ru(III)(NH_3)_4(HIm)_2]^{3+}$), antimicrobials (e.g. organic drugs coordinated to ruthenium centres, such as $[Ru(II)Cl_2(chloroquine)_2]$ against malaria and others for the treatment of Chaga´s disease), antibiotics (ruthenium complexes of organic antibiotic compounds, e.g. the Ru(III) derivative of thiosemicarbazone against *Salmonella typhi* and *Enterobacteria faecalis*), nitrosyl delivery/scavenger tools (e.g. the Ru(III) polyaminocarboxylates known as AMD6245 and AMD1226 to treat stroke, septic shock, arthritis, epilepsy and diabetes), vasodilator/vasoconstrictor agents and, as above mentioned, as drugs for cancer chemotherapy [51].

1.3.1 Ruthenium properties that make it suitable for biological applications:

- **Ligand exchange kinetics:**

Ruthenium(II) and ruthenium(III) complexes have similar ligand-exchange kinetics to those of platinum(II) complexes. This property makes them the first choice in the search for compounds that display similar biological effects to platinum(II) drugs [51,52]. Very few metal drugs reach the biological target without being modified, which makes ligand exchange animportant determinant of biological activity. Most metallodrugs undergo interactions with macromolecules such as proteins, or with small S-donor compounds, or even with water. Some interactions are essential for inducing the desired therapeutic properties of the complexes. As the rate of ligand exchange is dependent on the concentration of the exchanging ligands in the surrounding solution, diseases that alter these concentrations in cells or in the surrounding tissues may have an effect on the activity of the drug.

- **Physiologically accessible oxidation states:**

The range of accessible oxidation states of ruthenium under physiological conditions makes this metal unique amongst the platinum group. The ruthenium centre,

predominantly octahedral, can be Ru(II), Ru(III) or Ru(IV). Ru(III) complexes tend to be more biologically inert than related Ru(II) and Ru(IV) complexes. The redox potential of a metal complex can be modified by varying the ligands. In the biological systems glutathione, ascorbate and single-electron-transfer proteins, like those involved in the mitochondrial electron-transfer chain, are able to reduce Ru(III) and Ru(IV) [53], while molecular dioxygen and cytochrome oxidase can oxidize Ru(II) in certain complexes [54-56].

The redox potential of ruthenium compounds can be exploited to improve the effectiveness of Ru-based drugs in the clinic [51,52]. In many cases the altered metabolism associated with cancer and microbial infection results in lower oxygen concentration (hypoxia) in these tissues in comparison to healthy ones [57]. In a healthy cell the reduction of Ru(III) to Ru(II) by glutathione is a very slow process. Besides, the Ru(II) product is readily oxidized back to Ru(III) by the dioxygen that is present in the tissue. However, the reduction of relatively inert Ru(III) complexes by glutathione is more important in the hypoxic environment of solid tumours (*Fig. 1.6*). The reduction of Ru(III) to Ru(II) can be catalysed by mitochondrial and microsomal single-electron-transfer proteins, amongst others. The mitochondrial proteins are of particular interest in drug design, as they can initiate apoptosis [51].

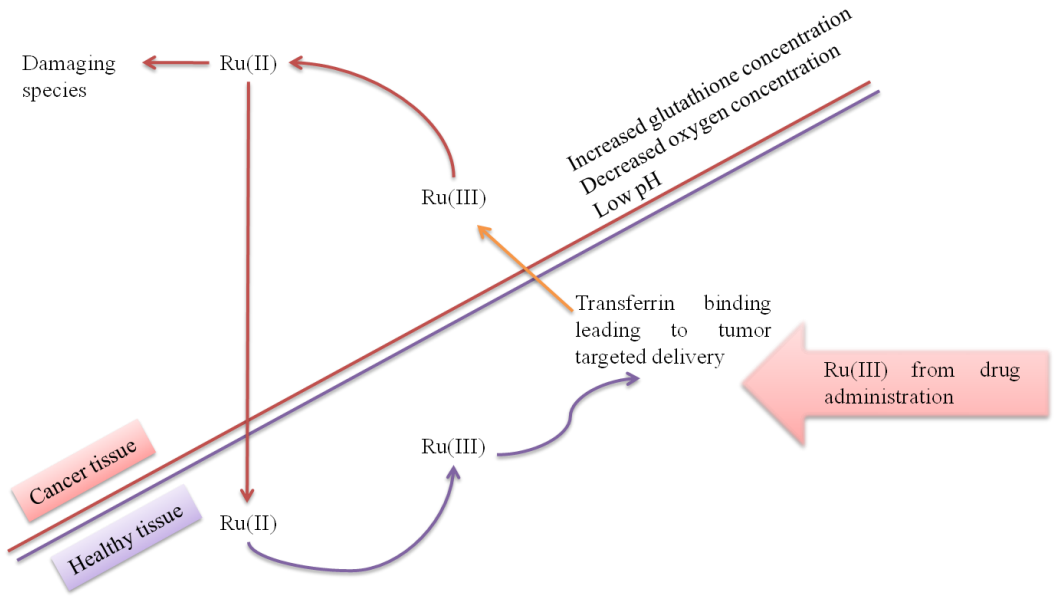

Fig. 1.6: *The oxidation state changes of ruthenium in cancer and healthy cells. The reductive environment of cancer cells favors Ru(II) which is more biologically active than Ru(III). Hence Ru(III) compounds are essentially prodrugs that become activated by reduction on reaching the cancer cell*

