Ruthenium polypyridyl complexes with anticancer properties

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1. Introduction

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

1.1. Metals in medicine. The discovery of cisplatin as an anticancer agent

Precious metals have been used for medicinal purposes for at least 3500 years, when records show that gold was included in a variety of medicines in Arabia and China.[1] However, the motivation for the use of these metals often had a superstitious or a religious origin, and was derived from the reasoning: if a metal is rare, it must mean it has special properties. Life was thought to be built exclusively from organic "bricks". In the late 1800´s, experiments carried out with blood samples revealed the existence of iron-containing compounds in this fluid.[2] The presence of metals in different enzymes was proven[3] and bioinorganic chemistry was granted the status of a separate discipline in the 1970´s.[4] 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 PO_4^{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.

In modern history, the first compound containing an inorganic element that was described to be used in the cure of a disease was salvarsan, an arsenic compound used in the treatment of syphilis, which was synthesized and tested in the beginning of the 20th century by Ehrlich (see Fig.1.1).[5, 6] Ehrlich, who was awarded the Nobel Prize in 1908 for his discovery of immunochemistry, is considered the founder of chemotherapy, which he defined as "the use of drugs to injure an invading organism without injury to the host". Ehrlich introduced the "magic bullet" concept, also known as "drug targeting", nowadays the object of extensive research worldwide.

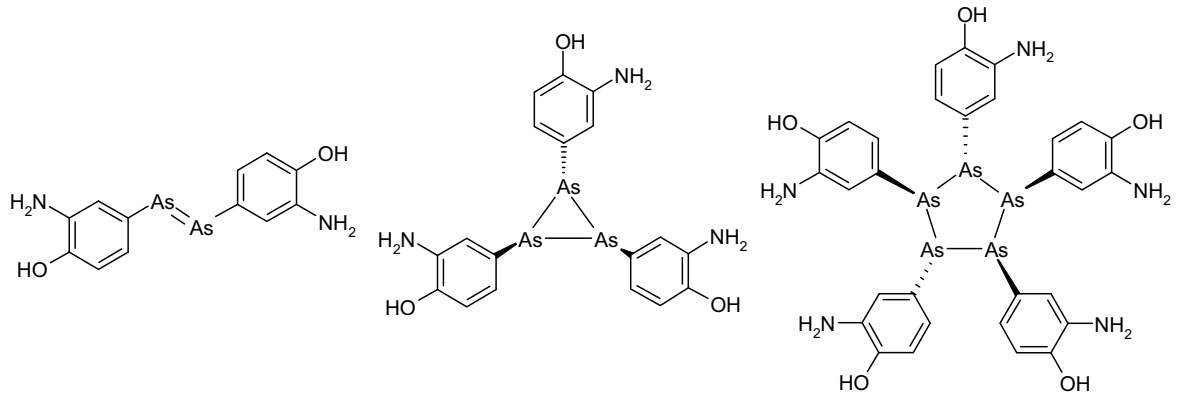

Fig.1.1. Molecular structure of the arsenic drug salvarsan as proposed by Ehrlich (left). In 2005, salvarsan was proven to consist of a mixture of cyclic species (centre and right).[7]

Medicinal inorganic chemistry as a discipline is considered to have boosted with the discovery of the anticancer properties of cisplatin.[1] 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. Medicinal inorganic chemistry comprises not only the intentional introduction of a metal ion into a biological system, but also the rescue of a metal ion that has been introduced in a biological system by accident. Examples of the first case are the administration of essential elements and mineral supplements (e.g. iron, copper, zinc, selenium), the use of diagnostic agents (e.g. gadolinium and manganese for MRI, barium and iodine for X-ray), and therapeutic agents (e.g. lithium for bipolar disorder, platinum compounds in anticancer chemistry, gold compounds for arthritis and bismuth for ulcers), as well as the use of radiopharmaceuticals for diagnosis (^{99m}Tc) and therapy (^{186}Re), and the use of enzyme inhibitors.[8] Chelation therapy is most widely used in the treatment of poisoning by an inorganic (not necessarily metallic) element (e.g. 2,3-dimercapto-1-propanol, known as BAL, used for mercury, arsenic, antimony or nickel poisoning; Na_2H_2edta, used for lead removal).

History of cisplatin, a leading anti-cancer drug

cis-diamminedichloridoplatinum(II) was first described by Peyrone in 1845.[9] 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.[10, 11] 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.[12]

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 avoid the action of cisplatin, being this resistance 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.

1.2. Cisplatin: mechanism of action

Cisplatin administration protocols currently include an intravenous infusion. Since this method is far from ideal, requiring patient hospitalization, research has been carried out to find an alternative administration route. A release-controlled formulation of cisplatin with reduced toxicity has recently been developed.[13] The complex is encapsulated inside nano-scale liposomal carriers and administered to the patient via nebulization. This new approach is currently undergoing phase I clinical trials.[13]

In the blood, the high physiological chloride concentration (ca. 100 mM) ensures that the complex remains neutral until it enters the cell. This passage was classically thought to occur mainly by passive diffusion. However, the debate about the importance of the participation of an active transport mechanism in this process was re-opened when cisplatin uptake was discovered to be mediated by the copper transporter Ctr1p both in yeast and in mammals.[14] Once in the cytosol, hydrolysis occurs due to the lower chloride concentration (ca. 4mM).

Cisplatin can bind to nucleic acids, proteins and sulfur-containing biomolecules, such as glutathione (GSH). The ultimate target of cisplatin, which triggers its cytotoxicity, is generally accepted to be DNA.[15]

DNA adducts formed by coordination of cisplatin

The DNA coordination sites of cisplatin after hydrolysis are, in order of preference, the N7 atom of guanine, the N7 atom of adenine, the N1 of adenine and N3 of cytosine. Two types of platinum-DNA binding have been found: monofunctional and bifunctional. Monofunctional binding is unlikely to be responsible for the cytotoxic action of cisplatin, since transplatin is as capable of forming this kind of adducts as cisplatin, while being inactive. Bifunctional binding results in chelation and subsequent formation of various adducts in DNA. Intrastrand 1,2-d(GpG) cross-links are the most abundant Pt-DNA adducts (60-65% of the platinum bound to DNA is in that form),[16] followed by intrastrand d(ApG)

cross-links (around 20% of the bound platinum). Only about 1.5% of the cisplatin was found to be involved in interstrand adducts; some minor DNA-protein cross-links were also formed (see Fig.1.2).[15, 17]

