Platinum Complexes as Anticancer Agents

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Abstract: The application of inorganic chemistry to medicine is a rapidly developing field, and novel therapeutic and diagnostic metal complexes are now having an impact on medical practice. Advances in biocoordination chemistry are crucial for improving the design of compounds to reduce toxic side effects and understand their mechanisms of action. Cisplatin, as one of the leading metal-based drugs, is widely used in the treatment of cancer. Significant side effects and drug resistance, however, have limited its clinical applications. Biological carriers conjugated to cisplatin analogs have improved specificity for tumor tissue, thereby reducing side effects and drug resistance. Platinum complexes with distinctively different DNA binding modes from that of cisplatin also exhibit promising pharmacological properties. This review focuses on recent advances in developing platinum anticancer agents with an emphasis on platinum coordination complexes.

Keywords: *cis*-Diaminedichloro-platinum(II), Pt(II); Pt(IV), *cis*-, *trans*-, amine, diamine coordination complexes, multinuclear complexes, DNA, cytotoxic activity, tumor cell lines.

INTRODUCTION

Medicinal application of metals can be traced back almost 5000 years [1]. The development of modern medicinal inorganic chemistry, stimulated by the discovery of cisplatin, has been facilitated by the inorganic chemist's extensive knowledge of the coordination and redox properties of metal ions. Metal centers, being positively charged, are favored to bind to negatively charged biomolecules; the constituents of proteins and nucleic acids offer excellent ligands for binding to metal ions. The pharmaceutical use of metal complexes therefore has excellent potential. A broad array of medicinal applications of metal complexes has been investigated, and several recent reviews summarize advances in these fields [2-6]. A lot of different coordination compounds and the mechanism of cytotoxic action have been discussed with regard to the development of new antitumor agents [7, 8].

Developing metal complexes as drugs, however, is not an easy task. Accumulation of metal ions in the body can lead to deleterious effects. Thus biodistribution and clearance of the metal complexes as well as its pharmacological specificity are to be considered. Favorable physiological responses of candidate drugs need to be demonstrated by *in vitro* study with targeted biomolecules and tissues as well as *in vivo* investigation before they enter clinical trials. A mechanistic understanding of how metal complexes achieve their activities is crucial to their clinical success, as well as to the rational design of new compounds with improved potency.

Cancer is caused when genetic damage to the cells prevents them from being responsive to normal tissue controls. The cancer spreads when affected cells multiply rapidly, forming tumors of varying degrees. Different therapies can be used, depending on how far the cancer has spread [4-8]. In the case of cancer, a chemotherapeutic agent is one that kills the rapidly dividing cells, thus slowing and stopping the cancer from spreading. Anticancer drugs have originated from a variety of sources, including dyestuffs and chemical warfare agents, and from natural products such as plants, microbes and fungi. Metal ions and metal coordination compounds are known to affect cellular processes in a dramatic way. This metal effect influences not only natural processes, such as cell division and gene expression, but also non-natural processes, such as toxicity, carcinogenicity, and antitumor chemistry. In chemotherapy, the key issue is killing the tumor cells, without causing too much harm to healthy cells.

Over the past 30 years, platinum-based drugs, notably cisplatin and carboplatin, have dominated the treatment of various cancers by chemical agents. However, because these drugs cause serious side effects, chemists are looking to other platinum complexes as potential anticancer agents. This review focuses on recent advances in developing platinum anticancer agents with an emphasis on platinum coordination complexes.

PLATINUM COORDINATION COMPOUNDS

Structure-activity relationships for a class of platinum coordination compounds confirmed that only those compounds having *cis* geometry block cell growth. The most active complex, cisplatin (**1**), was found to exhibit antitumor activity, whereas its *trans* isomer showed no such activity [7-8]. Many derivatives of cisplatin also inhibit growth, and these compounds have at least one N-H group, which is responsible for important hydrogen-bond donor properties, either in the approach of the biological target or the final structure. Most of the well-known platinum anticancer complexes have the general formula *cis*-$[PtX_2(NHR_2)_2]$, in which R = organic fragment and X = leaving group, such as

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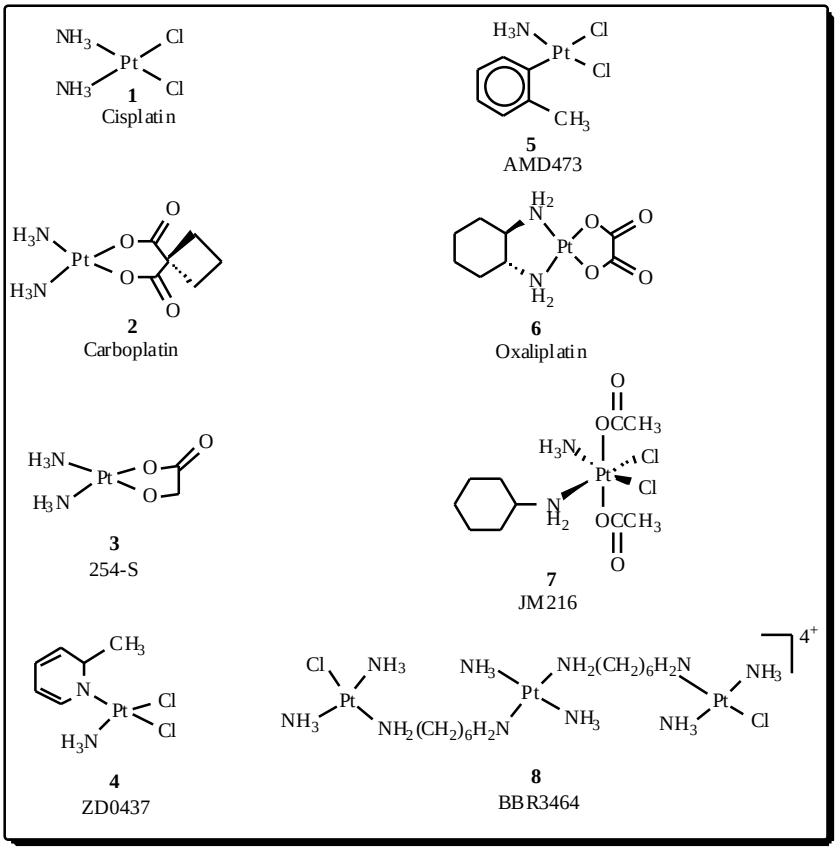
chloride or (chelating *bis*)carboxylate. Many other active Pt(II) compounds are known now, even with *trans* geometries, and these will be dealt with below.

The development of cisplatin as a successful antitumor drug is often seen as the prototypical success story. The large number of patients who have been cured after cisplatin treatment of cancer is impressive. However, the fact that the precise mechanism of action remains elusive has resulted in great interest in metal DNA binding generally and cisplatin and its analogs' binding properties particularly. As a consequence, cisplatin chemistry has provided a fertile ground for exciting bioinorganic chemistry research.

It is very important to note that similar coordination complexes from other groups of metals do not yield active compounds. The key factor explaining why Pt is most useful clearly relates to ligand-exchange kinetics. An important property of the platinum coordination compounds is the fact that the Pt-ligand bond, which has the thermodynamic strength of a typical coordination bond, is much weaker than (covalent) C-C and C-N or C-O single and double bonds. However, the ligand-exchange behavior of Pt compounds is quite slow, which gives them a high kinetic stability and results in ligand-exchange reactions of minutes to days, rather than microseconds to seconds for many other coordination compounds. Another unusual phenomenon deals with the preferred ligands for Pt ions. Pt(II) has a strong thermodynamic preference for binding to S-donor ligands. For that reason, one would predict that platinum compounds would perhaps never reach DNA, with many cellular platinophiles (S-donor ligands, such as glutathione, methionine) as competing ligands in the cytosol. Finally, the so-called kinetic *trans* effect should be mentioned, which is responsible for ligand-exchange reactions on metalsions. The effect is most pronounced for Pt(II) compounds, where it has been studied in great detail [7,8]. The rule can be quite simply formulated as*:* ligands located *trans* to another ligand with a strong *trans* effect (such as many soft ligands) are more rapidly substituted than ligands in *cis* positions.

Despite the success of cisplatin, however, it lacks selectivity for tumor tissue, which leads to severe side effects. These include renal impairment, neurotoxicity and ototoxicity (loss of balance/hearing), which are only partially reversible when the treatment is stopped. With long-term or high-dose therapy, severe anemia may develop. To address these problems, modified versions of cisplatin, leading to second and third generation platinum-based drugs have been synthesized over the past 30 years. Several platinum complexes (**2-8**) are currently in clinical trials, but some of these new complexes have not yet demonstrated such significant advantages over cisplatin.

The second-generation platinum drug carboplatin, $[Pt(C_6H_6O_4)(NH_3)_2]$ (**2**), has fewer toxic side effects than cisplatin and is more easily used in combination therapy. Its low reactivity allows a higher dose to be administered. Carboplatin is used more for ovarian cancer treatment, whereas oxaliplatin (**6**) is known to be most effective in colon cancer treatment.



More recent developments have shown that spontaneous (intrinsic) drug resistance may develop in certain tumors, which is one of the main limitations when treating patients. Such resistance is easily detected in tumor cell lines, so that new drugs can now be rapidly screened. As a result, a new group of compounds with different amines and lacking the classical *cis*-diamine structure with two leaving groups has evolved during the last decade. These compounds are often considered the so-called third-generation drugs.

Combining drugs with different modes of action often synergizes their effects, so the scientists will continue to probe the various metallopharmaceutical mechanisms in the hope that together they might yield an even wider range of effective chemotherapeutic agents [9-12]. Combination therapy could be the way forward in the fight against cancer.

cis-DIAMINEDICHLORO-PLATINUM(II)

The successful development of metal-containing anticancer drugs clearly starts with *cis*-[$PtCl_2(NH_3)_2$], often referred to as cisplatin (**1**). Although the compound was first described in 1845, its anticancer properties were not discovered until 1964. *cis*-Diaminedichloro-platinum(II) is one of the most potent and effective antitumor agents discovered in the last century serendipitously by Barnett Rosenberg. The antitumor and toxic effects of this drug have long been discussed [13]. It has displayed encouraging results in testicular tumors. The drug's therapeutic effectiveness has also been recognized in a variety of other solid tumors, particularly ovarian, bladder, and head and neck malignancies.

Cisplatin is usually administered intravenously rather than orally because of solubility problems. Once in the bloodstream, cisplatin diffuses across the cell membranes into the cytoplasm. The intracellular Cl^- concentration is less than that beyond the cell walls, so a complex equilibrium process is set up, see Fig. (**1**). Cationic platinum complexes, such as $[Pt(NH_3)_2(OH_2)Cl]^+$, are formed when a water molecule attacks the platinum metal centre, thus eliminating a chloride ion which acts as a non-coordinating anion. The cell essentially traps the cisplatin by transforming it into a cationic component of a neutral molecule. After losing two Cl^- ions, hydrolyzed cisplatin reacts with DNA, forming coordinative bonds to nitrogen atoms of the nucleobases. The active species in the cell is thus $(NH_3)_2Pt^{2+}$, not cisplatin.

The binding of $(NH_3)_2Pt^{2+}$ to DNA leads to changes in the DNA structure. NMR studies indicate that the Pt^{2+} binds to N7 atoms of a pair of guanine (G) bases on adjacent strands of DNA. A pathway for GG intrastrand cross-linking of DNA by cisplatin is shown in Fig. (**2**): aquation is followed by monofunctional adduct formation, and then ring closure to give the bifunctional GG macrochelate. In the GG chelate, the two G bases are in the head-to-head conformation. $(NH_3)_2Pt^{2+}$ creates a unique junction between the strands. This 'local distortion' leads to an impairment in the processing of DNA in tumor cells. The distortion is 'characterized' by a High Mobility Group (HMG), i.e. an 80 amino acid sequence found in many proteins that bend DNA dramatically [14]. In an effort to elucidate the nature of the DNA-HMG complex, it was discovered that the HMG protein binds to the DNA-cisplatin adducts in a 1:1 ratio and protects them from DNA repair enzymes. So it is not simply the distortion of DNA by the $(NH_3)_2Pt^{2+}$ that disrupts the function of the DNA, but also the ability of the platinated-DNA function to attract HMGs.

Cisplatin enters cells by passive diffusion [15] and also, as recently discovered, by active transport mediated by the copper transporter Ctr1p in yeast and mammals [16, 17]. Details about this latter mechanism remain to be elucidated. The cytotoxicity of cisplatin originates from its binding to DNA and the formation of covalent cross-links. The 1,2-intrastrand d(GpG) cross-link is the major adduct. Binding of cisplatin to DNA causes significant distortion of helical structure and results in inhibition of DNA replication and transcription [15, 18]. The distorted, platinated DNA structure also serves as a recognition binding site for cellular proteins [19, 20], such as repair enzymes, transcription factors, histones and HMG-domain proteins. Binding of the HMG-domain proteins to cisplatin-DNA lesions has been

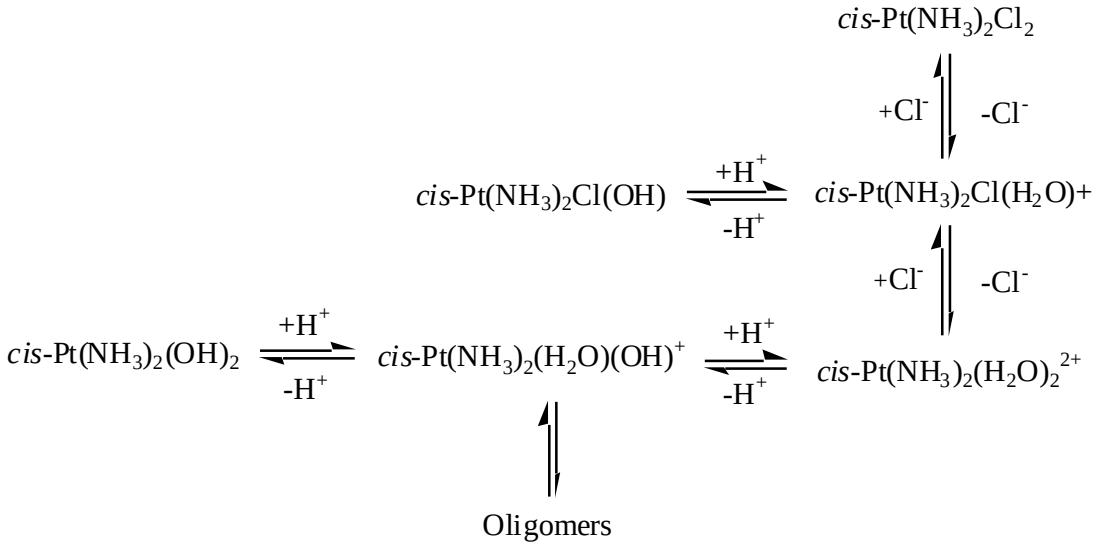


Fig. (1). Equilibrium process for cisplatin in cancer cells.

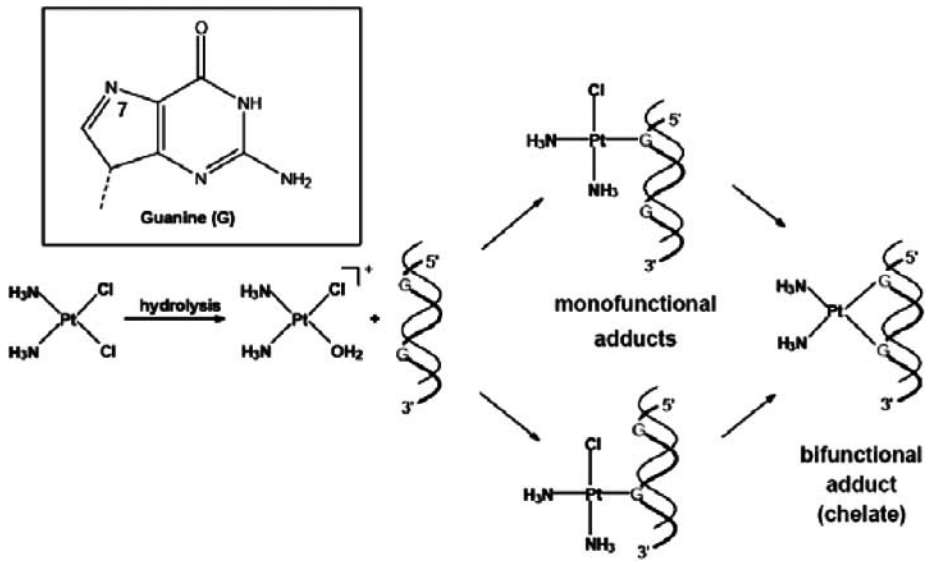


Fig. (2). Pathways for GG intrastrand crosslinking of DNA by cisplatin. The insert shows the structure of guanine and the position of N7, the major Pt binding site.

suggested to mediate the antitumor activity of the drugs [14, 21-23]. The anticancer efficacy of cisplatin is also influenced by the efficiency of cisplatin-DNA adduct removal by the cellular repair machinery, with nucleotide excision repair being a major pathway. The repair of platinum-DNA cross-links is retarded when the DNA is bound to the histones in a nucleosome core particle [24, 25]. Mello *et al*. [26] have shown that the human mismatch-repair protein, hMSH2, also binds specifically to DNA containing cisplatin adducts and displays selectivity for the DNA adducts of therapeutically active platinum complexes. These results suggest a role for hMSH2 in mediating cisplatin toxicity. Various adducts are formed upon interaction of platinum complexes with nucleotides, but contribution of individual adducts to antitumor activity and toxicity of platinum complexes still remains to be examined. Warnke *et al*. [27] investigated the formation of adducts following the reaction of *cis*-diaminedichloroplatinum (II) (cisplatin) with various DNA nucleotides. Formation and conversion of distinct species were confirmed. The potential applications comprised studies of novel platinum complexes, investigations of platinum-adduct formation with DNA, and determination of platinum-DNA adducts in cells. Isolated nucleic acids which can confer on a cell at least a 5-fold increase in cisplatin resistance relative to a cisplatin sensitive cell were disclosed recently by Yokoyama [28-30]. The nucleic acids of the invention can further confer on a cell resistance to heavy metals such as cadmium and copper. The isolated nucleic acids and proteins of the invention were useful for conferring cisplatin resistance on a cell, for example non-malignant cells in a tumor bearing subject being treated with cisplatin. The above inventions also disclosed methods for identifying substances which inhibit cisplatin resistance in a cell or which are chemosensitizers of cisplatin.

