CHAPTER 1

Design, Synthesis, Characterization and Biological Studies of Ruthenium and Gold Compounds with anticancer properties

1.1 Introduction

Cancer is a major health problem. Numbers are evident: 10 million cases are diagnosed each year [1], and in 2020, new cancer cases are predicted to have doubled to a 20 million per year [3]. Certainly several improvements have been achieved, in the understanding, diagnostics, treatment and prevention of cancer through the investment in biological and chemical research.

Treatment options for cancer include surgery, chemotherapy and radiotherapy, and the choice of treatment depends on the type of cancer, stage, health status and co-morbidity of the patient. With the use of these therapies half of the patients can be cured, while the other half may have a prolonged survival or even no benefit at all [4].

Cancer chemotherapy formally started with the discovery of the cytotoxic effect of N-mustards in some cancer types and further development in this field was a constant goal due to lack of cytotoxic activity on several other cancer types.

Later on, the undeniable success of cisplatin in the treatment of testicular and ovarian cancer attracted research attention to metal-based antineoplastic agents and cisplatin analogues like carboplatin and oxaliplatin were designed based on the chemical and biological advantages and disadvantages of cisplatin as an anticancer agent. However, undesirable side effects, drug resistance (intrinsic or developed) and narrow application in the wide range of cancer types have prompted a search for other metal-based antitumour agents. Metal-based compounds with titanium, germanium, rhodium, rhenium, gallium, gold, ruthenium, tin, cobalt and copper, have been studied and many of them have shown promising results and have even been included in clinical trials.

The use of metal-based compounds is of particular interest due to their physical and chemical properties. Properties like ligand exchange rates, redox properties, oxidation states, coordination affinities, solubility, biodisponibility and biodistribution could be modified in order to increase the cytotoxic effects and to reduce the side effects. In particular, several ruthenium and gold complexes have shown potential application as anticancer agents and the study of their chemical and biological properties will facilitate the elucidation of a clear structure-activity relationship that in the near future may be used for the design, synthesis and characterization of more effective anticancer agents with reduced side effects.

This thesis describes important chemical, physical and biological properties from selected ruthenium and gold compounds in the search of more effective cytotoxic compounds and a better understanding of structure-activity relationships.

The first chapter comprises general information related to cancer and its impact in the world as well as the most important chemical and biological findings in the field of ruthenium and gold cytotoxic complexes with potential application in the treatment of cancer. The final part of this chapter will introduce the aims and the general outline of this work.

"I never see what has been done; I only see what remains to be done"

Marie Curie, scientist (1897-1956)
Chapter 1

1.2 Cancer and its statistics. Definition and actual trends

Cancer calls for a larger degree of personal concern than does any other disease. In fact, of the many challenges that medicine has faced, none of them has had a more controversial beginning and none has experimented more hard fought progress than the treatment and cure of cancer.

In modern society, cancer is considered one of the most feared diseases throughout the world, supplanting the "white death" or tuberculosis that was the most feared during the last century; the "black death" or bubonic plague that killed thousands of people during the middle ages or leprosy during the biblical times. Cancer attacks 1 in 5 people in prosperous countries and it is often resistant to chemo- or radiation- therapies, the mostly used treatments in the control of this disease. For instance, in the United States, cancer was the second cause of death on 2003, with a 22.7% of all deaths [2]. With people on average getting older, the frequency of cancer is expected to increase further.

In the past cancer was, with a few exceptions, the equivalent of a sentence to death. Certainly, several improvements have been reached, in the understanding, diagnosis, treatment and prevention of cancer through the investment in biological and chemical research. The fact is that over the past four generations the progress of medical sciences has made it possible to transfer one kind of cancer after another from the category of "incurable disease" to that of "curable". This is not to say that all cancers could be cured. Researchers are not only learning more about what causes cancer, and how it grows and progresses but also they are looking for new and better ways to prevent, detect, and treat it as well as looking for ways to improve the quality of life for people with cancer during and after their treatment.

In healthy humans, cells grow and divide to form new cells as the body needs them. When cells grow old, they die, and new cells take their place. But opposite to the physiological process just described, cancer cells escape from the control mechanisms that normally regulate their growth and division. These extra cells can form a mass of tissue called a growth or tumour. Many contributing factors have been identified in the onset of cancer, including exposures to certain carcinogens in our diet and environment (tobacco, alcohol, sunlight, etc). Certain genes normally regulate cell growth and division, and mutations that alter the expression of those genes in somatic cells can lead to cancer. Ageing, ionizing radiations, some viruses and bacteria, certain hormones and even a poor diet, lack of physical activity and overweight are also considered as important contributing factors. Besides, several forms of cancer have been found to have familial tendencies. Certain cancers appear to arise primarily through inherited genetic alterations, while others develop as a result of both genetic and environmental interactions. Many of these risk factors can be avoided. Others, such as family history, cannot be avoided.

Most recent estimations [4] (2003) showed that in the United States, 477.2 new cases occurred per 100000 people per year (Figure 1.2.1). The causes of cancer vary worldwide. In developed countries, tobacco is a major origin, causing 1 in 3 cancer deaths. In the developing world, infection plays a major role; it is responsible for almost 1 in 4 cancer deaths [5]. The most common cancers (in descending order) in the developed world are those of the lung, colorectal, breast, stomach, and prostate. In the developing world the most common cancers are those of stomach, lung, liver, breast and cervix.

Although lung cancer rates in women have recently stabilized, lung cancer remains the leading cause of cancer death in women. The recent stabilization in new breast cancers is largely unexplained and further studies should be developed. Although most major cancers are occurring less frequently, some are on the rise and require greater efforts to control. These include non-Hodgkin lymphoma, leukaemia, melanoma of skin, and cancers of the thyroid, kidney, and pancreas in both, men and women. The incidence of some relatively rare cancers, including those of the liver, oesophagus and myeloma, is also increasing.

In spite of the high occurrence of new cancer cases, improvements in the survival rates have been observed. These improvements could be the result of early detection, improvements in the detection tests and better treatments. For adults diagnosed with cancer (all cases) in 1997, 65% had survived their cancer for at least 5 years [4].

Concerning death rates, the statistics developed by the National Institute of Cancer (USA) showed that they increased through 1990, then stabilized until 1993, and finally fell slightly
Most deaths from cancer are due to metastasis, the spread of cancer cells. Although overall death rates are on the decline, deaths from some cancers, such as oesophageal, liver, and thyroid cancers, are increasing.

1.3. Cancer therapeutics

In general terms, cancer treatment is improving, saving lives and extending survival for people with cancer, including breast and colon, and for people with leukaemias, lymphomas, and paediatric cancers.

Sometimes, the treatment goal is to cure the cancer. In other cases, the goal is to control the disease or to reduce symptoms for as long as possible. Most treatment plans include surgery, radiation therapy or chemotherapy. Some involve hormone therapy or biological therapy. In addition, stem cell transplantation may be used, so that a patient can receive very high doses of chemotherapy or radiation therapy. Some cancers respond best to a single type of treatment. Many others may respond best to a combination of treatments.

Once the cancer process has been clearly detected, the treatment plan should be designed. This plan depends mainly on the type of cancer and the stage of the disease, but also the patient's age and general health has to be considered. In case of chemotherapy, the most important factors to be considered are the side effect profiles, use of concurrent radiotherapy, performance status of the patient and total cost difference between the various chemotherapy regimens, as these will have an impact on the choice of therapy.

Treatments may work in a specific area (local therapy) or throughout the body (systemic therapy) [4]:

a. In the local therapy the removal or destruction of cancer takes part in just one part of the body. Surgery to remove a tumour is local therapy. Radiation to shrink or destroy a tumour also is usually local therapy.

b. By the contrary in the systemic therapy drugs or substances are sent through the bloodstream to destroy cancer cells all over the body. They kill or slow the growth of cancer cells that may have spread beyond the original tumour. Chemotherapy, hormone therapy, and biological therapy are usually systemic therapy. They can be used in combination with radiotherapy and surgery.
Several types of drugs could be used to treat cancer. Among them are certain drugs that block the effect of the hormones in the body (hormone therapy). Biological therapy is a treatment with substances that boost the body's own immune system against cancer. These substances can be made in the laboratory and given to patients to destroy cancer cells, or change the way the body reacts to a tumour. They may also help the body to repair or even make new cells destroyed by chemotherapy.

1.4. Chemotherapy and metal-based anticancer compounds

Although the neoplastic process has been recognized for a very long time, little was known about the biological mechanisms of transformation and tumour progression until the advent of molecular biology in the second half of the 20th century. About 60 years ago drug therapies became the focus in the efforts to cure cancer. Use of chemotherapy is still improving but advancements are needed in education and funding.

Chemotherapy is the use of drugs that kill cancer cells. The typical administration routes in patients are either by mouth, or through a vein. Independently of the route, the drugs eventually enter the bloodstream and can affect cancer and healthy cells all over the body.

Depending on the type of cancer and how advanced it is, chemotherapy can be used to cure the cancer. Cancer is considered cured when the patient remains free of evidence of cancer cells. Chemotherapy also could be meant to control the cancer. This is done by keeping the cancer from spreading; slowing the cancer's growth; and killing cancer cells that may have spread to other parts of the body from the original tumour. Finally, chemotherapy can be used to relieve symptoms that the cancer may cause. Relieving symptoms, such as pain can help patients live more comfortably.

Most of the clinically-used anticancer drugs are systemic anti-proliferative agents also called cytotoxins (cytotoxic therapy), that preferentially kill dividing cells, primarily by attacking their DNA at some level (synthesis, replication or processing). These cytotoxins have many advantages as anticancer drugs, specially the ability to kill large numbers of tumour cells with constant proportion kinetics. However, these drugs are not truly selective for cancer cells, and their therapeutic efficacy is limited by the damage they also cause to proliferating normal cells such as those in the bone marrow and gut epithelia. This is particularly true in the treatment of solid tumours, where the majority of tumour cells are not dividing rapidly [6]. Because cancer treatments often damage healthy cells and tissues, side effects are common. Side effects depend mainly on the type and extent of the treatment, but they also vary from person to person, and they may even change from one treatment session to the next. When drugs damage healthy blood cells (cells that divide rapidly), an increased chance of getting infections, bruise or bleed easily could be observed in patients. Weakness and tiredness are also commonly described. Chemotherapy can also cause hair loss (cells in hair roots divide rapidly) and poor appetite, nausea and vomiting, diarrhoea, or mouth and lip sores because of the effect in the cells that line the digestive tract. Some drugs can affect fertility. Although the side effects of chemotherapy can be distressing, most of them are temporary.

The dawning of cancer chemotherapy has been generally accepted as the serendipitous discovery of the mustard family of agents after the First World War. From the first experiments with nitrogen mustards [7] till the current attempts to develop drugs for specific cancer-related targets, researchers from multiple disciplines have joined together in the search of more effective cancer drugs.

The discovery of mustards is not the only example in which serendipity and chemistry have together led to the discovery of clinically effective anticancer agents [8].

Cisplatin was discovered in 1965 [9] through studies developed by Rosenberg and co-workers on the passage of an electric current (using platinum electrodes) through suspensions of *Escherichia coli* bacteria using ammonium chloride as an electrolyte. Analytical chemical expertise was then used to establish that the platinum electrodes used in the experiments had reacted with constituents of the culture medium to form cis-diamminedichloridoplatinum(II), \([\text{Pt(NH}_3]_2\text{Cl}_2]\), which inhibits division of bacterial cells (Figure 1.4.1). Rosenberg *et al*. then hypothesized that the precursor compound cisplatin would also affect cell division in mammalian systems, and found that it showed selective toxicity both in vitro and in vivo against experimental tumours [10-12].
These results were particularly promising, because at that time the researchers did not know about any other anticancer drug capable of having this effect. After the evidence of cisplatin in curing tumours in mice, and considering the toxicity information obtained from studies on dogs and monkeys, the hypothesis that cisplatin might be effective as an antitumour agent in people with cancer was the following research challenge.

Cisplatin is known to produce responses in approximately 80% of patients with testicular cancer, greater than 90% of patients with ovarian carcinomas, roughly 40% of patients with head and neck cancers, around 40% of patients with some lymphomas and any activity in colon carcinoma [11, 13-21]. The unexpected success of cisplatin in treating a fairly wide variety of cancers, however, was slightly obscured by the evidence of serious kidney toxicity and other side effects, natural and acquired resistance to cisplatin and the reduced therapeutic indexes that could be used considering toxicity limitations [22].