❖ **Iron mimic:**

One more property of ruthenium that makes it very significant in medicinal chemistry is its tendency to selectively bind biomolecules, which partly accounts for the low toxicity of ruthenium drugs [51,52]. Transferrin and albumin are two proteins used by mammals to solubilise and transport iron, thereby reducing its toxicity. The ability of some ruthenium drugs to bind to transferrin has been proven [58-62]. Since rapidly dividing cells, such as microbially infected or cancer cells, have a greater requirement of iron, they increase the number of transferrin receptors on their surfaces. This implies that the amount of ruthenium taken up by these infected or cancerous cells is greater than the amount taken up by healthy cells. This selectivity of the drug towards the diseased cells accounts for a reduction of its general toxicity.

1.3.2 Anticancer activity:

Two approaches are commonly used for the design of new anticancer compounds. The trial-and-error approach consists of synthesizing as many complexes as possible that are analogous to a complex of known activity, but which has drawbacks that need to be solved. These new complexes are then tested for anticancer activity, both *in vitro* and *in vivo*. The second approach is based on thorough studies of the properties of some particular complexes, with the final aim of reaching some knowledge about their mechanisms of action. The chemical, physical, pharmacological properties, the uptake of the drug, its biodistribution and its detoxifying processes are subject of study. This implies a multidisciplinary task in which collaboration of scientists from different fields is necessary. Step by step novel derivatives are developed as potential drugs in anticancer therapy. The first generation of ruthenium compounds synthesized for anticancer purposes consists on a series of complexes that mimic platinum drugs and target DNA, just like cisplatin is generally accepted to do.

1.3.3 Classification of ruthenium complexes with anticancer properties:

❖ **Ammine-chlorido derivatives**

The first ruthenium complexes to be tested in search for anticancer properties were close imitators of cisplatin: several ammine and chlorido ligands were coordinated to Ru(II) and Ru(III) to form complexes with general formula $[Ru(NH_3)_{6-x}Cl_x]^{n+}$. Those complexes in which the oxidation state of the ruthenium ion was (+2) were expected to bind to DNA in an analogous way to cisplatin [63-65]. Interestingly, both *cis*-$[Ru(III)(NH_3)_4Cl_2]^+$ and especially *fac*-$[Ru(III)(NH_3)_3Cl_3]$ displayed a comparable antitumour activity to that of cisplatin in a few selected cell lines [60,66]. It has been

hypothesized that these complexes, once inside the cell, are reduced to less inert Ru(II) species, which bind to DNA after hydrolysis [53].

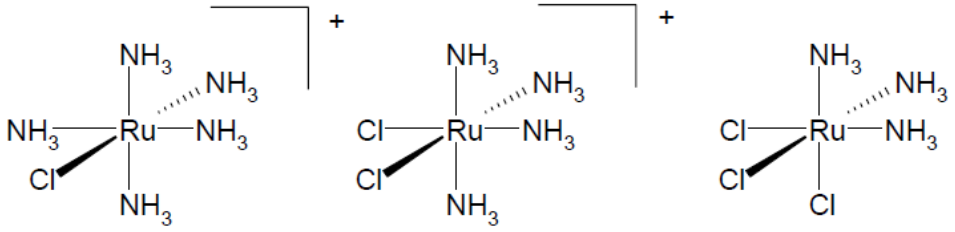

Fig.1.7: *Ammine-chlorido derivatives. From left to right, $[Ru(II)(NH_3)_5Cl]^+$,cis-$[Ru(III)(NH_3)_4Cl_2]^+$ and fac-$[Ru(III)(NH_3)_3Cl_3]$.*

❖ **Dimethylsulfoxide complexes**

The substitution of the ammine ligands by dmso molecules yields compounds with improved solubility. Both *cis*- and *trans*-$[Ru(II)Cl_2(dmso)_4]$ (*Fig.1.8*) were shown to be able to coordinate to guanine residues of DNA via the N7 position [67]. The better activity displayed by the *trans* complex with respect to its *cis* analogue, both *in vitro* and *in vivo*, in cytotoxicity tests, was explained by means of differences in kinetics. This *trans* isomer also seemed to overcome cisplatin resistance, as seen in the case of the P388 leukaemia cell line. This observation, together with the fact that *trans*-$[Ru(II)Cl_2(dmso)_4]$ shows a good antimetastatic activity [68], suggests that the *trans*-ruthenium complexes could be an interesting alternative to cisplatin, by acting through a different mechanism of action. A series of dimethyl sulfoxide-ruthenium complexes was designed, which were inspired on the above-mentioned promising compound. Noteworthy are the compounds Na{*trans*-$[Ru(III)Cl_4(dmso)(Him)]$}, (Him = imidazole), nicknamed NAMI, and the more stable $[H_2Im]$[*trans*-$Ru(III)Cl_4(dmso)(Him)$], also known as NAMI-A (*Fig.1.8*). The dmso ligand is in both cases bound via the S atom. NAMI-A is the first ruthenium complex to have ever reached clinical testing for anticancer activity, of which it has recently completed phase-I studies. Nowadays, when surgical removal of primary cancers is efficient and successful, a complex such as NAMI-A, which presents an antimetastatic activity in a broad range of tumors including lung metastasis, is becoming of utmost interest [69,70].