Thus, the key elements in the effects of cisplatin (and derivatives) on DNA are its controlled hydrolysis, transport to and within the cell, and binding to DNA; specific binding at adjacent guanine bases; and especially a specific distortion of DNA, changing its interactions with proteins, leading to either repair of the damage, or cell killing by apoptosis.

PLATINUM-BASED ANTICANCER AGENTS

Besides cisplatin, the second-generation drug, carboplatin (diamine[1,1-cyclobutanedicarboxylato(2-)]-*O,O´*-platinum (II)) (**2**) has been introduced into oncotherapy. The observed pharmacokinetic differences between cisplatin and carbo-platin depend primarily on the slower rate of conversion of carboplatin to reactive species. Studies on the interaction of carboplatin with DNA indicate that the reaction proceeds *via* ring-opening in carboplatin and subsequent binding with DNA constituents. Replacement of the chloride groups in the cisplatin molecule by cyclobutanedicarboxylate ligand significantly diminished the nephrotoxic effects of the formed carboplatin, without affecting its antitumor potency. Due to the reduced spectrum of adverse side effects, carboplatin is better tolerated by patients and can be used at several-fold higher doses than cisplatin. It should be noted that among more than thirty platinum antineoplastic agents studied in clinical trials, only carboplatin has been accepted worldwide [31]. Thus, further investigations are carried out to synthesize "the second generation platinum drugs" with improved toxicological profiles and "third generation drugs" overcoming cisplatin resistance.

Oxaliplatin (**6**) has been approved for clinical use in Europe, China and, for colorectal cancer, the United States. Strategies for developing new platinum anticancer agents include the incorporation of carrier groups that can target tumor cells with high specificity. Also of interest is to develop platinum complexes that bind to DNA in a fundamentally different manner than cisplatin in an attempt to overcome the resistance pathways that have evolved to eliminate the drug. These complexes may provide a broader spectrum of antitumor activity.

Here it is focused on recent efforts to prepare novel Pt(II) complexes using the strategies described above and review some mechanistic insights into the cytotoxic effects of these

complexes. Pt(IV) compounds are also discussed here, although most of them have been recently reviewed [32].

Based on mechanistic findings, coordination chemists are designing and synthesizing new compounds. A novel DNA-binding metal compound with antitumor activity and clinical efficacy must fulfill the following key requirements: (*1*) good intrinsic properties, including saline solubility and enough stability to arrive intact at the cellular target; (*2*) efficient transport properties in blood and through membranes; (*3*) efficient DNA-binding properties but slow reactivity with proteins; (*4*) the ability to differentiate between cancerous and normal cells; and (*5*) activity against tumors that are, or have become, resistant to cisplatin and derivatives. This latter requirement usually implies a structure that is distinct from cisplatin-type species.

CISPLATIN ANALOGS WITH CARRIER GROUPS

Drug delivery systems that can target a tumor site and/or prevent binding to non-pharmacological targets are beneficial in reducing drug toxicity and resistance. An important clinical limitation is resistance to cisplatin. Some types of cancer are known to be intrinsically insensitive to cisplatin treatment, whereas other cancers develop resistance only during chemotherapy. Therefore, the applicability of cisplatin is limited to a relatively narrow range of tumors [15]. The cisplatin-resistance mechanism appears to be multifactorial, with at least three factors identified as potential modulators of cellular resistance: 1) Decreased uptake, as in many cisplatin-resistant cell lines, reduced intracellular accumulation; 2) increased intracellular detoxification by glutathione reacting with platinum drugs forms deactivated adducts that are known to be excreted by a glutathione S-conjugate export pump; and 3) enhanced DNA repair has been observed in some cisplatin-resistant cell lines. Most recently, decreased apoptosis has been associated with drug resistance.

Since the early seminal work in the preclinical and clinical development of cisplatin, several thousand analogs have been synthesized and tested for properties that would enhance the therapeutic index of cisplatin [33]. The mechanisms of cisplatin resistance have been under intense study, and many of the cisplatin analogs synthesized in the past decade have been designed specifically with the hope of overcoming platinum resistance. The mechanism of the cytotoxic activity of platinum complexes has also been studied intensely. Recently synthesized analogs have been designed to interact with DNA in a manner different from cisplatin and carboplatin, with the desire of finding new structures with a superior or wider spectrum of antitumor efficacy. Most recently, water soluble platinum complexes that retain antitumor activity, but that can be effectively absorbed after oral administration, have been synthesized with the goal of improving patient quality of life. A lot of platinum analogs are currently in clinical trials around the world. Some of these analogs only represent attempts to reduce cisplatin toxicity and/or allow administration without forced hydration and diuresis, which carboplatin already does. Others are 'third generation' complexes shown to have limited or no cross-resistance with cisplatin in preclinical studies. They are being tested clinically with particular attention to this highly desirable property.

Polimer-coated micelles can protect platinum from intracellular thiols and result in prolonged circulation time and reduced lymphatic clearance; polymeric compounds tend to accumulate in tumor tissue, enhancing delivery of cisplatin to tumor sites. Recently, a poly(ethylene glycol) micelle was prepared containing a poly(aspartic acid) block to provide the chelating and leaving groups for platinum ions. It displayed a significantly longer circulation time in the bloodstream and higher accumulation in tumors, as demonstrated by an *in vivo* biodistribution assay of Lewis lung carcinoma-bearing mice [34]. Reduced accumulation in kidney was also observed, resulting in low nephrotoxicity, one of the major side effects of cisplatin [35]. Similarly, a series of platinum-polymer conjugates with *trans*-1,2-diaminocyclohexane as spectator ligands was investigated. *In vitro* cell survival tests of there conjugates showed their cytotoxicities to be 10-fold higher than that of cisplatin against Colo320 DM cells, a multi-drug-resistant cell line [36]. New polymeric platinum complexes conjugated to a cyclotriphosphazene have been invented recently by Sohn *et al*. [37-39]. A biocompatible graft co-polymer adduct including a reversibly linked Pt(II) compound has been reported by Bogdanov *et al*. [40].

A homing moiety such as galactose [41, 42] and bile acid [43, 44] for liver and estrogen derivatives for estrogen receptor (ER) positive tissues [45] such as breast have been utilized to achieve tissue specificity. For example, platinum - polymer conjugates with attached galactose exhibited cell-specific cytotoxic activity against human hepatoma cells. The cytotoxicity was suggested to be mediated by galactose receptors expressed on the surface of the cells [41, 42]. Similarly, a platinum-estrogen linked compound showed effective binding both as isolated receptor and in whole cell assays [45]. The antitumor activity of this complex, however, was not evaluated.

Other strategies to improve the antitumor efficacy include the use of porphyrin-platinum conjugates. The porphyrin enhances tumor specificity of the conjugates by its preferable accumulation in neoplastic tissues. In addition, porphyrins are commonly used in photodynamic therapy [4]. Thus, by linking a platinum complex to a porphyrin moiety, additional toxicity against tumor cells can be achieved upon irradiation. Indeed, porphyrin-platinum complexes derivatives with either a hematoporphyrin [46] or a tetraarylporphyrin [47] exhibited enhanced cellular uptake and additional antitumor activity by the photo-induced mechanism.

Because DNA is a key pharmacological target of platinum compounds, DNA-targeting groups such as intercalators were conjugated to the metal over a decade ago [48-50]. Such compounds exhibit enhanced antitumor activity. Renewed interest in platinum complexes with appended intercalators has produced some promising results. A series of *cis*-ethylenediamineplatinum(II) complexes with tethered 9-aminoacridine-4-carboxamides was able to overcome cross-resistance in human ovarian carcinoma cell lines *in vitro* [51]. Altered DNA sequence specificity and increased DNA binding rates compared with those of cisplatin were observed for these intercalator-platinum conjugates [52]. The review [53] summarizes major advancements in the chemistry and biology of platinum-

intercalators from 1984 to 2004, with emphasis being placed on the interplay between chemical structure, mechanism of DNA binding, and biological properties. The majority of DNA-targeted anticancer agents bind through covalent interactions, non-covalent intercalation or groove binding, or hybrid binding modes. The sequence and regiospecificity of these interactions and the resulting structural alterations within the biopolymer play an important role in the mechanism of action of these drugs. DNA-binding proteins and/or DNA-processing enzymes, which also interact with DNA in a sequence- and groove-specific manner, are mediators of the cytotoxic effect produced by these agents. Thus, one major goal in the design of new clinical agents of this type is to produce new types of adducts on DNA, which may lead to unprecedented cell kill mechanisms. Platinum-intercalator conjugates are such a class of hybrid agents acting through a dual DNA binding mode. The platinum center (usually a *cis*-diaminedichloro Pt(II) unit) dominates the DNA adduct profiles in the majority of these species-the result of the metal's tendency to form cross-links in runs of consecutive guanine bases in the major groove of DNA [53]. This paradigm has been broken recently for the first time with the design of cytotoxic platinum-acridinylthiourea conjugates, a class of adenine-affinic minor-groove directed agents [53].

A variety of studies have dealt with several typical classes of ligands suitable for preparation of platinum complexes, showing favorable cytotoxicity against cancer cells.

AMINE PLATINUM COMPLEXES

Most of the well-known platinum anticancer complexes have amines as ligands in their general formula. Some of such active compounds are discussed below in view of their application as cytotoxic agents. Platinum coordination compounds comprising at least one amine ligand and use of such compounds in the treatment of cancer were described by Heffernan *et al*. [54, 55]. Compositions of matter were provided wherein *cis*-platinum (II) moiety having amine substituents is bonded to anionic macromolecular entities [56, 57]. They were effective antitumor agents in mammals. Cisplatin analogs which possess antitumor activity were presented in the following disclosures [58-60]. These inventions related to platinum coordination compounds and to pharmaceutical compositions containing them. Novel platinum(II) complexes of 1,2-diaminocyclohexane, 1-aminomethylcyclooctylamine and 1,2-diamino-2,4-dimethylpentane were provided [61, 62]. Such complexes were of use in inhibiting the growth of certain mammalian tumors.

A series of structurally related mixed-amine dichloroplatinum complexes (*cis*-coordinated with amine and various diphenylmethylamines and 1,2-diphenylethylamines) has been prepared [63]. Cytotoxicity was determined in two human breast cancer cell lines (MDA-MB-231 and MCF-7) and one human ovarian cancer cell line (SK-OV-3). There is no apparent relationship between the hydrophobicities of the compounds and their cytotoxic potencies.

The asymmetric platinum complexes *cis*-Pt(LL´)Cl_2 (L = NH_3, L´ = CH_3NH_2, $(CH_3)_2NH$, $C_2H_5NH_2$ and $(C_2H_5)_2NH$ and LL´ = N,N-dimethylethylenediamine) were synthesized and their cytotoxic effects have been measured using L-1210 cells [64]. The IC_{50} values of the asymmetric platinum complexes are almost comparable to the corresponding value of *cis*-Pt$(NH_3)_2Cl_2$. The amounts of platinum taken into the L-1210 cells are little affected by the alkylamino substitution. The results suggest that the bifunctional platinum binding to the target molecule may be responsible for the cytotoxicity.

Structure-cytotoxicity relationships for six alicyclic *cis*-(NH_3)(R-NH_2)Cl_2Pt(II) complexes, where R=C_3H_5, C_4H_7, C_5H_9, C_6H_{11}, C_7H_{13} and C_8H_{15} (complexes abbreviated C3, C4, C5, C6, C7 and C8, respectively), were evaluated against four sensitive (L1210/0, A2780, FSaIIC and Colon 26), two cisplatin-resistant (L1210/DDP and 2780CP) and two tetraplatin-resistant (L1210/DACH and 2780TP) murine and human tumor cell lines [65]. The studies demonstrated that in general, the structure of C6 was optimal within the homologous series for cytotoxic potency against these tumor models. Biochemical pharmacological studies indicated that the greater sensitivity of cells to C6 could be correlated with their low tolerance to DNA damage induced by this homolog. These results provide evidence for the alicyclic ring size as a structural determinant of DNA damage tolerance and antitumor activity in sensitive and resistant tumor cells.

A new series of highly water-soluble aminoalkanol platinum(II) complexes have been synthesized and characterized [66]. Preliminary *in vitro* and *in vivo* screening tests for antitumor activities of these complexes against L1210 murine leukemia were performed. In general, these compounds were far less cytotoxic than cisplatin and possessed only a moderate degree of antitumor activity.

The synthesis, physical properties, antitumor activity, structure-activity relationships, and nephrotoxicity of a series of [2-substituted-4,5-*bis*(aminomethyl)-1,3-dioxolane]platinum(II) complexes were described [67]. Most members of this series showed the excellent antitumor activity against murine L1210 leukemia cells transplanted in mice and were superior to cisplatin and carboplatin. The (malonato) platinum(II) complex and the (glycolato)platinum(II) complexes were selected for further studies based on the greater *in vivo* and *in vitro* antitumor activity and desirable physical properties. Novel platinum complexes of glycolic acid type having potent antitumor activity and high water solubility with low nephrotoxicity and a pharmaceutical composition containing one or more said compounds together with one or more carriers, diluents or excipients were provided [68, 69]. Malonato platinum coordination compounds have been presented by Cleare *et al*. [70, 71]. Amino-substituted-malonato platinum(II) complexes with high antitumor activity in mice, chemical stability and solubility in aqueous fluids for i.v. administration have been synthesized [72]. The invention provided also a preferred method for their preparation. Disclosed were water-soluble salts of the known coordination compounds 2-hydroxymalo-natodiammine platinum (II), 2-hydroxymalonato(1,2-diamino-cyclohexane) platinum (II) and 2-hydroxymalonato (1,1-diaminomethyl-cyclohexane)platinum (II) [73]. The novel sodium and ammonium salts of the present invention possess high water-solubility, thus allowing intravenous dosage forms to be prepared.

Amine/amine dichloroplatinum(II) complexes have been evaluated for structure-activity relationship in wild-type L1210/0, 185-fold cisplatin-resistant L1210/DDP and 39-fold tetraplatin-resistant L1210/DACH murine leukemia cells [74]. The mechanism of resistance in these cell lines is multifactorial, with DNA repair playing a dominant role. The amines incorporated in the complexes were selected from the alicyclic, heterocyclic and isoaliphatic class, and contained 3, 4, 5 or 6 carbon atoms. The studies demonstrated that ascending each of the homologous series increased cytotoxic potency against sensitive and cisplatin-resistant cell lines and, more importantly, reduced the cross-resistance of cisplatin-resistant cells. In L1210/DACH cells, the potency remained similar across the alicyclic and isoaliphatic series, while there was a consistent decrease in activity in the heterocyclic series for each stepwise increase in amine size [74]. Furthermore, the relationship between structure and resistance factor in L1210/DACH cells was in direct contrast to that seen in the L1210/DDP model in that the factors increased on ascending the homologous series stepwise. The lower members of the alicyclic and heterocyclic series and cisplatin had comparable resistance factors in the L1210/DACH line; higher members displayed resistance factors that were comparable to or greater than that of tetraplatin [74]. These results provide evidence for amine class and size as factors that can modulate the potency and capacity of amine/amine platinum complexes to circumvent cisplatin or tetraplatin resistance.

Cis-diamineplatinum(II) orthophosphate complexes [75] and *cis*-diamineplatinum(II) organophosphate complexes [76] were prepared. The resulting complexes possess pronounced antitumor activity in mice and low toxicity; consequently, they have high therapeutic indices. There were described platinum-polyhydroxylated amine compounds which exhibit antitumor activity in mammalian species [77]. The products were highly soluble in aqueous solutions and they may be administered either orally or in parenteral form. Platinum complexes of polyhydroxylated alkylamines and 2-polyhydroxylated alkyl-1,2-diaminoethanes were useful for inducing regression and/or palliation of cancer diseases in mammals [78]. Yolles S. has reported a therapeutic coordination compound, the reaction product of a hydroxy quinone and a *cis*-platinum (II) compound substituted with chloro ligands and ammonia or derivatized ammonia ligands [79].

DIAMINE PLATINUM COMPLEXES

Diamine platinum complexes have attracted significant attention for many years. The invention [80] relates to a novel platinum-diamine complex with an excellent antitumor activity and a lower renal toxicity than cisplatin. Platinum(II) complexes comprising platinum(II), a diamine, a ligand of D-gluconic acid and an inorganic or organic anion or ligand, and effecting superior antineoplastic activity at a smaller dose were presented [81]. Processes for the preparation of platinum-diamine complexes for the treatment of malignant tumors were described and exemplified [82-84]. The disclosure [85] described platinum complexes of heterocyclic-1,2-diamines, which possess the property of inhibiting the growth of tumors in mammals. The inventions [86, 87] provided novel bis-naphthalimides characterized by having a linker containing a heteroatom, their preparation, pharmaceutical compositions thereof, and various methods of using the bis-naphthalimides. The bis-naphthalimides have exceptional DNA binding properties and demonstrate cytotoxicity in both *in vitro* and *in vivo* tumor models, in particular, against melanoma.

Recently, thirteen newly synthesized or resynthesized diamine-platinum(II) complexes were characterized, and their cytotoxic activities were tested on parental and resistant ovarian cancer cell lines [88]. They represent models of conjugates between biologically active vectors and cytotoxic Pt(II) moieties within the "drug targeting and delivery strategy". The quantitative structure-activity relationship approach provides simple regression models able to predict $\log(1/IC_{50})$ of diamine-platinum(II) complexes on both parental and resistant ovarian cancer cell lines. Several new platinum(II) complexes were synthesized from a common triphenylethylene precursor using various diamines [89]. The cytotoxicity of the compounds, evaluated on human breast cancer cell lines (MCF-7 and MDA-MB-231), was greatly influenced by the nature of the diamine ligand. The synthesis of a pair of enantiomeric Pt(II) complexes, [Pt(*R*,*R*-eap)Cl_2] and [Pt(*S*,*S*-eap)Cl_2] (eap = N,N-diethyl-2,4-pentanediamine) was described [90]. The *in vitro* cytotoxicity of each of the enantiomers toward murine leukemia and human bladder tumor cells has been measured. The *R,R* enantiomer was found to be more active in the leukemia cells. In the bladder tumor cell line, no significant difference in activity was found. New [Pt(diamine)$_2$]CBDCA complexes of 1,4-diaminebutane and 1,3-diaminepropane (CBDCA = 1,1-cyclobutanedicarboxylate) have been synthesized [91]. Cytotoxicity data show that the complexes exhibit remarkable cytotoxic properties. Thus, in contrast with carboplatin, the compounds, which also contain the CBDCA ligand, are able to circumvent cisplatin resistance in several tumor cells.