Cisplatin also presents little solubility in aqueous solutions and is therefore administered intravenously, another inconvenience to outpatient treatment. Newer platinum analogues are continuously emerging which are expanding the spectrum of activity of the original drug, or at least reducing the side effects and resistance. Over the past 35 years, pre-clinical screening of several thousand new molecules based on platinum complexes has resulted in the identification of around 28 compounds that have entered clinical development [15, 18, 20, 22-25]. Of these, seven (Figure 1.4.2) are currently approved in clinics. Cisplatin and carboplatin (all around the world, approved, 1978 and 1985 respectively) [18], oxaliplatin (few countries only, approved 1996) [18], lobaplatin (China, approved 2001) [18], nedaplatin (Japan, approved 1995) [18], heptaplatin(SKI2053, South Korea, approved 1999) [26, 27], all them with Pt(II) as metal centre and satraplatin (JM216, USA, approved 2007) [28]. This last one is the first platinum-containing anticancer agent expressly developed for oral administration with Pt(IV) approved in clinics. Cisplatin, carboplatin and oxaliplatin are highly effective metal-based anticancer agents used in 50 % of all tumour therapies all over the world [29].

Nevertheless, despite limitations in its medical application, the paramount importance of cisplatin came from the attention attracted to the study of metal-based drugs and the design of efficient metal-base therapeutics.

The employment of metals in the treatment of different diseases can be traced back almost 5000 years [30]. As far back as 3000 BC papyrus records from ancient Egypt reveal that copper was used to treat infections and sterilize water. Also, well documented is the use of gold in a variety of drugs by Arabians and Chinese, 3500 years ago. Various iron remedies were used in Egypt about 1500 BC, around the same time that zinc was discovered to promote the healing of wounds.

In the Renaissance era in Europe, mercury(I) chloride was used as a diuretic and the nutritional essentiality of iron was discovered. Paracelsus (1493-1541), considered by some researchers as the true father of modern metallotherapy, used alchemical mixtures of various heavy metals, such as iron, cadmium, mercury, arsenic and antimony to treat patients with different diseases, including even cancer. Almost three hundred years later, in 1865, Lissauer reported the treatment of two leukaemia patients with an arsenical formulation (Fowler's mixture). It is in the last 100 years, however, that the medicinal activity of inorganic compounds has slowly been developed in an analytic manner, probably starting in the early 1900s with K[Au(CN)₂] for
tuberculosis treatment, several antimony compounds for leishmaniasis, and the antibacterial activity of various gold salts [31].

In the past, inorganic compounds were applied in an empirical fashion with little attempt to design the compounds used and with little or no understanding of the molecular basis of their mechanism of action. The development of modern medicinal inorganic chemistry has been made easy by the inorganic chemist's extensive knowledge on coordination and redox properties of metal ions. Then, systematic consideration of specific properties of metal ions, their patterns of tissue uptake and distribution in organisms and their preferred coordination in complexes has opened up the possibility for inorganic chemists to contribute to the health and well-being of man.

An astounding number of metals occur naturally in biological systems and play, in fact, a crucial role in several biological processes without them life would not be possible. Metals as cations are favoured to bind to negatively charged biomolecules (electron rich) as the constituents of proteins and nucleic acids. For example, magnesium is found in chlorophyll (Figure 1.4.3), which is necessary for photosynthesis; both chlorophyll and iron-containing heme groups are found in the photosynthetic reaction centre. Cobalt is found in coenzyme B12, which is essential for the transfer of alkyl groups from one molecule to another in biological systems, as well as the reduction of the ribose ring in ribonucleotides that make up RNA to the deoxyribose ring in deoxyribonucleotides that make up DNA. Nickel is found in the coenzyme F430, which is required for methanogenesis, a process used by the archaeobacteria in which the simple gases, such as H2, CO, and CO2, are used to provide both energy and a carbon source. Iron is found in a variety of iron-sulphur clusters, which are necessary for electron transport and for nitrogen fixation, as well as in heme groups, found in haemoglobin, which is used for dioxygen transport and storage in the body.

Metals then perform a wide variety of tasks in the living systems such as assembly of hard structures (endo-or exoskeletons, membrane integrity and even molecular stabilizations as in case of DNA); charged carriers for very fast information transfer; formation, metabolism and degradation of organic compounds; the transfer of electrons and activation of molecules (catalysis) [32].

Inorganic or metal-containing medicinal compounds then may contain either chemical elements essential to life, or non essential/toxic elements that carry out specific medicinal purposes that could include diagnostic and therapeutic functions in the study or treatment of a wide variety of diseases and metabolic disorders [31, 33, 34]. For example, once recognized primarily as a toxic element, selenium is now incorporated in most multivitamin formulations and has known essential biochemical functions in selenoproteins and selenoenzymes in humans.

The pharmaceutical use of metal complexes therefore has excellent potential. Metals have also been introduced into biological systems to probe structure and function of those systems. For example, heavy metals such as mercury and platinum are employed to help to determine the
structure of macromolecules by X-ray crystallography and electron microscopy. Furthermore, metal-containing compounds are used to diagnose a variety of conditions.

![Chemical structures](image)

**Figure 1.4.3** Schematic representation of (a) Chlorophyll a, (b) Heme group on haemoglobin, c) coenzyme B12 and d) coenzyme F430 [35].

Sadler [36] pointed out that most of the elements of the periodic table up to and including bismuth (Z=83) are potentially useful in the design of new drugs and diagnostic agents and even though the radioactivity associated with elements of higher atomic number poses serious toxicity problems, they could be effective at low doses for diagnosis and therapy. More examples of metal compounds used in medicine are summarized in Table 1.4.1.

However, developing drugs with metals incorporated in the structure is not an easy task. It is necessary to determine which parts of the compound are essential for activity: the metal itself, the ligands, or the entire complex (metal plus at least some of the ligands). Many metallo-drugs are "prodrugs" as they undergo ligand substitution and/or redox reactions before they reach the target site. The exact amount of drug (dose) and the right metal-ligand combination are also important facts to be considered. In a metal-containing compound, the ligand is often, but not always, an organic compound that binds the metal ion(s) and modifies the physical and chemical properties of the ion. An important feature of inorganic drug design is how the ligand affects bioavailability, where bioavailability is the amount of a dose that is functionally usable by an organism. Also important to be considered is the accumulation of metal ions in the body because the accumulation can have toxic effects. Thus, biodistribution and clearance of the metal-based drugs, as well as its pharmacological specificity have to be considered.

Favourable physiological responses of the potential drug need to be demonstrated by *in vitro* studies with targeted biomolecules and tissues, as well as *in vivo* research with xenografts and animal models before they are acceptable to enter clinical trials. Further challenges in the field are to develop more efficient predictive methods for metal-based compounds of therapeutic interest.
Table 1.4.1 Some examples of inorganic elements and compounds with medicinal purposes [37].

<table>
<thead>
<tr>
<th>Element</th>
<th>Example of a Product Name</th>
<th>Active Compound in the Product</th>
<th>Medicinal Usage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li</td>
<td>Camcolit</td>
<td>Li₂CO₃</td>
<td>Manic depression</td>
</tr>
<tr>
<td>N</td>
<td>Laughing gas</td>
<td>N₂O (nitrous oxide)</td>
<td>Anaesthetic</td>
</tr>
<tr>
<td>F</td>
<td>Magnesia</td>
<td>MgO</td>
<td>Antacid, laxative</td>
</tr>
<tr>
<td>Fe</td>
<td>Fe(II)-fumarate or succinate</td>
<td></td>
<td>Dietary iron supplement</td>
</tr>
<tr>
<td>Co</td>
<td>Cobaltamin S</td>
<td>Coenzyme vitamin B₁₂</td>
<td>Dietary vitamin supplement</td>
</tr>
<tr>
<td>Zn</td>
<td>Calamine</td>
<td>ZnO and 0.5% of Fe₂O₃</td>
<td>Skin ointment</td>
</tr>
<tr>
<td>Zn</td>
<td>Zn undecanoate</td>
<td></td>
<td>Antifungal (athlete’s foot)</td>
</tr>
<tr>
<td>Br</td>
<td>NaBr</td>
<td></td>
<td>Sedative</td>
</tr>
<tr>
<td>Tc</td>
<td>Technescan PYP</td>
<td>⁹⁹mTc-pyrophosphate</td>
<td>Bone scanning</td>
</tr>
<tr>
<td>Sb</td>
<td>Triostam</td>
<td>NaSb⁺⁻ gluconate</td>
<td>Anti leishmanial (antiprotozoal)</td>
</tr>
<tr>
<td>I</td>
<td>Baridol</td>
<td>BaSO₄</td>
<td>Anti infective, disinfectant</td>
</tr>
<tr>
<td>Ba</td>
<td>De-Nol K₃</td>
<td>[Bi III (citrate)₂]</td>
<td>Anti acid, anti ulcer</td>
</tr>
</tbody>
</table>

Not a long time ago, Abrams and Murrer [38] considered the field of Medicinal inorganic chemistry as one having many important applications, but with still few principles keeping the field together. Now it is clear that multidisciplinary research is needed to define the main factors involved in the structure-activity relationship of all drugs that later will help in an increasingly purposeful design of new and more effective metal-based therapeutics.

The current development tendencies in the field of platinum anticancer compounds are focused on reducing the toxicity toward healthy cells and increasing the spectrum of activity of these complexes against a wide range of cancer types. The new tendencies are related to the incorporation of carrier groups with high specificity to target tumour cells. Also of interest is the chemical modification of the platinum-agents that interact with DNA in order to overcome resistance. It is also important to mention that the understanding of the chemical reactivity of cisplatin-like compounds is not enough. A better understanding of the cellular mechanisms of resistance to cisplatin has been obtained thanks to the preclinical laboratory-based investigations using different cancer cell lines. In particular, significant progress in the platinum anticancer field and in chemotherapy in general have been achieved through understanding the mechanism of DNA binding and the pharmacological effects triggered by cisplatin [39].

The search for an agent with increased anticancer activity, reduced side effects and lack of drug-resistance phenomena still remains an elusive goal; therefore several metal-based coordination compounds have been studied.

During the past decades since the discovery of cisplatin as an effective anticancer agent, much more work has been done in the field of antitumour-active metal complexes than before this time. Initially most efforts were concentrated on platinum as the central metal. Thousands of platinum complexes were synthesized for this reason and more than 1000 platinum compounds were investigated in preclinical tests for antitumour activity. A modest success was achieved with a few derivatives, though significant progress in platinum based anticancer agents has been achieved.

Nowadays a growing research interest is concentrated in the study of polynuclear platinum compounds [40-47], as well as the design of platinum compounds with bioactive ligands (acridine derivatives, doxorubicin, oestrogen analogues, aminoacids, sugars, etc). The successful approach in platinum antitumour drug design, where the metal centres are interconnected by bridging linkers, is based on the ability of such compounds to form DNA adducts with promising anticancer properties [47-49].

Preclinical and clinical investigations have shown that the development of new metal agents with modes of action different from cisplatin is possible. Thus, metal-based compounds with titanium, ruthenium and gallium have already been evaluated in phase I and phase II trials, while complexes with iron, cobalt, or gold have shown promising results in preclinical trials [50-52]. These non-platinum anticancer agents have been studied, since most direct derivatives of...
cisplatin were found not as active as the original compound and specific chemical reactivity has been pointed out as a main limitation in case of platinum derivatives.

Complexes containing ruthenium, titanium [53-55], gallium [56-64], and germanium [65-71] have entered the clinical trial stage [52] and some structures are depicted in Figure 1.4.4.

![Titanocene dichloride](Image)

![Budotitane](Image)

![Spirogermanium](Image)

![Galium nitrate](Image)

Figure 1.4.4 Schematic representations of some non-platinum anticancer agents having been in clinical trials.

Other transition metal complexes with 2 cis-oriented chloride ligands have been tested for antitumour activity. The palladium analogue cis-[Pd(NH₃)Cl₂] is inactive, probably due to the high kinetic lability of Pd(II) compared to Pt(II), as a result of which, isomerisation is facile. Later, complexes of more inert metal ions such as Rh(III and II) [72, 73], Ru(II) were synthesized and tested. The spectrum of investigated metals also comprises main group metals [74, 75], as bismuth and tin, and also transition metals [76-81] as vanadium, iron and cobalt, as well as cerium [82, 83]. In general terms, the studied agents have been classified as classical inorganic compounds, complexes with ionic/neutral organic ligands but also organometallic species are known [84].

As could be concluded, the design of new antitumour agents is one of the most active areas in medicinal chemistry. Nevertheless, the number of drugs for the treatment of this disease is still very limited.

Finally, the next stage in drugs design has to be the development of high-complex drugs that deal successfully with transport (though membranes), survival in the cell, binding to DNA and excretion mechanisms with minimum side effects where both metal coordination and hydrogen bonding more likely are the key factors.