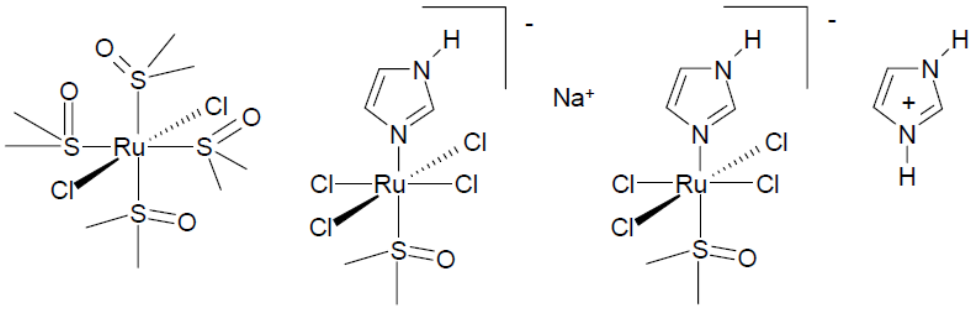

Fig.1.8: *Dimethylsulfoxide complexes. From left to right, trans-[Ru(II)(dmso)$_4$Cl$_2$], Na{trans-[Ru(III)Cl$_4$(dmso)(Him)]} (NAMI) and [H$_2$Im]{trans-[Ru(III)Cl$_4$(dmso)(Him)]} (NAMI-A).*

It is possible that these complexes are reduced to Ru(II) once inside the cell. It has been shown that NAMI loses two of its chlorido ligands, which are substituted by aqua ligands. This hydrated species could bind to several biomolecules, including DNA [71,72]. However, the main mechanism of action of both NAMI and NAMI-A is thought not to be directly related to binding to DNA, but these molecules would exert their action via different ways than cisplatin [72-74]. A series of NAMI-A analogues bearing a weakly basic heterocyclic nitrogen ligand *trans*- to dmso was synthesized [69]. These complexes were found to be more stable than NAMI-A in slightly acidic solution, and their *in vivo* effectiveness appeared to be slightly better than that of the parent compound. NAMI-A, as well as these analogues, were proven to have an effect on cell distribution among cell cycle phases. In the case of the parent compound a cell cycle arrest is induced in the G(2)-M phase, an effect which does not take place in the experiments carried out with the NAMI-A analogues [69].

❖ **Complexes with other heterocyclic ligands**

Keppler and co-workers prepared a group of complexes, the so-called "Keppler-type" compounds. These are anionic ruthenium(III) complexes with monodentate heterocyclic nitrogen donor ligands, the most successful of which have the formula *trans*-[RuCl$_4$(L)$_2$]$^-$, where L is imidazole (KP418) or indazole (KP1019 and KP1339), and the counterion (LH)$^+$ or Na$^+$ (*Fig.1.9*). KP1019 and KP1339 were reported effective in inhibiting platinum resistant colorectal carcinomas in rats [75]; KP1019 recently completed phase-I clinical trials [61].

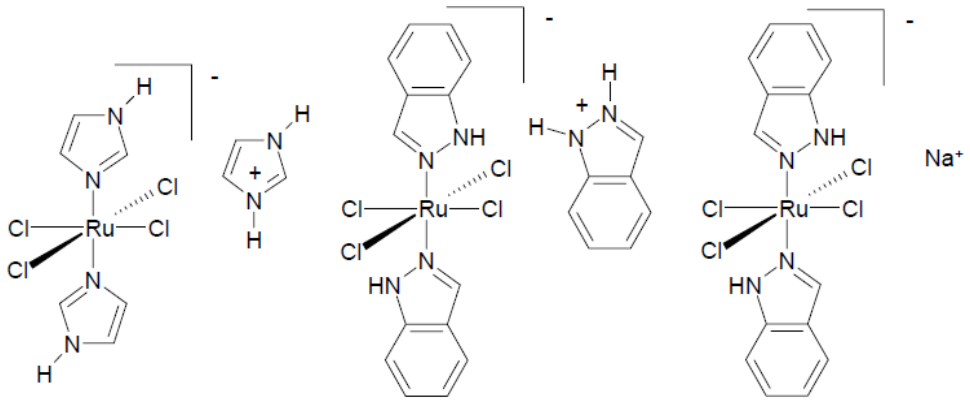

Fig.1.9: *Molecular formula of the ruthenium(III) complexes imidazolium trans-[tetrachloridobis(imidazole)ruthenate(III)] (KP418), indazolium trans-[tetrachloridobis(indazole)ruthenate(III)] (KP1019) and sodium trans-[tetrachloridobis(indazole)ruthenate(III)] (KP1339).*

The mechanism of action of these complexes is thought to differ considerably from that of cisplatin. The involvement of the "activation-by-reduction" process and the transferrin-mediated transport into the cells seem to play a very important role in the efficiency of the "Keppler-type" complexes [61,75], as in the case of NAMI-A.

Several ruthenium polypyridyl complexes (*Fig.1.10*) were synthesised, their *in vitro* DNA binding was studied and their antitumour activity in murine L1210 leukaemia and human cervix carcinoma HeLa cells was investigated. The only complex of this kind which was reported to be antitumour active was *mer*-[Ru(III)(tpy)Cl_3], where tpy is 2,2´:6´,2"-terpyridine [76]. This complex was also the only one of this group that showed significant bifunctional DNA binding, therefore its cytotoxicity was thought to be related to the possibility of interstrand DNA cross-link formation [77,78].