A number of new Pt(II) complexes is described having the general formula PtCl(2)(LL), where LL is a chelating diamine ligand [92]. Ligands LL were chosen as D,L-2,3-diaminopropionic acid and its ethyl ester, and D,L-2,4-diaminobutyric acid and its ethyl ester. The compounds were characterized using analytical and spectroscopic methods. The influence of the size of the chelate ring and its functionalization on the biological properties was studied. It was demonstrated by circular dichroism (CD) that the effects on the secondary structure of DNA induced by the four complexes are different. The interaction takes place at the N7 position of the purine bases, as shown by NMR studies. The survey from the literature shows that the effects of 2,3-diaminopropionic acid and 2,4 diaminobutyric acid on base-stacking have not been reported so far. The platinum complexes of 2,3-diaminopropionic acid and 2,4-diaminobutyric acid are able to form intrastrand adducts with DNA and to distort the double helix by changing the base stacking. The ethyl ester derivatives uncoil the DNA from the B form to the C form. The interactions with 5'-GMP and DNA were compared with their antitumor activity. The platinum complexes of diaminocarboxylic acids exhibit cytotoxic activity in the A431, HeLa, and HL-60 cell lines in a dose- and time-dependent manner.

A series of intercalator-tethered platinum(II) complexes $PtLCl_2$ have been prepared, where L are the diamine ligands

N-[2- [(aminoethyl)amino]ethyl]-phenazine-1-carboxamide, *N*-[3-[(2-aminoethyl)amino]propyl]-phenazine-1-carboxamide, *N*-[4-[(2-aminoethyl)-amino]butyl]-phenazine-1-carboxamide and *N*-[5-[(aminoethyl)amino]pentyl]-phenazine-1-carboxamide [93]. The platinum(II) complexes where the polymethylene linker chain contains three, four or five carbon atoms are considerably more cytotoxic against murine P388/W than either cisplatin or the metal-free ligands themselves. Four platinum(II) aminobenzamidine complexes have been prepared and tested for their ability to interact with the nicked and closed circular forms of the pUC8 plasmid DNA [94]. The results show that the complexes of formula $[Pt(LH)_2Cl_2]2X$ have a *cis*- geometry with an amino-Pt bonding, where L is either p- or m-aminobenzamidine and where 2X is $2Cl^-$ or $PtCl_4^{2-}$. The synthesized compounds were assayed for antitumor activity *in vitro* against colon (CX-1), lung (LX-1), and mammary (MX-1) human tumor cells. The results show that these complexes inhibited the multiplication of the tumor cells and that they show higher specificity for lung cells.

Diaminocyclohexane platinum complexes and their use in the treatment of tumors were widely studied [95-98]. The invention [99] comprised a water-soluble square-planar *cis*-platinum(II) four-coordinate complexes of 1,2-diaminocyclohexane with antitumor activity. The disclosure [100] described hydroxylated 1,2-diaminocyclohexane platinum complexes which possess the property of inhibiting the growth of tumors in mammals. Organoplatinum complexes having antineoplastic activity against the L1210 mouse leukemia test system and having sufficient water-solubility for use in aqueous i.v. fluids have been presented [101]. The organoplatinum complexes include malonato (1,2-diaminocyclohexane) platinum (II) (**9**), hydroxymalo-nato (1,2-diaminocyclohexane) platinum (II), dinitrato (1,2-diaminocyclohexane) platinum (II), sulfato (1,2-diaminocyclohexane) platinum (II), and hydroxonitrato (1,2-diaminocyclohexane) platinum (II). 4-Carboxyphthalato(1,2-diaminocyclohexane)-platinum(II) complex was also prepared [102]. An organic complex of platinum, *cis*-isocitrato (1,2 diaminocyclohexane) platinum (II) having improved anti-tumor activity and water-solubility was presented [103]. The complex is administered in the form of a pharmaceutical composition. The invention [104] related to compounds that are complexes of diaminocyclohexane platinum glucuronic acid halide and the use of these compounds as antitumor agents for lower animals. (N-Phosphonacetyl-L-aspartato)(1,2-diaminocyclchexane) platinum(II) [105] and (2,2,-*bis*(aminomethyl)-1,3-propanediol-N,N')platinum complexes [106] were presented. Diastereomeric mono- and di-substituted diaminocyclohexane compounds and novel antineoplastic Pt(II) complexes derived from the stereoisomers were described [107]. Mono- and di-hydroxyl substitution on the cyclohexane ring renders the organoplatinum complex relatively more water soluble, thereby facilitating intravenous administration. The Pt(II) complexes of the invention are less nephrotoxic than cisplatin and are readily excreted via the kidney due to their enhanced water solubility. In a composition aspect, the present invention encompasses novel pharmaceutical compositions comprising the novel Pt(II) complexes in an amount sufficient to have an antineoplastic effect in an animal or patient. Novel 1,4-diaminocyclohexane platinum II and platinum IV complexes were synthesized, which show activity *in vivo* against tumor models resistant to cisplatin and tetraplatin [108]. The novel complexes include the sulfato, chloro, hydroxyl, acetato methylmalonato, tartronato and 1,1-cyclobutane dicarboxy-lato as leaving ligands and 1,4-DACH amine as non-leaving ligands. The complexes showed good *in vitro* cytotoxic activity against murine leukemia L1210/0 and human ovarian A2780 cell lines. High *in vivo* activity was shown against L1210 leukemia cells and against cisplatin resistant L1210/DDP and tetraplatin resistant L1210/DACH. Excell-ent antitumor activity against M5076 was also exhibited by the new complexes. Additionally, the platinum complexes did not elicit any indication of nephrotoxicity in the *in vivo* tests. Seven new water-soluble cationic complexes of general formula [Pt(2-pyc)(N-N)]+ (where N-N is $2NH_3$, ethylene- diamine, 1,2-diaminopropane, 1,3-diaminopropane, (+/-) *trans*-1,2-diaminocyclohaxane (DACH), 2,2'-dipyridylamine or 1,10-phenanthroline, and 2-pyridinecarboxylate anion) have been prepared [109]. These compounds inhibit the growth of P388 lymphocytic leukemia cells. They show ID_{50} value comparable to cisplatin. The gel electrophoresis studies suggest that these complexes bind to DNA, and this binding leads to a conformational change in DNA. Novel platinum(II) complexes of 1,2-diaminocyclohexane, 2,2'-bipiperidine, 1,2-diamino-2,4-dimethylpentane, 1,2-diaminocyclooctane, 3,4-diamino-2,5-dimethylhexane and 1-aminomethyl-cyclooctylamine were provided [110]. Such complexes are of use in inhibiting the growth of certain mammalian tumors. New complexes of formula [Pt(NN)(XO_3)] (where NN is 2,2'-bipyridine, 1,10-phenanthroline, 2,2'-dipyridylamine, ethylenediamine or (+/-)*trans*-1,2-diaminocyclohexane, and XO_3^{2-} is SeO_3^{2-} or TeO_3^{2-}) have been synthesized [111]. These complexes inhibited the growth of P 388 lymphocytic leukemia cells.

Another class of diamine compounds with antitumor activity is the group of ethylenediamine-based platinum complexes (**10**). A series of dichloro(ethylenediamine)-type platinum complexes bearing ester-, amide- and ether-bonded alkyl straight chains was prepared as a model for the prodrug of *cis*-diamminedichloroplatinum [112] and the cytotoxic activity of the complexes against the S-180 cell line was investigated. Schonenberger *et al*. presented antitumor active (1,2-diphenylethylenediamine)-platinum (II) complex compounds [113]. Ring-substituted diaqua(1,2-diphenyl-ethylenediamine)platinum(II) sulfate was prepared [114] and mode of binding to the DNA was studied. [1,2-*bis*(4-hydroxyphenyl)ethylenediamine] dichloroplatinum (II) complexes with one substituent in the 2-position (CH_3, CF_3, F, Cl, Br, I: meso- and d,l-1-$PtCl_2$, meso-(3-5)-$PtCl_2$, meso-(7 and 8)-$PtCl_2$) or two substituents in the 2,6-positions (CH_3, Cl: meso-2-$PtCl_2$, meso- and d,l-6-$PtCl_2$) in both benzene rings were synthesized and tested for estrogenic and cytotoxic activities [115]. Two complexes (meso-6-$PtCl_2$ and meso-7-$PtCl_2$) possess both effects. A series of isomeric [1,2-*bis*(difluorophenyl)ethylenediamine] dichloroplatinum (II) complexes and cisplatin were tested on the P388 leukemia and on the murine mammary carcinoma for evaluating antineoplastic activity against breast cancer *in vivo* [116]. The activity of 1, 2-*bis*(2, 6-difluoro-3-hydroxy-

phenyl)ethylenediamine] platinum(II) complexes against breast cancer was investigated [117, 118]. Cytotoxic effects, which are poorly pronounced in experiments on several breast cancer cell lines (e.g. MCF-7), do not significantly contribute to the antibreast cancer activity of the compounds. Ethylenediamine platinum(II) and 1,2-diaminocyclohexane platinum(II) pyrophosphate complexes with pronounced anti-tumor activity and low toxicity were prepared [119]. The search for platinum (II)-based comp-ounds with improved therapeutic properties prompted researchers to design and synthesize a new family of water-soluble, third generation *cis*-diaminedichloroplatinum (II) complexes linked to uracil and uridine. Synthesis and antitumor evaluation of *cis*-(1,2-diaminoethane) dichloropla-tinum (II) complexes linked to 5- and 6-methyleneuracil and -uridine analogs have been presented [120]. The cytotoxic activities were evaluated against three cell lines (FM-3A, P-388 and J-82) and none of the synthesized compounds showed any significant activity. A series of acridine-2- and -4-carboxamide-linked analogs of (1,2-diaminoethane) dichloroplatinum(II) has been prepared and evaluated for biological activity against several tumor cell lines *in vitro* and *in vivo* [121]. The platinum complexes were generally more cytotoxic than the corresponding ligands against wild-type P388 leukemia cells *in vitro*, with acridine-4-carboxamide complexes being the more effective. In contrast to cisplatin and (1,2-diaminoethane) dichloroplatinum(II), the complexes were equally active *in vitro* against both wild-type and cisplatin-resistant P388 lines. The 4-carboxamide complexes showed high levels of *in vivo* activity against wild-type P388 using a single-dose protocol, and one compound was also significantly active *in vivo* in a cisplatin-resistant line, against which cisplatin and (1,2-diamino-ethane)dichloroplatinum(II) are inactive.

Platinum complexes of amino acids and of carboxylic acids have attracted much interest. The binding studies of several complexes $[Pt(NN)(AA)]^+$ (where NN is 2,2'-bipyridine or 1,10-phenanthrodine, and AA is an anion of amino acid glycine, L-alanine, L-leucine, L-phenylalanine, L-tyrosine, L-tryptophan, L-valine, L-proline, or L-serine) with calf thymus DNA have been carried out and screened for cytotoxicity in P388 lymphocytic cells [122]. The mode of binding of the above complexes to DNA suggests the involvement of the hydrogen bonding between them. New platinum(II) complexes of formula [Pt(dipy)(AA)]+ (where dipy is 2,2'-dipyridylamine, AA is an anion of glycine or L-alanine) have been synthesized and characterized with amino acids binding as bidentate ligands [123]. Of the above complexes, the L-alanine complex shows ID_{50} values against P388 lymphocytic leukemia cells lower than *cis*-diaminedichloroplatinum(II), whereas the glycine complex shows ID_{50} values higher than cisplatin. The interaction of calf thymus DNA with the above complexes shows significant spectral changes in the presence of [Pt(dipy)(gly)]Cl and [Pt(dipy)(ala)]Cl and the mode of binding between these complexes and DNA seems to be noncovalent. Six new Pt(II) complexes are described having the general formula $PtCl_2$(LL), in which LL is a chelating diamine ligand bearing an amino acid as substituent [124]. The amino acids chosen are l-alanine and its methyl ester, and l-phenylalanine. The influence on the biological properties of the size of the chelate ring and the structure of the amino acid substituent has been studied. In all cases, the interaction takes place at the N7 position of the purine bases. The complexes with l-alanine and l-phenylalanine exhibit cytotoxic activity in HeLa and HL-60 cell lines, in a dose- and time-dependent manner. No cytotoxic activity of the methyl ester derivatives have been determined because of their low solubility in aqueous solution. Three novel Pt(II) complexes [PtLCl] (where L = glycine-*N'*-8-quinolylamide, l-alanine-N'-8-quinolylamide and *N*-(tert-butoxycarbonyl)-l-methionine-N'-8-quinolylamide) have been synthesized and characterized [125]. These complexes have been tested against a wide range of tumor cell lines including BEL-7402, HCT-116, SPC-A4, MOLT-4, P388, HL-60, A-549, SGC-7901, MKN-28, and HO-8910. Amundsen and Stern described ascorbate complexes of platinum(II) coordinated to ammonia, a monodentate amine ligand or a bidentate amine ligand as, for example, an alkylamine, an alkylenediamine or a cycloalkylamine [126, 127]. The complexes are useful in the treatment of malignant tumors in animals and they are characterized by high solubility in water and low toxicity. *cis*-Platinum(II) amine lactate complexes also were prepared [128]. These complexes possess pronounced antitumor activity and low toxicity and thus have high therapeutic indices. They are also highly soluble in water. Novel platinum complex compounds of amines with dibasic acids which have antineoplastic activity and method of using the compounds to treat tumors in mammals were presented [129]. Platinum complexes of aliphatic tricarboxylic acids useful for inducing regression and/or palliation of cancer diseases in mammals were also described [130]. Turkevich *et al*. [131] prepared complexes of square planar platinum(II) compounds and *N*-methyl glucamine, which were effective antitumor agents.

trans- PLATINUM COMPLEXES

Hitherto, it has been generally accepted as a paradigm of the biochemical pharmacology of platinum antitumor drugs that a *cis*-configuration of the leaving groups is necessary for antitumor activity of platinum compounds. However, it has been recently observed that certain *trans*-platinum complexes have both *in vitro* and *in vivo* antitumor activity. Platinum complexes with distinctively different DNA binding modes from that of cisplatin may provide higher antitumor activity against cisplatin-resistant cancer cells. Among such complexes are those with amine ligands having *trans* stereochemistry. The *trans* analog of cisplatin, *trans*-diaminedichloroplatinum(II) (*trans*-DDP), is inactive, but its inertness may originate in part from kinetic instability and consequent susceptibility to deactivation. Substitution of one or both ammine ligands in *trans*-DDP with more bulky ligands can retard ligand substitution reactions of the two chloride ions, thereby reducing undesired reactions between platinum and cellular components and facilitating its interaction with DNA. Discovery of these properties has stimulated the development of additional complexes with *trans* geometry. Several classes of *trans* platinum complexes have been characterized, showing favorable cytotoxicity against cancer cells, especially cisplatin-resistant cells [132]. The spectator ligands in these complexes can be classified into three groups: planar aromatic amines, alkylamines and iminoethers. These compounds in general are more active

than their *cis* analogs against cisplatin-resistant cell lines. A large body of novel platinum complexes, in both the *cis*- and *trans*-forms, with various donor ligands, e.g. beta-carboline alkaloids, pyrazoles, DMSO, ferrocenylphosphines, etc. have been tested for their antitumor activity against number of fluid suspension (P388, L1210, K562, and Raji) and solid tumor (KB, T47D, SW948, HeLa, A549, L929, Hep-2, RD) cell lines [133]. Remarkable cytotoxic effects against these cell lines were observed by some of these complexes. These results are preliminary, but encouraging, since they are in a disagreement with the previous studies that *cis*-isomers are more active than *trans*-ones; the complexes which have not received the required attention from the vast number of researchers in this field. Water soluble *trans*-platinum complexes with antitumor activity and method of using them were presented [134]. These new water soluble 1,2 - diaminocyclohexane (DACH) platinum complexes demonstrate a broader spectrum of activity, increased antitumor activity, and reduced toxicity in mouse models. Other highly water soluble analogs show a distinct superiority over existing platinum complexes by virtue of overcoming more than one mechanism of resistance. In animal models, they have been shown to be active against cisplatin and tetraplatin resistant cell lines (DDP-resistant L1210, DACH-resistant L1210, and M5076). No other analog appears to have activity against both resistant cell lines.

The discovery in the 1990s of several *trans*-Pt complexes with *in vitro* and *in vivo* activity against tumor cells sensitive and/or resistant to cisplatin has forced the re-evaluation of the structure-activity relationships for platinum antitumor drugs. Because the determinant factors of cytotoxic activity of *trans*-platinum complexes do not follow the same patterns as those found for cisplatin and its analogs, the differences in cellular and biochemical pharmacology between *trans*-platinum antitumor complexes and cisplatin might be systematically exploited to design novel *trans*-platinum complexes with a clinical profile complementary to that of cisplatin and related analogs. Therefore, there may exist a novel molecular rationale for new platinum antitumor drugs development in the twenty-first century [135]. The interest in *trans*-amine Pt compounds with amines is very rapidly growing. The global modification of mammalian and plasmid DNAs by novel platinum compounds, *trans*-$[PtCl_2(NH_3)(Am)]$, where Am=2-methylbutylamine or sec-butylamine was investigated [136]. These modifications were analyzed in the context of the activity of these new compounds in several tumor cell lines including those resistant to antitumor cisplatin. The results showed that the replacement of one amine group by 2-methylbutylamine or sec-butylamine ligand in clinically ineffective transplatin resulted in a radical enhancement of its activity in tumor cell lines so that they are more cytotoxic than cisplatin and exhibited significant antitumor activity including activity in cisplatin-resistant tumor cells. Importantly, this replacement also markedly altered DNA binding mode of transplatin and reduced the efficiency of repair systems to remove the adducts of the new analogs from DNA. The results support the view that one strategy to activate *trans* geometry in bifunctional platinum(II) compounds including circumvention of resistance to cisplatin may consist in a chemical modification of the ineffective transplatin, which results in an increased efficiency to form DNA interstrand cross-links. The synthesis and chemical characterization of three new *trans*-platinum complexes of structural formula *trans*-$[PtCl_2(amine)(isopropylamine)]$ (amine = n,n-dimethylamine, propylamine, and butylamine), were described [137]. Cytotoxicity tests in tumor cell lines sensitive to *cis*-DDP (Jurkat, Hela, and Vero) and also in tumor cell lines overexpressing ras oncogenes and resistant to *cis*-DDP (HL-60 and Pam 212-ras) show that the complexes have higher cytotoxic activity than cisplatin. These results suggest that *trans*-$PtCl_2$ complexes with asymmetric aliphatic amines may be considered a new class of biologically active *trans*-platinum drugs. Perez *et al*. reported the synthesis, characterization and cytotoxic activity against ras-transformed cells of several *trans*-$[PtCl_2LL']$ complexes, where L and L' are asymmetric aliphatic amines (L = dimethylamine and butylamine, L' = isopropylamine) [138]. The results show that the compounds *trans*-$[PtCl_2(isopropylamine)(dimethylamine)]$ and *trans*-$[PtCl_2(isopropylamine)(butylamine)]$ kill Pam 212-ras cisplatin resistant cells through apoptosis induction. Farrell *et al*. described efforts to elucidate the cellular mechanism of action of a novel *trans*-platinum compound, *trans*-(dichloroamminethiazole)platinum(II) (ATZ), which demonstrates antiproliferative and cytotoxic effects against both MCF-7 human breast and A2780 human ovarian carcinoma cells in culture [139]. Binding of ATZ to DNA was similar for the two cell lines. The invention presented by Farrell [140] provided recently a method for enhancing the water solubility of cytotoxic trans-platinum complexes. The invention also provided a method for killing tumor cells, and a method for the treatment of tumors by the administration of a cytotoxic platinum coordination complexes.