The following two sections will be focus on relevant results obtained in the field of chemotherapy in the treatment of cancer with gold and ruthenium compounds

1.5. Gold compounds as potential anticancer therapeutics

The application of gold in medicine dates back to ancient times through reports of gold preparations used to treat a variety of ailments in Arabic and Chinese documents [75]. Most probably, the exceptional chemical and physical properties of gold should induce man to seek medicinal applications for it. The earliest medical use of gold can be traced to China around 2500 BC. In form of amulets and medallions, it was used to ward off disease and evil spirits. In many cases, brews containing gold powders were administered to patients [85].

In medieval times in Europe, alchemists learned to use aqua regia to dissolve gold and then gold compounds, as well as elemental gold were used in medicinal treatments as numerous recipes for an elixir known as aurum potabile were described, but their healing effects are uncertain [86].

A gold syrup could be found in the new pharmacopoeias of the 17th century and was advocated by Nicholas Culpepper for the treatment of ailments caused by a decrease in the vital spirit, such as melancholy, fainting, fevers and falling sickness. It was during this period that contradictory opinions about the medicinal properties of gold were discussed and its use was in slight decline. The medicinal use of gold, that was extensive since its introduction by the alchemists, dropped to almost nothing during the 18th century. Later in the 19th century a mixture of gold chloride and sodium chloride, “muriate of gold and soda” Na[AuCl₄] was used to treat syphilis [85, 87] in the reasoning that it may have an action similar to that of mercury. Leslie I.
Keeley, an American physician designed gold’s cure for alcoholism, one of the greatest use of medicinal gold in history since around 100000 patients were treated.

Use of gold compounds in the 20th century started after 1890 with the discovery, by the German bacteriologist, Robert Koch, of the bacteriostatic properties of potassium dicyanidoaurate, K[Au(CN)2], towards tubercle bacillus. Tuberculosis treatment with gold therapy was subsequently introduced in the 1920s although later controlled clinical trials demonstrated its inefficacy [88].

As the symptoms of rheumatoid arthritis and tuberculosis are similar, a hypothesis established in those days pointed out that the tubercle bacillus was responsible of rheumatoid arthritis, so the application of this gold compound in the treatment of rheumatoid arthritis was deeply studied by Jacques Forestier [89-94]. Later, this K[Au(CN)2] was switched to the less toxic “gold thiolates”. After a thirty-year debate a clinical study sponsored by the Empire Rheumatism Council confirmed the effectiveness of gold compounds against rheumatoid arthritis [95-98]. Also Sigler et al. [99] reported that gold decreased the rate of disease progression [100-103]. Since that time gold drugs have also been tested to treat a variety of other rheumatic diseases [87], including psoriatic arthritis, juvenile arthritis, palindromic rheumatism and discoid lupus erythematosus [104-106] and various inflammatory skin disorders such as pemphigus, urticaria and psoriasis [107-109].

The radioactive gold-198 was also used during the last century in the treatment of malignancies. Nowadays, other radioisotopes, notably iridium-125 and iodine-125 have replaced gold-198 colloid as a neoplastic suppressant [110, 111].

Chrysotherapy, treatment that uses gold based drugs (from Greek word for gold, *chrysos*) is now an accepted part of modern medicine [75, 86, 87, 112]. The term was first popularized when gold salts, usually gold thiolates, were used for the treatment of rheumatoid arthritis but nowadays it is considered as the use of gold salts to treat medical conditions, especially rheumatoid arthritis.

Of the many gold thiolates used for the treatment of rheumatoid arthritis, two remain in active clinical use in the United States: gold sodium thiomalate and gold thioglucose, sold under the trade names Myochrysine or Aurolate and Solganol. In Europe, sodium bis(thiosulfate)gold(I) (Sanochrysine®) and sodium thiopropanolsulfonate-S-gold(I) (Allochrysine® or Aurothioprol®) are also used for treatment in humans. The only new compound introduced into clinical use in the last 30 years has been auranofin(Ridaura®), triethylphosphine(2,3,4,6-tetra-O-acetyl—1-D-thiopyranosato-S)gold(I) (approved on 1985, USA) [86, 112, 113]. All the schematic representations are shown in Figure 1.5.1.

The antiarthritic Au(I) thiolate complexes are formulated as approximate [1:1] complexes, but their structures in solution are complicated [114]. Au(I) must be at least two-coordinate and thiolate sulfur acts as a bridge between Au(I) ions: -S-Au-S-Au-. Chains and cyclic structures are possible but X-ray evidence suggested a double helical geometry in the solid state [114, 115].

In general terms these chemical compounds could be classified in two classes [112], the Au(I) thiolates and phosphanegold(I) thiolates. The first class is formed by polymeric, charged and water soluble molecules. By contrast, the second class of compounds comprises monomeric, neutral and lipophilic species. With the exception of Sanochrysine®, the precise molecular structures of the class-I drugs are not known, but the gold atoms in these complexes exist in linear coordination geometries defined by two sulphur atoms. Examples of class-I are sodium aurothiomalate, sodium aurothioglucose, sodium thiopropanolsulfonate-S-gold(I) and sodium bis(thiosulfate)gold(I). Auranofin, the only member of the class-II drugs, has some advantages over previous gold drugs, the most important, it can be taken orally [86, 115]. With this administration form, the serum gold levels are reduced and maintained for longer, so less retention of gold in the tissues is observed and therefore, renal toxicity is significantly reduced. These advantages are, however, diminished by the reduction in its therapeutic efficiency, when compared with the earlier oligomeric gold(I) thiolates.

The compounds used in the treatment of rheumatoid diseases are the major clinical use for gold compounds to date and there have been no main changes in this field since the introduction of auranofin in 1985. Undoubtedly some of the most interesting advances in the understanding of the chemical reactivity and pharmacology of gold drugs have emerged from studies of their mechanism of action and further applications were hypothesized from this knowledge.
During 1970 and 1980, gold drugs were the standard of care in treating moderate to severe active rheumatoid arthritis. As newer treatments have become available with superior benefit and less risk, gold drugs are less prescribed and that is why companies have stopped making the medication [116]. In patients with inflammatory arthritis, such as adult and juvenile rheumatoid arthritis, gold salts can decrease the inflammation of the joint lining. This effect can prevent destruction of bone and cartilage [117-121]. Gold salts are called second-line drugs because they are often considered when the arthritis progresses in spite of anti-inflammatory drugs (NSAIDs and corticosteroids). Head to head comparisons between the treatment with gold and methotrexate (preferential treatment in rheumatoid arthritis) demonstrated no significant difference but some advantages for gold [122]. Gold treatment is significantly less often discontinued for lack or loss of efficacy than methotrexate in controlled clinical trials [123] and induce the most long-lasting remissions [124-129] with improvement in the functional capacity and quality of life [125, 130-135]. The increasing knowledge in the rheumatoid arthritis treatment accumulated in the last 75 years, the changes in the methodologies of clinical trials during the last decade [136] and the growing knowledge of the pathophysiology of rheumatoid inflammation demand further comparison studies for establishing the most efficient treatment.

Exactly how gold salts work is not well understood. The cellular and molecular evidence recovered in the last decades could not be more complicated; Gold may have inhibitory as well as activating effects on different cell functions which means that several mechanisms of action are possible [104, 122, 137]. There is some evidence underlying that gold drugs are in fact “pro-drugs” which upon administration in the patient, metabolize with bond cleavage because ligand substitution reactions are relatively facile on Au(I) [86, 104, 109, 138]. Au(I) compounds have low activation energies and proceed via three-coordinate intermediates [86, 139]. Thiol exchange reactions are important in vivo. The initial ligands in the gold drugs are displaced (substitution of thiols and/or displacement and oxidation of PEt3 to OPEt3 in case of auranofin). In the blood, most of the Au(I) is carried by the thiol in cysteine-34 of albumin. Gold concentrations in blood can rise to about 20-40 μM after injection of gold drugs [86, 140]. The half-life for gold excretion is about 5-31 days, but gold may also remain in the body for many years [109, 122, 138]. A major deposit site is in lysosomes (aurosomes) [141-143], the membrane-bound intracellular compartments that house destructive enzymes. The inhibition of enzymes that destroy joint tissue may be the key function of the antiarthritic activity of gold, although the cause of rheumatoid arthritis itself is unknown. Patients who smoke and are treated with gold drugs, attain much higher concentrations.
of gold in their red blood cells than non-smokers. Inhaled tobacco smoke contains up to 1700 ppm HCN, and gold has a very high affinity for cyanide (log \( \beta \) 36.6). Cyanide reacts with the administered gold drug to form \([Au(CN)_{2}]^–\), which readily passes through cell membranes. Traces of cyanide appear to be present naturally in the body (formed from SCN\(^–\)), and \([Au(CN)_{2}]^–\) is a metabolite of gold drugs even in patients who are not smokers, reaching levels of 5-560 nM in their urine [86, 144].

The newest hypothesis in the gold drugs mechanism of action comprises the immunology factor involved in the rheumatoid arthritis processes. Class II major histocompatibility complex (MHC) proteins are essential for the normal immune system function but also drive many autoimmune responses. They bind peptide antigens in endosomes and present them on the cell surface for recognition by CD4(+) T cells [145]. Presentation of these molecules alerts other specialized recognition cells of the immune system called lymphocytes, which starts the normal immune response. Usually this response is limited to harmful bacteria and viruses, but sometimes this process goes awry and the immune system turns towards the body itself causing autoimmune diseases such as juvenile diabetes, lupus, and rheumatoid arthritis. In cell culture experiments, Dedecker et al. [146], have proved that gold compounds strip peptides from human class II MHC proteins by an allosteric mechanism [147]. Biochemical experiments indicate the metal-bound MHC protein adopts a 'peptide-empty' conformation that resembles the transition state of peptide loading [148]. Furthermore, this metal inhibitor (and other noble metals like Pt) blocks the ability of antigen-presenting cells to activate T cells. This unknown allosteric mechanism may help resolve how gold(I) drugs affect the progress of rheumatoid arthritis and may provide a basis for developing a new class of anti-autoimmune drugs. Further research is needed where the mechanism of gold drugs action has to be tested and explored directly in diseased tissues.

Independently of the mechanism of action, the potential benefits of using gold-based drugs in patients with inflammatory diseases, rheumatoid arthritis and other autoimmune diseases should be weighed against the potential risks of gold toxicity on organ systems and the difficulty in quickly detecting and correctly attributing the toxic effects. Absolute identification of patients at risk of having side effect is not possible, but dosage reduction and intense monitoring of laboratory and clinical signs may prevent its occurrence. In fact gold's most adverse events affect the skin and mucous membranes predominantly. They are harmless and occur most often during blinded clinical trials which have no possibilities of further adjustment of the dose [122].

The formation of Au(III) may be responsible for some of the toxic side-effects of gold drugs. Although most of the gold \textit{in vivo} is present as Au(I), powerful oxidants such as hypochlorous acid (HClO), which can oxidise Au(I) to Au(III), are generated at sites of inflammation, so white blood cells from patients treated with gold drugs become sensitive to Au(III). Further understanding of the redox cycling of gold may lead to a better understanding of these side-effects.

Besides their established use to treat arthritis gold complexes exhibiting anticancer potency have evolved.

Gold can exist in a number of oxidation states: \(-I, 0, I, II, III, IV\) and \(V\), but only gold \(0, I\) and \(III\) are stable in aqueous systems, and, therefore, in biological environments. In contrast, the oxidation states \(-I, II, IV\) and \(V\) are less common. Stability of the \(-I\) and \(V\) states in water is improbable, given their redox properties, which suggest that they will not play important roles in biological systems. Both gold\(I\) and gold\(III\) are unstable with respect to gold\(0\) and are readily reduced by mild reducing agents. Gold\(I\) is thermodynamically more stable than gold\(III\). Many gold\(III\) complexes are strong oxidizing agents, being reduced to Au(I), and this means that they could be toxic [112].

While gold\(III\) is usually regarded as oxidizing and the body reducing, the appropriate choice of ligand donor set can stabilize the higher oxidation state of gold, controlling then, the relatively instability, light sensibility and reduction to metallic gold under physiological conditions. Thus, increasingly, gold\(III\) complexes have been evaluated for their potential antitumour activity.

The design and testing of gold complexes for antitumour activity over the past several decades has been based on four underlying principles [86]: 1) analogies between square planar complexes of Pt\(II\) and Au\(III\), both of which are d\(^6\) ions, this means isoelectronic and isostructural to platinum\(II\); 2) analogy to the immunomodulatory effects of gold\(I\) antiarthritic agents; 3) coordination of gold\(I\) and gold\(III\) with known antitumour agents to form new
compounds with enhanced activity and 4) some gold(III) complexes present good enough stability in physiological environments.