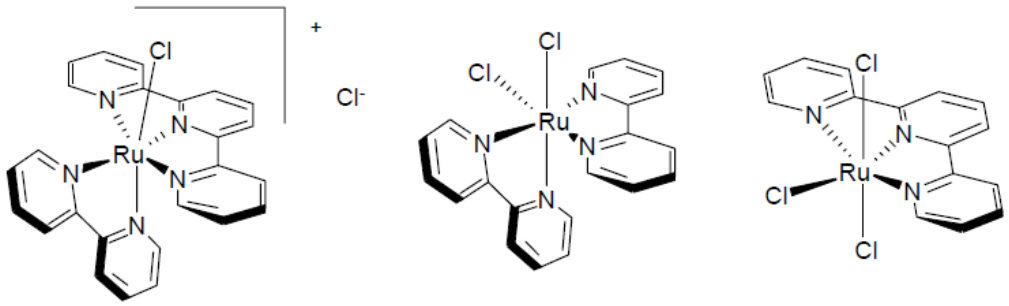

Fig.1.10 *Molecular formula of the ruthenium polypyridyl complexes [Ru(II)(bpy)(tpy)Cl]Cl, cis-[Ru(II)(bpy)$_2$$Cl_2$], and mer-[Ru(III)(tpy)$Cl_3$] (bpy = 2,2'-bipyridine, tpy = 2,2':6'2''-terpyridine).*

❖ **Organoruthenium complexes**

The mononuclear ruthenium(II) arene complexes of the type [(η^6-arene)Ru(II)(en)X][PF_6], where en is ethylenediamine and X is chloride or iodide (*Fig.1.11*), constitute a group that is believed to exert an antitumour action via mechanisms different from those of other ruthenium(III) complexes such as NAMI-A or KP1019 [79-82]. The chlorido or iodido ligand is readily lost to yield the more reactive aqua species [83]. 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 [84,85]. [(η^6-toluene)Ru(II)(pta)Cl_2] (RAPTA-T), where pta is 1,3,5-triaza-7-phosphaadamantane (*Fig.1.11*), is the parent compound from which a group of water-soluble selective DNA-binding antimetastatic drugs were synthesized [86,87]. The RAPTA compounds exhibit pH dependent DNA binding, almost no toxicity towards cancer cells *in vitro* and no toxicity at all towards healthy cells, also *in vitro*. However, RAPTA-T was found to inhibit lung metastases in mice bearing a mammary carcinoma, again with only mild effects on the primary tumours. The mechanism of action of the RAPTA compounds is only starting to be investigated [88].

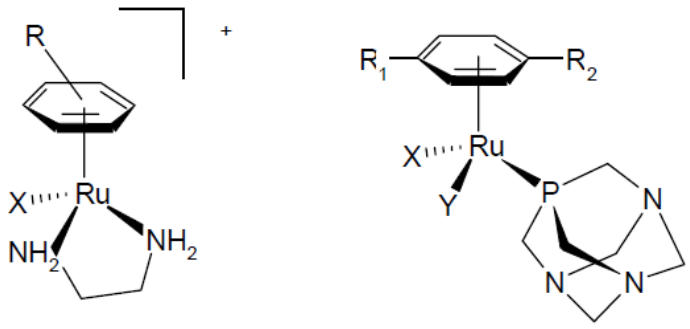

Fig.1.11: *General formula of two groups of organometallic ruthenium(II) complexes with modified arene ligands. On the left, [(η^6-arene)Ru(II)(en)X]$^+$, where the arene can be benzene, p-cymene, biphenyl, 5,8,9,10-tetrahydroanthracene or 9,10-dihydroanthracene. X is Cl or I. On the right, [(η^6-arene)Ru(II)(pta)XY] (RAPTA complexes). R1, R2 are alkyl groups; X and Y can be Cl or different μ-dicarboxylate ligands.*

1.3.4 How these drugs work: mechanisms of action

In the past two decades a new approach to treating cancer, known as targeted therapy, has started to emerge [89]. While classical chemotherapy involves drugs interfering with replication and mitotic processes of tumour cells, their "target" being thus DNA, a more recent strategy involves targeting cellular signalling pathways of cancer cells, yielding highly effective cancer treatments with less severe side effects [90]. The recent discovery of receptors and growth factors, such as epidermal growth factor receptor (EGFR),

vascular endothelial growth factor (VEGF), or cyclin-dependent kinases (CDK) that are upregulated in cancer cells provides new possible targets for cancer therapy [90]. The high specificity of targeted therapies accounts for a more manageable toxicity profile of the drugs. Its main drawback is that most targeted therapeutic drugs are only effective in specific types of cancer (e.g. Imatinib mesylate for chronic myelogenous leukaemia, Erlotinib for advanced non-small cell lung cancer, etc), which limits their applicability [89]. In recent years, ruthenium-based drug research is moving from classical chemotherapy into the nonconventional approaches.

Classical ruthenium anticancer therapy is based on the capability of ruthenium to coordinatively bind to DNA via some of the nitrogen atoms of the nucleic bases, in particular via the nitrogen N7 of guanine. This is also the action expected from the first ruthenium complexes designed as anticancer drugs, the ammine-chloro derivatives. The novelty is that these complexes are thought to act as ruthenium(III) prodrugs, which would be inactive until the ruthenium gets reduced in the cytosol [53,63-65].