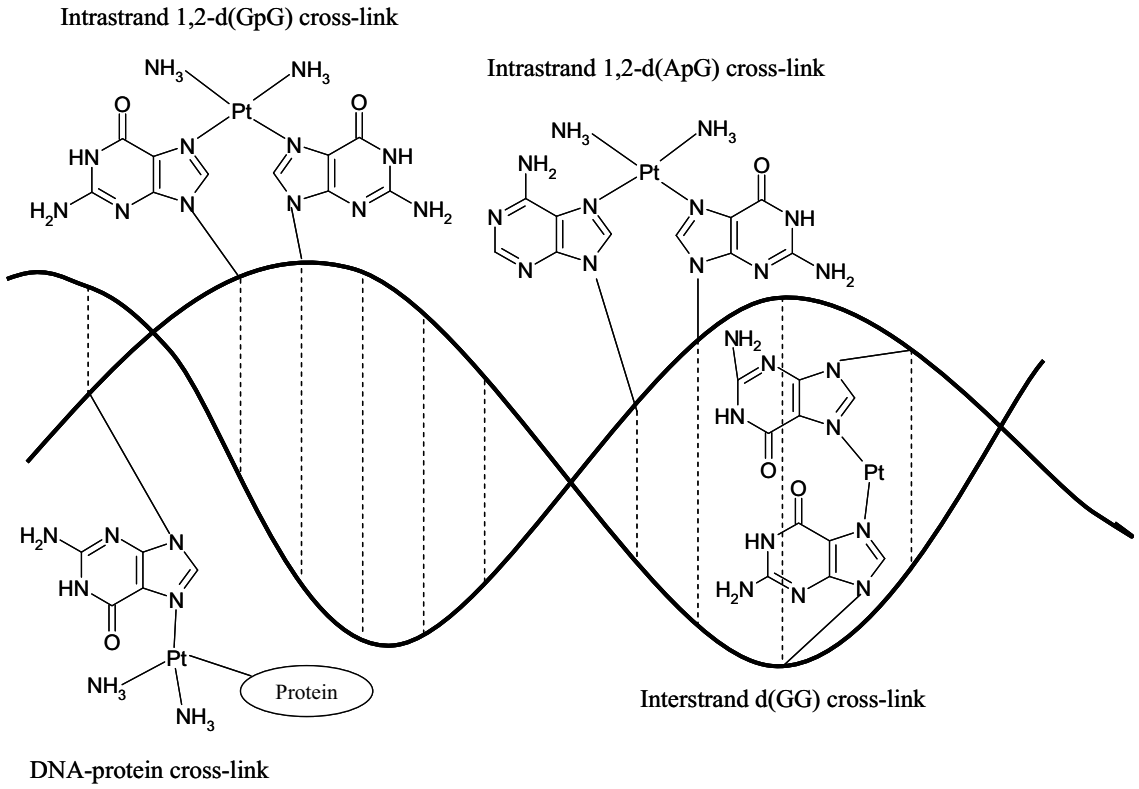

Fig.1.2. Schematic view of a double-stranded DNA, depicting some of the most commonly occurring Pt-DNA adducts. Geometry considerations (HH, HT orientation) have been ignored.

Cisplatin-DNA adducts inhibit DNA replication, block transcription by RNA polymerase II and trigger programmed cell death or apoptosis.[15, 18] Experiments carried out to study the kinetics of the Pt-DNA interaction, amongst others, pointed out that the two most abundant adducts, *i.e.* intrastrand 1,2-d(GpG) and d(ApG) cross-links, are responsible for the cytotoxic effects of cisplatin. However, the results obtained in these studies are not unambiguous.[15]

The formation of the above-mentioned cisplatin-DNA cross-links structurally distorts the DNA, resulting in a loss of helix stability and a structural change.[19-22] NMR studies in solution have tried to predict the structural changes provoked by cisplatin in various DNA

fragments (see Fig.1.3); a few crystal structures have also been obtained (see Fig.1.3) that basically agree with the geometries proposed from the NMR spectra.

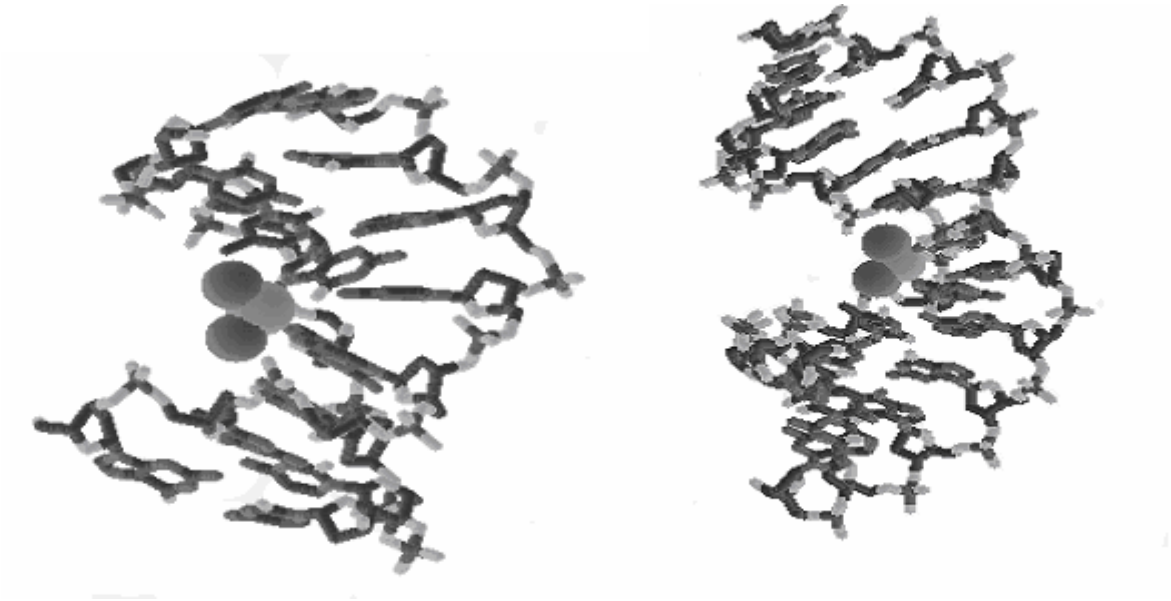

Fig.1.3. Structure of a DNA double helix fragment containing a 1,2-d(GpG) intrastrand cross-link: NMR-solution structure (left)[23] and schematic crystal structure (right).[24]

The dihedral angle between the guanine rings in the Pt adduct ranges from 76° to 87°, reflecting distortion of base stacking. All the complementary base-pairing interactions remain, however, intact, even within the G-C base pairs directly involved in the Pt-binding.[15] A bending of the DNA is observed with a kink of 40-80° towards the major groove. Simultaneously an unwinding of the helix is observed of about 20°, provoking a compression of the major groove and opening up the minor groove.[25-27] The cisplatin–DNA adducts may be stabilized by the formation of a hydrogen bond between one of the platinum ammine ligands and an oxygen atom on the 5'-phosphate group of DNA, which may be crucial for the activity of cisplatin.[28-31]

The resulting wide and shallow minor groove opposite the platinum adduct is recognised by a number of cellular proteins, including DNA repair proteins, histones and high mobility group (HMG) domain proteins such as HMGB1 (see Fig.1.4).[32]

DNA repair mechanism

Cisplatin–DNA lesions are repaired in cells primarily through the nucleotide excision repair (NER) pathway, which consists on a group of proteins with enzymatic functions.[33-35] In NER, an enzyme system first recognizes the lesion and then hydrolyzes two phosphodiester bonds, one on either side of the lesion, to generate an oligonucleotide carrying the damage. The gap is then filled in and ligated by a DNA ligase.[35]

The importance of the role of these proteins in the mechanism of action of cisplatin is underlined by the observation that the sensitivity to cisplatin increases in those cells deficient in DNA repair, while the DNA repair is more efficient in some cisplatin-resistant cell lines.[36]

Numerous HMG-domain proteins have been found to specifically recognize and bind to cisplatin-modified DNA. Examples of these proteins are TBP, TATA-binding protein[37-39] and the transcription factor FACT (Facilitates Chromatin Transcription).[40]

HMGB1 and other cellular proteins that recognize platinum-DNA adducts (see Fig.1.4) may play a role in the mechanism of action of cisplatin, according to two main hypotheses.[41] The first of these hypotheses proposes that cisplatin-damaged DNA hijacks proteins away from their natural binding sites, leading to cellular stress and eventually cell death. The second hypothesis suggests that binding by cellular proteins shields cisplatin adducts from nucleotide excision repair (NER), allowing them to persist and drive apoptosis.[42, 43] These two mechanisms are not mutually exclusive. Although many studies have demonstrated that HMG-domain proteins enhance cisplatin antitumour efficiency, others reached the opposite conclusion.[44-46] It seems, therefore, that the effect of these proteins in modulating the activity of cisplatin depends upon the cell type and context.