The discovery that auranofin(Au(I)) has activity against HeLa cells in vitro and P388 leukaemia cells in vivo led to the screening of many auranofin analogues, but the spectrum of activity was found limited [75]. Chlorido(triethylphosphane)gold(I) (Figure 1.5.2) showed potent cytotoxic activity in vitro, but less antitumour activity in vivo compared with auranofin [149].

More promising results were achieved with a series of gold phosphane complexes. The lead compound was [(AuCl)2dppe] [150-154] (dppe, bis(diphenylphosphane)ethane) (Figure 1.5.2). The dppe ligand exhibits antitumour activity by itself and it was suggested that gold serves to protect the ligand from oxidation and aids in the delivery of the active species. The observation that gold drugs are indeed pro-drugs, lead to the consideration that gold can be used as a platform to deliver anticancer agents into tumours, as the coordination of drugs will alter the normal metabolic pathways and release mechanism, leading in favourable cases, to greater efficacy.

A particularly interesting behaviour was the rearrangement (in solution and biological media) observed on some of the diphosphane compounds to produce a rare coordination geometry for gold(I) based on a tetrahedral arrangement [155] of four phosphorous donor atoms around gold as illustrated on Figure 1.5.2 and Figure 1.5.3.

Mechanistic studies suggest that, in contrast to cisplatin, DNA is not the primary target of these complexes. Rather, the cytotoxicity is mediated by their ability to slow down mitochondrial function [156] and inhibit protein synthesis. Bis(diphosphane)gold(I) complexes, in general, are active against various types of cancer and kill cells via damage to mitochondria. Heart toxicity [157, 158] highlighted during pre-clinical studies, has so far prevented their clinical use, but it may be possible to circumvent this problem by a careful selection of substituents on the phosphane moiety and by tuning the lipophilicity of the cation, approximations running nowadays.

The coordination of gold by phosphane ligands with the three different phosphorus-bond substituents, leading to chiral phosphorous coordination compounds, has also attracted attention. It was reported that potency is increased with an increasing number of coordinated phosphorus atoms but higher potency related to chirality was not observed [75, 86, 159].

Figure 1.5.2 Schematic representation of cytotoxic gold(I) compounds.
Given the fact that the clinically used gold compounds are pro-drugs, as mentioned above, a logical extension was to couple phosphanegold(I) species to biologically active thiols. Thiols, such as 6-mercaptopurine [160] and 6-thioguanine [161] (Figure 1.5.2) with well-known anticancer activity against human leukaemia were tested. The results lead to the conclusion that the presence of the phosphanegold(I) entity enhances the potency of the biological active free thiols. Several findings arose from these studies and more are to be expected as the research is ongoing [75].

Gold(I) drugs have proved effectiveness in many diseases but even after 70 years of clinical use, only a small improvement in the knowledge related to the mechanism of action has been achieved. This lack of knowledge in part is a result of the wide dispersion of gold compounds in the body and the absence of effective high-affinity target sites of action. Over the years several hypotheses have been formulated. Two of them have received considerable attention. One proposal describes the formation of [Au(CN)₂]⁻ species (dicyanidoaurate(I) species, aurocyanide species), which targets certain immune cells involved in the inflammatory response [162]. The generation of gold(III) under in vivo conditions comprises the second proposal that attracted attention [154].

The transformations of gold complexes in biological systems, especially mammals, have been delineated and some metabolites identified and studied. Further research in these transformations, metabolites and their ability to affect biological processes is strongly needed in order to test the gold(III) and aurocyanide hypothesis.

Even though the exact mechanism of gold(I)-derivatives-cytotoxicity is unclear, several lines of evidence stress the involvement of mitochondria, where the elements involved in the oxidative phosphorylation, could be the primary intracellular targets.

Additionally, in vivo as well as in vitro studies indicate that gold binds to lymphocyte membranes and accumulates within lymphocytic cells, altering their normal functions. From this evidence, and considering that certain tumours elicit an immune (B lymphocyte) response, in which, the antibodies cover or block tumour determinants that would otherwise, be attacked by killer or cytotoxic T lymphocytes, another hypothesis of the biological activity of gold compounds states that, suppression of the B lymphocyte function by gold compounds could prevent the formation of this blocking antibodies that protect tumours and thereby facilitate the tumour destruction by T cells [109].

Whereas the majority of gold(I) compounds described above feature gold in a coordination geometry defined by soft (easily polarisable) sulphur and/or phosphorus atoms, gold(III) compounds generally feature hard atom donors such as nitrogen, oxygen and carbon. Four-coordinated gold(III) is found in square-planar geometries and in this regard resembles the geometry found for cisplatin.

The cytotoxic/antitumour screening of gold(III) compounds formally started in the mid-1970s. Just until mid-1990s a growing interest has been evident as judged from the number of recent publications on the subject. In some cases important systemic toxic effects, produced by
gold(III) complexes have been reported and significant differences in the spectrum of action have been noticed compared to cisplatin.

Several dimethylgold(III)-based complexes examined shortly after the discovery of the antitumour potential of cisplatin showed only modest activity [86, 163].

As in the case of gold(I), several Au(III) coordination compounds incorporating biologically active molecules have been synthesized and their cytotoxic activity has been tested. Streptonigrin [164], uracil [165], glycylylhistidine [166](Figure 1.5.4, d) derivatives among others were studied. Moderate cytotoxic activity was observed.

\[
\begin{align*}
\text{Figure 1.5.4} & \quad \text{Schematic representation of cytotoxic gold(III) compounds and cationic species.}
\end{align*}
\]

Gold(III) compounds with imine donors were also prepared and biologically tested. Some of the most interesting cytotoxic effects were reported for the complexes schematically described on Figure 1.5.4. Most of them contain ligand donors derived from one element or the combination of chloride, nitrogen, oxygen and carbon. Several gold(III) complexes have shown a high cytotoxic activity [167] (Figure 1.5.4, a, b, c). In some cases resistance to cisplatin was clearly overcome.

One approach followed was the synthesis of complexes with the mononegative bidentate ligand, damp, (2-[(dimethylamino)methyl]phenyl), and two monodentate anionic ligands, chloride, Cl or acetate, O\textsubscript{2}CCH\textsubscript{3}. The damp ligand forms part of a five-membered chelate ring in which the nitrogen of the amine group and the carbon of the aryl ring bind to the metal (Figure 1.5.4, l, m). The monodentate ligands are readily hydrolyzed and are available for substitution. These gold(III) complexes have been evaluated against an \textit{in vitro} panel of human tumour cell lines comprising cells of different tissue types and different responses to cisplatin. Initial \textit{in vitro} studies indicated that the breast carcinoma cell line ZR-75-1 is sensitive to the compounds [87, 168, 169]. Further
tests in vivo against a xenograft of the same tumour cells demonstrate modest antitumour activity. Analogues of this complex have been evaluated in the same way [168].

Also in vitro cytotoxicity studies showed promising activity of two Au(III) complexes with bipyridyl-related ligands, [Au(bpy)(OH)2]PF6 (bpy=2,2’-dipyridyl) and [Au(bpy2-1H)(OH)]PF6 (bpy2=6-(1,1-dimethylbenzyl)-2,2’-bipyridine) [170] (Figure 1.5.4, j, k). Low cisplatin cross-resistance was observed. Both complexes are quite stable under physiological conditions, with [Au(bpy-H)(OH)]PF6 being resistant to sodium ascorbate reduction.

Cases to be underlined are [Au(phen)Cl2]Cl (phen=1,10-phenanthroline) (Figure 1.5.4, g) and [Au(tpy)Cl]Cl2 (tpy=2,2’:6’,2”-terpyridine))(Figure 1.5.4, i). These compounds exhibit a profile of cytotoxicity similar to that of cisplatin on the sensitive line, although the concentrations that are needed to achieve the same effect are three times higher. Surprisingly, [Au(tpy)Cl]Cl2 largely overcomes resistance to cisplatin as it is at least three times more effective than cisplatin itself on the resistant line [171].

Analysis of the cytotoxicity data permits formulation of some preliminary structure/function relationships that are summarized below [172]:

a) the cytotoxicity of these gold(III) complexes is strictly related to the presence of the gold(III) centre ( [Au(en)2]3+ (en=ethylenediamine) (Figure 1.5.4, e) and [Au(dien)Cl]2+ (dien=diethylenetriamine) (Figure 1.5.4, f) are significantly more cytotoxic than the corresponding platinum compounds)

b) the presence of hydrolysable chloride atoms in the gold(III) centre or, in general, of good leaving groups, does not represent an essential requirement for cytotoxicity

c) excessive stabilization of the gold(III) centre results in loss of biological activity as was already observed in case of [Au(cyclam)]3+ (cyclam=1, 4, 8, 11-tetraazacyclotetradecane) (Figure 1.5.4, h)

d) the amount of gold(III) that enters in the cells is roughly proportional to the exposure time, at least during the first hours

Many of the gold(III) compounds are able to overcome to a large extent resistance to cisplatin, suggesting that a different mechanism of action is taking part. In addition, the studies suggest that the in vitro interactions of gold(III) complexes with calf thymus DNA are weak, and to produce only modest modifications of the double helix, whereas significant binding to model proteins takes place, confirming that different mechanisms compared to cisplatin [167, 173, 174] could occur. Preliminary pharmacological investigation of some representative gold(III) complexes have been extended to their effect on the cell cycle and to reveal induction of apoptosis [175, 176]. By flow cytometry, even though not significant modifications of the cell cycle phases were observed after 48 h-incubation of the gold compounds and the cells, a relative intense sub-G1 peak appeared, which represents cells undergoing apoptosis [176].

Based on newly obtained experimental evidence, it is tempting to propose that gold(III) compounds may exert their cytotoxic effects by causing direct mitochondrial damage through modification of specific proteins. It has been observed that a few gold(III) complexes are tight inhibitors of the selenoenzyme thioredoxin reductase (TxR), a crucial enzyme in the cells for protection against the oxidative stress damages and then causing big perturbation in the mitochondrial functions [176-178]. This hypothesis is further reinforced by the observation that antiarthritic gold(I) compounds, like auranofin, have shown to induce apoptosis via the selective and potent inhibition of the mitochondrial isoform of thioredoxin reductase. In fact, Gold(I) compounds are among the most effective known inhibitors of this protein.

It is clear, after all this evidence, that gold(III) complexes represent an interesting family of cytotoxic agents due to the peculiar chemical and biological properties and further research has to be developed.

1.6. Ruthenium compounds as potential anticancer therapeutics

For similar reasons that the chemistry of gold compounds attracted the attention of researchers, ruthenium complexes were studied in hopes of discovering new drugs with improved antitumour properties, reduced side effects, lack of cross resistance (inhered or acquired) and if possible better physical and pharmacological properties like solubility and stability [179]. Active compounds with metals other than platinum offer the possibility to have mechanisms of action,
Chapter 1

biodistribution, and toxicity different from platinum drugs and might be therefore active against human malignancies that are resistant to them and luckily with reduced toxic effects.

Ruthenium has proved to be one of the most promising among all the investigated metals [78, 180-185]. Over the past 5 decades a number of attempts have been made to develop ruthenium-containing pharmaceuticals. Most of these agents, independently of the ligand composition, have shown encouraging biological properties and some were found to be less toxic than cisplatin, allowing higher therapeutic doses. Especially in recent years the main efforts have been directed to the search of ruthenium complexes with ligands of biological interest for the treatment of cancer.

Ruthenium has been subject of study due to its unique chemistry among the platinum metals [186, 187]. Ruthenium complexes present relative low rate of ligand exchange which is comparable to Pt(II) and Pt(IV), good stability of several oxidation states, pronounced tendency to form multinuclear complexes and ability to mimic iron in binding to biological molecules like transferrin [188].

Ruthenium occurs in aqueous solution predominantly as Ru(II), Ru(III) or Ru(IV). Ru(III) and Ru(II) are almost invariably six coordinate with octahedral geometry and are generally inert to substitution when bond to nitrogen bases (with half-lives that may be as long as months to years at low temperature) [189]. Ru(III) complexes present less reactivity than related Ru(II) and Ru(IV) complexes. For instance, the loss of ammines and heterocyclic nitrogen bases from [Ru(II)L(NH3)5] (where L=NH3 or heterocyclic nitrogen) is somewhat faster, but still proceeds fairly slow with half-lives on the order of a day under physiological conditions. It is well known that ligand exchange is an important determinant of biological activity, as very few metal drugs reach the biological target without being chemically modified. Metal interaction with macromolecules (nucleic acids, proteins), small sulphur- or oxygen-donor compounds (glutathione, ascorbate, for example) and/or water could take part in the cells under physiological conditions. Some of these interactions are essential for inducing the therapeutic effect.