Binding to DNA via an additional mode was achieved when an intercalating polypyridyl ligand was added to the ruthenium system. Additional properties that make polypyridyl groups desirable ligands for ruthenium, are their photoluminescence, which makes them suitable as DNA probes, as well as enhance stability of the complexes amongst others [89]. An often encountered problem is the poor water solubility of many of these complexes. While ruthenium(II) dimethylsulfoxide complexes were conceived as water-soluble versions of the above-mentioned ammine-chlorido derivatives, the good antimetastatic activity of *trans*-[Ru(II)Cl_2(dmso)$_4$] soon became apparent, as well as its capability to overcome cisplatin resistance in certain cell lines. These two observations suggested a mechanism of action different to the by then widely accepted mechanism of cisplatin [68].

DNA also seems to be a target for the organometallic arene-ruthenium complexes. The coordination of the ruthenium atom to the nucleic bases was seen to be enhanced through H-bonding interactions or weakened because of steric interactions, suggesting the possibility to design compounds to target specific nucleotides [91]. The binding of the complex to DNA appeared to be promoted by hydrophobic arene-purine base π-π stacking interactions when large ring systems were used [84].

Finally, the photoreactive ruthenium compounds can also be considered within the classical therapy, as well as most of the dinuclear ruthenium compounds as their target is still DNA.

One of the most successful ruthenium-based anticancer drug to date, NAMI-A, displays a unique behaviour. Its lack of cytotoxicity *in vitro*, together with its *in vivo*

ability to reduce metastases weight while the primary tumor remains unaffected, appear to exclude DNA as the primary target. NAMI-A binds strongly to serum proteins, including the iron transporter transferrin, and it induces cell arrest in the premitotic G(2)-M phase [69]. Studies carried out with NAMI-A analogues suggest that the imidazole fragment is not essential for the antimetastatic activity. On the other hand, the reinforcement of the axis dmso-Ru-N-donor ligand by using N-containing heterocycles that are less basic than imidazole reduce the loss of dmso from the complex, increasing at the same time the antitumour action [69].

The only ruthenium drug other than NAMI-A currently undergoing clinical trials, KP1019 (see Fig.1.13), is significantly cytotoxic *in vitro* against colorectal cell lines SW480 and HT29 by inducing apoptosis [75]. The drug was also found to be highly effective in *in vivo* tests in which cisplatin had been inactive. The mechanism of action of the "Keppler-type" complexes is thought to be due to at least two factors, namely the "activation-by-reduction" process and the transferrin-mediated transport into the cells [61,75].

KP1019 is capable of forming crosslinks with DNA that are different to those originated by cisplatin. DNA is not completely excluded as a target for KP1019. However, it induces apoptosis in colorectal cell lines mainly via the intrinsic mitochondria pathway [61,92]. An increase in the number of indazole ligands of these complexes improved significantly the *in vitro* cytotoxicity in several cell lines, allegedly because the cellular uptake is facilitated and the reduction potential is increased [93].

Although DNA appears to be a target for the organometallic arene-ruthenium complexes (*vide supra*), the RAPTA complexes constitute a particular case (see Fig.1.16). Parting from the observation that the complex RAPTA-T displayed a similar *in vivo* activity to NAMI-A, albeit with lower systemic toxicity, a group of derivatives from this parent compound was synthesised, which were then tested *in vitro* for interactions with different biological molecules and *in vivo* for antitumour and antimetastatic activity [88,94]. Several of these complexes showed a reduction in lung metastases in mice, while leaving the primary tumour mostly unaffected. Moreover some specific protein-binding interactions were detected [88,94]. A proteomic-based analytical approach based on 2D PAGE and laser ablation inductively-coupled mass spectrometry (ICP-MS) appears to be a promising tool to identify the specific proteins interacting with ruthenium-based drugs [95-97].

1.4 Summary:

It can be summarized from the vast literature that ruthenium drugs are particularly important in the clinic due to their low toxicity. These complexes appear in some cases to function in a different way to classical chemotherapies. For this reason the conventional tests used to screen new compounds for anticancer activity should be treated with caution, and new assays for potential drug candidates are needed. Methods are required to rapidly locate drug interactions with key protein targets. Finally, even when metal drugs are not found directly active, they may interact with the proteins that regulate apoptosis, thereby modifying cell behaviour.

1.5 Aim and outline of this thesis:

Ruthenium compounds are second generation (post-platinum) transition metal chemotherapeutics that possess unique properties granting them, at least in preclinical studies, more selective entry into tumor cells with fewer toxic effects to normal cells. A wealth of laboratory data now demonstrates that numerous ruthenium(II) and (III) complexes show antitumor activity in a variety of cancer cell lines and animal models. Taking insights from the clinical progress of NAMI-A, KP1019 and RAPTA type ruthenium complexes, a group of related complexes have been presented here along with detailed studies of their biological activities.

The subject is first introduced earlier in **Chapter 1** with an overview about medicinal inorganic chemistry, in particular about platinum and ruthenium anticancer agents. Special attention is given to the mechanisms of action of these antitumour drugs, as well as the structure-activity relationships that are known to date.

Chapter 2 encompasses synthesis and detailed characterization of four different ligands series which have been chosen with special focus on their biological relevance. These ligands were to be used in the preparation of mixed ligand ruthenium(II) complexes.