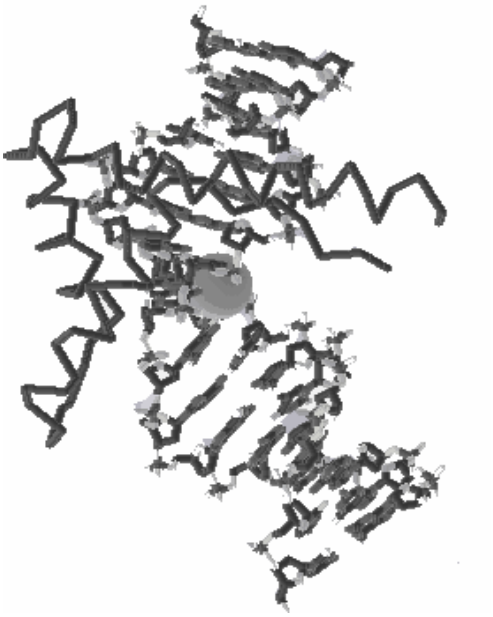

Fig.1.4. Schematic crystal structure of the HMGB1a protein bound to a cisplatin-modified DNA duplex.[32]

1.3. 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).[12] 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 (See Fig.1.5). 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.[47, 48]

Two other second- and third-generation compounds have been approved for clinical use. *cis*-diammine(glycolato)platinum(II) (nedaplatin)[49] (see Fig.1.5) was approved in 1995

by the Health and Welfare Ministry in Japan[50] 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.[51-56] (1R,2R-diaminocyclohexane)oxalatoplatinum(II) (oxaliplatin)[57] (see Fig.1.5) was approved in France[50] 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.[50]

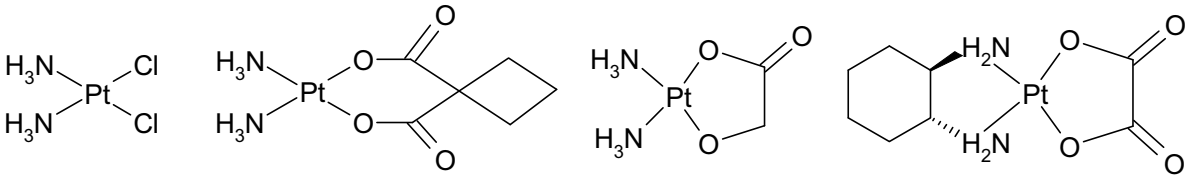

Fig.1.5. Molecular structure of a few selected platinum drugs. From left to right: cisplatin, carboplatin, nedaplatin and oxaliplatin.

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.

Platinum(IV) complexes

The design of platinum(IV) complexes yielded a new concept in platinum anticancer therapy. These compounds with lipophilic groups at axial positions would facilitate intestinal absorption of the drug, making oral administration possible.[58] Moreover they would act as pro-drugs, which get reduced to platinum(II) by intracellular glutathione, ascorbic acid or other reducing agents. The platinum(II) would bind subsequently to DNA and exert the desired action.[59, 60] The most successful Pt(IV) complex is bis(acetato)-amminedichlorido(cyclohexylamine)platinum(IV) (see Fig.1.6), also known as satraplatin or JM216. Phase II trials of this drug have been completed by GPC-Biotech in hormone-refractory prostate cancer (HRPC), ovarian cancer and small cell lung cancer.[61] Phase III evaluation of satraplatin combined with prednisone is ongoing as a second-line

chemotherapy treatment for patients with HRPC. Other trials evaluating the effects of satraplatin in combination with radiation therapy, in combination with other cancer therapies and in various other cancers are underway or planned.[61] Satraplatin also shows *in vivo* oral antitumour activity against a variety of murine and human subcutaneous tumour models, comparable to the activity of cisplatin. In addition, it has a relatively mild toxicity profile, being myelosuppression instead of nephrotoxicity the dose- limiting factor.[62]

Sterically hindered *cis*-platinum(II) complexes

In the search for platinum drugs that show activity in those cell lines in which cisplatin is inefficient, a strategy was tried which consisted on designing complexes with sterically crowded non-leaving groups. These compounds would react preferentially with nucleic acids over sulfur-containing biomolecules, thus avoiding inactivation by GSH and others. *cis*-amminedichlorido(2-methylpyridine)platinum(II) (ZD0473 or AMD473; see Fig.1.6) exhibited no cross-resistance to cisplatin in *in vitro* tests carried out with human ovarian carcinoma cells,[63] so it was selected for clinical trials. Phase-II clinical trials carried out with lung and metastatic breast cancer patients showed a good tolerability of the drug, but no greater efficacy over existing agents in platinum-resistant patients.[64, 65] Studies are ongoing using the drug in combination with other drugs, including docetaxel.[65, 66] The results obtained in phase II clinical trials with ovarian cancer patients also suggested that ZD0473 may not completely circumvent the platinum-resistance mechanisms.[67] Studies are ongoing of combined therapy with liposomal doxorubicin or paclitaxel.[67]

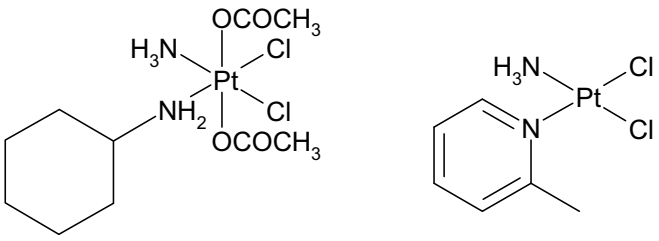

Fig.1.6. Molecular structure of the anticancer platinum complexes satraplatin or JM216 (a Pt(IV) complex, on the left) and ZD0473 (a Pt(II) complex, on the right).

***trans*- platinum(II) complexes**

Since transplatin displays no antitumour activity, one of the early conclusions drawn in the SAR´s was that the *cis*- geometry was an essential requisite. On the other hand a

complex that reacts exactly like cisplatin will never overcome resistance to it. In the search for complexes that followed a different mechanism to cisplatin the first SAR-rule was revised. Indeed a series of active *trans*-Pt(II) compounds was found.[68]

The *trans*-Pt(II) complexes that have been synthesised so far can be divided into several groups that respond to the general formula *trans*-[$PtCl_2$(L)(L')]. The pioneers were Farrell and his group, with complexes where L = a pyridine-like ligand and L´= an ammine, a sulfoxide or a pyridine-like group.[69-72] Following his example, other groups synthesised more *trans*-Pt(II) complexes, finding in some cases very good anticancer activities. Navarro-Ranninger and her group focused on complexes with L = L´ = branched aliphatic amines.[73, 74] Gibson and others reported that the replacement of one of transplatin´s ammine ligands by a heterocyclic ligand, such as piperidine, piperazine or 4-picoline, resulted in a radical enhancement of the cytotoxicity.[75, 76] Finally the group of Natile and Coluccia synthesised complexes where L = an iminoether ligand and L´ = an amine or one more iminoether ligands.[77, 78]