In fact, the redox potential of a coordination compound is modified by ligand exchange. In physiological conditions, glutathione, ascorbate and single-electron transfer proteins can reduce Ru(III) and Ru(IV), while molecular oxygen and cytochrome oxidase can oxidize Ru(II). Then, the redox potential of ruthenium compounds could be an effective tool in a search for biological activity. In the case of Ru(II) complexes, the reduction potentials vary with the ligands present. In general terms, anionic, $\sigma$-donor ligands lower the reduction potential, while neutral or cationic, $\pi$-acceptor ligands raise it. In the case of the ammineruthenium(II) ions, ligand substitution is controlled by the rate of water exchange, which occurs with a half-life of about 0.1s.

Ruthenium(IV) compounds generally require oxide or sulphide ligands for stabilization [190, 191].

Due to the transcendental importance related with the mechanism of biological activity, several studies have been focussed in the interaction between the active ruthenium complexes and their likely biological targets (DNA, RNA, proteins, mainly transferrin and albumin but also, cytochrome c and other specific receptors) [51, 192-198]. Even interactions of ruthenium compounds with the mitochondrion and the cell surface have been a focus of study [199].

From a chemical point of view ruthenium compounds offer a promising approach to the development of new anticancer agents, as they interact with DNA at the same initial sites (N7-guanine) as platinum [198, 200-204], but they are more likely to undergo redox chemistry in vivo. Ru(III)-DNA interaction, if occurs, is weaker than the interaction between platinum and DNA. This subtle difference produces significantly smaller structural and conformational modifications in the DNA double helix once ruthenium is attached.

The importance of this interaction, when talking about the anticancer properties, is now under critical study, due to several observed discrepancies. It is generally accepted that the cytotoxicity of Ru(III)/Ru(II) complexes is related to their ability to bind DNA [194, 205], although some exceptions have been reported [30]. It has been also proved that some Ru compounds are able to inhibit DNA replication, produce mutagenic effects, induce SOS repair, bind to DNA and reduce RNA synthesis, all of them suggesting that a DNA interaction is taking part. Detailed research still has to be developed, but valuable evidence has been obtained through the study of novel Ru compounds and their interaction with DNA, which in time will help to produce more effective Ru anticancer drugs.
Several ruthenium complexes induce DNA strand cleavage, probably through Fenton chemistry. Purine oxidation and hydrolysis reactions of N(7)-bound \([\text{Ru}^{III}(\text{NH}_3)_5]\)-nucleoside complexes occur simultaneously in the mid-pH range [198]. It should be mentioned that Barton has shown that Ru(II) complexes with large, bidentate aromatic ligands intercalate into DNA to a degree dependent on their chirality and that of the nucleic acid [206, 207]. Enantiomers of [Ru \((\text{dip})_3\)]^{2+} (dip=4,7-diphenylphenanthroline) distinguish between right- and left-handed DNA helices, with the D enantiomer preferentially binding to right-handed B-DNA. While both enantiomers bind equally to left-handed Z-DNA, space-filling models suggest that a left-handed DNA with a tighter helix should preferentially bind the \(\Lambda\) enantiomer [199, 204]. Migration of Ru between DNA sites was also observed [189, 208], a behaviour which is pH-dependent. Also important to be mentioned is that coordination of [Ru^{III}(\text{NH}_3)_5\text{Cl}]^{2+} to DNA is facilitated by glutathione (GSH) reduction to the more substitution-labile [Ru^{II}(\text{H}_2\text{O})(\text{NH}_3)_5]^{2+} at \([\text{GSH}] / [\text{Ru}^{III}] \leq 1\) concentrations. However at ratios \([\text{GSH}] / [\text{Ru}^{III}] \geq 1\) DNA binding is inhibited by GSH, which coordinates the Ru II and facilitates its oxidation back to Ru^{III}.

On the other hand, other important biological targets for Ru have been studied. Under physiological conditions, antitumour Ru(III) complexes bind tightly plasma proteins (albumin and transferrin) [209], with a preferred tendency of coordination to imidazole groups (from the essential amino acid histidine); thus very likely, protein binding of ruthenium(III) complexes has a large impact in pharmacodynamics and pharmacokinetics of these experimental drugs. Since Ru is immediately below Fe in the periodic table and has a high affinity for phenolate ligands, which are involved in the transferrin Fe-binding site, it is not surprising that Ru also has a high affinity for this plasma protein. Ruthenium’s ability to mimic iron in binding transferrin and albumin has been described as the reason for the reduced toxicity observed in some ruthenium-based anticancer drugs These two proteins, albumin and transferrin are used by mammals to transport iron. Since rapidly dividing cells, for example microbial-infected cells or cancer cells, have a greater requirement for iron, these cells increase the number of transferrin receptors located on their surfaces, thereby sequestering more of the circulating metal-loaded transferrin. Tissue distribution studies [210-212] of several \(^{103}\text{Ru}\) and \(^{97}\text{Ru}\)-labelled complexes indicate a fair degree of tumour localization, which has been attributed to two, perhaps not unrelated, physiological mechanisms [179, 213-215]. While tumour localization is not a prerequisite for chemotherapeutic activity, in many cases it is desirable in order to increase the specificity of the drug. In vivo, the exact increase in radio-labeled ruthenium compounds in cancer cells, compared to healthy cells, has been shown to range from 2-12 fold, depending on the cell type. As the drug is targeted to cancer cells, its overall toxicity is reduced, because less of it will reach healthy cells.

It has been shown that the major fraction of Ru(III) species (80-90%) is bound to albumin and a much smaller amount to transferrin [216]. Even though the Ru compounds are attached in a major proportion to albumin, it is believed that transferring uptake is the most important mode of transport to the tumour. It has been proposed that cell surface transferrin receptors bind ruthenium-loaded transferrin with high affinity; the transferrin–receptors complexes are subsequently endocytosed and transferred to acidic non-lysosomal compartments where ruthenium is released [217]. Binding of Ru(III) species has a strong impact on albumin structure and influences considerably its binding of other molecules including drugs. The preferred binding sites for the Ru(III) complexes are at histidine residues of the protein, presumably following the loss of one or more of the chloride ligands [216].

Some mechanistic proposals have been briefly described to explain the antitumour activity of Ru drugs with the experimental evidence available. In particular, it is believed that Ru(III) complexes remain oxidatively intact (inactive and unreactive) in the body, until they reach the tumour site, where the reducing environment and acidic pH allow reduction to the more reactive Ru(II) core. That is why the selective toxicity of Ru(III) drugs takes part. This binding capacity provides a possibility to target Ru(III) complexes to tumours with high transferrin receptor densities. However, to be active in vivo, the complexes must have a biologically accessible reduction potential, which depends on the ligands present [51, 218].

It is also conceivable that some of the anticancer activity of Ru involves depleting Fe from cells and proteins [51, 188].

Another likely biological target for Ru drugs is cytochrome c. Cytochrome c is a mitochondrial peripheral membrane protein functioning in the respiratory chain in the inner
mitochondrial membrane, shutting electrons from cytochrome c reductase to cytochrome c oxidase. It was found that cytochrome c, when released from mitochondria to the cytosol, activates apoptosis [219] which is a mechanism altered in cancer cells. It is known that at pH=7 a stable complex between Ru(III) and histidine-33 in ferricytochrome c is formed [47]. Then, the Ru-complex binding to cytochrome c may change considerably the structure of the protein and affects its biological functions.

Perhaps, other important protein transport systems could be affected by interaction with Ru compounds. It was observed that calcium levels inside mitochondria are altered after interaction of mononuclear and polynuclear Ru complexes to the calcium ion carrier [220].

It is clear that the interactions taking part between cells and Ru compounds are not by far simple. Although many research groups have been working in the study of the biological activity of ruthenium-based antitumour drugs, a conclusive mode of action has not been found yet.

Since the early work of Rosenberg and other laboratories, the determination of structure-activity relationships (SAR) by systematic research proved to be problematic. This topic is still a big challenge, because such structure activity relationships depend on several factors, to be mentioned are: the kind of screening, methodology (sites of administration of tumour and compound, dose schedule, number of tumour cells, etc) and the activity criteria employed; i.e., different screens and/or screening labs, can rank the same group of compounds differently [179].

Also, biological reasons could be mentioned as the state of knowledge of the aetiology of cancer, the limitation of the available techniques in the study of the cell biology, the influence of screening methodology on outcome, the highly variable response of different screens of the chemical structure of a compound. But all of them are affected entirely by chemical problems, some of which are unique in metal compounds [179]. There is at least some indication that the antitumour activity is not limited to compounds that contain the established SAR’s. The rational design of new structures obviously will be greatly aided by an improved understanding of the tumour cell biology, the development of more predictive screens and a more detailed knowledge of the mechanism of action of cisplatin and others.

Many ruthenium complexes with oxidation state of 2+ or 3+ display antitumour activity, especially against metastatic cancers for which cisplatin and platinum derivatives are not active. Some of these compounds are under intensive preclinical and clinical investigation [221].

Probably, the first Ru compounds described in the literature as promising anticancer compounds were the family of ammineruthenium(III) and amineruthenium(III) complexes of which some structural formulas are depicted in figure 1.6.1. It was suggested [222] that am(m)ineruthenium(III) complexes could behave in a similar way than cisplatin (cis-[Pt(NH₃)₂Cl₂]), in particular, in the mode of binding to helical DNA and with the additional quality of preferential toxicity to cancer tissue [223]. They showed particularly good activity against murine-P388 leukaemia and HeLa (cervical human cancer model) [224]. In addition, several studies of the interaction between this family of compounds and biological important proteins were described in literature. Further development of these molecules was stopped due to their poor solubility in water [224].

Figure 1.6.1 Schematic representation of am(m)ine ruthenium(III) agents with known cytotoxic properties.
A trinuclear μ-oxido bridged mixed-valence compound, ruthenium red ([(NH₃)₅RuIII-O-RuIV(NH₃)₄-O-RuIII(NH₃)₅]₆⁺) [225, 226], which certainly affects calcium metabolism [220, 227-230] also was shown to inhibit tumour growth [231, 232] and DNA synthesis [233, 234]. Later a common dinuclear (μ-O-[Ru(NH₃)₄(HCOO)]²⁺) impurity present in commercial ruthenium red, was described as responsible for most of the calcium transport inhibition in mitochondria [228, 235].

Another important family of compounds with cytotoxic properties involving cis and trans isomers of ruthenium(II) with general formula, [Ru(II)(dmso)₄X₂] (where X=Cl or Br and dmso=dimethyl sulfoxide), showed potential clinical application (Figure 1.6.2) [236, 237]. While just mildly active, the reduced toxicity and better inhibition in metastases encouraged further research. The sulfinyl group is an excellent acceptor site for the π-electrons from Ru(II) [238, 239].

Initially the low blastogenic activity of [Ru(dmso)₄Cl₂] and [Ru(dmso)₄(phen)]Cl₂ [240] and intense growth inhibition of E. coli [241, 242] comparable to the one developed by cisplatin, and even greater toxicity for DNA damage repair-deficient strains of E. coli [243] indicated good expectations on this family. The study of their effects on primary tumour and on metastasis has revealed antimetastatic activities superior to effects on primary tumour. The original studies were developed with cis-[Ru(II)(dmso)₄Cl₂] because its similarities with cisplatin. However comparisons of the antitumour effects between cis and trans derivatives have revealed enhanced activity for the last one [244, 245]. The trans isomer of [Ru(II)(dmso)₄X₂] strongly inhibits metastases of Lewis lung carcinoma (a 20 times higher effect than the cis isomer).

In aqueous solution, the cis complex immediately loses the only O-bound dmso ligand, while the trans isomer quickly loses two S-bonded cis-dmso ligands, yielding in both cases aqua species. Both hydrolyzed isomers later undergo slow reversible chloride dissociation forming cationic compounds. At this point it is suggested that the trans compound possesses three reactive groups while the cis isomer has just two. This fact and also the steric effect developed by the three dmso ligands still coordinated to the cis isomer could be correlated with the higher effect developed by the trans compound. Both cis and trans compounds bind to DNA in cell-free media [246, 247]. The trans isomer binds to DNA much more rapidly and to a higher degree than the cis isomer. The conformation changes observed once coordinated to DNA are subtle in case of the cis complex, but the trans isomer significantly alters the conformation of B-DNA [237]. The preferred site for binding in case of both isomers appears to be the N7 of guanine with a possible involvement by the phosphate. The binding mode to DNA by the trans isomer includes formation of bifunctional adducts such as intrastrand cross-links between neighbouring purine residues and less than 1% forms interstrand cross-links [199], while the cis isomers just form monofunctional lesions on natural DNA. DNA adducts of the trans isomer are able to inhibiting RNA synthesis by DNA-dependent RNA polymerases, but those of the cis isomer are not.