Novakova *et al*. have shown that the replacement of amine ligands by iminoether in transplatin (*trans*-$[PtCl_2(NH_3)_2]$) results in a marked enhancement of its cytotoxicity so that it is more cytotoxic than its *cis* isomer and exhibits significant antitumor activity, including activity in cisplatin-resistant tumor cells [141]. In addition, they have also shown that this new *trans* compound (*trans*-$[PtCl_2(E\text{-}iminoether)_2]$) (**11**) forms mainly monofunctional adducts at guanine residues on DNA, which is generally accepted to be the cellular target of platinum drugs. In order to shed light on the mechanism underlying the antitumor activity of *trans*-$[PtCl_2(E\text{-}iminoether)_2]$ they examined oligodeoxyribonucleotide duplexes containing a single, site-specific, monofunctional adduct of this transplatin analog. The results indicate that major monofunctional adducts of *trans*-$[PtCl_2(E\text{-}iminoether)_2]$, locally distort DNA, bend the DNA axis by 21 degrees toward the minor groove, are not recognized by HMGB1 proteins and are readily removed from DNA by nucleotide excision repair (NER). In addition, the monofunctional adducts of *trans*-$[PtCl_2(E\text{-}iminoether)_2]$ readily cross-link proteins, which markedly enhances the efficiency of this adduct to terminate DNA polymerization by DNA polymerases *in vitro* and to inhibit removal of this adduct from DNA by NER. It is suggested that DNA-protein ternary cross-links produced by *trans*-$[PtCl_2(E\text{-}iminoether)_2]$ could persist considerably longer than the non-cross-linked monofunctional adducts, which would potentiate toxicity of this antitumor platinum compound toward tumor cells

sensitive to this drug. Thus, *trans*-[$PtCl_2$(*E*-iminoether)$_2$] represents quite a new class of platinum antitumor drugs in which activation of *trans* geometry is associated with an increased efficiency to form DNA-protein ternary cross-links, thereby acting by a different mechanism from 'classical' cisplatin and its analogs. In order to widen knowledge on antitumor *trans*-[$PtCl_2$(iminoether)$_2$] complexes, Coluccia *et al*. have synthesised new derivatives, which differ in the configuration of the iminoether ligands [142]. The substitution of iminoethers for ammines determines a major lipophilicity and cellular uptake of the platinum drug. The complexes were active *in vivo* against the murine P388 system. The results confirm the antitumor activity of *trans*-[$PtCl_2$(iminoether)$_2$] complexes, and indicate that the configuration of the iminoether ligands may affect the pharmacological properties of this class of complexes. Cytotoxicity and DNA binding mode of new platinum-iminoether derivatives with different configuration at the iminoether ligands have been investigated by Boccarelli *et al*. [143]. The study gives new insight into the mechanism of action of *trans* platinum-iminoether complexes, enabling for the first time comparison between different ligand isomers.

Very recently, a series of *trans*-Pt(II)-piperazine compounds were reported that displayed significant cytotoxicity against cisplatin-resistant ovarian cancer cells [144]. These cationic complexes are more water soluble and bind more rapidly to DNA compared with cisplatin and *trans*-DDP, whereas their interactions with two cellular proteins, ubiquitin and myoglobin, are much slower than those of cisplatin and their neutral analogs. A series of new platinum complexes with homopiperazine have been synthesized and characterized [145]. The complexes are of the type: [PtLX] (where L = homopiperazine, 1-methyl-homopiperazine or 1,4-dimethylhomopiperazine, and X = 1,1-cyclobutanedicarboxylato (CBDCA), or methylmalonato ligand). Some of these synthesized complexes have good *in vitro* cytotoxic activity against the cisplatin-sensitive human ovarian A2780 (IC_{50} = 0.083-17.8 μM) and the isogenic cisplatin-resistant 2780CP (IC_{50} = 20.1-118.1 μM) cell lines. The synthesis, chemical characterization, and interaction with cells of new sterically hindered *trans*- and *cis*-diaminedichloroplatinum(II) complexes are described [146]. The amine ligands include monofunctional piperidine and piperazine. *In vitro* evaluation in OV-1063 and C-26 tumor cells revealed that replacing one NH_3 of the inactive transplatin by an aromatic planar ligand (4-picoline) or by an aliphatic nonplanar heterocyclic ligand (piperidine) or replacing both NH_3 groups with these ligands significantly increases the cytotoxic activity of the complexes. In contrast, replacing one NH_3 of the *cis* isomer by an aromatic planar ligand or by an aliphatic amine lowered their cytotoxiciy in comparison to cisplatin. Cell penetration and Pt-DNA adduct formation were also evaluated. The results suggest that these novel mixed nonclassical *trans*-Pt(II) complexes are biologically and mechanistically distinct from known Pt complexes and deserve evaluation of their efficacy in tumor-bearing animals. A series of new platinum complexes of the type [Pt(mmap)X] (where mmap, 1-methyl-4-(methylamino) piperidine and X, 1,1-cyclobutanedicarboxylato (CBDCA), oxalato, malonato, methylmalonato, dimethylmalonato, ethylmalonato, diethylmalonato or 2,3-naphthalene dicarboxylato (NDCA)) have been synthesized and characterized [147]. The complexes were evaluated for their cytotoxic potential against the sensitive A2780 tumor model and cisplatin-resistant clone derived *in vitro* from potential cells. Nguewa *et al*. have evaluated the cytotoxic properties against the protozoan *Leishmania infantum* of four water soluble cationic *trans*-Pt(II)Cl_2 compounds containing as inert groups NH_3 and piperazine, 4-picoline and piperazine, n-butylamine and piperazine, and NH_3 and 4-piperidino-piperidine [148]. In contrast to *cis*-diamminedichloroplatinum(II), binding of these compounds to calf thymus DNA induces conformational changes more similar to those of *trans*-diamminedichloroplatinum(II) that may be attributed to denaturation of the double helix.

MULTINUCLEAR PLATINUM COMPLEXES

Another class of platinum complexes that bind to DNA in a manner different from that of cisplatin is multinuclear complexes. These compounds contain two, three or four platinum centers with both *cis* and/or *trans* configurations. Polyamines are generally utilized as linkers to connect the platinum centers. A representative trinuclear complex, BBR3464 (**8**), has entered a phase II clinical trial and exhibits activity against pancreatic, lung and melanoma cancers. Furthermore, this complex is effective against human tumor mouse xenografts containing mutant p53 gene [149]. The p53 gene is a tumor suppressor encoding a nuclear phosphoprotein that mediates cellular response towards genotoxic stress including cisplatin treatment [150]. Over 60% of human cancers are characterized by nonfunctional p53. Gatti *et al*. investigated the cellular effects of exposure to two platinum compounds (cisplatin and BBR3464), in the osteosarcoma cell line, U2-OS, carrying the wild-type p53 gene and capable of undergoing apoptosis or cell cycle arrest in response to diverse genotoxic stresses [151]. Taken together, the results indicate that cellular response (i.e. apoptosis or growth arrest) to different genotoxic lesions is associated with a specific recognition of DNA damage and different p53-mediated signaling pathways. Multinuclear platinum complexes could be regarded as useful tools for investigating the p53-mediated process of cell cycle arrest in response to DNA damage. Therefore, the activity of BBR3464 against cells with mutant p53 renders it a potent anticancer drug. BBR3464 is a highly charged 4^+ species. It binds to DNA rapidly, forming various long-range interstrand and intrastrand cross-links. The interstrand adducts account for ~20% of the BBR3464-mediated DNA adducts. Recent mechanistic studies suggest that the interstrand cross-links, rather than intrastrand adducts, are important to the antitumor activity [152, 153]. The hypersensitivity of BBR3464 to tumors with mutant p53 was investigated by a p53 binding assay [154, 155], suggesting that BBR3464 bypasses p53-mediated pathways. Antitumour efficacy studies of BBR3464 were performed in a panel of human tumor xenografts refractory or poorly responsive to cisplatin [156]. The platinum compound exhibited efficacy in all tested tumors and an impressive efficacy (including complete tumor regressions) was displayed in two lung carcinoma models, CaLu-3 and POCS. In contrast to cisplatin, the above triplatinum complex was very effective as an inducer of apoptosis in a lung carcinoma cell line carrying mutant p53. Manzotti *et al*. describe the

preclinical evaluation of BBR3464 in a series of human tumor cell lines and tumor xenografts, with special emphasis on tumor types known to be resistant to cisplatin [157]. In a panel of seven human tumor cell lines naturally resistant to cisplatin (three ovarian and four melanomas), BBR3464 was extremely potent with IC_{50} values at least 20-fold lower than cisplatin. In an attempt to examine the cellular basis of the preclinical antitumor efficacy of BBR3464 in the treatment of cisplatin-resistant tumors, Perego *et al*. [158, 159] have performed a comparative study of cisplatin and BBR3464 in a human osteosarcoma cell line (U2-OS) and in an *in vitro* selected cisplatin-resistant subline (U2-OS/Pt). A marked increase of cytotoxic potency of BBR3464 in comparison with cisplatin in U2-OS cells and a complete lack of cross-resistance in U2-OS/Pt cells were found. A detailed analysis of the cisplatin-resistant phenotype indicated that it was associated with reduced cisplatin accumulation, reduced interstrand cross-link (ICL) formation and DNA platination, microsatellite instability, and reduced expression of the DNA mismatch repair protein PMS2. Despite BBR3464 charge and molecular size, in U2-OS and U2-OS/Pt cells, BBR3464 accumulation and DNA-bound platinum were much higher than those observed for cisplatin. Study of the cellular pharmacology of the dinuclear platinum complexes, BBR3005 and BBR3171 and the trinuclear platinum complex, BBR3464 was undertaken in wild type and cisplatin-resistant L1210 murine leukemia cell lines [160]. All complexes are potent cytotoxic agents against the wild type cell line. Only BBR3464 shows enhanced activity against the cisplatin-resistant cell line following a brief exposure. The cisplatin-resistant cell line is relatively tolerant of DNA adducts induced by both cisplatin and BBR3464, but BBR3464 is much less affected. All complexes induce DNA interstrand cross-links. Di/trinuclear complex-induced interstrand cross-linking peaks early, suggesting rapid genomic access and interaction. Subsequent decay suggests susceptibility to DNA repair mechanisms. BBR3464 has been selected for clinical development largely on the basis of results from *in vivo* activity and toxicity studies. These results show BBR3464 to have unique properties in the context of acquired cisplatin-resistance that enhance its candidacy as a potential anticancer agent.

Bis-platinum compounds are part of an important class of anticancer agents known as platinates, currently used as standard treatment for lung, ovarian and colorectal cancer, among others [161, 162]. Though, currently marketed platinum drugs are standard in the treatment of many common cancers, patients often develop resistance to them, limiting their overall clinical benefit. In contrast to standard platinum agents, which contain a single platinum molecule, *bis*-platinum compounds contain two platinum molecules which may help to stem tumor resistance to platinum. It is generally believed that standard platinum agents fight tumors by binding between DNA, a mechanism known as intermolecular binding. With two platinum molecules, *bis*-platinum compounds cause two different types of binding, intermolecular binding between the DNA strand, and intramolecular binding within the DNA strand.

A series of platinum(II) tri-n-butylphosphine complexes having the formulas *cis*-$[PtCl_2L_2]$, $[PtCl(en)L]Cl$, $[Pt(en)L_2](ClO_4)_2$, *sym-trans*-$[Pt_2Cl_4L_2]$, $[Pt_2Cl_2L_4](ClO_4)_2$, *trans,trans*-$[PtCl_2L(mu\text{-}N_2H_4)PtCl_2L]$ *trans,trans*-$[PtCl_2L(mu\text{-}en)PtCl_2L]$, and *cis,cis*-$[PtClL_2(mu\text{-}N_2H_4)PtClL_2](ClO_4)_2$ (L = tri-n-butylphosphine; en = ethylenediamine) have been synthesized and their cytotoxic activity *in vitro* and *in vivo* has been studied [163]. All investigated dinuclear diamine-bridged platinum(II) compounds were cytotoxic *in vitro* against L1210 cells and showed no cross-resistance to cisplatin. On the other hand, no antitumor activity was observed vs L1210 leukemia in DBA2 mice. Cesar *et al*. described the synthesis and characterization of six new dinuclear platinum complexes having N,N'-di-(2-aminoethyl)-1,3-diamino-2-propanol, aryl substituted N-benzyl-1,4-butanediamines and N-benzyl-1,6-hexanedia-mines as ligands [164]. They reported the cytotoxic activity and cellular accumulation of these complexes in a human small-cell lung carcinoma cell line and its resistant subline. Resistant cells exhibited a lesser degree of cross-resistance to these compounds when compared to cisplatin. A series of dimers of the monofunctional platinum species $[Pt(dien)\ Cl]^+$, linked by a variety of flexible (polymethylene) and more rigid chains, was prepared and evaluated for DNA interactions and cytotoxic activity [165]. Solutions of the *bis*$(Pt(dien)Cl)^{2+}$ complexes were stable, but solid products could not be isolated. All of the *bis*$(Pt(dien)Cl)^{2+}$ complexes unwound closed circular supercoiled DNA more efficiently than the monomer, and were more efficient than the difunctional platinum complex cisplatin at cross-linking linearized plasmid DNA, as measured on non-denaturing agarose gels. None of the *bis*$(Pt(dien)Cl)^{2+}$ complexes were as cytotoxic as cisplatin in both the wild-type and platinum-resistant P388 murine leukemia cell lines. The more rigid analogs were equitoxic in both sensitive and cisplatin-resistant cells, but none showed *in vitro* activity against the P388 tumor. New derivatives of the cytotoxic azole-bridged dinuclear platinum(II) complexes have been prepared and structurally characterized [166, 167]. A cytotoxicity assay of these dinuclear platinum(II) compounds on human tumor cell lines was performed. In most of the cell lines, they showed much higher cytotoxicity than those of cisplatin. Implications of these findings are discussed in the context of a structure-activity relationship. Six related dinuclear *trans*-platinum complexes, with the formula $[[trans\text{-}PtCl_2(NH_3)(L)]_2(mu\text{-}H_2N(CH_2)_nNH_2)]^{2+}$ (L = pyridine, 2-picoline, 4-picoline; n = 4, 6) and chloride or nitrate anions, are compared with known cytotoxic dinuclear compounds (L = NH_3; n = 4, 6) that overcome cisplatin resistance [168]. The cytotoxicity of the compounds was determined in L1210 murine leukemia and L1210/2, a cisplatin-resistant derivative. In general, the substituted compounds are less cytotoxic than their NH_3 counterparts. The amounts of platinum bound to DNA do not correlate with the cytotoxicity data. As DNA is considered to be the cellular target of platinum antitumor drugs, structural details of the DNA adducts probably account for the differences in cytotoxic activity.

Several polynuclear Pt(II) chelates with biogenic polyamines were synthesized and screened for their potential antiproliferative and cytotoxic activity in different human cancer cell lines [169]. Distinct effects were found for different cell lines (HeLa and HSC-3 epithelial-type cells, THP-1, MOLT-3, and CCRF-CEM leukemia cell lines) and different structural characteristics of the complexes.

Cytotoxicity and cell growth inhibition studies were performed for three distinct polynuclear platinum(II) complexes of spermidine, which showed to have significant cytotoxic and antiproliferative properties on the HeLa cancer cell line [170]. The chemical environment of the metal centres in the drugs, as well as the coordination pattern of the ligand, were found to be strongly determinant of their cytotoxic ability. In the light of the results gathered, the most effective anticancer compound among the ones tested (IC_{50}=5 μM) was found to be the one displaying three difunctional ($PtCl_2N_2$) moieties ($(PtCl_2)_3(spd)_2$). Both the cytotoxic activity and the antiproliferative properties of the complexes studied showed to be irreversible for all the concentrations tested.