Mixed ligand ruthenium(II) polypyridyl complexes is presented in **Chapter 3**. A complete description is given of the synthesis and characterization by several methods employing 1,10-phenanthroline as the ancillary ligand with a general structural formula $[Ru(II)(phen)_2(L)]ClO_4$ (*phen* = 1,10-phenanthroline; *L* = N, O and O, O donor ligands discussed in previous chapter).

Piano stool type organometallic ruthenium(II) complexes with a general structural formula $Ru(\eta^6\text{-}p\text{-}cym)(L)Cl]$ (*p-cym* = *p*-cymene $MeC_6H_4Pr^i$; *L* = N, O and O, O donor ligands discussed in chapter 2) were synthesized and well characterized in **Chapter 4** with an aim to check for their bioactivities and compare them with those of ruthenium polypyridyl complexes discussed in previous chapter.

In **Chapter 5** the binding interactions of the synthesized ruthenium(II) complexes with two important biomolecules; DNA and Serum albumin; were looked into using spectroscopic techniques. The aim of the thesis to study the anticancer activities of the ruthenium complexes was followed starting from cytotoxicity assay on human lung and breast cancer cell lines. The results so obtained prompted us to further investigate the mode of cell death being caused by the compounds under study.

Finally **Chapter 6** offers a summary and a cumulative discussion of the results presented in this thesis.

1.6 References:

[1] Schreiber, S. L. *Nat. Chem. Biol.* **2005**, *1*, 64.(2) Doudna, J. A. *Nat. Chem. Biol.* **2005**, *1*, 300.

[2] Tomi, K. S. *Chem. Biol. Drug Des.* **2006**, *67*, 196.

[3] Raymond, K. N., Bioinorganic chemistry II. Washington, **1977**.

[4] Dixon, N. E.; Gazzola, C.; Blakeley, R. L.; Zerner, B., *J. Am. Chem. Soc.* **1975,** *97*, 4131-4133.

[5] Kendrick, M. J.; May, M. T.; Plishka, M. J.; Robinson, K. D., Metals in biological systems. Chichester, **1992**.

[6] Antman, K. H., *Oncologist* **2001,** *6*, 1-2. Waxman, S.; Anderson, K. C., *Oncologist* **2001,** *6*, 3-10.

[7] Lippard, S. J. *Nat. Chem. Biol.* **2006**, *2*, 504.

[8] David, S. S.; Meggers, E. *Curr. Opin. Chem. Biol.* **2008**, *12*, 194.

[9] Cohen, S. M. *Curr. Opin. Chem. Biol.* **2007**, *11*, 115.

[10] Bertini, I.; Gray, H. B.; Stiefel, E. I.; Valentine, J. S. *Biological Inorganic Chemistry: Structure and Reactivity*; Eds.; University Science Books: Sausalito, CA, **2007**.

[11] Frau`sto da Silva, J. J. R.; Williams, R. J. P. *The Biological Chemistry of the Elements*, 2nd ed.; Oxford University Press: Oxford, U.K., **2001**.

[12] Lippard, S. J.; Berg, J. M. *Principles of Bioinorganic Chemistry*; University Science Books: Mill Valley, CA, **1994**.

[13] Sun, H. Z.; Zhang, L.; Szeto, K. Y. *Met. Ions Biol. Syst.*, **2004**, *41*, 333.

[14] Thompson, K. H.; Orvig, C. *J. Chem. Soc. Dalton Trans.*, **2000**, 2885.

[15] Ward, J. R.; Williams, H. J.; Egger, M. J.; Reading, J. C.; Boyce, E.; Altz-Smith, M.; Samuelson Jr., C. O.; Willkens, R. F.; Solsky, M. A.; Hayes, S. P.; Blocka, K. L. Weinstein, A.; Meenan, R. F.; Guttadauria, M.; Kaplan, S. B.; Klippel, *J. Arthritis & Rheumatism*, **1983**, *26*, 1303.

[16] Rosenberg, B.; Vancamp, L.; Krigas, T. *Nature*, **1965**, *205*, 698.

[17] Hermann, P.; Kotek, J.; Kubicek, V.; Lukes, I. *Dalton Trans.*, **2008**, 3027.

[18] Terreno, E.; Castelli, D. D.; Viale, A.; Aime, S. *Chem. Rev.*, **2010**, *110*, 3019.

[19] Correia, J. D. G.; Paulo, A.; Raposinho, P. D.; Santos, I. *Dalton Trans.*, **2011**, *40*, 6144.

[20] Thorp-Greenwood, F. L.; Coogan, M. P. *Dalton Trans.*, **2011**, *40*, 6129.

[21] Thompson, K. H.; Orvig, C. *Science*, **2003**, *300*, 936.

[22] Orvig, C.; Abrams, M. J. *Chem. Rev.*, **1999**, *99*, 2201.

[23] Peyrone, M. *Ann. Chemie Pharm.*, **1845**, *51*, 1.

[24] Rosenberg, B.; van Camp, L.; Trosko, J. E.; Mansour, V. H. *Nature* , **1969**, *222*, 385.

[25] Reedijk, J. *Pure Appl. Chem.*, **1987**, *59*, 181.

[26] Wang, D.; Lippard, S. J. *Nat. Rev. Drug Discov.*, **2005**, *4*, 307.