The cytotoxicity of both isomers in a human melanoma cell line (SK-MEL 188) was tested. The cell growth inhibition was higher for the trans isomer. The antiproliferative activity of both isomers was significantly enhanced after irradiation with UVA light in comparison with their activity in the dark [248]. Due to the similar chloride substitution rates of these complexes in comparison with the cisplatin case, an analogous mechanism of action might be expected. Nevertheless their anticancer activity against cisplatin-resistant cell lines suggests some differences in the overall mechanism of action [249].

Dimethyl sulfoxide complexes of Ru(III) have been under investigation, exhibiting antitumour activity as well [51]. No further studies had been developed about these Ru anticancer compounds, presumably due to the higher attention that a new family of promising Ru compounds attracted and which will be briefly described next.

Initially motivated by the success of ruthenium compounds with general formula, mer-[RuCl₃L] and trans-(LH)[RuCl₂(L₂)], ruthenium(III)-dmso complexes as trans-
(dmso)$_2$H[RuCl$_4$(dmso)$_2$] and mer,cis-[RuCl$_3$(dmso)$_4$L] were synthesised. Although too labile for pharmacological purposes these compounds were useful starting materials for the synthesis of complexes of the type trans-Na[RuCl$_4$(dmso)L] and mer,cis-[RuCl$_3$(dmso)L] with L being a monodentate ligand (water, ammine or heterocyclic N-donor ligands, like imidazole, indazole, benzimidazole or oxazole) [250]. Despite some interesting results, the poor solubility was found to be an important disadvantage. To solve this problem, new complex salts were synthesised and anionic compounds developed. The anionic compound with the general formula trans-Na[RuCl$_4$(dmso)L] was studied, but in particular the imidazole (Him) complex trans-Na[RuCl$_4$(dmso)(Him)], (NAMI) [251], showed a high effect on lung metastasis rather than on the primary tumour. This compound is highly hygroscopic and difficult to be reproduced, but has been tested thoroughly in different animal studies and then entered clinical trials. NAMI is much less effective than cisplatin at altering DNA conformation, affecting DNA electrophoretic mobility and inhibiting DNA recognition and cleavage by restriction enzymes [193, 194].

Later, a more stable and reproducible compound, NAMI-A, (H$_2$im)trans-[RuCl$_4$(dmso)(Him)] was synthesised (Figure 1.6.3), exhibiting similar pharmacological properties [252, 253]. It is relatively non-toxic in vitro against tumour cells, while NAMI-A shows remarkable in vivo activity against metastases and has recently (2004) and successfully completed a Phase I trial (the first ruthenium complex ever that reached clinical trials) and is schedule to enter Phase II in the near future [254]. Remarkable inhibiting activity was detected against colorectal cancer and also on metastatic tumour growth in leukaemia P388 and L1210 and subcutaneous transplanted B16 melanoma [255]. But the best NAMI-A antitumour activity has been reported in lung cancer where the half-life elimination is around 8 times longer than that in the primary tumour mass. This effect it is attributed to efficient interaction between NAMI-A and collagen that is present in high concentration.

![Figure 1.6.3](image-url)

Figure 1.6.3: Schematic representation of NAMI and NAMI-A anticancer agents on clinical trials.

Although the precise mechanism of action has not been proved yet, several observations have been accumulated. NAMI-A presents strong binding to serum proteins suggesting that the drug could potentially exploit receptor-mediated delivery by transferrin for selective target of cancer cells [256]. It is considered that the mechanism of metastasis control is due to two combined factors, anti-angiogenic [257, 258] and anti-invasive [259] properties of NAMI-A on tumour cells and on blood vessels. The control of angiogenesis is believed to result of the scavenging properties of NAMI-A on the nitric oxide produced by endothelial cells and induction of apoptosis. NAMI-A also inhibits matrix metalloproteinases involved in angiogenesis [258]. Some evidence suggests that NAMI-A interferes in the cell-cycle regulation resulting in a transient accumulation of cells in the G$_2$-M phase [260-262]. NAMI-A binds coordinatively to DNA [263], but in lower rate when compared with cisplatin, which is possible due to intracellular inactivation. In spite of this observation, DNA-binding could not be excluded as a possible cytotoxic mechanism. Under physiological conditions, NAMI and NAMI-A present various equilibriums [51, 264] which are strongly dependent of pH [236]. Loss of dmso and imidazole following the chloride dissociation produces polyoxo complexes of ruthenium. At 25 °C, hydrolysis of the first chloride is observed within an hour while the second takes more than twice as long. At physiological pH, trans-NAMI, [RuCl$_4$(dmso)(Him)]$, is more labile to substitution than trans-[Ru(Him)$_2$Cl$_4$]$^+$ (t$_{1/2}$ = 19.7h, 25 °C) [265]. Chloride loss for the former is catalyzed by reduction to Ru(II), which is expected to occur under physiological conditions and could be enhanced in vitro by traces of biological reductants, such as ascorbic acid, glutatone or cysteine [266, 267]. Consequently, a redox-catalytic (activation by reduction) mechanism is suspected. Past studies indicated that the antitumour activity of Ru(III) complexes also depends on their reduction to Ru(II) species [224]. The in vitro
activity of a homologous series of Ru(III) complexes was reported to increase with increasing ease of reduction [268].

Going further, the synthesis and testing of NAMI-A analogues with anticancer properties using different nitrogen donors, like acyclovir [269], guanine [269], 5,7-dimethyl[1,2,4]triazolo[1,5-a]pyrimidine [184], pyrazine, pyrazole, 4,4'-bipyridine, 1,2-bis(4,4'-pyridyl)ethane, etc, with important cytotoxic activity, suggest that the imidazole fragment is not an essential feature for the antimetastatic property of NAMI-A [270-272]. On the contrary some evidence describes that the presence of dmso should be a prerequisite for the antimetastatic activity of this class of ruthenium complexes [260].

Several NAMI-type complexes derived from NAMI-A by changing the nature of the N-ligand, were then developed and from them, the second ruthenium compound right now in clinical trials was developed. Indazolium trans-tetrachloridobis(1H-indazole)ruthenate(III)(Figure 1.6.4), abbreviated as KP1019 (FFC14A) is an anionic compound showing antitumour activity against colon carcinomas and a variety of primary explanted human tumours [185, 263, 273] where NAMI was not found active at all. The activity of this compound is superior to the standard drug 5-flourouracil in experimental therapy of autochthonous colorectal carcinoma of the rat.

\[
\text{KP1019 (FFC14A)} \quad \text{KP1339 (FFC14)}
\]

**Figure 1.6.4** Schematic representation of KP1019 and KP1339 anticancer agents on clinical trials.

Under physiological conditions, KP1019 is just moderately soluble and therefore the sodium salt of it, KP1339 (FFC14)(Figure 1.6.4) is much more soluble in water(35 times more soluble) and is used in clinical trials for in situ preparation of KP1019. It was found that the hydrolysis of KP1019 occurs with exchange of the four chloride ligands for hydroxide ions or aqua molecules [273]. The hydrolysis proceeds slower than NAMI-A hydrolysis but leads temporary also to aqua complexes. It is clear that KP1019 can be efficiently taken up into cells, it is bound tightly to albumin and transferrin (within a few seconds); once KP1019 enters the cell, apoptosis is activated and oxidative stress and DNA damage is observed. Fruhauf and Zeller [205] observed that KP1019 brings about antitumour activity by interacting with DNA and inhibiting DNA synthesis. More experiments gave insight into the manner in which an excess of indazole affected the cytotoxicity and it was observed that the tumour inhibiting activity could be further increased by the addition of an excess of indazole [274, 275]. The effect of KP1019 on cytochrome c was studied and the binding of the ruthenium complex to the cytochrome was shown to induce a conformational change of the protein with a loss of the tertiary structure, clearly changing the heme group state, and increasing the α-helical content of apocytochrome c [276]. It seems plausible that the coordination of KP1019 and the consequent conformational changes could influence the biological functions of the protein [218]. Even though further research needs to be done, it is probably that the mechanism of action involves the accumulation in transferrin receptor-(over)expressing tumour cells via the transferrin receptor, reduction to Ru(II) species, reaction with DNA and apoptosis induction though the intrinsic mitochondrial pathway [50, 273, 277, 278].

Inspired by this NAMI-A derivative, the chemical and biological properties of other NAMI-A type complexes have been studied [183, 184, 279]. Complexes derived from changes in the N-ligand, dinuclear NAMI-A type compounds containing heterocyclic bridging N-N ligands [280], and new Ru-dmso nitrosyls compounds have been synthesised [281] and several of them have been found to have antimetastatic activity comparable to, or even better than NAMI-A.

Cisplatin shows antitumour activity against experimental tumours, being cytotoxic against tumour cells in vitro as well. Therefore, compounds like NAMI-A, are expected to behave in the
same way, as comparisons with cisplatin are normally established. On the contrary, it has been shown that NAMI-A shows the same action as cisplatin (or even better) in vivo on solid metastasizing tumours, but is virtually devoid of cytotoxicity against tumour cells in vitro. Keppler underlined the fact that due to the use of wrong tumour models, i.e. by using screening models in which cisplatin showed high activity, ruthenium compounds normally failed to show a better effect than cisplatin and were too early rejected as potential drugs [251]. Finally the property that renders ruthenium complexes unique among other antineoplastic agents is principally the lack of evident direct cell cytotoxicity at doses that increase the lifetime expectancy in tumour-bearing hosts, which means a low or absence of bone marrow or epithelial toxicity [262, 282, 283]. The high biological activity of NAMI-A against metastasis should stimulate laboratory studies with appropriate experimental models to predict clinical activity, since the use of experimental conditions closely similar to those of human tumours should help the identification of more active compounds [284].

A third important group of ruthenium compounds with promising cytotoxic properties are the ruthenium complexes of so-called polypyridine ligands(Figure 1.6.5) [196]. Polypyridyl-Ru complexes have been exploited extensively as molecular DNA probes, given their photoluminescence properties [285, 286], the ability of pyridyl ligands to intercalate DNA [287-292] and the DNA-cleavage properties [293]. The shape, size and rigidity of multidentate polypyridyl ligands confer shape and chirality to the ruthenium complexes that have been exploited to achieve customised DNA-binding properties, apart from the extra stability provided by the aromatic rings to the metal-ligand structures. These properties have provided a strong motivation for the developing of so-called polypyridyl-Ru complexes as DNA-targeting anticancer agents and a large number of these complexes have been screened for anticancer activity.

Typical oligo and polypyridyl ring ligands include 2,2'-bipyridine (bpy), 1,10-phenanthroline (phen) and 2,2'6'2''-terpyridine (tpy) as they are commercially available and readily form stable complexes with ruthenium(II/III). Some of the earliest polypyridyl-Ru complexes studied for potential anticancer properties include cis-[Ru(II)(bpy)2Cl2] (Figure 1.6.5) (both Λ and Δ enantiomers), mer-[Ru(III)(tpy)Cl3] (Figure 1.6.5) and [Ru(II)(bpy)(tpy)Cl]Cl(Figure 1.6.5). In vitro, mer-[Ru(III)(tpy)Cl3] was found to be significantly more cytotoxic (L1210, HeLa) than the others; this property matches with the in vivo data in mice [294-297]. This trend also correlates to the ability of mer-[Ru(III)(tpy)Cl3] to form DNA-interstrand crosslinks, whereas the inactive cis-[Ru(II)(bpy)2Cl2] appears not to exhibit such interactions [294, 296]. Although the cis-[Ru(II)(bpy)2Cl2] compound possesses two potentially free coordination sites after hydrolysis of the chloride ions, it appears it is not able to coordinate bifunctionally to two DNA bases. Besides mer-[Ru(III)(tpy)Cl3] showed both, in vitro and in vivo activity, but the poor solubility in aqueous solution hampered further development as antitumour agent.

There have also been numerous examples of polypyridyl-Ru complexes comprising one or more [Ru(II)(bpy)L]2+ or [Ru(II)(phen)L]2+ units [where L=derivatised quinolines, 2,6-(2'-benzyimidazolyl)pyridine/chalcone, aryl diazo-β-diketonate, 4-substituted thiosemicarbazides, 4-substituted thiopicolinanalides, 2-phenylazoimidazoles, etc.] [196, 218] (Figure 1.6.5) in an attempt to improve the DNA-intercalating properties of the complexes [298-306]. Important improvements in this field have been accomplished, but still numerous attempts to advance these compounds into clinical trials have not been reported.

A series of DNA-binding ruthenium(II) complexes with the tetradequate ligand, cyclam, and different DNA-intecalating quinonediimine ligands were studied in vitro (human cervix carcinoma, KB-3-1, and its multidrug-resistant subclone,KB-V1). For this family of compounds, it was noted that the cytotoxicity was linked to the ability of the quinonediimine ligands to intercalate. The IC50 values were considered too high to be of further interest [307].