NONCLASSICAL PLATINUM COMPOUNDS

Studies over the last few years have shown that the range of platinum complexes with useful cytotoxicity and antitumor activity is not strictly limited to structural analogs of cisplatin. In general, we can expect that cells will process structurally different species in different manners. The metabolic chemistry and DNA binding will be altered in comparison to the *cis*-$[PtX_2(amine)_2]$ class. This point is of particular importance because any altered pattern of antitumor activity of structural analogs of cisplatin is likely to be due to unpredictable pharmacokinetic, rather than truly mechanistic factors. The fact that discrete cisplatin-DNA adducts vary in their biological activity further supports the hypothesis that complexes structurally dissimilar to cisplatin may produce biological activity complementary to the parent drugs. The mechanism of action of nonclassical complexes is different from that of cisplatin and its analogs. Their pattern of antitumor activity is also altered with respect to cisplatin. Thus, not all platinum-containing drugs need necessarily be similar in their clinical profile to cisplatin. Both the dinuclear *bis*-platinum complexes and the *trans* complexes give their own distinct patterns of tumor specificity different from cisplatin and each other [171]. New cytotoxic mechanisms for platinum complexes may also be placed in context with cisplatin resistance. Modes of overcoming cisplatin resistance may reside at the various levels of uptake, interaction with "detoxifying" intracellular thiols, and DNA repair. Likewise complexes with novel mechanisms of action may circumvent resistance by more than one unique route. From the discovery of cisplatin, the development of analogs has essentially been an empirical exercise. Because of their similar mechanism of action, much comparison has been made between platinum complexes and the classic alkylating agents. Yet, the alkylating agents represent a good example where a number of structurally distinct drugs with different anticancer activities are clinically available. This desirable feature may be achieved for platinum complexes by emphasis on complexes structurally dissimilar to the presently used agents. The dinuclear *bis*-platinum complexes and mononuclear complexes in the *trans* geometry are of special interest. Comparison of common features and differences between different classes may point to guidelines for the rational design of complexes with a different spectrum of clinical antitumor activity to cisplatin and activity against cisplatin-resistant tumors.

In the search for *cis*-aminedichloro(2-methylpyridine)-platinum(II) (ZD0473, **4**) derivatives with improved antitumor activity, unexpected monofunctional platinum(II) complexes with one normal and one cyclometalated 2-phenylpyridine ligand (**12**) were discovered that exhibited high antitumor efficacy against cisplatin-resistant mouse sarcoma 180 (S-180*cis*R) cell lines [172]. Consistent with higher activity in the resistant cells, more efficient cellular uptake of the new complexes compared with cisplatin was demonstrated. Reduced accumulation of cisplatin mediated by P-glycoprotein (P-gP) efflux was suggested to be one of the pathways for cisplatin resistance in S-180*cis*R cells. As a monofunctional complex, the platinum-phenylpyridine compound cannot form DNA cross-links, indicating a different binding mode from that of cisplatin unless a ligand is displaced intracellularly. Its high cytotoxicity in cisplatin-resistant cells may possibly be a consequence of diminished DNA repair.

Pyridines have attracted significant attention as appropriate ligands for synthesis of new platinum coordination compounds. A number of platinum coordina-tion complexes with different ligands, which include aniline or pyridine, were prepared, characterized and tested for their *in vitro* cytotoxic effects on KB cells and for their antitumor activity against some tumor systems (L1210 and P388 leukemia, ADJ/PC6A plasma cell tumour and Yoshida sarcoma) [173]. Water soluble platinum(II) complexes have been synthesized that contain the N,O-chelate pyridin-2-yl acetate (PyAc) as a novel structural motif in platinum antitumor complexes [174]. Possible implications for the DNA binding and cytotoxic effect of the compounds are discussed.

New platinum(II) complexes of 2-acetyl pyridine and pyridine-2-carbaldehyde N(4)-ethyl-thiosemicarbazones have been characterized [175, 176]. The cytotoxic activity for the platinum(II) complexes in comparison to that of cisplatin and thiosemicarbazones was evaluated in a pair of cisplatin-sensitive and -resistant ovarian cancer cell lines A2780 and A2780/Cp8. The platinum(II) complexes showed a cytotoxic potency in a very low micromolar range and were found able to overcome the cisplatin resistance of A2780/Cp8 cells. Cytotoxicity tests in tumor cells sensitive to *cis*-DDP (HL-60, JURKAT, Hela and 3T3) and in tumor cells transformed by ras oncogenes and resistant to *cis*-DDP (Pam 212-ras) show that cyclometallated complexes of *p*-isopropylbenzaldehyde N-protected thiosemicarbazones may be endowed with specific cytotoxic properties [177]. In fact, these novel metal-thiosemicarbazone compounds kill Pam 212-ras cells through apoptosis induction. These results, together with others recently published [178-180], indicate that the design and synthesis of metallated-thiose-micarbazone compounds may lead to the discovery of novel antitumor agents able to circumvent *cis*-DDP resistance, in particular tumor cell lines. Quiroga *et al*. reported the synthesis and characterization of two new metallic complexes derived from phenylacetaldehyde thiose-mincarbazone: $Pt(C_9H_{11}N_3S)Cl_2$ and $Pd(C_9H_{11}N_3S)Cl_2$ [181]. The testing of the cytotoxic activity of these compounds against several human and murine cell lines sensitive and resistant to *cis*-DDP suggests that the compounds may be considered potential anticancer agents since they exhibit $1C_{50}$ values in a μM range similar to cisplatin (*cis*-DDP).

The cytotoxic activity of these compounds is higher in *cis*-DDP-resistant tumor cells than that of other antitumor drugs such as etoposide and adriamycin. On the other hand, the analysis of the interaction of these compounds with linear plasmid DNA indicate, that both compounds have an enhanced capacity to form DNA interstrand cross-links in comparison with *cis*-DDP.

The alkylating ability of amidoesters, diethyl pyridylmethylphosphonate esters and their *cis*-platinum(II) complexes has been investigated [182]. The highest activity was found for *cis*-$[PtCl_2(4\text{-pmpe})_2]$ where 4-pmpe is diethyl 4-pyridylmethylphosphonate. The results show a correlation between alkylating activity *in vitro* and cytotoxic activity *in vivo* for platinum(II) complexes. Brzezinska *et al*. investigated the effect of *cis*-$[PtCl_2(4\text{-pmpe})_2]$ on murine mast cells [183]. It was able to evoke histamine release from murine mast cells. The histamine secretion was dependent on the concentration of compound and on the time and temperature of the reaction. The results indicate that *cis*-platinum (II) complexes activate mast cells to secrete histamine via a non-cytotoxic, active secretory process.

Carrara *et al*. studied the cytotoxic effect of new Pt mercaptopyridine complexes on several tumoral cell lines (F10, Fohn, LoVo and HeLa) as well as on the fibroblast cell line (3T3) [184]. The data reported make evident that the presence of ligands with sulfur donor atoms may be of particular importance in confirming the antitumor properties of Pt complexes. Since it has been widely demonstrated that platinum-based drugs, like cisplatin, carboplatin and oxaliplatin, bind preferentially to guanine in N7 position and that telomerase assemblage includes a RNA portion rich in guanine, Colangelo *et al*. designed and synthesized a series of new complexes with a cytotoxic $[Pt(II)Cl_2]$ moiety, with the aim of selecting carrier ligands able to inhibit telomerase enzyme [185]. Among these compounds, [*cis*-dichloropyridine-5-isoquinolinesulfonic acid Pt(II)] showed the most significant inhibition of telomerase in a cell-free biochemical assay. The authors report the biological effects of this compound on *in vitro* tumor model (MCF-7) and the biochemical effects on non-neoplastic NIH3T3 cells. The results highlight its possible role as a selective antitelomerase tool for cancer treatment.

New binuclear complexes of formula $[Pt_2(bipy)_2(BAA)]Cl_2$ (where bipy is 2,2'-bipyridine and BAA is a dianion of meso-α-α'-diaminoadipic acid or meso- α-α' -diaminosuberic acid) have been synthesized [186]. These complexes have been tested against P388 lymphocytic leukemia cells and their target is DNA molecules. Binding studies of the complexes to calf thymus DNA have been carried out and the mode of binding seems to be hydrogen bonding. Three new crown ester-linked bipyridine homologs with three, four or five ethylene glycol units, which are bulky and soluble in both hydrophilic and lipophilic media, were synthesized [187]. These complexes were converted to carboplatin analogs by exchange of the leaving group. Some of platinum complexes showed a moderate cytotoxic effect on both murine leukemia L1210 and P388 even though they do not have any NH proton. The neutral complex of formula [Pt(bpy)(cbdca)] (**13**) [where bpy is 2,2'-bipyridine and cbdca is anion of 1,1-cyclobutanedicarboxylic acid] has been synthesized [188]. The complex inhibits the growth of P388 lymphocytic leukemia cells and its target is DNA.

Physicochemical factors for cytotoxic activity in platinum dinuclear complexes with pyrimidine and imide ligands have been examined [189]. It is concluded that inside cells the reactivity of the platinum complexes having imide ligands is higher than that of *cis*-DDP. The accumulation of platinum into cells is dominated by the hydrophobicity and the charge of platinum complexes. Highly hydrophobic complexes are thought to be adsorbed in cell membranes, resulting in low cytotoxic activity since they cannot reach DNA. A schematic model of the interaction between platinum complexes and serum proteins reveals that more hydrophobic complexes tend to bind to serum proteins more stably. At least three possible paths of the cellular platinum accumulation are suggested: direct accumulation of the platinum complexes, incorporation in the form of *cis*-DDP produced from the complexes, and incorporation through protein-platinum complexes, although the contribution of the third one may be small.

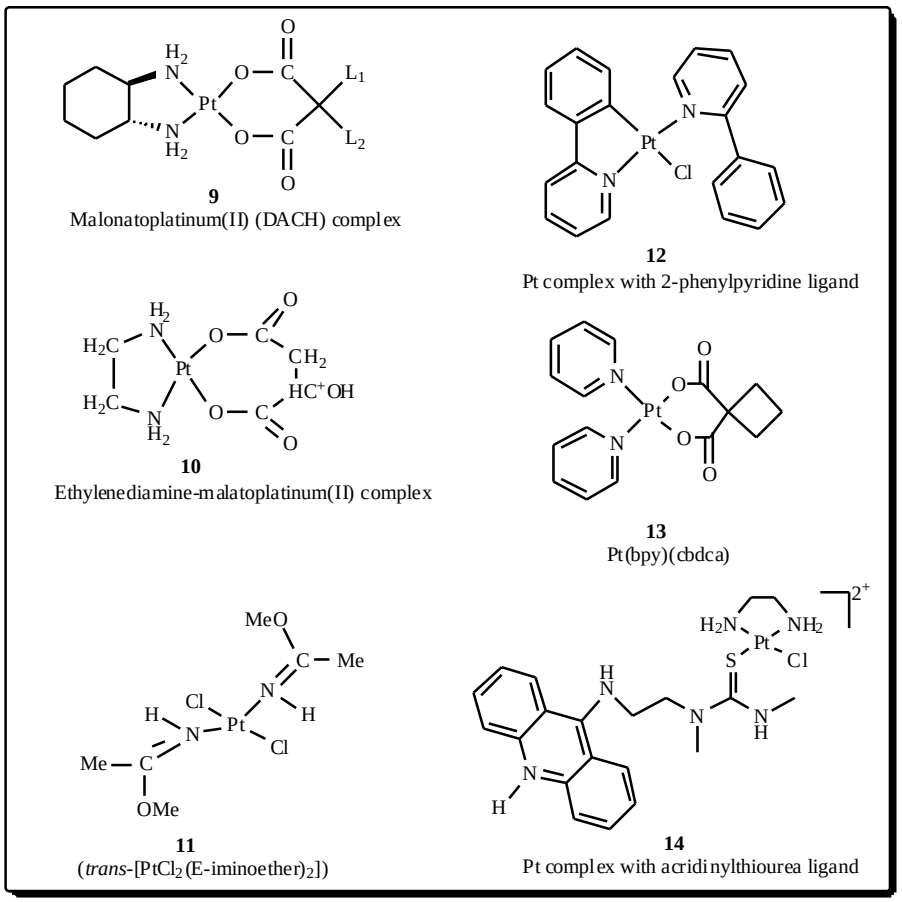
Novel platinum(II) complexes with 5,7-disubstituted-1,2,4-triazolo[1,5-α]pyrimidines have been synthesized and characterized [190]. The complexes are of two types: $[PtCl_2(L)_2]$ and $[PtCl_2(NH_3)(L)]$, where L=5,7-diphenyl-1,2,4-triazolo[1,5-α]pyrimidine and 5,7-ditertbutyl-1,2,4-triazolo[1,5-a]pyrimidine. The antiproliferative activity *in vitro* of the complexes have been tested against the cells of four human cell lines: SW707 rectal adenocarcinoma, A549 non-small cell lung carcinoma, T47D breast cancer and HCV29T bladder cancer. A number of coordination compounds of Pt(II) with ligands incorporating azole and pyrimidine rings has been synthesized by Wisniewski *et al*. [191]. The *in vitro* cell proliferation-inhibitory activity of these compounds was examined against human cancer cell lines: A 549 (lung carcinoma), LS-180 (colon cancer) and MCF-7 (breast cancer). Pt(II) complex of formulae $[PtLCl_2]$, where L = mepirizole, was synthesized and characterized [192]. The cytotoxic activity of Pt complex was checked for six different tumor cell lines and was lower than that of cisplatin. The Pt bound to DNA was also checked and only a low percentage is able to cross the cell membrane. Drewa *et al*. assessed cytotoxic effect of four new platinum compounds on B16 and CIS91 melanoma cells *in vitro* [193]. The following complexes were tested: Tetrachloro*bis* (5,7-dimethyl-1,2,4-triazol [1,5α] pirymidine) platinum (IV), *trans*-dichloro (dimethylsulfoxide) (5,7-dimethyl-1,2,4-triazol-[1,5α] pirymidine) platinum(II), *cis*-dichloro (dimethylsulfoxide)(1-β-D-ribofuranosyl-1,2,4-triazol-3-carboxamide) platinum(II), and chloro(dimethylsulfoxide)(1-β-D-ribofuranosyl-1,2,4-triazol-3-carboxamide) platinum(II). Cytotoxic and soluble properties of the compounds could be modified and improved.

Recently, a platinum(II) complex with a thiourea ligand (**14**) was reported that showed excellent cytotoxicity against a leukemia cell line. The complex may bind to DNA in a dual manner involving platinum coordination and acridine intercalation. The complex exhibited activity against two ovarian cancer cell lines at micromolar concentrations, but slightly less activity than that of the free ligand [194, 195].

Pt(IV) COORDINATION COMPLEXES

Platinum(II)-based anticancer drugs are associated with high reactivity and thus a poor biological stability. The platinum(IV)-complexes display potential advantages due to their greater stability and bioreductive activation, thereby allowing a greater proportion of the drug to arrive at the target intact. An important question is whether such compounds are reduced before entering the cell, inside the cell, or perhaps not at all. It is not completely clear, although most evidence points toward slowly formed Pt(IV) adducts. The so-called JM-216 (**7**), a Pt(IV) compound, has been in routine clinical use as an orally administered drug. Hall *et al*. [196] studied comparative efficacy of novel platinum(IV) compounds with established chemotherapeutic drugs in solid tumor models. All compounds tested were able to produce cytotoxicity in monolayer cell cultures, however, the potencies of platinum(IV) drugs were lower than that observed for the platinum(II) compounds or established organic chemotherapeutic agents. There was no significant alteration in the potency of platinum(II) or (IV) compounds to produce cytotoxicity in multicellular tumor spheroids (MCTS) compared to monolayer cultures. All the organic and platinum-based cytotoxic agents produced, to varying degrees, either a retardation or reduction in MCTS growth. Histology indicated that the predominant morphological change was that of apoptosis, although there were some drug-dependent effects such as the metaphase arrest produced by vinblastine and chromatin dispersal to the periphery of nuclei produced by doxorubicin. In summary, whilst the platinum(IV) derivatives were able to produce cytotoxicity via apoptosis, the introduction of a stable axial group significantly retarded the rate at which this occurred.

The use of molecular biological methodologies has provided a greater understanding of the cytotoxic effects of cisplatin and the underlying mechanisms of tumor cell resistance. Resistance to cisplatin is often multifocal with plasma membrane, cytosolic and nuclear components. Cisplatin-DNA adducts appear to be recognized by specific damage recognition proteins. Proteins associated with the transport of platinum through plasma membranes and genes associated with cisplatin resistance appear to be close to being elucidated. Current Phase I and Phase II clinical trials with platinum-containing complexes largely focus on the 1,2 diaminocyclohexane (DACH) carrier ligand, the dicarboxylatocyclobutane leaving group and complexes which circumvent cisplatin resistance in murine leukemia models. At present, the trials are at too early a stage to allow comment on their clinical utility and, consequently, the relevance of the murine leukemia-based preclinical observations [197]. On the horizon, orally active platinum (IV) amine/amine dicarboxylate dichloride coordination complexes with preclinical toxicological profiles similar to carboplatin enter clinical trial. New platinum (IV)-amine complexes with high antitumor activity have been presented in the inventions [198, 199]. Novel platinum (IV) complexes having potent antitumor activity and high water solubility



9
Malonatoplatinum(II) (DACH) complex

10
Ethylenediamine-malatoplatinum(II) complex

11
(*trans*-[$PtCl_2$(E-iminoether)$_2$])

12
Pt complex with 2-phenylpyridine ligand

13
Pt(bpy)(cbdca)

14
Pt complex with acridinylthiourea ligand

with low toxicity and pharmaceutical compositions were provided [200].

Seventeen alkylamine ammine dicarboxylatodichloroplatinum(IV) complexes of general structure c,t,c-[$PtCl_2(OCOR1)_2NH_3(RNH_2)$], where R = aliphatic or alicyclic and R1 = aliphatic or aromatic, have been evaluated against L1210 cell lines with acquired resistance to cisplatin (10-fold), tetraplatin (34-fold) or carboplatin (14-fold) [201]. All of these compounds overcame cisplatin, tetraplatin and carboplatin resistance. Potency increased as the number of carbon atoms in the axial aliphatic ligands (R1) increased. The most active compounds were those possessing aromatic ligands at R1, regardless of whether R = aliphatic or alicyclic. The lipophilic properties of some of these platinum(IV) dicarboxylates may contribute to both the potency and circumvention of resistance by these compounds. Using a panel of six human ovarian carcinoma cell lines varying by two orders of magnitude in terms of cisplatin cytotoxicity, Kelland *et al*. [202] have investigated the *in vitro* antitumor activity of a series of novel alkylamine ammine dicarboxylatodichloroplatinum(IV) complexes. A clear relationship existed between increasing the number of carbons in the R1 substituent and increasing cytotoxicity up to R1 = C_5H_{11}. In terms of changing the R group, maximum cytotoxic effects were conferred by alicyclic substituents. Furthermore, increasing the alicyclic ring size from cyclobutane through to cycloheptane resulted in increasing cytotoxicity. The agents with longer axial chains were significantly more cytotoxic than cisplatin and, moreover, exhibited a selective cytotoxic effect against the most intrinsically cisplatin-resistant cell lines. This novel class of platinum compound represents a valuable lead in the development of a "third-generation" agent capable of exhibiting activity against clinical disease currently resistant to cisplatin. Turanek *et al*. [203] compared antitumor potency of platinum(IV) complexes with increasing hydro-phobicity of their ligands. Alkylamine ligands with increasing hydrophobicity were: isopropylamine, cyclo-hexylamine, 1-adamantylamine and 3,5-dimethyl-1-adaman-tylamine. The synthesis, characterization, and antitumor properties of a group of platinum (IV) complexes, formed by oxidation of *cis*-dichlorodiammineplatinum (II) or its *cis*-dihydroxo analog, were presented [204]. It appears that the antitumor activity of the compounds is either due to Pt(IV) binding via ligand displacement to important cellular components or through the ability of the compounds to undergo *in vivo* reduction to platinum (II) species.