All these groups have reported that the cytotoxic ability of the above-described *trans*-platinum complexes with bulky non-leaving groups is in some cases superior to that shown by cisplatin, and often better than the cytotoxicity of their respective *cis*- analogues. These *trans*- complexes are characterized by a spectrum of activity different from cisplatin and they often overcome resistance. The background concept for designing these complexes is that sterically crowded carrier ligands slow down the reaction between the platinum centre and the biomolecules.[68] In addition, these complexes will cause different DNA alterations from those generated by *cis*-platinum complexes.[71, 79] Finally, the cellular response towards these *trans* complexes is expected to be different than the response towards the cisplatin analogues.[80] This is a mechanistically crucial point, which requires further investigation from a molecular pharmacology point of view.[80, 81]

Polynuclear platinum drugs

In the search for platinum complexes that interact with DNA in a drastically different way to cisplatin, several dinuclear compounds were studied.[82] This new approach allowed many variations to be introduced, to fine-tune or drastically change the DNA binding modes and the biological activity of these complexes. Symmetric complexes have been synthesised and also complexes with two inequivalent coordination spheres;[82] the compounds can vary from bifunctional to tetrafunctional; flexible amine linkers were used,

as well as rigid bridges. These dinuclear complexes were later amplified, becoming trinuclear, tetranuclear and even pentanuclear complexes. The interaction between each of these complexes, with its characteristic size and charge, and DNA is expected to be unique, as is the cellular processing of each drug. The final aim is the synthesis of a heterogeneous group of compounds some of which could overcome both intrinsic and acquired resistance to cisplatin.[82]

A comparative study involving several dinuclear bifunctional and trifunctional platinum(II) complexes (see Fig.1.7) was carried out to investigate the effects of geometry and polyfunctionality on their biological activity.[83] The results obtained showed that some of the complexes display a good antitumour activity, in various cases improving that of cisplatin. More interestingly, some of these complexes overcome cisplatin resistance. Mechanistically these compounds are expected to interact with DNA in different ways.

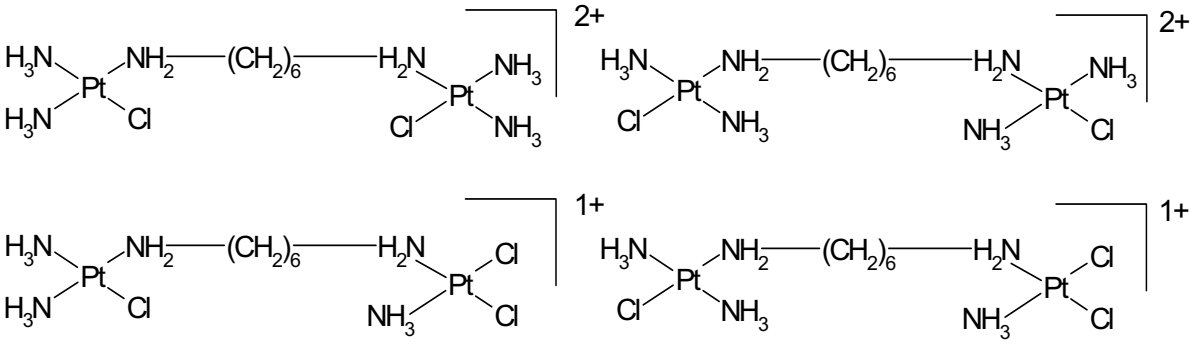

Fig.1.7. The platinum(II) dinuclear complexes 1,1/c,c (above, left), 1,1/t,t (above, right), 1,2/c,c (below, left) and 1,2/t,t (below, right). Counterions are not shown in the picture.

Dinuclear (and trinuclear) complexes incorporating the 4,4′-dipyrazolylmethane (dpzm) ligand have been reported by Collins *et al* (see Fig.1.8).[84] The presence of the heteroaromatic rings in the dpzm group could allow for favourable van der Waals interactions and hydrogen bonding within the DNA minor groove. These compounds display, however, less cytotoxicity than the dinuclear complexes with straight-chain diamine linkers.

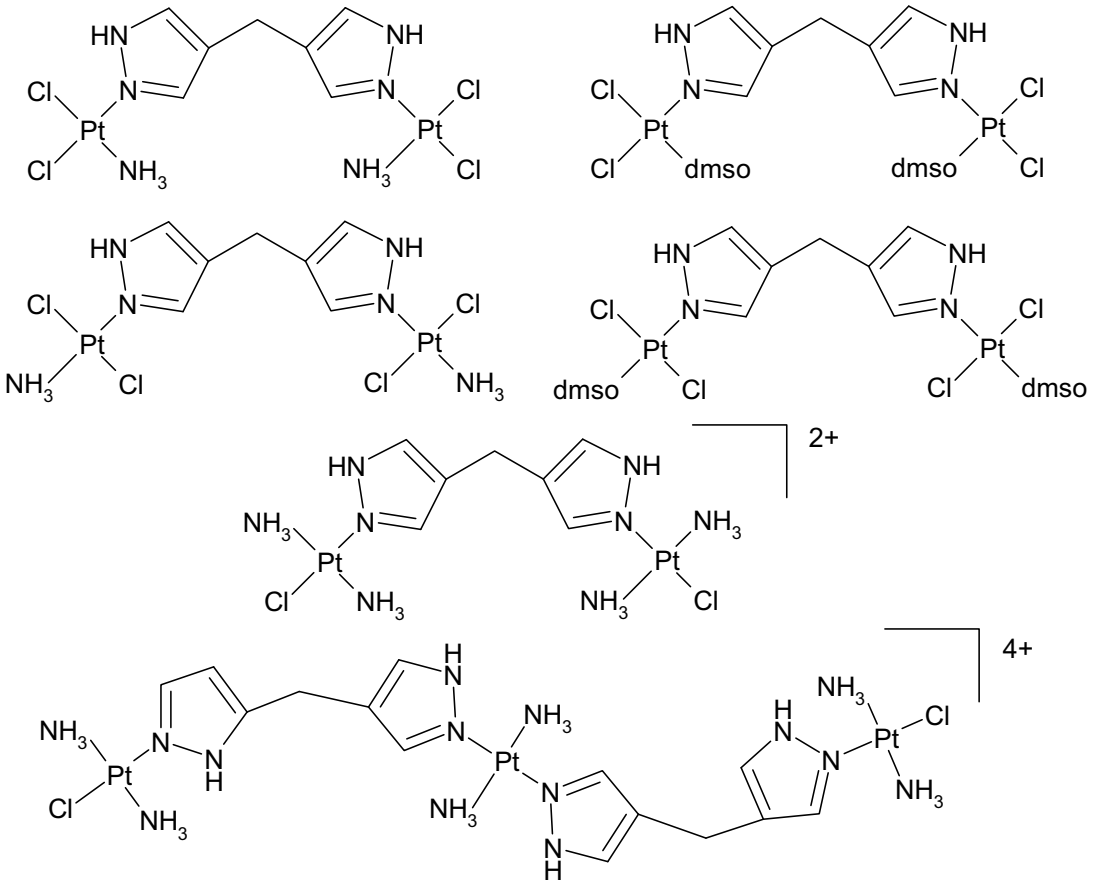

Fig.1.8. Singularly bridged, multi-nuclear platinum complexes linked by the 4,4'-dipyrazolylmethane (dpzm) ligand.