Another family of mononuclear compounds with general formula [Ru(II)(tpy)LCl]+ (where L=bidentate nitrogen ligand), was synthesised and characterized [308]. From all the compounds tested, [Ru(tpy)(tmephen)Cl]+ (tmephen=3,4,7,8-tetramethyl-1,10-phenanthroline) shows the highest inhibition effect of bacterial cell growth. It was also proved that the compound could coordinate to DNA with preference at the purine residues. It appears that the increase in liability of the chloride ligand improves the reactivity of these ruthenium compounds towards the coordination bond formation in Ru-DNA adducts which results in significant inhibition effect in cell growth. No further cytotoxic studies have been published as yet.
The complex cis-(Cl,Cl)-[Ru\textsuperscript{II}(tpy)(NO)Cl\textsubscript{2}]Cl (Figure 1.6.5) has been synthesised and characterized showing good toxicity towards a human carcinoma cell line (A2780) and higher than that of mer-[Ru\textsuperscript{III}(tpy)Cl\textsubscript{3}], cisplatin or carboplatin [309]. This compound was shown to liberate NO upon mercury lamp radiation, an observation that could potentially be applied into anticancer phototherapy.

![Chemical structures and metal complexes](image)

**Figure 1.6.5** Schematic representation of pyridine-based Ru agents with cytotoxic properties.

Metal complexes of active drugs have been developed in the recent years and could have important pharmacological activities, mainly because complexation with the metal can protect the drug against enzymatic degradation, can produce more convenient hydrophobicity/hydrophilicity properties, can improve transport processes and/or can give extra organ-specificity. Finally, the biological activity can be reinforced by the combination of effects from the ligands and from the metal moiety.

In 2002, Harding et al. reported the synthesis and characterization of the complexes Ru(L1-CH\textsubscript{3})(CO\textsubscript{3})Cl, RuL\textsubscript{2}(CO\textsubscript{2})\textsubscript{2}Cl\textsubscript{2}, and RuL\textsubscript{3}(CO\textsubscript{2})\textsubscript{2}Cl\textsubscript{2} (L1=6-methoxy-5,8-quinolinedione, L2=7-amino-6-methoxy-5,8-quinolinedione, L3=6,6'-dimethoxycarbonyl-2,2'-bipyridine) [310] (Figure 1.6.5). The ligands studied are analogues of streptonigrin, a highly active anticancer compound against a variety of human cancers. On the basis of structural activity studies for streptonigrin, the researchers identified the key structures responsible for its activity and synthesized quinolidine and bipyridine analogues to mimic the active sites. No biological studies have been performed yet.

With the same purpose, Ru(III)/Ru(II) complexes with ketoconazol have been studied and showed important anticancer activity and limited toxicity. Ketoconazole (1-[4-[4-[[2S,4R]-2-(2,4-dichlorophenyl)-2-(imidazol-1-ylmethyl)-1,3-dioxolan-4-yl][methoxy][phenyl]piperazin-1-yl]ethanone, C\textsubscript{26}H\textsubscript{28}Cl\textsubscript{2}N\textsubscript{4}O\textsubscript{4}) has been used as a second line agent in hormone-refractory cancer therapy [311]. Also the synthesis, structural characterization electrochemical evaluation and estimation of selected biological parameters of ruthenium complexes with thiopurines and thiopyrimidines have been reported by Cini et al. [221]. Thiopurines are currently used as antileukemic and antiviral agents and as treatments against several types of other serious disorders, such as Crohn’s disease. Thiopyrimidines and their derivatives are also investigated for their antiviral potential, as well as for their photochemical properties which could be applied in phototherapy. From this family
of compounds two Ru-thiopurine complexes have shown significant cytotoxic activity towards ovarian carcinoma cell lines (A2780, cisplatin-sensitive and cisplatin-resistant lines).

The field of anticancer polynuclear ruthenium compounds is much less explored than the anticancer polynuclear platinum field, and it appears that the ruthenium complexes are less reactive than the corresponding platinum compounds [199, 270, 272, 312]. Most of the efforts have been focused on polynuclear polypyridyl ruthenium compounds as photoprobes and photoreagents of DNA, as tools for the determination of local structures and topologies of DNA in order to relate them to their functions. Of course, the development of such molecules can also lead to new potential antitumour drugs based on metallic compounds, as is the case with the platinum(II) complexes [313].

Recently, a heteronuclear Pt-Ru complex, involving a Ru(tpy) core and a highly flexible bridging chain was developed [314, 315]. The design of this molecule is supported on a platinum moiety, which could be bound to DNA and the Ru(tpy) moiety that it is proposed to have an intercalating behaviour, thereby providing additional anchor support. Also more rigid bridging ligands have been used in the synthesis of dinuclear Ru-Pt compounds, where the interaction of these dinuclear compounds with DNA proves to be of interest as potential chemotherapeutic agents [316, 317].

Another family of mononuclear ruthenium complexes which has attracted big attention includes several arylazopyridine ruthenium(II) complexes [318-322] (Figures 1.6.6 and 1.6.7). The three isomeric dichloridoruthenium(II) complexes $\alpha$-, $\beta$-, and $\gamma$-[Ru$^\text{II}(\text{azpy})_2\text{Cl}_2$] (azpy=2-(phenylazo)pyridine) have been investigated for their cytotoxic properties against a series of tumour cell lines [323]. Nevertheless the close chemical and structural similarities among them, the structural characteristics have a significant impact on the efficacy of the compounds as cytotoxic agents. The complexes $\alpha$-[Ru$^\text{II}(\text{azpy})_2\text{Cl}_2$] and trans-$\gamma$-[Ru$^\text{II}(\text{azpy})_2\text{Cl}_2$] exhibit a very high cytotoxicity which stands in contrast to the much lower cytotoxicity of the cis-$\beta$-[Ru$^\text{II}(\text{azpy})_2\text{Cl}_2$] isomer. The high cytotoxic values of the $\alpha$- and $\gamma$- isomers in vitro (A498, EVSA-T, H226, IGROV, MCF-7, WIDR and M19) were comparable to those of cisplatin and 5-fluorouracil [318, 324].

![Figure 1.6.6 Schematic representation of the arylazopyridine ligands.](image)

The binding of $\alpha$-[Ru$^\text{II}(\text{azpy})_2\text{Cl}_2$] to monomeric 9-ethylguanine and guanosine has been studied and compared with the binding of cis-[Ru(bpy)$_2$Cl$_2$] [325]. 9-Ethylguanine and guanosine form monofunctional adducts. The guanine derivatives of the azpy-Ru complexes, present more possible orientations, than the orientations available for cis-[Ru(bpy)$_2$Cl$_2$]. This factor is considered of main importance in the binding of $\alpha$-[Ru$^\text{II}(\text{azpy})_2\text{Cl}_2$] to DNA and very likely related to the high cytotoxicity of this compound. The slight modification of azpy by addition of methyl groups to either the pyridine or phenyl moiety, as in [Ru$^\text{II}(\text{tazpy})_2\text{Cl}_2$] and [Ru$^\text{II}(4\text{mazpy})_2\text{Cl}_2$] (tazpy=o-tolylazopyridine, 4mazpy=2-(phenylazo)-4-methylpyridine) does not alter the trend, thereby validating the structure-activity relationship of the isomers when talking about cytotoxicity [324]. DFT calculations suggest that the ability of the [Ru$^\text{II}(4\text{mazpy})_2\text{Cl}_2$] isomers to intercalate to DNA decreases from $\gamma$-$\alpha$-$\beta$ isomers on the basis of the geometric and electronic factors, which correlates with the observed cytotoxicity [326]. The $\gamma$-isomer has the most preferential geometric arrangement of 4mazpy for DNA intercalation, as well as the lowest LUMO energy level and smallest HOMO-LUMO energy gap, clearly becoming the most reactive towards DNA. A mixed-ligand ruthenium analogue, cis-[Ru$^\text{II}(\text{azpy})(\text{bpy})\text{Cl}_2$], which is structurally similar to $\alpha$-
[RuII(azpy)2Cl2] was found to be 2-10 fold more in vitro-active (A498, EVSA-T, H226, IGROV, MCF-7, WIDR and M19) than cis-[RuIII(bpy)2Cl2], but much less toxic (>50 times) than either α- or β-[RuII(azpy)2Cl2] [327]. The tris-ligand complexes, [RuL3](PF6)2 (L=2-(phenylazo)pyridine or o-tolylazopyridine) and mixed-ligand complexes, [RuII(azpy)3n-1(bpy)n](PF6)2, where the chloride ligands are replaced, have been synthesized, structurally characterized and tested for cytotoxic activity [323]. These complexes were designed to test the hypothesis that α-[RuII(azpy)2Cl2] exhibits a high cytotoxicity due to its two cis-chloride ligands, which may be exchanged for biological targets like DNA as in the case of cisplatin. Remarkably, the cytotoxicity of the tris-ligand compounds was found to be moderate (but not higher than the parent α-[RuII(azpy)2Cl2]); so even though the chloride ligands are not present, cytotoxic activity is observed. This could imply a different mechanism of action.

\[ \text{Figure 1.6.7 Schematic representation of the three isomeric arylazopyridine ruthenium(II) complexes.} \]

Some water-soluble derivatives of α-[RuII(azpy)2Cl2], where the chloride ligands are replaced by bridging carboxylate ligands, like oxalate, malonate or 1,1-cyclobutanedicarboxylate, were synthesized and characterized [328]. Although the compounds are 5-10 fold less cytotoxic than α-[RuII(azpy)2Cl2] (in A2780 cisplatin-sensitive and cisplatin-resistant cell lines) and slightly less cytotoxic than cisplatin, its cytotoxicity is comparable to that of carboplatin. Another water-soluble analogue, [NEt4]2[RuII(sazpy)2Cl2] (where sazpy=2-phenylazopyridine-5-sulfonate), with a sulfonate functionality on the pyridyl moiety, showed 100 times less cytotoxic activity (A2780 cisplatin-sensitive and cisplatin-resistant cell lines) than α-[RuII(azpy)2Cl2] [329].

More recently, dinuclear analogues with bridging azpy ligands (bisazopyridine, Figure 1.6.6), comprising two azpy units joined at the para position of the phenyl rings by a bridging methylene group, have been reported [330]. Each supramolecular complex contains two [RuII(azpy)2Cl2] moieties arranged in either the α or γ isomers. Three isomers have been isolated that contains either α/α-, α/γ- or γ/γ-[RuII(azpy)2Cl2] units and their structures have been confirmed by X-ray diffraction studies. The α/γ and γ/γ isomers were tested in vitro (T47D, HBL-100) with the γ/γ isofrom exhibiting the highest cytotoxicity, >30-fold higher than that of cisplatin. Ruthenium complexes with polyaminopolycarboxylic chelating ligands, constitute, another important promising group of metal-based cytotoxic compounds [331-333] (Figure 1.6.8). The use of polyaminopolycarboxylate ligands in metallopharmaceutical applications is of growing interest not only because of their ability of strong binding to metal centres, or because their amino and carboxylate binding entities are akin to those in biological systems, but also because the complexes formed are, in general terms, six-coordinated, octahedral and highly water soluble. In [Ru(cdta)Cl2], where cdta=1,2-cyclohexanediaminotetraacetate, the chloride ligands are cis to each other and the carboxilates appear to be labile(Figure 1.6.8). Ru(IV/III) reduction potential occurs at 0.78V, while the Ru(III/II) couple is at -0.01V [332], which means that Ru(III) and Ru(II) could be present under physiological conditions. This also suggests that the transport by transferrin could have an important role in the biological activity. [Ru(cdta)Cl2] is the first Ru(IV)-compound with cytotoxic activity reported [334].
The compound \textit{cis}-H[Ru\textsuperscript{III}(ppta)Cl\textsubscript{2}], where ppta=1,2-propylenediaminetetraacetate, also shows good antitumour activity [335] (Figure 1.6.8). It is believed that the chlorides dissociate to produce a number of reactive Ru\textsuperscript{II}-species. The complex rapidly binds to albumin, apotransferrin or diferric transferrin producing relatively stable adducts in which Ru\textsuperscript{III}(ppta)-moiety is probably bound to protein surface. Electrophoretic assays show that \textit{cis}-H[Ru\textsuperscript{III}(ppta)Cl\textsubscript{2}] damages nuclear DNA and significantly alters the conformation of the plasmid pUC19 DNA. Moreover, this compound inhibits DNA recognition and DNA lysis by restriction enzymes. \textit{cis}-H[Ru\textsuperscript{III}(ppta)Cl\textsubscript{2}] shows antitumour activity \textit{in vivo} (EAT, L1210, P388, MX-1, M5076) with low systemic toxicity [336]. The ethylenediaminetetraacetate-Ru(III) complex(Figure 1.6.8) also displays \textit{in vitro} cytotoxicity (MFC-7, NCI-H460 and SF-268). On the basis of spectrophotometric, electrochemical and kinetic data and comparing the reactivity of [Ru\textsuperscript{III}(edta)(H\textsubscript{2}O)] with DNA bases and DNA (calf-thymus) itself, it is proposed that the interaction of [Ru\textsuperscript{III}(edta)(H\textsubscript{2}O)] with DNA takes place through the adenine base unit in a kinetically preferred pathway [337]. A related compound K[Ru\textsuperscript{III}(eddp)Cl\textsubscript{2}](where eddp=ethylenediamine-N,N-di-3-propionate) also displays cytotoxicity \textit{in vitro} (HeLa, BT-20, HT29) and induces DNA cleavage [338].