An original approach intended to facilitate the intratumoral activation of Pt(IV) diamines by illumination with visible light to form photolysis products that irreversibly bind to DNA and are cytotoxic to human cancer cells is reported [205]. The novel Pt(IV) complex *trans*,*cis*-[$Pt(OAc)_2I_2$-(en)] was prepared. The conformation of the acetato groups around the O-Pt-O axis deviated significantly from the conformation of the acetato groups in the X-ray crystal structure reported for the *cis*-dichloro analog, which may explain the very different aqueous solubilities of the two compounds. The photolysis of *trans*,*cis*-[$Pt(OH)_2I_2$(en)] with visible light resulted in a 22% enhancement of antiproliferative activity. Platinum (IV)-diamine complexes, a process for the preparation of pharmaceutical compositions and a method of treating malignant tumors in mice were presented [206, 207]. Diamine platinum(IV) complexes having mixed carboxylate ligands have been found to have desirable antitumor activity, as well as relatively low levels of toxicity [208].

As part of a drug discovery program to discover more effective platinum-based anticancer drugs, a series of platinum complexes of *trans* coordination geometry centered on *trans*-ammine(cyclohexylaminedichlorodihydroxo) platinum(IV) has been evaluated *in vitro* against a panel of cisplatin-sensitive and cisplatin-resistant human tumor cell lines (predominantly ovarian) [209]. *In vitro*, against 5 human ovarian carcinoma cell lines, the complex was comparably cytotoxic to cisplatin itself and over 50-fold more potent than transplatin. It exhibited a different cross-resistance pattern to that of its *cis* isomer. Preliminary intracellular DNA binding studies showed that in contrast to transplatin, the above complex was efficient at forming DNA-DNA interstrand cross-links. It is the first *trans*-platinum complex to demonstrate marked antitumor efficacy against both murine and human tumor models and represents a significant structural lead to complexes capable of circumventing cross-resistance to cisplatin. Platinum(IV) amine/cycloalkylamine homologous series was evaluated for cytotoxicity and biochemical pharmacology in murine leukemia L1210/0, *cis*-DDP-resistant L1210/DDP, and diaminocyclohexaneplatinum-resistant L1210/1,2-diamino-cyclohexane (DACH) cells [210]. Within each series, which contained 4 homologs with differing alicyclic (cycloalkyl) ring size (cyclopropane, cyclobutane, cyclopentane, or cyclohexane), cytotoxicity increased with increasing ring size. This appeared to be due to an increase in partition coefficient, and the resulting increase in drug accumulation and intracellular DNA adducts in ascending each of the series. There were exceptions to this generalization, predominantly in L1210/DACH cells, where the biochemical pharmacology was not entirely consistent with the cytotoxic response and suggested that other factors may be at play. The results have demonstrated high dependencies on ring size of the carrier amine ligand, valence state of platinum, and the nature of the axial ligand for modulation of potency, cross-resistance property, and biochemical pharmacology of amine/cycloalkylamine complexes.

The *trans*-1,2-diaminocyclohexaneplatinum(II) complexes, (DACH)Pt(II), have attracted significant attention for many years because they do not show cross-resistance with cisplatin, probably as a result of inducing Pt-DNA adducts that are poorly repaired in resistant cells, even though they are identical to those induced by cisplatin, or of inhibiting essential processes such as replication or transcription. In a series of studies, (DACH)Pt(II) complexes having antitumor activity were designed with a wide range of lipophilicity and hydrophilicity [211, 212]. A series of platinum(II) and (IV) monoadducts of the type [Pt(II)(DACH)LCl]NO_3 and [Pt(IV)(DACH)*trans*-$(X)_2$LCl] NO_3 (where DACH=*trans*-1R,2R-diaminocyclohexane, L=adenine, guanine, hypoxanthine, cytosine, adenosine, guanosine, inosine, cytidine, 9-ethylguanine (9-EtGua), or 1-methylcytosine and X=hydroxo or acetato ligand) have been synthesized and characterized [213]. Some of these synthesized models of DACH-Pt-DNA adducts have good *in vitro* cytotoxic activity against the

cisplatin-sensitive human cancer ovarian A2780 cell line (IC_{50}=1-8 μM). Interestingly, a substituted nucleobase (9-ethylguanine) adduct was over 6-fold more potent than regular adducts. The results suggested that DNA adducts of DACH-Pt are cytotoxic with low cross-resistance. Several Pt(IV) complexes containing 1*R*,2*R*-cyclohexanediamine (1*R*,2*R*-DACH) as a carrier ligand were synthesized [214]. The cytotoxicities and the uptake of the platinum complexes by leukemia L1210 cells were compared in order to study the correlation between their structures and cytotoxicities. [Pt(IV)Cl_4(1*R*,2*R*-DACH)], *trans*(Cl)-[Pt(IV)Cl_2(oxalato)(1*R*,2*R*-DACH)], and *trans*(Cl)-[Pt(IV) Cl_2(malonato)(1*R*,2*R*-DACH)] had high cytotoxicities. In addition, *trans*(OH)-[Pt(IV)$(OH)_2Y_2$(1*R*,2*R*-DACH)] (Y_2: oxalato or malonato) did not exhibit cytotoxicity towards leukemia L1210 cells, whereas *trans*(Cl)-[Pt(IV)Cl_2Y_2(1*R*,2*R*-DACH)] (Y_2: oxalato or malonato) were highly cytotoxic. The accumulation of *trans*(OH)-[Pt(IV)$(OH)_2Y_2$(1*R*,2*R*-DACH)] in leukemia L1210 cells was much lower than that of *trans*(Cl)-[Pt(IV)Cl_2Y_2(1*R*,2*R*-DACH)]. Platinum(IV) complexes, in which leaving groups are replaced by hydroxide groups, have decreased cytotoxic activity, because the hydroxide groups of the platinum(IV) complex reduce the uptake of platinum by the cells. *trans*(OH),*cis*(Cl)-[Pt(IV)$(OH)_2Cl_2$(1*R*,2*R*-DACH)], which has hydroxide and chloride groups, was easily incorporated into the cells and exhibited the high cytotoxic activity. This behavior indicates that the chloride group apparently overcomes the ameliorating effect of the hydroxide group. The synthesis, characterization, and antitumor activity of a series of platinum(IV) complexes of the type DACH-Pt$IV(X)_2Y$ (where DACH = *trans*-dl, or *trans*-l-1,2-diaminocyclohexane, X = OH or Cl, and Y = oxalato, malonato, methylmalonato, tartronato, ketomalonato, 1,1-cyclopropanedicarboxylato, or 1,1-cyclobutanedicarboxy-lato), were described by Khokhar *et al*. [215]. The complexes had good *in vitro* cytotoxic activity (IC_{50} = 0.14-7.6 μg/ml) and were highly active *in vivo* against leukemia L1210 cells. In addition, excellent *in vivo* antitumor activities against B16 melanoma, M5076 reticulosarcoma and cisplatin-resistant L1210/DDP cell lines were also exhibited by an analog selected for further evaluation. Water soluble 1,2-diaminocyclohexane platinum (IV) complexes as antitumor agents have been found to have desirable antitumor activity, as well as relatively low levels of toxicity [216, 217]. A series of new platinum(IV) complexes of the type [PtIV(DACH)*trans*$(L)_2Cl_2$] (where DACH = *trans*-1*R*,2*R*-diaminocyclohexane, and L = acetate, propionate, butyrate, valerate, hexanoate, or heptanoate) bearing the carboxylate groups in the axial positions have been synthesized and characterized [218]. These analogues were evaluated *in vitro* and demonstrated cytotoxic activity against the human ovarian 2008 tumor cell line (IC_{50} = 0.001-0.06 μM). Structure-activity study revealed that activity was highest for the analogue where L = butyrate. New platinum (IV) complexes were provided, which exhibit antitumor activity as shown by the tests on mouse leukemia, L-1210 cell in mice [219]. These new platinum (IV) complexes contain 1,2-cyclohexanediamine or 2-(amino-methyl)cyclohexyl-amine as a ligand. The invention [220] described a method of preparing tetrachlorodiamino-cyclohexane platinum (IV) complexes by reacting a diamine selected from the group consisting of 1,2-diamino-cyclohexane and operable isomers thereof in the form of a dihydrohalide with alkali metal or hydrogen hexachloro-platinate. New substantially isomerically pure tetrahalo (1,2-diaminocyclohexane) Pt(IV) complexes having antineo-plastic activity were disclosed [221]. Novel 1,4 and 1,2-diaminocyclohexane platinum IV complexes have been synthesized that show activity *in vivo* against tumor models resistant to cisplatin and tetraplatin [222]. The novel complexes include the chloro, acetate, trifluoroacetate, propionate, butyrate, pentanoate, hexanoate and heptanoate as leaving ligands and 1,4 or 1,2-DACH-amine ligands. The complexes showed good *in vitro* cytotoxic activity against murine leukemia L1210/0, L1210/DDP and L1210/DACH. High *in vivo* activity was shown against L1210 leukemia cells and against cisplatin resistant L1210/DDP. Excellent antitumor activity against M5076 was also exhibited by the new complexes. Additionally, the platinum complexes did not elicit any indication of nephrotoxicity in the *in vivo* tests.

CURRENT AND FUTURE DEVELOPMENTS

Coordination chemistry in living systems is more than just a matter of metal-ligand bond formation and metal-ligand stability. Control of metal binding to DNA, by simultaneous coordination and hydrogen bonding has been crucial to research. It needs no discussion that the above-presented highlights and outlook provide fascinating new possibilities for research in the coming decade. New techniques, which follow the reactions of Pt complexes and nucleic acids and proteins, will allow the detection of otherwise invisible intermediate products.

In summary, it is generally appreciated that enormous progress has been made in the understanding of the mode of action of cisplatin. Application of this knowledge in drug design is close, and it is generally expected that in the next decade improved antitumor drugs will be developed based on the knowledge of the Pt-DNA interactions (and their repair) and on the kinetics of binding of Pt compounds to proteins and DNA. Although questions have been raised about whether the intrinsically weak metal-ligand coordination bond will ever lead to new drug applications, the kinetic control of stability is likely to overcome this.

The need for new platinum antitumor drugs was underscored by the usefulness of cisplatin and carboplatin in chemotherapy and the resistance of many tumors to these compounds. Combinatorial chemistry could aid in the search for cisplatin analogs if fast, high-throughput assays were available. The goal is to develop rapid cell-based assays suitable for high-throughput screening that accurately predicts the cytotoxicity of platinum complexes.

The next stage in drug design is likely to be the development of dedicated drugs that comprise the transport (through the membranes), survival in the cell, binding to the DNA, and eventually, excretion from the body with minimum side effects. In this process, both metal coordination and hydrogen bonding will be key factors at the molecular level.

CONCLUSION

Recent advances in medicinal inorganic chemistry demonstrate significant prospects for the utilization of metal

complexes as drugs, presenting a flourishing arena for inorganic chemistry. Significant progress in platinum based anticancer agents has been achieved, based in part on a mechanistic understanding of the DNA-binding and pharmacological effects of cisplatin. A lot of new compounds with reduced toxicity and high specificity have been developed. The future development of medicinal inorganic chemistry requires an understanding of the physiological processing of metal complexes, to provide a rational basis for the design of new metal-based drugs. Application of new methodologies such as combinatorial chemistry, extensively used in organic drug discovery, will be beneficial for the development of inorganic compounds as therapeutics.

REFERENCES

[1] Orvig C, Abrams MJ. Medicinal inorganic chemistry: introduction. Chem Rev 1999; 99: 2201-3.

[2] Sakurai H, Kojima Y, Yoshikawa Y, Kawabe K, Yasui H. Antidiabetic vanadium(IV) and zinc(II) complexes. Coord Chem Rev 2002; 226: 187-98.

[3] Sadler PJ, Li H, Sun H. Coordination chemistry of metals in medicine: target sites for bismuth. Coord Chem Rev 1999; 185-186: 689-709.

[4] Ali H, van Lier JE. Metal complexes as photo- and radiosensitizers. Chem Rev 1999; 99: 2379-450.

[5] Louie AY, Meade TJ. Metal complexes as enzyme inhibitors. Chem Rev 1999; 99: 2711-34.

[6] Volkert WA, Hoffman TJ. Therapeutic radiopharmaceuticals. Chem Rev 1999; 99: 2269-92.

[7] Grunicke H, Doppler W, Helliger W, *et al*. Tumor biochemistry as basis for advances in tumor chemotherapy. Arch Geschwulstforsch 1986; 56: 193-201.

[8] Zhukova OS, Dobrynin IaV. Current results and perspectives of the use of human tumor cell lines for antitumor drug screening. Vopr Onkol 2001; 47: 706-9.

[9] Hasegawa, K., Mori, K., Ishii, N., Aoki, Y.: CA 2478640 (**2003**).

[10] Essigmann, J.M., Croy, R.G., Yarema, K.J., Morningstar, M.: US6500669 (**2002**).

[11] Essigmann, J.M., Croy, R.G., Chen, Z.: US5882941 (**1999**).

[12] Essigmann, J.M., Croy, R.G., Yarema, K.J., Morningstar, M.: US5882941 (**1999**).

[13] Rozencweig M, von Hoff DD, Slavik M, Muggia FM. Cis-diamminedichloroplatinum (II). A new anticancer drug. Ann Intern Med 1977; 86: 803-12.

[14] Kane SA, Lippard SJ. Photoreactivity of platinum(II) in cisplatin-modified DNA affords specific cross-links to HMG domain proteins. Biochemistry 1996; 35: 2180-8.

[15] Jamieson ER, Lippard SJ. Structure, recognition, and processing of cisplatin-DNA adducts. Chem Rev 1999; 99: 2467-98.

[16] Ishida S, Lee J, Thiele DJ, Herskowitz I. Uptake of the anticancer drug cisplatin mediated by the copper transporter Ctr1 in yeast and mammals. Proc Natl Acad Sci USA 2002; 99: 14298-302.

[17] Lin X, Okuda T, Holzer A, Howell SB. The copper transporter CTR1 regulates cisplatin uptake in Saccharomyces cerevisiae. Mol Pharmacol 2002; 62: 1154-9.

[18] Lee K-B, Wang D, Lippard SJ, Sharp PA. Transcription-coupled and DNA damage-dependent ubiquitination of RNA polymerase II *in vitro*. Proc Natl Acad Sci USA 2002; 99: 4239-44.

[19] Cohen SM, Lippard SJ. Cisplatin: from DNA damage to cancer chemotherapy. Prog Nucleic Acid Res Mol Biol 2001; 67: 93-130.

[20] Kartalou M, Essigmann JM. Recognition of cisplatin adducts by cellular proteins. Mutat Res 2001; 478: 1-21.

[21] He Q, Liang CH, Lippard SJ. Steroid hormones induce HMG1 overexpression and sensitize breast cancer cells to cisplatin and carboplatin. Proc Natl Acad Sci USA 2000; 97: 5768-72.

[22] Zamble DB, Mikata Y, Eng CH, Sandman KE, Lippard SJ. Testisspecific HMG-domain protein alters the responses of cells to cisplatin. J Inorg Biochem 2002; 91: 451-62.

[23] Wong B, Masse JE, Yen Y-M, Giannikoupolous P, Feigon J, Johnson RC. Binding of cisplatin-modified DNA by Saccharomyces cerevisiae HMGB Protein Nhp6A. Biochemistry 2002; 41: 5404-14.

[24] Wang D, Hara R, Singh G, Sancar A, Lippard SJ. Nucleotide excision repair from a site-specifically platinum-modified nucleosomes. Biochemistry 2003; 42: 6747-53.

[25] Carte N, Legendre F, Leize E, *et al*. Determination by electrospray mass spectrometry of the outersphere association constants of DNA/platinum complexes using 20-mer oligonucleotides and ($[Pt(NH_3)_4]^{2+}$, $2Cl^-$) or ($[Pt(py)_4]^{2+}$, $2Cl^-$). Anal Biochem 2000; 284: 77-86.

[26] Mello JA, Acharya S, Fishel R, Essigmann JM. The mismatch-repair protein hMSH2 binds selectively to DNA adducts of the anticancer drug cisplatin. Chem Biol 1996; 3: 579-89.

[27] Warnke U, Gysler J, Hofte B, *et al*. Separation and identification of platinum adducts with DNA nucleotides by capillary zone electrophoresis and capillary zone electrophoresis coupled to mass spectrometry. Electrophoresis 2001; 22: 97-103.

*[28] Yokoyama, S.: US6046044 (**2000**).

[29] Yokoyama, S.: US5846725 (**1998**).

[30] Yokoyama, S.: US5646011 (**1997**).

[31] Jakupec MA, Galanski M, Keppler BK. Tumor-inhibiting platinum complexes – state of the art and future perspectives. Rev Physiol Biochem Pharmacol 2003; 146: 1–53.

[32] Hall MD, Hambley TW. Platinum(IV) antitumor compounds: their bioinorganic chemistry. Coord Chem Rev 2002; 232: 49-67.

[33] Weiss RB, Christian MC. New cisplatin analogues in development. A review. Drugs 1993; 46: 360-77.

[34] Nishiyama N, Kato Y, Sugiyama Y, Kataoka K. Cisplatin-loaded polymer-metal complex micelle with time-modulated decaying property as a novel drug delivery system. Pharm Res 2001; 18: 1035-41.

[35] Courjault F, Leroy D, Coquery L, Toutain H. Platinum complex-induced dysfunction of cultured renal proximal tubule cells. A comparative study of carboplatin and transplatin with cisplatin. Arch Toxicol 1993; 67: 338-46.