Within the group of dinuclear platinum(II) complexes, a remarkable example consists in the use of pyrazole and triazole as rigid bridging ligands. The groups of Chikuma and Reedijk synthesised dinuclear platinum(II) complexes (see Fig.1.9) that display much higher *in vitro* cytotoxicity than cisplatin on several human tumour cell lines and largely overcome cross-resistance to cisplatin.[85, 86]

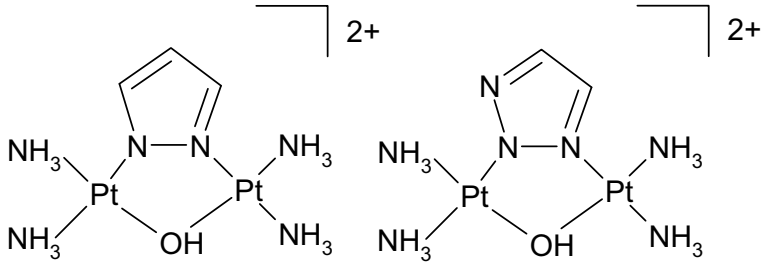

Fig.1.9. Molecular structure of two azole-bridged dinuclear platinum(II) complexes.

The trinuclear platinum(II) complex 1,0,1/*t,t,t* or BBR3464 (see Fig.1.10) was selected for phase II trials once promising pre-clinical data had been obtained.[87] BBR3464, which provides long-range intrastrand crosslink upon DNA, was found to be very potent as a cytotoxic agent, besides being effective against cisplatin-resistant tumour cells. Notable features are the potency, the ten-fold lower maximum tolerated dose (MTD) in comparison to cisplatin and the broad spectrum of tumours sensitive to this agent. Importantly, BBR3464 also displays high antitumour activity in human tumour xenografts characterized as mutant p53, tumours that are known to be insensitive to drug intervention.

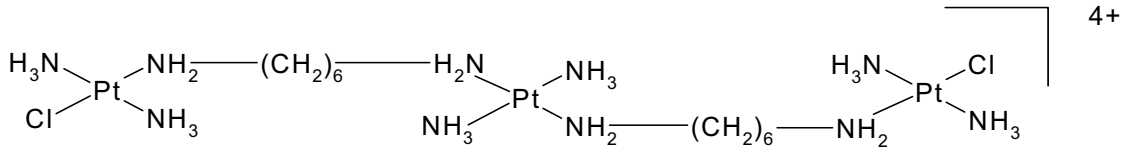

Fig.1.10. The platinum(II) trinuclear complex 1,0,1/t,t,t.

1.4. A possible alternative to platinum therapy: ruthenium chemistry

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.[88, 89] 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.[90]

Ruthenium properties that make it suitable for biological applications

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.[90, 91] Very few metal drugs reach the biological target without being modified, which makes ligand exchange an important 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.

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 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),[92] always depending on the nature of the ligands, while molecular dioxygen and cytochrome oxidase can oxidize Ru(II) in certain complexes.[93-95]

The redox potential of ruthenium compounds can be exploited to improve the effectiveness of Ru-based drugs in the clinic.[90, 91] 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.[96] 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.[97]

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.[90]

One more property of ruthenium that makes it very appreciated in medicinal chemistry is its tendency to selectively bind biomolecules, which partly accounts for the low toxicity of ruthenium drugs.[90, 91] 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.[97-101] 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 on its general toxicity.

Anticancer activity

Two approaches are commonly used for the design of new anticancer compounds. The trial-and-error approach consists on synthesizing as many compounds as possible that are analogous to a complex of known activity, but which has drawbacks that need to be solved. These new compounds 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.

1.5. 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 $[Ru(NH_3)_{6-x}Cl_x]^{Y+}$. Those complexes in which the oxidation state of the ruthenium ion was (II) were expected to bind to DNA in an analogous way to cisplatin, and indeed the first experiments performed with the complexes $[Ru(II)(NH_3)_5Cl]^+$ (see Fig.1.11) and $[Ru(II)(NH_3)_5(H_2O)]^{2+}$ fulfilled this expectation.[102-104] The cytotoxicity tests carried out with these species yielded however disappointing results. 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.[99, 105] 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.[92] The trichloride complex, being the most promising of all these compounds, was discarded for further investigation due to its poor water solubility.

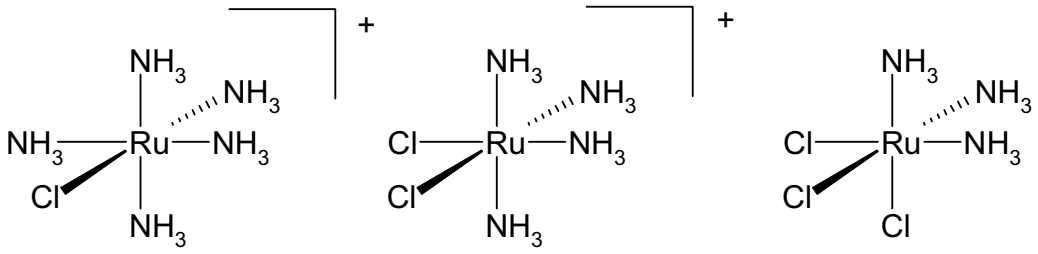

Fig.1.11. Ammine-chlorido derivatives. From left to right, $[Ru(II)(NH_3)_5Cl]^+$, cis-$[Ru(III)(NH_3)_4Cl_2]^+$ and fac-$[Ru(III)(NH_3)_3Cl_3]$.

Dimethylsulfoxide complexes

The substitution of the ammine ligands by dmso molecules yields compounds with improved solubility. Both *cis*- and *trans*-$[Ru(II)Cl_2(dmso)_4]$ (see Fig.1.12) were shown to be able to coordinate to guanine residues of DNA via the N7 position.[106] 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.[107] This observation, together with the fact that *trans*-$[Ru(II)Cl_2(dmso)_4]$ shows a good

antimetastatic activity,[107] 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 (see Fig.1.12). 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 tumours including lung metastasis, is becoming of utmost interest.[108, 109]

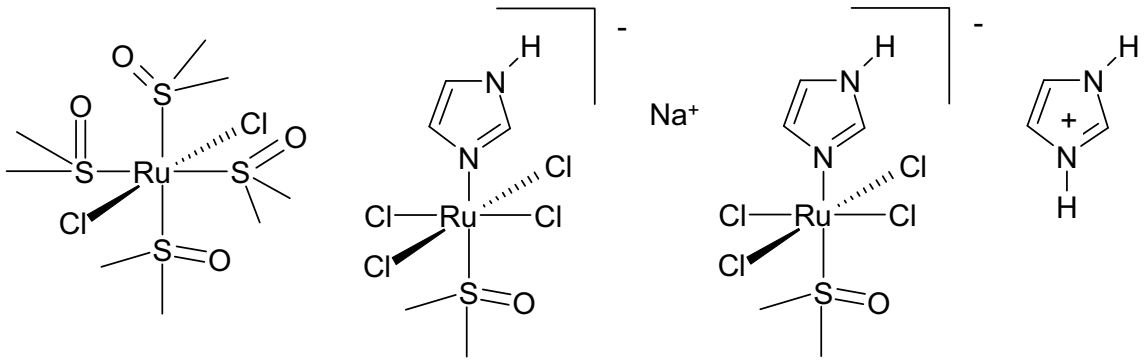

Fig.1.12. Dimethylsulfoxide complexes. From left to right, trans-[Ru(II)(dmso)$_4$Cl$_2$], Na{trans-[Ru(III)Cl$_4$(dmso)(Him)]} (NAMI) and [H$_2$Im]{trans-[Ru(III)Cl$_4$(dmso)(Him)]} (NAMI-A).