[27] Jamieson, E. R.; Lippard, S. J. *Chem. Rev.*, **1999**, *99*, 2467.

[28] Rabik C. A.; Dolan, M. E. *Cancer Treat. Rev.*, **2007**, *33*, 9.

[29] Kelland, L. *Nat. Rev. Cancer*, **2007**, *7*, 573.

[30] Thatcher, N.; Lind, M. *Semin. Oncol.*, **1990**, *17*, 40.

[31] Alberts, D. S.; Green, S.; Hannigan, E. V.; Otoole, R.; Stocknovack, D.; Anderson, P.; Surwit, E. A.; Malvlya, V. K.; Nahhas, W. A.; Jolles, C. J. *J. Clin. Oncol.* **1992**, *10*, 706.

[32] Uchida, N.; Takeda, Y.; Kasai, H.; Maekawa, R.; Sugita, K.; Yoshioka, T. *Anticancer Res.*, **1998**, *18*, 3375.

[33] Lebwohl, D.; Canetta, R. *Eur. J. Cancer*, **1998**, *34*, 1522.

[34] Hasegawa, Y.; Takanashi, S.; Okudera, K.; Aoki, M.; Basaki, K.; Kondo, H.; Takahata, T.; Yasui-Furukori, N.; Tateishi, T.; Abe, Y.; Okumura, K. *Jpn. J. Clin. Oncol.*, **2004,** *34*, 647.

[35] Kawase, S.; Okuda, T.; Ikeda, M.; Ishihara, S.; Itoh, Y.; Yanagawa, S.; Ishigaki, T. *Gynecol. Oncol.*, **2006**, *102*, 493.

[36] Takaoka, E. I.; Kawai, K.; Ando, S.; Shimazui, T.; Akaza, H. *Jpn. J. Clin. Oncol.*, **2006**, *36*, 60.

[37] Sato, Y.; Takayama, T.; Sagawa, T.; Okamoto, T.; Miyanishi, K.; Sato, T.; Araki, H.; Iyama, S.; Abe, S.; Murase, K.; Takimoto, R.; Nagakura, H.; Hareyama, M.; Kato, J.; Niitsu, Y. *Cancer Chemother. Pharmacol.*, **2006**, *58*, 570.

[38] Shirai, T.; Hirose, T.; Noda, M.; Ando, K.; Lshida, H.; Hosaka, T.; Ozawa, T.; Okuda, K.; Ohnishi, T.; Ohmori, T.; Horichi, N.; Adachi, M. *Lung Cancer*, **2006**, *52*, 181.

[39] Shinohara, N.; Harabayashi, T.; Suzuki, S.; Nagao, K.; Nagamori, S.; Matsuyama, H.; Naito, K.; Nonomura, K. *J. Clin. Oncol.*, **2006**, *24*, 236S.

[40] Misset, J. L. *Br. J. Cancer*, **1998**, *77*, 4.

[41] Hall, M. D.; Hambley, T. W. *Coord. Chem. Rev.*, **2002**, *232*, 49.

[42] Hall, M. D.; Mellor, H. R.; Callaghan, R.; Hambley, T. W. *J. Med. Chem.*, **2007**, *50*, 3403.

[43] Wheate, N. J.; Walker, S.; Craig, G. E.; Oun R. *Dalton Trans.*, **2010**, *39*, 8113.

[44] Vollano, J. F.; Al-Baker, S.; Dabrowiak, J. C.; Schurig, J. E. *J. Med. Chem.*, **1987**, *30*, 716.

[45] Kauffman, G. B.; Slusarczuk, G.; Kirschner, S. *Inorg. Synth.*, **1967**, *7*, 236.

[46] Reithofer, M.; Galanski, M.; Roller, A.; Keppler, B. K. *Eur. J. Inorg. Chem.*, **2006**, 2612.

[47] Ang, W. H.; Isam Khalaila, I.; Allardyce, C. S.; Juillerat-Jeanneret, L.; Dyson, P. J. *J. Am. Chem. Soc.*, **2005**, *127*, 1382.

[48] Yuan, Y.; Kwok, R. T. K; Tang, B. Z.; Liu, B. *J. Am. Chem. Soc.*, **2014**, *136***,** 2546.

[49] Zanzi, I.; Srivastava, S. C.; Meinken, G. E.; Robeson, W.; Mausner, L. F.; Fairchild, R. G.; Margouleff, D. *Nucl. Med. Biol.*, **1989**, *16*, 397.

[50] Srivastava, S. C. *Semin. Nucl. Med.*, **1996**, *26*, 119.

[51] Allardyce, C. S.; Dyson, P. J., *Platinum Metals Rev.* **2001,** *45*, 62-69

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

[53] Clarke, M. J.; Bitler, S.; Rennert, D.; Buchbinder, M.; Kelman, A. D. *J. Inorg. Biochem.*, **1980**, *12*, 79.

[54] Stanbury, D. M.; Haas, O.; Taube, H. *Inorg. Chem.*, **1980**, *19*, 518.

[55] Stanbury, D. M.; Mulac, W. A.; Sullivan, J. C.; Taube, H. *Inorg. Chem.*, **1980**, *19*, 3735.

[56] Stanbury, D. M.; Gaswick, D.; Brown, G. M.; Taube, H. *Inorg. Chem.* **1983**, *22*, 1975.