[36] Neuse EW, Mphephu N, Netshifhefhe HM, Johnson MT. Synthesis and preliminary *in vitro* evaluation of polymeric dicarboxylatoand dihydroxylatoplatinum(II) chelates as antiproliferative agents. Polym Adv Technol 2002; 13: 884-95.

*[37] Sohn, Y.S., Baek, H.G., Lee, C.O.: US6221906 (**2001**).

[38] Sohn, Y.S., Baek, H., Cho, Y.H., Jung, O.-S.: US5665343 (**1997**).

[39] Sohn, Y.S., Song, S.-C., Lee, S.B.: US6333422 (**2001**).

*[40] Bogdanov, A., Weissleder, R., Brady, T. J.: US5871710 (**1999**).

[41] Ohya Y, Oue H, Nagatomi K, Ouchi T. Design of macromolecular prodrug of cisplatin using dextran with branched galactose units as targeting moieties to hepatoma cells. Biomacromolecules 2001; 2: 927-33.

[42] Ohya Y, Nagatomi K, Ouchi T. Synthesis and cytotoxic activity of macromolecular prodrug of cisplatin using poly(ethylene glycol) with galactose residues or antennary galactose units. Macromol Biosci 2001; 1: 355-63.

[43] Briz O, Serrano MA, Rebollo N, *et al*. Carriers involved in targeting the cytostatic bile acid-cisplatin derivatives cis-diammine-chlorocholylglycinate-platinum(II) and cis-diamminebisursodeoxycholate-platinum(II) toward liver cells. Mol Pharmacol 2002; 61: 853-60.

[44] Paschke R, Kalbitz J, Paetz C, *et al*. Cholic acid-carboplatin compounds (CarboChAPt) as models for specific drug delivery: synthesis of novel carboplatin analogous derivatives and comparison of the cytotoxic properties with corresponding cisplatin compounds. J Inorg Biochem 2003; 94: 335-42.

[45] Jackson A, Davis J, Pither RJ, Rodger A, Hannon MJ. Estrogen derived steroidal metal complexes: agents for cellular delivery of metal centers to estrogen receptor-positive Cells. Inorg Chem 2001; 40: 3964-73.

[46] Lottner C, Bart K-C, Bernhardt G, Brunner H. Hemato-porphyrinderived soluble porphyrin-platinum conjugates with combined cytotoxic and phototoxic antitumor activity. J Med Chem 2002; 45: 2064-78.

[47] Lottner C, Bart K-C, Bernhardt G, Brunner H. Soluble tetraarylporphyrin-platinum conjugates as cytotoxic and phototoxic antitumor agents. J Med Chem 2002; 45: 2079-89.

[48] Sundquist WI, Bancroft DP, Chassot L, Lippard SJ. DNA promotes the reaction of *cis*-diaminedichloroplatinum(II) with the exocyclic amino groups of ethidium bromide. J Am Chem Soc 1988; 110: 8559-60.
[49] Bowler BE, Ahmed KJ, Sundquist WI, Hollis LS, Whang EE, Lippard SJ. Synthesis, characterization, and DNA-binding properties of (1,2-diaminoethane)platinum(II) complexes linked to the DNA intercalator acridine orange by trimethylene and hexamethylene chains. J Am Chem Soc 1989; 111: 1299-306.
[50] Sundquist WI, Bancroft DP, Lippard SJ. Synthesis, characterization, and biological activity of cisdiamineplatinum(II) complexes of the DNA intercalators 9-aminoacridine and chloroquine. J Am Chem Soc 1990, 112:1590-1596.
[51] Holmes RJ, McKeage MJ, Murray V, Denny WA, McFadyen WD: *cis*-Dichloroplatinum(II) complexes tethered to 9-aminoacridine-4-carboxamides: synthesis and action in resistant cell lines *in vitro*. J Inorg Biochem 2001; 85: 209-17.
[52] Temple MD, Recabarren P, McFadyen WD, Holmes RJ, Denny WA, Murray V. The interaction of DNA-targeted 9-aminoacridine-4-carboxamide platinum complexes with DNA in intact human cells. Biochim Biophys Acta 2002; 1574: 223-30.
[53] Baruah H, Barry CG, Bierbach U. Platinum-intercalator conjugates: from DNA-targeted cisplatin derivatives to adenine binding complexes as potential modulators of gene regulation. Curr Top Med Chem 2004; 4: 1537-49.
*[54] Heffernan, J.G., Cleare, M.J., Picker, D.H.: US4760156 (**1988**).
*[55] Heffernan, J.G., Hydes, P.C., Picker, D.H.: US4760155 (**1988**).
[56] Smith, G.D., Brown, D.S., Bernstein, P., Weller, J.E.: US4673754 (**1987**).
[57] Smith, G.D., Brown, D.S., Bernstein, P., Weller; J.E.: US4584392 (**1986**).
[58] Bitha, P., Hlavka, J.J., Lin; Y.-I.: US4808730 (**1989**).
[59] Rosenberg, B., VanCamp, L., Krigas, T.: US4339437 (**1982**).
[60] Hydes, P.D., Watkins, D.M.: US4250189 (**1981**).
*[61] Speer, R.J., Stewart, D.P.: US4594418 (**1986**).
*[62] Speer, R.J., Stewart; D.P.: US4562275 (**1985**).
[63] Bednarski PJ. Relationships between the aqueous chemistry and the *in vitro* cytotoxic activities of mixed-amine cisplatin analogues. Biochem Pharmacol 1992; 43: 2609-20.
[64] Hirano T, Inagaki K, Fukai T, Alink M, Nakahara H, Kidani Y. Cytotoxicity of asymmetric platinum complexes against L-1210 cells. Effect of bulky substituents. Chem Pharm Bull (Tokyo) 1990; 38: 2850-2.
[65] Yoshida M, Khokhar AR, Siddik ZH. Cytotoxicity and tolerance to DNA adducts of alicyclic mixed amine platinum(II) homologs in tumor models sensitive and resistant to cisplatin or tetraplatin. Oncol Rep 1998; 5: 1281-7.
[66] Khokhar AR, Xu QY, Newman RA, Siddik ZH. Preparation, characterization, and antitumor activity of water-soluble aminoalkanol platinum(II) complexes. J Inorg Biochem 1991; 43: 57-63.
[67] Kim DK, Kim G, Gam J, *et al*. Synthesis and antitumor activity of a series of [2-substituted-4,5-bis(aminomethyl)-1,3-dioxolane] platinum(II) complexes. J Med Chem 1994; 37: 1471-85.
[68] Totani, T., Aono, K.: US4577038 (**1986**).
[69] Totani, T., Aono, K., Komura, M.: US4560781 (**1985**).
[70] Cleare, M.J., Hoeschele, J.D., Rosenberg; B., Van Camp, L.L.: US4657927 (**1987**).
[71] Cleare, M.J., Hoeschele, J.D., Rosenberg, B., Van Camp, L.L.: US4140707 (**1979**).
[72] Gandolfi, O.: US4614811 (**1986**).
*[73] Kaplan, M.A., Granatek, A.P.: US4322362 (**1982**).
[74] Yoshida M, Khokhar AR, Siddik ZH. Circumvention of platinum resistance: structure-activity relationship for homologous series of ammine/amine platinum(II) complexes in L1210 cell lines. Anticancer Drug Des 1994; 9: 425-34.
[75] Hoeschele, J.D., Amundsen, A.R.: US4248840 (**1981**).
[76] Hoeschele, J.D., Amundsen, A.R.: US4234499 (**1980**).
[77] Amundsen, A.R., Stern, E.W.: US4500465 (**1985**).
[78] Hlavka, J.J., Child, R.G., Bitha, P., Lin, Y.I.: US4587331 (**1986**).
[79] Yolles, S.: US4283342 (**1981**).
*[80] Nakayama, Y., Iwata, K., Takahashi, K.: US4607114 (**1986**)**.**
*[81] Kidani, Y., Noji, M.: US4477387 (**1984**).
*[82] Verbeek, F., Bulten, E.J., Berg, J.: US4466924 (**1984**).
*[83] Nowatari, H., Hayami, H., Kuroda, Y., Yoda, S., Takahashi, K.: US4861905 (**1989**).
*[84] Berg, J., Verbeek, F., Bluten, E.J.: US4410544 (**1983**).
[85] Bitha, P., Child, R.G., Hlavka, J.J., Lin, Y.-I.: US4716157 (**1987**).
[86] Weis, A.L., Chen, S.-F., Reedy, P.S., Mittakanti, M., Dexter, D.L.: US5604095 (**1997**).
[87] Weis, A.L., Chen, S.-F., Reddy, P.S., Mittakanti, M., Dexter, D. L., Woynarowski, J.M.: US5561042 (**1996**).
[88] Monti E, Gariboldi M, Maiocchi A, *et al*. Cytotoxicity of cis-platinum(II) conjugate models. The effect of chelating arms and leaving groups on cytotoxicity: a quantitative structure-activity relationship approach. J Med Chem 2005; 48: 857-66.
[89] Sene A, Berube G, Gaudreault RC. Synthesis and preliminary *in vitro* cytotoxic activity of novel cisplatin and carboplatin analogues. Drug Des Discov 1998; 15: 277-85.
[90] Vickery K, Bonin AM, Fenton RR, *et al*. Preparation, characterization, cytotoxicity, and mutagenicity of a pair of enantiomeric platinum(II) complexes with the potential to bind enantioselectively to DNA. J Med Chem 1993; 36: 3663-8.
[91] Alvarez-Valdes A, Perez JM, Lopez-Solera I, *et al*. Preparation and characterization of platinum(II) and (IV) complexes of 1,3-diamenepropane and 1,4-diaminebutane: circumvention of cisplatin resistance and DNA interstrand cross-link formation in CH1cisR ovarian tumor cells. J Med Chem 2002; 45: 1835-44.
[92] Moradell S, Lorenzo J, Rovira A, *et al*. Platinum complexes of diaminocarboxylic acids and their ethyl ester derivatives: the effect of the chelate ring size on antitumor activity and interactions with GMP and DNA. J Inorg Biochem 2003; 96: 493-502.
[93] Perrin LC, Prenzler PD, Cullinane C, Phillips DR, Denny WA, McFadyen WD. DNA targeted platinum complexes: synthesis, cytotoxicity and DNA interactions of cis-dichloroplatinum(II) complexes tethered to phenazine-1-carboxamides. J Inorg Biochem 2000; 81: 111-7.
[94] Navarro-Ranninger C, Ochoa PA, Perez JM, Rodriguez JH, Masaguer JR, Alonso C. Platinum complexes of *p*- and *m*-aminobenzamidine. Synthesis, characterization, and cytotoxic activity. J Inorg Biochem 1992; 48: 163-71.
*[95] Brown, d. D.B., Khokhar, A.R., Hacker, M.P., McCormack, J.J.: US4758588 (**1988**).
[96] Kidani, Y., Inagaki, K.: US4169846 (**1979**).
[97] Kidani, Y., Noji, M.: US4200583 (**1980**).
[98] Kidani, Y., Noji, M.: US4256652 (**1981**).
*[99] Khokhar, A.R., Newman, R.A., Krakoff, I.H.: US5011959 (**1991**).
*[100] Hlavka, J.J., Lin, Y.-I. Bitha, P.: US4670458 (**1987**).
[101] Gale, G.R., Meischen, S.J.: US4115418 (**1978**).
[102] Gale, G.R., Schwartz, P.: US4137248 (**1979**).
[103] Macquet, J.P.: US4452812 (**1984**).
[104] Kidani, Y., Noji, M.: US4551524 (**1985**).
[105] Meischen, S.J., Gale, G.R., Naff, M.B.: US4284579 (**1981**).
[106] Child, R.G., Bitha, P., Hlavka, J.J., Lin, Y.-I.: US4760157 (**1988**).
[107] Witiak, D.T., Rotella, D.P.: US5206400 (**1993**).
*[108] Khokhar, A.R., Siddik, Z.H.: US5393909 (**1995**).
[109] Paul AK, Srivastava TS, Chavan SJ, Chitnis MP, Desai S, Rao KK. Synthesis, characterization, cytotoxic, and DNA binding studies of some platinum (II) complexes of 1,2-diamine and alpha-diimine with 2-pyridinecarboxylate anion. J Inorg Biochem 1996; 61: 179-96.
*[110] Speer, R.J., Stewart, D.P.: US4739087 (**1988**).
[111] Mansuri-Torshizi H, Mital R, Srivastava TS, Parekh H, Chitnis MP. Synthesis, characterization, and cytotoxic studies of alpha-diimine/1,2-diamine platinum(II) and palladium(II) complexes of selenite and tellurite and binding of some of these complexes to DNA. J Inorg Biochem 1991; 44: 239-47.
[112] Kageyama Y, Yamazaki Y, Okuno H. Novel approaches to prodrugs of anticancer diaminodichloroplatinum(II) complexes activated by stereoselective enzymatic ester hydrolysis. J Inorg Biochem 1998; 70: 25-32.
[113] Schonenberger, H., Wappes, B., Jennerwein, M., von Angerer, E., Engel, J.: US4598091 (**1986**).
[114] Bernges F, Holler E. Ring-substituted diaqua(1,2-diphenyl-ethylenediamine)platinum(II) sulfate reacts with DNA through a dissociable complex. Eur J Biochem 1992; 208: 573-9.
[115] Karl J, Gust R, Spruss T, *et al*. Ring-substituted [1,2-bis(4-hydroxyphenyl)ethylenediamine]dichloroplatinum (II)

complexes: compounds with a selective effect on the hormone-dependent mammary carcinoma. J Med Chem 1988; 31: 72-83.

[116] Spruss T, Bernhardt G, Schickaneder E, Schonenberger H. Different response of murine and human mammary tumour models to a series of diastereoisomeric [1,2-bis(difluorophenyl) ethyl-enediamine]dichloroplatinum(II) complexes. J Cancer Res Clin Oncol 1991; 117: 435-43.

[117] Schertl S, Hartmann RW, Batzl-Hartmann C, *et al*. [1, 2-Bis(2, 6-difluoro-3-hydroxyphenyl)ethylenediamine]platinum(II) complexes, compounds for the endocrine therapy of breast cancer - mode of action II: contribution of drug inactivation, cellular drug uptake and sterical factors in the drug-target interaction to the antitumor activity. Arch Pharm (Weinheim) 2004; 337: 349-59.

[118] Schertl S, Hartmann RW, Batzl-Hartmann C, *et al*. [1, 2-Bis(2, 6-difluoro-3-hydroxyphenyl)ethylenediamine]platinum(II) complexes, compounds for the endocrine therapy of breast cancer - mode of action I: antitumor activity due to the reduction of the endogenous estrogen level. Arch Pharm (Weinheim) 2004; 337: 335-48.

*[119] Hoeschele, J.D., Amundsen, A.R.: US4234500 (**1980**).

[120] Kim JC, Lee MH, Choi SK. Synthesis and antitumor evaluation of *cis*-(1,2-diaminoethane) dichloroplatinum (II) complexes linked to 5- and 6-methyleneuracil and -uridine analogues. Arch Pharm Res 1998; 21: 465-9.

[121] Lee HH, Palmer BD, Baguley BC, *et al*. DNA-directed alkylating agents. 5. Acridinecarboxamide derivatives of (1,2-diaminoethane)dichloroplatinum(II). J Med Chem 1992; 35: 2983-7.

[122] Mital R, Srivastava TS, Parekh HK, Chitnis MP. Synthesis, characterization, DNA binding, and cytotoxic studies of some mixed-ligand palladium(II) and platinum(II) complexes of alpha-diimine and amino acids. J Inorg Biochem 1991; 41: 93-103.

[123] Paul AK, Mansuri-Torshizi H, Srivastava TS, Chavan SJ, Chitnis MP. Some potential antitumor 2,2'-dipyridylamine Pt(II)/Pd(II) complexes with amino acids: their synthesis, spectroscopy, DNA binding, and cytotoxic studies. J Inorg Biochem 1993; 50: 9-20.

[124] Moradell S, Lorenzo J, Rovira A, *et al*. Water-soluble platinum(II) complexes of diamine chelating ligands bearing amino-acid type substituents: the effect of the linked amino acid and the diamine chelate ring size on antitumor activity, and interactions with 5'-GMP and DNA. J Inorg Biochem 2004; 98: 1933-46.

[125] Zhang J, Wang X, Tu C, *et al*. Monofunctional platinum complexes showing potent cytotoxicity against human liver carcinoma cell line BEL-7402. J Med Chem 2003; 46: 3502-7.

[126] Amundsen, A.R., Stern, E.W.: US4457926 (**1984**).

[127] Amundsen, A.R., Stern, E.W.: US4462998 (**1984**).

[128] Amundsen, A.R., Stern, E.W.: US4271085 (**1981**).

*[129] Bitha, P., Child, R.G., Hlavka, J.J., Lin, Y.-I.: US4675336 (**1987**).

[130] Bitha, P., Child, R.G., Hlavka, J.J., Lin, Y.-I.: US4665210 (**1987**).

[131] Turkevich, J., Burchenal, J.H.: US4376782 (**1983**).

[132] Natile G, Coluccia M. Current status of *trans*-platinum compounds in cancer therapy. Coord Chem Rev 2001; 216-217: 383-410.

[133] Al-Allaf TA, Rashan LJ. *Cis*- and *trans*-platinum and palladium complexes: a comparative study review as antitumour agents. Boll Chim Farm 2001; 140: 205-10.

*[134] Farrell, N., Bierbach, U.: US6001872 (**1999**).

[135] Perez JM, Fuertes MA, Alonso C, Navarro-Ranninger C. Current status of the development of *trans*-platinum antitumor drugs. Crit Rev Oncol Hematol 2000; 35: 109-20.

[136] Prokop R, Kasparkova J, Novakova O, *et al*. DNA interactions of new antitumor platinum complexes with trans geometry activated by a 2-metylbutylamine or sec-butylamine ligand. Biochem Pharmacol 2004; 67: 1097-109.

[137] Montero EI, Diaz S, Gonzalez-Vadillo AM, Perez JM, Alonso C, Navarro-Ranninger C. Preparation and characterization of novel trans-[$PtCl_2$(amine)(isopropylamine)] compounds: cytotoxic activity and apoptosis induction in ras-transformed cells. J Med Chem 1999; 42: 4264-8.

[138] Perez JM, Montero EI, Gonzalez AM, Alvarez-Valdes A, Alonso C, Navarro-Ranninger C. Apoptosis induction and inhibition of H-ras overexpression by novel trans-[$PtCl_2$(isopropylamine)(amine')] complexes. J Inorg Biochem 1999; 77: 37-42.