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.[110, 111] 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.[111-113]

A series of NAMI-A analogues bearing a weakly basic heterocyclic nitrogen ligand *trans*- to dmso was synthesized.[108] 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.[108]

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^+ (see Fig.1.13). KP1019 and KP1339 were reported effective in inhibiting platinum-resistant colorectal carcinomas in rats;[114] KP1019 recently completed phase-I clinical trials.[100]

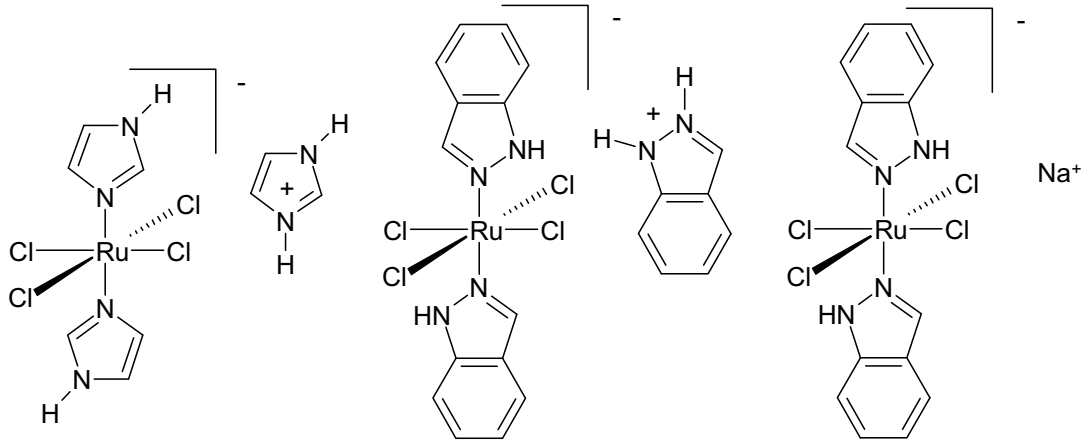

Fig.1.13. Molecular formula of the ruthenium(III) complexes imidazolium trans-[tetrachloridobis(imidazole)ruthenate(III)] (KP418), indazolium trans-[tetrachloridobis(indazole)ruthenate(III)] (KP1019) and sodium trans-[tetrachloridobis(indazole)ruthenate(III)] (KP1339).

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,[100, 114] as in the case of NAMI-A.

Several ruthenium polypyridyl complexes (see Fig.1.14) 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.[115] 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.[116, 117] Its poor water solubility, however, hampered its further progress into the clinical trials.

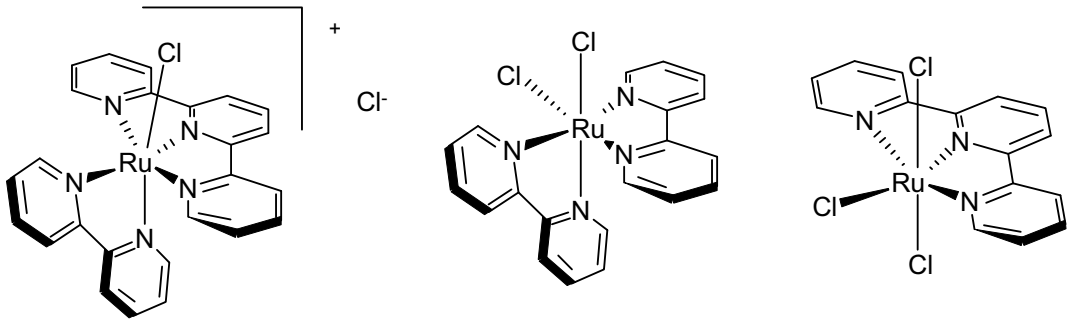

Fig.1.14. Molecular formula of the ruthenium polypyridyl complexes [Ru(II)(bpy)(tpy)Cl]Cl, cis-[Ru(II)(bpy)$_2$Cl$_2$], and mer-[Ru(III)(tpy)Cl$_3$] (bpy = 2,2'-bipyridine, tpy = 2,2':6'2''-terpyridine).

Ten years later an X-ray structure was reported of the *cis*-[Ru(II)$(bpy)_2]^{2+}$ fragment (bpy = 2,2′-bipyridine) bifunctionally binding to two DNA model bases.[118] However, the ruthenium(II) precursor *cis*-[Ru(II)$(bpy)_2Cl_2$] had been proven mostly inactive in the above-described biological tests.[115] The fact that this complex can bind two model bases (after chloride removal) but it is inactive *in vitro* questions the relation that has been established between the possibility of bifunctionally binding to DNA and the cytotoxicity of ruthenium polypyridyl complexes.

As a last noteworthy example of *in vitro* antitumour-active ruthenium complexes with heterocyclic ligands, one of the isomers of *cis*-[Ru(II)$(azpy)_2Cl_2$] (see Fig.1.15), where azpy = 2-phenylazopyridine, showed a remarkably high cytotoxicity against fast-growing cell lines.[119, 120] The higher activity of *cis*-[Ru(II)$(azpy)_2Cl_2$] with respect to *cis*-[Ru(II)$(bpy)_2Cl_2$] has been related to a higher flexibility of the azpy ligand, which

allows an easier substitution of the chloride ligand and thus the binding of the complex to even two DNA bases.[119]

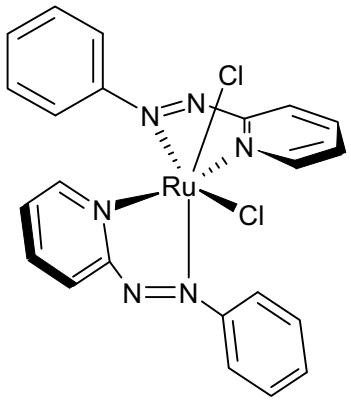

Fig.1.15. Molecular formula of the most active isomer of cis-[Ru(II)(azpy)$_2$Cl$_2$].

Ruthenium polyaminocarboxylate complexes

There has been a wide interest in the redox properties of ruthenium(III/IV) complexes with polydentate mixed-donor ligands. Ligands like ethylenediaminetetraacetate (edta), 1,2-cyclo-hexanediaminotetraacetate (cdta), 1,2-propylendiaminetraacetate (pdta), triethylenetraminehexaacetate (ttha), N,N,N′,N′-tetrakis(2-pyridyl)adipamide (tpda), N-hydroxyethylethylenediaminetriacetate (hedtra) and others from the H_4edta family have been coordinated to ruthenium to form complexes with acid-base and redox properties that have been thoroughly studied.[121-125]

Some of these complexes were found to be able to bind to DNA model bases, as well as to blood proteins, such as albumin and transferrin, which suggested that they might have an antitumour activity.[97, 110, 126-128] While this is still under study, the complex containing cdta was the first Ru(IV) compound reported to display cytotoxic activity.[129, 130]

Organoruthenium complexes

The monodentate 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 (see Fig.1.16), 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.[131-134] The chlorido or iodido ligand is readily lost to yield the more reactive aqua species.[135] 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.[136, 137]