[57] Galeotti, T.; Cittadini, A.; Neri, G.; Scarpa, A. *Membrane in cancer cells*. New York, **1988**, *551*

[58] Clarke, M. J. *Coord. Chem. Rev.*, **2003,** *236*, 209.

[59] Smith, C. A.; Sutherland-Smith, A. J.; Keppler, B. K.; Kratz, F.; Baker, E. N. *J. Biol. Inorg. Chem.*, **1996**, *1*, 424.

[60] Clarke, M. J.; Zhu, F. C.; Frasca, D. R. *Chem. Rev.*, **1999**, *99*, 2511.

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

[62] Polec-Pawlak, K.; Abramski, J. K.; Semenova, O.; Hartinger, C. G.; Timerbaev, A. R.; Keppler, B. K.; Jarosz, M. *Electrophoresis*, **2006**, *27*, 1128.

[63] Clarke, M. J.; Buchbinder, M.; Kelman, A. D. *Inorg. Chim. Acta*, **1978**, *27*, L87.

[64] Clarke, M. J.; Jansen, B.; Marx, K. A.; Kruger, R. *Inorg. Chim. Acta*, **1986**, *124*, 13.

[65] Rodriguez-Bailey, V. M.; La Chance-Galang, K. J.; Doan, P. E.; Clarke, M. J. *Inorg. Chem.*, **1997**, *36*, 1873.

[66] Durig, J. R.; Danneman, J.; Behnke, W. D.; Mercer, E. E. *Chem.-Biol. Interact.*, **1976**, *13*, 287.

[67] Alessio, E.; Mestroni, G.; Nardin, G.; Attia, W. M.; Calligaris, M.; Sava, G.; Zorzet, S. *Inorg. Chem.*, **1988**, *27*, 4099.

[68] Coluccia, M.; Sava, G.; Loseto, F.; Nassi, A.; Boccarelli, A.; Giordano, D.; Alessio, E.; Mestroni, G. *Eur. J. Cancer*, **1993**, *29A*, 1873.

[69] Bergamo, A.; Gava, B.; Alessio, E.; Mestroni, G.; Serli, B.; Cocchietto, M.; Zorzet, S.; Sava, G. *Int. J. Oncol.*, **2002**, *21*, 1331.

[70] Kostova, I. *Curr. Med. Chem.*, **2006**, *13*, 1085.

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

[72] Messori, L.; Orioli, P.; Vullo, D.; Alessio, E.; Iengo, E. *Eur. J. Biochem.*, **2000**, *267*, 1206.

[73] Barca, A.; Pani, B.; Tamaro, M.; Russo, E., *Mutat. Res.-Fundam. Mol. Mech. Mutagen.*, **1999**, *423*, 171.

[74] Sanna, B.; Debidda, M.; Pintus, G.; Tadolini, B.; Posadino, A. M.; Bennardini, F.; Sava, G.; Ventura, C. *Arch. Biochem. Biophys.*, **2002**, *403*, 209.

[75] Kapitza, S.; Pongratz, M.; Jakupec, M. A.; Heffeter, P.; Berger, W.; Lackinger, L.; Keppler, B. K.; Marian, B. *J. Cancer Res. Clin. Oncol.*, **2005**, *131*, 101.

[76] Nováková, O.; Kaspárková, J.; Vrána, O.; van Vliet, P. M.; Reedijk, J.; Brabec, V. *Biochemistry*, **1995**, *34*, 12369.

[77] van Vliet, P. M.; Haasnoot, J. G.; Reedijk, J. *Inorg. Chem.*, **1994**, *33*, 1934.

[78] van Vliet, P. M.; Toekimin, S. M. S.; Haasnoot, J. G.; Reedijk, J.; Nováková, O.; Vrána, O.; Brabec, V. *Inorg. Chim. Acta*, **1995**, *231*, 57.

[79] Morris, R. E.; Aird, R. E.; Murdoch, P. D.; Chen, H. M.; Cummings, J.; Hughes, N. D.; Parsons, S.; Parkin, A.; Boyd, G.; Jodrell, D. I.; Sadler, P. J. *J. Med. Chem.*, **2001**, *44*, 3616.

[80] Aird, R. E.; Cummings, J.; Ritchie, A. A.; Muir, M.; Morris, R. E.; Chen, H.; Sadler, P. J.; Jodrell, D. I. *Br. J. Cancer*, **2002**, *86*, 1652.

[81] Nováková, O.; Chen, H. M.; Vrána, O.; Rodger, A.; Sadler, P. J.; Brabec, V. *Biochemistry*, **2003**, *42*, 11544.

[82] Nováková, O.; Kaspárková, J.; Bursova, V.; Hofr, C.; Vojtiskova, M.; Chen, H. M.; Sadler, P. J.; Brabec, V. *Chem. Biol.*, **2005**, *12*, 121.

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

[84] Chen, H. M.; Parkinson, J. A.; Parsons, S.; Coxall, R. A.; Gould, R. O.; Sadler, P. J. *J. Am. Chem. Soc.*, **2002**, *124*, 3064.

[85] Wang, F. Y.; Bella, J.; Parkinson, J. A.; Sadler, P. J. *J. Biol. Inorg. Chem.*, **2005**, *10*, 147.

[86] Allardyce, C. S.; Dyson, P. J.; Ellis, D. J.; Heath, S. L. *Chem. Commun.*, **2001**, 1396.