More recent studies are focussed in the design of polyaminopolycarboxylate-Ru complexes that could function as effective NO scavengers in biological systems [339-341]. Probably one disadvantage of this family is the negative charge that these complexes could have, which could reduce the binding function to DNA.

Organometallic sandwich and half-sandwich complexes offer an important potential in drug design. Due to the fast growth of organometallic chemistry theories during the second half of the 20th century, a better understanding of the structure-property relationships at the atomic level of organometallic compounds has been achieved. A rational design of new potential organometallic drugs, with increased biological activities and the potential to overcome resistance, selectivity issues and toxicity, is possible nowadays due to the large diversity of structure and bonding modes (π-coordination, metal-carbon multiple bonds, etc) that can be tuned [342].

Organometallic Ru complexes with arene ligands represent one of the most recent groups of ruthenium compounds with antitumour properties. Since arenes are known to stabilize ruthenium(II) which, by the way, is considered the most probable oxidation state of ruthenium in the active anticancer compounds once introduced in the body (activation by reduction theory), a large family of organometallic Ru(II) complexes has been synthesised, characterized and biologically tested in the past years [196, 199, 218, 343-346]. Other advantages for this class of compounds stem from the good aqueous solubility of half-sandwich Ru(II) mono-arene complexes(clinical use) and the relative inertness towards displacement of the arene moiety.

Since the initial discovery that [Ru(η\textsuperscript{6}-C\textsubscript{6}H\textsubscript{6})(dmso)Cl\textsubscript{2}] can inhibit topoisomerase II activity, multiple derivatives have been prepared by replacing dmso with different ligands like 3-aminopyrididine, p-aminobenzoic acid and aminoguanidine among many others. These analogues show enhanced efficacy of topoisomerase II inhibition and higher cytotoxicity against breast and colon carcinoma cells compared to the parent compound [30, 347]. It was suggested that [Ru(η\textsuperscript{6}-C\textsubscript{6}H\textsubscript{6})(dmso)Cl\textsubscript{2}] interacts with DNA and forms cross-links with topoisomerase II. The complex exhibited antiproliferative activity \textit{in vitro} (Critt-2), but a direct link between its ability to inhibit topoisomerase II, and the antiproliferative effect, is not clear. Arenes-ruthenium complexes containing essentially, the bidentate sulfoxide ligand, 1,2-bis(ethylsulfinyl)ethane (BESE), have

![Figure 1.6.8 Schematic representation of polyaminocarboxylate-Ru(III) complexes.](image-url)
been tested *in vitro* (MDA-MB-435s), but their cytotoxicities were found to be more than 5-fold lower than that of cisplatin or carboplatin; so any further research was cancelled [348].

Closely related Ru(II) arene compounds with the formula \([\text{Ru}^{II}(\eta^6\text{-arene})(\text{en})X]^+ \) (\(X=\text{Cl} \) or \(\text{I}; \eta^6\text{-arene}=\text{p-cumene or biphenyl}; \text{en}=\text{ethylenediamine or N-ethyl ethylenediamine}\) (Figure 1.6.9) were demonstrated to inhibit the proliferation of ovarian cancer cells. Some of the IC\(_{50}\) values were found comparable with carboplatin [349], although these complexes do not inhibit topoisomerase II activity. One compound was probed to bind strongly to DNA, forming monofunctional adducts selectively with guanine bases. More analogues of this family of compounds, with general formula \([\text{Ru}^{II}(\eta^6\text{-arene})(\text{YZ})X]^+ \) were prepared by changing the nature of the bidentate ligand(\(\text{YZ}\)), as the presence of a bidentate ligand seems to be advantageous for the anticancer activity [342, 343, 349].

![Figure 1.6.9](image.png) Schematic representation of a family of organometallic Ru(II) complexes.

The structure of Ru(II) half-sandwich complexes allows for variation of the three main building blocks, the monodentate ligand, \(X\), the bidentate ligand (\(\text{YZ}\)) and the arene to fine-tune the pharmacological properties of these complexes. It appears that mainly the bidentate ligand(\(\text{YZ}\)) can help to control the stability and ligand-exchange kinetics of the complex; the nature of the arene can help to influence the cell uptake and interactions with biological targets and finally the monodentate ligand, \(X\), also considered the leaving group, typically chloride, can help to control the timing of activation of these class of complexes [342]. Some preliminary structure-activity relationships have been proposed based on biological test. It appears that a more hydrophobic arene ligand and a single ligand exchange site (occupation of the other two coordination sites by a stable bidentate chelating ligand) are associated with high cytotoxicity [350]. Initial studies on amino acids and nucleotides suggest that kinetic and thermodynamic control over a wide spectrum of reactions of Ru(II) arene complexes with biomolecules can be achieved. Extensive oligonucleotide studies have been carried out and \([\text{Ru}^{II}(\eta^6\text{-cymene})(\text{en})X]^+ \) has been found to preferentially bind to guanine bases to form monofunctional DNA adducts [351]. With large arene ring systems, the metal complexes bind to nucleotides bases by hydrophobic arene-purine base \(\pi-\pi\) stacking interactions, which could explain the enhanced cytotoxicity of those derivatives [352, 353]. When comparing the cytotoxicity in cell lines sensitive and resistant to cisplatin (A2780 and A2780R), they show equivalent IC\(_{50}\) values which may suggest that the mechanism of action is quite different from that of cisplatin. In fact, these Ru(II) complexes present an altered profile of biological activity when compared with metal-based anticancer complexes currently in clinical use or in clinical trials. The patterns of activity established *in vitro* for \([\text{Ru}^{II}(\eta^6\text{-arene})(\text{en})X]^+ \) are comparable to a large degree *in vivo*, with the compounds effecting significant
growth delays against both A2780 and A2780R tumours grafted on mice (xenografts). Mice are also able to tolerate the ruthenium complex better than cisplatin (up to 25 mg per kg body mass of [Ru$^{II}$(η$^6$-byphenyl)(en)Cl]PF$_6$ injected on days 1 and 5 without significant weight loss, compared to 10 mg per kg cisplatin as a single injection in one day) [342, 354]. Further research is needed mainly to complete and optimize the pharmacological profiles of these complexes.

The synthesis and characterization of dinuclear Ru(II)-arene analogues have been reported recently using 2,3-bis(2-pyridyl)pyrazine or linear amines as bridging ligand (Figure 1.6.10). The photoactivation of these compounds appears to simultaneously produce a high reactive Ru species (mainly mononuclear Ru(II) arenes, independently proven to be cytotoxic) that can bind to DNA and a fluorescent marker (a free arene). Therefore these complexes have the potential to combine both photoinduced cell death and fluorescence imaging of the location and efficiency of the photoactivation process. Cytotoxic studies have not been reported yet [355].

![Figure 1.6.10 Schematic representation of dinuclear Ru(II)-arene analogues.](image)

Important biological activity was observed also in ruthenium complexes of the RAPTA type. The so-called RAPTA compounds comprises a class of organometallic ruthenium(II) complexes with a monodentate 1,3,5-triazene-7-phosphatricyclo[3.3.1.1]decane (pta) ligand and a η$^6$-arene ligand [196] (for a selection see Figure 1.6.11). RAPTA compounds are generally stable under atmospheric conditions with good thermodynamic stability. The pta ligand can also be readily derivatised to form acetylated species or methylated species, further expanding the scope of possible RAPTA compounds [356]. RAPTA is also unusual when compared with other phosphane-ruthenium complexes, because RAPTA compounds are not only soluble on polar organic solvents, but also in water. RAPTA compounds are considered as effective drugs because are highly selective towards tumour cells with a low systemic toxicity. Also of importance is the fact that RAPTA compounds interacts with proteins in a specific way, which could be the basis for new therapeutic targets beyond DNA. In particular,
growing interest is focused in order to explain why RAPTA-T and NAMI-A present a remarkably similar \textit{in vivo} activity which suggest that they could respond to similar targets \cite{345}. The development of complementary proteomic techniques, as well as rational drug design for RAPTA complexes has produced more than 20 RAPTA complexes that could give more information in order to propose a clear structure-activity relationship.

Despite low cell-growth inhibitory activity \textit{in vitro} on cultured cells they lowered the growth of lung metastases in mice bearing mammary carcinoma. This happened even in absence of a corresponding response at the primary tumour site \cite{345, 357}. The briefly described relationships between structure and activity have been accepted to be empirical in nature, but initially allowed the synthesis and study of a manageable number of complexes, albeit of similar structure.

1.7 Aim and contents of this thesis

A widespread interest exists in the effect of metal-based compounds in biological systems, particularly, due to the major advances in the molecular and cellular metabolism knowledge, but also due to improvement in the detection techniques and clinical trials tools used to study this biologic effect. As this knowledge increases, the design of new metal-based compounds either as metallodrugs, or as heavy atoms labels, will undoubtedly improve.

The general aim of this thesis is to develop a systematic knowledge in the search of alternative anticancer metal-based compounds with enhanced cytotoxic properties and establishment of structure-activity relationships. The long term goal for the research projects here started is to develop a systematic approach in the synthesis of gold and ruthenium based anticancer compounds capable of overcoming the inherent problems related to the cisplatin treatment, but also with wider application to different tumour types.

This research in particular describes the synthesis, characterization and biological activity of several metal-based systems of gold and ruthenium. This research is strongly motivated by exploring the relatively little knowledge that is known about the mechanism of action of gold drugs in particular in the field of cancer treatment and the promising role of Ru-anticancer drugs.

Chapter 1 comprises general information about cancer and its importance in our society. It also provides an overview about the most important metal-based drugs studied in the search of an efficient anticancer compound. Finally a micro-review into platinum, gold and ruthenium chemistry and their relation with anticancer properties is presented in an attempted to describe the most important findings in the field.

Chapter 2 describes the synthesis and characterization of novel mononuclear Gold(III) complexes with modified 2-(phenyazo)pyridine ligands.

Chapter 3 addresses the stability studies in solution of [Au(L)Cl₂]Cl·xH₂O complexes (where L=2-(phenylazo)pyridine, 2-(tolylazo)pyridine, 2-(phenylazo)-3-methylpyridine, 2-(phenylazo)-4-methylpyridine and 2-(tolylazo)-3-methylpyridine) and their biological studies. The cytotoxic activity and search of a structure-activity relationship is also discussed.

Chapter 4 deals with the synthesis of new Ru(III)-bis(arylimine)pyridine Schiff base ligand complexes. The characterization and elucidation of the paramagnetic structures was achieved by means of nuclear magnetic resonance. Also studies of the interaction of the Ru(III) compounds with a DNA-model base are included. Finally, the cytotoxic properties of this family of compounds are determined and structure activity relationships discussed.

Chapter 5 provides the synthesis and characterization of novel Ru(II)-bis(arylimino)pyridine complexes as chlorido-, 1,10-phenanthroline, 2,2'-dipyridyl-, 2-(phenylazo)pyridine-, 2-(phenylazo)-3-methylpyridine-, 2-(phenylazo)-3-methylpyridine- and 2-picolinate- adducts. The structural studies by X-ray crystallography for some of complexes with general formula, [RuLₓLyX]ClO₄ (Lₓ=bis(arylimino)pyridine Schiff-base ligands, Ly=bidentate ligands: azpy, 3maazpy, tazpy, bpy, phn, and X=Cl) are described. Chapter 5 also includes the \textit{in vitro} cytotoxic studies of these Ru(II) compounds in a selected group of cell lines. Some particular structure-activity relationships could be observed and are discussed in detail.

Finally important remarks about the findings in the projects described along this thesis, conclusions and some suggestions are developed in Chapter 6 and briefly discussed. Parts of this work has been published or submitted for publication \cite{358-360}.
1.8 References


Chapter 1