[(η^6-toluene)Ru(II)(pta)Cl_2] (RAPTA-T), where pta is 1,3,5-triaza-7-phospha-adamantane (see Fig.1.16), is the parent compound from which a group of water-soluble selective DNA-binding antimetastatic drugs was synthesized.[138, 139] 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.[140]

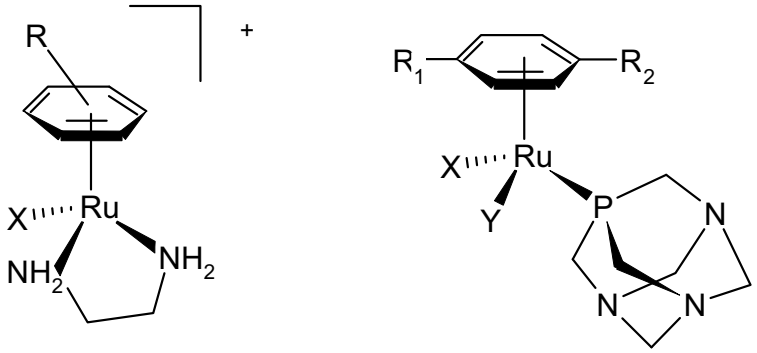

Fig.1.16. General formula of two groups of organometallic ruthenium(II) complexes with modified arene ligands. On the left, [(η^6-arene)Ru(II)(en)X]$^+$, where the arene can be benzene, p-cymene, biphenyl, 5,8,9,10-tetrahydroanthracene or 9,10-dihydroanthracene. X is Cl or I. On the right, [(η^6-arene)Ru(II)(pta)XY] (RAPTA complexes). R_1, R_2 are alkyl groups; X and Y can be Cl or different μ-dicarboxylate ligands.

Photoreactive ruthenium compounds that induce DNA cleavage

Recently some photoreactive ruthenium(II) complexes have been under study as potential anticancer agents.[91] In phototherapy, a photosensitizer absorbs light and it then reacts with a targeted endogenous molecule (O_2 or DNA) via energy or electron transfer.[91] Metal compounds such as polyazaaromatic ruthenium(II) complexes are good candidates as photosensitizers, with properties that can be modulated by introducing changes in the ligands.[141]

Once a photosensitizer is excited, it can react with a dioxygen molecule, leading to the production of singlet dioxygen.[141] This very reactive species may induce formation of

oxidizing agents, such as superoxide or hydroxyl radicals, that can damage DNA by oxidizing the guanine moiety or even cleaving the DNA strand.[141]

Important advances in this field are the discovery that singlet dioxygen production by $[Ru(II)(bpy)_2(phen)]^{2+}$ is able to block partially the activity of a bacteriophage RNA-polymerase,[142] as well as the encapsulation of the complex $\{Ru(II)[dip(SO_3Na)_2]_3\}$, where $dip(SO_3Na)_2$ is the sodium salt of disulfonated 4,7-diphenyl-1,10-phenantroline, into polyacrylamide nanoparticles for use in photodynamic therapy.[143] The main drawbacks of the *in vivo* treatments in photodynamic therapy are collateral damages to healthy cells, acquired resistance and limitation of light penetration in tissues.[143]

An electron-transfer process can also be involved in phototherapy leading to DNA cleavages. Ru(II)-2,3-naphthalocyanine compounds showed activity *in vivo* against cancer cells in absence of singlet oxygen.[144] Besides, it has been demonstrated that a photo-induced electron transfer takes place from a guanine to the excited state of some Ru(II) complexes containing π-deficient ligands such as TAP (1,4,5,8-tetraazaphenantrene), HAT (1,4,5,8,9,12-hexaazatriphenylene) or BPZ (2,2′-bipyrazine). The formation of the radical on the guanine is enough to provoke DNA cleavages.[141, 145]

Dinuclear ruthenium complexes

As explained in section 1.3, several dinuclear platinum complexes have been synthesised in search for compounds that interact with DNA in a drastically different way to cisplatin. The interaction of each of these complexes with DNA, as well as its cellular processing, are expected to be unique, involving long-range intrastrand cross-links upon DNA and van der Waals interactions within the minor groove, amongst others, with as final aim finding a drug capable of overcoming cisplatin resistance.

Although the electrochemical and photophysical properties of several cationic ruthenium dimeric complexes with heterocyclic bridging ligands had been extensively studied in the 1970s,[146] the testing of this kind of complexes in the oncological field was only reported in the last decade. A group of these complexes has the general formula $[\{trans\text{-}Ru(III)Cl_2(dmso)L_1L_2\}_2(\mu\text{-}L_3)]^{m-}$. L_1, L_2 are Cl or dmso. L_3 is a nitrogen heterocyclic ligand with at least two nitrogen atoms, like pyrazine (pyz), pyrimidine (pym), 4, 4′-bipyridine (bipy), 1,2-bis(4-pyridyl)ethane (etbipy), 1,2-bis(4-pyridyl)propane (prbipy) or *trans*-1,2-bis(4-pyridyl)ethylene (etilbipy). Finally, m is 0, 1 or 2 (see Fig.1.17). These complexes are based on the mononuclear NAMI-A.[147] The dinuclear complexes

obtained from this antimetastatic compound show a chemical stability that renders them very suitable for pharmacological formulation, as well as a high antimetastatic activity that indicates they could be helpful in the treatment of tumours with a high degree of metastatic diffusion, such as mammary, lung or digestive tract carcinomas.[148-151]

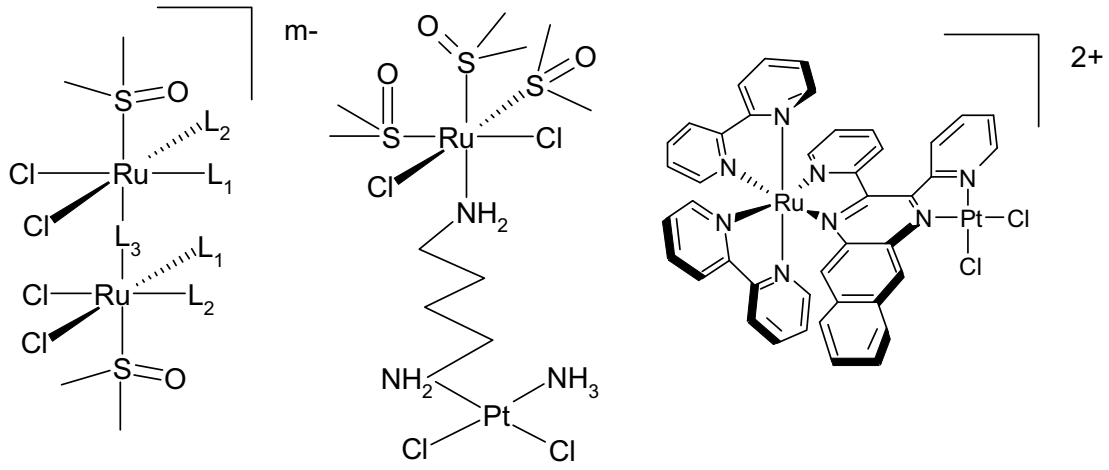

Fig.1.17. From left to right, the general formula of dinuclear Ru(III) complexes based on NAMI-A; a Ru(II)-Pt(II) heterodinuclear complex with an aliphatic linker and a Ru(II)-Pt(II) heterodinuclear complex with an intercalating linker.

In vitro studies carried out with these complexes showed that a G(2)-M cell cycle arrest was induced, which was dependant on the ruthenium concentration and on the cell line, while their cytotoxicity was only mild against human and murine cell lines. This behaviour is comparable to that of the parent mononuclear complex NAMI-A. Moreover the cell cycle-regulating protein cyclin B appears to be significantly modified.[152]

A variation of these compounds, where dmso is substituted by tetramethylene sulfoxide (tmso) is currently under study. So far only the mononuclear compounds have been described,[153, 154] as well as the anionic ruthenium(III) dinuclear versions of these complexes with pyrazine as bridging ligand.[155]

Mixed-valent ruthenium tetracarboxylate complexes were shown to have a mild antineoplastic activity against P388 leukaemia cell lines. However, these complexes are poorly water soluble.[109] Mixed-valent complexes of structural formula $[(RuL)_2(\mu\text{-}O_2CR)_4](PF_6)$ were tested for cytotoxicity against HeLa and multidrug resistant CoLo 320DM human cancer cells. L is an imidazole, a 1-methylimidazole or an aqua

ligand when R is a methyl group, and L is an ethanol when R is a ferrocenyl (Fc) or a Fc-CH=CH-. The related series of complexes with formula $M_3[Ru_2(\mu\text{-}O_2CR)_4(H_2O)_2]\cdot 4H_2O$, where M is Na^+ when R is *m*-$C_6H_4SO_3^-$ and M is K^+ when R is *p*-$C_6H_4SO_3^-$ were also tested for cytotoxicity against the above-mentioned HeLa and multidrug resistant CoLo 320DM human cancer cells. A few of these complexes show some cytotoxicity and, more interestingly, CoLo 320DM was found to be more sensitive to these complexes than HeLa, which is more sensitive than CoLo 320DM to cisplatin. This observation suggests that the mechanism of action of these complexes is different to that of the classical platinum drugs.[156]

Complexes with μ-N,N'-diphenylformamidinate and μ-(fluoroanilino)pyridinates have also been prepared. The compound μ-$[(F_3CCO_2)_4(F_3CCO_2)Ru_2]$ forms *cis*-$[\mu$-$(F_3CCO_2)_4$-μ-(9EtGua)$Ru_2(CH_3OH)_2]^{2+}$ where 9EtGua = 9-ethylguanine in which the guanines bridge between the two Ru(II) atoms in a N7-O6 head-to-tail fashion.[99, 157]

The compound μ-O-$[Ru(III)(bpy)_2(H_2O)_2]_2^{4+}$ is a borderline example. This dinuclear ruthenium(III) complex has been proven to be effective in double-stranded DNA cleavage. However, its action is thought to be due to the mononuclear $[Ru(III)(bpy)_2(H_2O)_2]^{2+}$, which is formed by intracellular reduction of the dinuclear complex.[158, 159]

The combination of metal moieties with different properties provides systems of great interest. Although the following three examples fall slightly out of the scope of the dinuclear ruthenium complexes, they are worth mentioning in relation with them. In general ruthenium complexes are less reactive than platinum compounds, and the design of ruthenium/platinum heterodinuclear complexes provides molecules that can selectively, sequentially react with particular DNA sequences and facilitate unique DNA modification.[160] The complex {[*cis*,*fac*-$Ru(II)Cl_2(dmso)_3$][μ-$NH_2(CH_2)_4NH_2$][*cis*-$PtCl_2(NH_3)$]} (see Fig.1.17) was the first of the series.[161] These anticancer compounds are suspected to exert their action via a novel mechanism of action, involving interstrand crosslinks in which each metal atom is coordinated to one strand of DNA. A second strategy is the coupling of a light absorber to a cisplatin moiety by a ligand capable of intercalative binding with DNA. The $\{[M(bpy)_2]_2(\mu\text{-dpb})[PtCl_2]\}^{2+}$ complexes, where M is Ru(II) or Os(II) and dpb is 2,3-bis(2-pyridyl)benzoquinoxaline, form primarily intrastrand crosslinks, but interstrand crosslinks were also formed (see Fig.1.17).[162, 163] Finally, the highly flexible heterodinuclear complex $[Ru(II)(tpy)](\mu\text{-dtdeg})[PtCl]^{3+}$, where dtdeg is bis[4′-(2,2′:6′,2″-terpyridyl)]-diethyleneglycol ether), was synthesized.[164] Modifications of

the linker are currently under study in search for a derivative of this complex with an increased antitumour activity.

1.6. 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.[165] 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.[166] 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.[166] 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.[165] In recent years, ruthenium-based drug research is moving from classical chemotherapy into the non-conventional 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.[92, 102-104]

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 ruthenium ligands are their photoluminescence, which makes them suitable as DNA probes, as well as the stability of the complexes that they can originate, amongst others.[165] 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.[107]

The use of polyaminocarboxylate ligands in metallopharmaceutical applications seemed a logical option due to their resemblance to biological molecules.[167] Several of these complexes turned out to be antitumour active with low systemic toxicity. Some of them were proven to bind to DNA, alter its conformation and even induce DNA cleavage.[110, 168] In addition, several of these complexes were found to be effective NO scavengers and protease inhibitors, thus they could be used to treat various diseases or serve as antiviral agents.[169]

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.[170] 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.[136]

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

One of the most successful ruthenium-based anticancer drugs 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 tumour 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.[108] 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.[108]

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.[114] 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.[100, 114] 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.[100, 171] 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.[172]

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.[140, 173] 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.[140, 173] 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.[174-176]

In conclusion, 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.

1.7. Aim and scope of this thesis

The existence of two common approaches for the design of new anticancer drugs has been mentioned in section 1.4. The first method, often known as "trial-and-error", is based on the synthesis and testing of libraries of closely-related complexes. This thesis is based on the second approach, which parts from the synthesis of very few compounds. These are thoroughly studied in order to gain some insight about the way they function and, subsequently, how they can be improved.

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.

A group of ruthenium(II) polypyridyl complexes is presented in **Chapter 2**. A complete description is given of the synthesis and characterization by several methods of three compounds derived from the cytotoxic, but poorly water-soluble complexes, Ru(III)(tpy)Cl_3 and α-Ru(II)$(azpy)_2Cl_2$.

With the purpose of proving or discarding DNA as a potential target of the newly-synthesised complexes, a study was carried out, which is included in **Chapter 3**. NMR is used as a basic tool to follow the reaction between each of the complexes and a DNA model base, allowing identifying kinetic differences amongst the three proposed compounds. A conformational investigation of the so-called ruthenium–model base adduct was found to be of theoretical interest.

Other modes of interaction between ruthenium complexes and DNA were looked into with the help of circular and linear dichroism. The question "is there a correlation between these interactions and the antitumour activity of the selected ruthenium(II) polypyridyl complexes?" has been dealt with in **Chapter 4**. The synthesis and characterisation of a ruthenium(II) homodinuclear complex are described. This compound, together with a few previously-known ruthenium(II) polypyridyl complexes, is investigated in the search for some structure-activity relationships.

In **Chapter 5** some suggestions for future directions in this work are given. The synthesis of a new ruthenium(II) homodinuclear complex, which is closely related to the other compounds herein described, raised several new questions.

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

Finally, a study carried out in relation with the work included in **Chapter 3**, in this case excluding the metal atom, is briefly described in the **Appendix**.

Parts of this thesis have been published[177-179] or submitted for publication.[180]

1.8. References

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