[139] Farrell N, Povirk LF, Dange Y, *et al*. Cytotoxicity, DNA strand breakage and DNA-protein crosslinking by a novel *trans* platinum compound in human A2780 ovarian and MCF-7 breast carcinoma cells. Biochem Pharmacol 2004; 68: 857-66.

*[140] Farrell, N.: US6350740 (**2002**).

[141] Novakova O, Kasparkova J, Malina J, Natile G, Brabec V. DNA-protein cross-linking by trans-[$PtCl_2$(E-iminoether)$_2$]. A concept for activation of the trans geometry in platinum antitumor complexes. Nucleic Acids Res 2003; 31: 6450-60.

[142] Coluccia M, Nassi A, Boccarelli A, *et al*. *In vitro* and *in vivo* antitumour activity and cellular pharmacological properties of new platinum-iminoether complexes with different configuration at the iminoether ligands. J Inorg Biochem 1999; 77: 31-5.

[143] Boccarelli A, Coluccia M, Intini FP, Natile G, Locker D, Leng M. Cytotoxicity and DNA binding mode of new platinum-iminoether derivatives with different configuration at the iminoether ligands. Anticancer Drug Des 1999; 14: 253-64.

[144] Najajreh Y, Perez JM, Navarro-Ranninger C, Gibson D. Novel soluble cationic *trans*-diaminedichloroplatinum(II) complexes that are active against cisplatin resistant ovarian cancer cell lines. J Med Chem 2002; 45: 5189-95.

[145] Ali MS, Whitmire KH, Toyomasu T, Siddik ZH, Khokhar AR. Preparation, characterization, and antitumor activity of new cisplatin analogs with homopiperazines: crystal structure of [PtII(1-methylhomopiperazine)(methylmalonato)].2H_2O. J Inorg Biochem 1999; 77: 231-8.

[146] Khazanov E, Barenholz Y, Gibson D, Najajreh Y. Novel apoptosis-inducing *trans*-platinum piperidine derivatives: synthesis and biological characterization. J Med Chem 2002; 45: 5196-204.

[147] Mukhopadhyay U, Thurston J, Whitmire KH, Siddik ZH, Khokhar AR. Preparation, characterization, and antitumor activity of new cisplatin analogues with 1-methyl-4-(methylamino)piperidine: crystal structure of [PtII(1-methyl-4-(methylamino) piperidine) (oxalate)]. J Inorg Biochem 2003; 94: 179-85.

[148] Nguewa PA, Fuertes MA, Iborra S, *et al.* Water soluble cationic *trans*-platinum complexes which induce programmed cell death in the protozoan parasite *Leishmania infantum*. J Inorg Biochem. 2005; 99: 727-36.

[149] Pratesi G, Perego P, Polizzi D, *et al*. A novel charged trinuclear platinum complex effective against cisplatin-resistant tumors: hypersensitivity of p53-mutant human tumor xenografts. Br J Cancer 1999; 80: 1912-9.

[150] Zamble DB, Jacks T, Lippard SJ. p53-dependent and -independent responses to cisplatin in mouse testicular teratocarcinoma cells. Proc Natl Acad Sci USA 1998; 95: 6163-8.

[151] Gatti L, Supino R, Perego P, *et al*. Apoptosis and growth arrest induced by platinum compounds in U2-OS cells reflect a specific DNA damage recognition associated with a different p53-mediated response. Cell Death Differ 2002; 9: 1352-9.

[152] Zehnulova J, Kasparkova J, Farrell N, Brabec V. Conformation, recognition by high mobility group domain proteins, and nucleotide excision repair of DNA intrastrand cross-links of novel antitumor trinuclear platinum complex BBR3464. J Biol Chem 2001; 276: 22191-9.

[153] Kasparkova J, Zehnulova J, Farrell N, Brabec V. DNA interstrand cross-links of the novel antitumor trinuclear platinum complex BBR3464. J Biol Chem 2002; 277: 48076-86.

[154] Brabec V, Kasparkova J. Molecular aspects of resistance to antitumor platinum drugs. Drug Resist Updat 2002; 5: 147-61.

[155] Kasparkova J, Pospisilova S, Brabec V. Different recognition of DNA modified by antitumor cisplatin and its clinically ineffective trans isomer by tumor suppressor protein p53. J Biol Chem 2001; 276: 16064-9.

[156] Pratesi G, Perego P, Polizzi D, *et al*. A novel charged trinuclear platinum complex effective against cisplatin-resistant tumours: hypersensitivity of p53-mutant human tumour xenografts. Br J Cancer 1999; 80: 1912-9.

[157] Manzotti C, Pratesi G, Menta E, *et al*. BBR 3464: A novel triplatinum complex, exhibiting a preclinical profile of antitumor efficacy different from cisplatin. Clin Cancer Res 2000; 6: 2626-34.

[158] Perego P, Caserini C, Gatti L, *et al*. A novel trinuclear platinum complex overcomes cisplatin resistance in an osteosarcoma cell system. Mol Pharmacol 1999; 55: 528-34.

[159] Perego P, Gatti L, Caserini C, *et al*. The cellular basis of the efficacy of the trinuclear platinum complex BBR 3464 against cisplatin-resistant cells. J Inorg Biochem 1999; 77: 59-64.

[160] Roberts JD, Peroutka J, Farrell N. Cellular pharmacology of polynuclear platinum anti-cancer agents. J Inorg Biochem 1999; 77: 51-7.

[161] Andrulis Jr. P.J., Schwartz, P.: US4565884 (**1986**).

*[162] Andrulis, Jr. P.J., Schwartz, P.: US4720504 (**1988**).

[163] Kozelka J, Segal E, Bois C. Cytotoxic activity of platinum (II) complexes with tri-n-butylphosphine. Crystal structure of the dinuclear hydrazine-bridged complex, cis,cis-[PtCl(PBu3n)$_2$(mu-N_2H_4)PtCl(PBu3n)$_2$] (ClO_4)$_2$.2$CHCl_3$. J Inorg Biochem 1992; 47: 67-80.

[164] Cesar ET, de Almeida MV, Fontes AP, *et al*. Synthesis, characterization, cytotoxic activity, and cellular accumulation of dinuclear platinum complexes derived from N,N'-di-(2-aminoethyl)-1,3-diamino-2-propanol, aryl substituted N-benzyl-1,4-butanediamines, and N-benzyl-1,6-hexanediamines. J Inorg Biochem 2003; 95: 297-305.

[165] Palmer BD, Wickham G, Craik DJ, *et al*. Synthesis, DNA binding interactions and biological activity of bis platinum (II) complexes of N,N,N',N'-tetrakis(2-aminoethyl)diamines. Anticancer Drug Des 1992; 7: 385-401.

[166] Komeda S, Lutz M, Spek AL, Chikuma M, Reedijk J. New antitumor-active azole-bridged dinuclear platinum(II) complexes: synthesis, characterization, crystal structures, and cytotoxic studies. Inorg Chem 2000; 39: 4230-6.

[167] Komeda S, Lutz M, Spek AL, *et al*. A novel isomerization on interaction of antitumor-active azole-bridged dinuclear platinum(II) complexes with 9-ethylguanine. Platinum(II) atom migration from N2 to N3 on 1,2,3-triazole. J Am Chem Soc 2002; 124: 4738-46.

[168] Jansen BA, van der Zwan J, den Dulk H, Brouwer J, Reedijk J. Dinuclear alkyldiamine platinum antitumor compounds: a structure-activity relationship study. J Med Chem 2001; 44: 245-9.

[169] Teixeira LJ, Seabra M, Reis E, *et al*. Cytotoxic activity of metal complexes of biogenic polyamines: polynuclear platinum(II) chelates. J Med Chem 2004; 47: 2917-25.

[170] Marques MP, Girao T, Pedroso De Lima MC, Gameiro A, Pereira E, Garcia P. Cytotoxic effects of metal complexes of biogenic polyamines. I. Platinum(II) spermidine compounds: prediction of their antitumour activity. Biochim Biophys Acta 2002; 1589: 63-70.

[171] Farrell N. Nonclassical platinum antitumor agents: perspectives for design and development of new drugs complementary to cisplatin. Cancer Invest. 1993; 11: 578-89.

[172] Okada T, El-Mehasseb IM, Kodaka M, Tomohiro T, Okamato K, Okuno H. Mononuclear platinum(II) complex with 2-phenylpyridine ligands showing high cytotoxicity against mouse sarcoma 180 cells acquiring high cisplatin resistance. J Med Chem 2001; 44: 4661-7.

[173] Craciunescu DG, Doadrio A, Furlani A, Scarcia V. Structure-antitumour activity relationships for new platinum complexes. Chem Biol Interact 1982; 42: 153-64.

[174] Bierbach U, Sabat M, Farrell N. Inversion of the *cis* geometry requirement for cytotoxicity in structurally novel platinum(II) complexes containing the bidentate N,O-donor pyridin-2-yl-acetate. Inorg Chem 2000; 39: 1882-90.

[175] Kovala-Demertzi D, Yadav PN, Demertzis MA, Coluccia M. Synthesis, crystal structure, spectral properties and cytotoxic activity of platinum(II) complexes of 2-acetyl pyridine and pyridine-2-carbaldehyde N(4)-ethyl-thiosemicarbazones. J Inorg Biochem 2000; 78: 347-54.

[176] Kovala-Demertzi D, Demertzis MA, Filiou E, *et al*. Platinum(II) and palladium(II) complexes with 2-acetyl pyridine 4N-ethyl thiosemicarbazone able to overcome the *cis*-platin resistance. Structure, antibacterial activity and DNA strand breakage. Biometals 2003; 16: 411-8.

[177] Quiroga AG, Perez JM, Montero EI, West DX, Alonso C, Navarro-Ranninger C. Synthesis and characterization of Pd(II) and Pt(II) complexes of p-isopropylbenzaldehyde N-protected thiosemi-carbazones. Cytotoxic activity against ras-transformed cells. J Inorg Biochem 1999; 75: 293-301.

[178] Quiroga AG, Perez JM, Lopez-Solera I, *et al*. Novel tetranuclear orthometalated complexes of Pd(II) and Pt(II) derived from *p*-isopropylbenzaldehyde thiosemicarbazone with cytotoxic activity in *cis*-DDP resistant tumor cell lines. Interaction of these complexes with DNA. J Med Chem 1998; 41: 1399-408.

[179] Quiroga AG, Perez JM, Lopez-Solera I, *et al*. Binuclear chloro-bridged palladated and platinated complexes derived from *p*-isopropylbenzaldehyde thiosemicarbazone with cytotoxicity against cisplatin resistant tumor cell lines. J Inorg Biochem 1998; 69: 275-81.

[180] Perez JM, Quiroga AG, Montero EI, Alonso C, Navarro-Ranninger C. A cycloplatinated compound of *p*-isopropyl-benzaldehyde thiosemicarbazone and its chloro-bridged derivative induce apoptosis in cis-DDP resistant cells which overexpress the H-ras oncogene. J Inorg Biochem 1999; 73: 235-43.

[181] Quiroga AG, Perez JM, Montero EI, Masaguer JR, Alonso C, Navarro-Ranninger C. Palladated and platinated complexes derived from phenylacetaldehyde thiosemicarbazone with cytotoxic activity in cis-DDP resistant tumor cells. Formation of DNA interstrand cross-links by these complexes. J Inorg Biochem 1998; 70: 117-23.

[182] Najman-Bronzewska L, Ochocki J. *In vitro* alkylating of alpha, beta-unsaturated amidoesters, diethyl pyridylmethylphos-phonates and their *cis*-Pt(II)complexes. Pharmazie 1997; 52: 198-202.

[183] Brzezinska-Blaszczyk E, Mincikiewicz M, Ochocki J. Effect of cisplatin and *cis*-platinum (II) phosphonate complex on murine mast cells. Eur J Pharmacol 1996; 298: 155-8.

[184] Carrara M, Berti T, D'Ancona S, Cherchi V, Sindellari L. *In vitro* effect of Pt and Pd mercaptopyridine complexes. Anticancer Res 1997; 17: 975-80.

[185] Colangelo D, Ghiglia AL, Viano I, Cavigiolio G, Osella D. *Cis*-[$Pt(Cl)_2$(pyridine)(5-SO3H-isoquinoline)] complex, a selective inhibitor of telomerase enzyme. Biometals 2003; 16: 553-60.

[186] Mansuri-Torshizi H, Srivastava TS, Parekh HK, Chitnis MP. Synthesis, spectroscopic, cytotoxic, and DNA binding studies of binuclear 2,2'-bipyridine-platinum(II) and -palladium(II) complexes of meso-alpha,alpha'-diaminoadipic and meso-alpha,alpha'-diaminosuberic acids. J Inorg Biochem 1992; 45: 135-48.

[187] Yoo J, Sohn YS, Do Y. Synthesis, structures and antitumor activity of the first crown ester-linked bipyridyl platinum complexes. J Inorg Biochem 1999; 73: 187-93.

[188] Mansuri-Torshizi H, Ghadimy S, Akbarzadeh N. Synthesis, characterization, DNA binding and cytotoxic studies of platinum(II) and palladium(II) complexes of the 2,2'-bipyridine and an anion of 1,1-cyclobutanedicarboxylic acid. Chem Pharm Bull (Tokyo) 2001; 49: 1517-20.

[189] Kodaka M, Dohta Y, Rekonen P, Okada T, Okuno H. Physicochemical factors for cytotoxic activity in platinum dinuclear complexes with pyrimidine and imide ligands. Biophys Chem 1998; 75: 259-70.

[190] Lakomska I, Szlyk E, Sitkowski J, *et al*. Multinuclear NMR spectroscopy and antitumor activity of novel platinum(II) complexes with 5,7-disubstituted-1,2,4-triazolo[1,5-a]pyrimidines. J Inorg Biochem 2004; 98: 167-72.

[191] Wisniewski MZ, Wietrzyk J, Opolski A. Novel Ru(III), Rh(III), Pd(II) and Pt(II) complexes with ligands incorporating azole and pyrimidine rings. I. Antiproliferative activity *in vitro*. Arch Immunol Ther Exp (Warsz) 2000; 48: 51-5.

[192] Onoa GB, Moreno V, Font-Bardia M, Solans X, Perez JM, Alonso C. Structural and cytotoxic study of new Pt(II) and Pd(II) complexes with the bi-heterocyclic ligand mepirizole. J Inorg Biochem 1999; 75: 205-12.

[193] Drewa T, Wozniak A, Olszewska D, *et al*. The *in vitro* study of influence of four novel platinum compounds on rodent melanoma cells. Acta Pol Pharm 2001; 58: 169-74.

[194] Martins ET, Baruah H, Kramarczyk J, *et al*. Design, synthesis, and biological activity of a novel non-cisplatin-type platinum-acridine pharmacophore. J Med Chem 2001; 44: 4492-6.

[195] Brow JM, Pleatman CR, Bierbach U. Cytotoxic acridinylthiourea and its platinum conjugate produce enzyme-mediated DNA strand breaks. Bioorg Med Chem Lett 2002; 12: 2953-5.

[196] Hall MD, Martin C, Ferguson DJ, Phillips RM, Hambley TW, Callaghan R. Comparative efficacy of novel platinum(IV) compounds with established chemotherapeutic drugs in solid tumour models. Biochem Pharmacol 2004; 67: 17-30.

[197] McKeage MJ, Higgins JD 3rd, Kelland LR. Platinum and other metal coordination compounds in cancer chemotherapy. A commentary on the sixth international symposium: San Diego, California, 23-26th January 1991. Br J Cancer 1991; 64: 788-92.

*[198] Abrams, M.J., Giandomenico, C., Murrer, B.A., Vollano, J.F.: US5072011 (**1991**).

*[199] Abrams, M.J., Giandomenico, C.M., Murrer, B.A., Vollano, J.F.: US5244919 (**1993**).

[200] Totani, T., Shiratori, O., Aono, K., Uchida, N.: US4658048 (**1987**).

[201] Orr RM, O'Neill CF, Nicolson MC, *et al*. Evaluation of novel amine/amine platinum (IV) dicarboxylates in L1210 murine leukaemia cells sensitive and resistant to cisplatin, tetraplatin or carboplatin. Br J Cancer 1994; 70: 415-20.

[202] Kelland LR, Murrer BA, Abel G, Giandomenico CM, Mistry P, Harrap KR. Amine/amine platinum(IV) dicarboxylates: a novel class of platinum complex exhibiting selective cytotoxicity to intrinsically cisplatin-resistant human ovarian carcinoma cell lines. Cancer Res 1992; 52: 822-8.

[203] Turanek J, Kasna A, Zaluska D, *et al*. New platinum(IV) complex with adamantylamine ligand as a promising anti-cancer drug: comparison of *in vitro* cytotoxic potential towards A2780/cisR cisplatin-resistant cell line within homologous series of platinum(IV) complexes. Anticancer Drugs 2004; 15: 537-43.

[204] Brandon RJ, Dabrowiak JC. Synthesis, characterization, and properties of a group of platinum (IV) complexes. J Med Chem 1984; 27: 861-5.

[205] Kratochwil NA, Zabel M, Range KJ, Bednarski PJ. Synthesis and X-ray crystal structure of *trans,cis*-[Pt(OAc)$_2$I$_2$(en)]: a novel type of cisplatin analog that can be photolyzed by visible light to DNA-binding and cytotoxic species *in vitro*. J Med Chem 1996; 39: 2499-507.

[206] Bulten, E.J., Verbeek, F.: US4482569 (**1984**).

[207] Bulten, E.J., Verbeek, F.: US4431666 (**1984**).

*[208] Khokhar, A. R., Siddik, Z. H. Al-Baker, S.: US5288887 (**1994**).

[209] Kelland LR, Barnard CF, Mellish KJ, *et al*. A novel trans-platinum coordination complex possessing *in vitro* and *in vivo* antitumor activity. Cancer Res 1994; 54: 5618-22.

[210] Yoshida M, Khokhar AR, Siddik ZH. Axial ligands and alicyclic ring size modulate the activity and biochemical pharmacology of ammine/cycloalkylamine-platinum(IV) complexes in tumor cells resistant to *cis*-diamminedichloroplatinum(II) or *trans*-1*R*,2*R*-1*S*,2*S*-diaminocyclohexane-tetrachloroplatinum(IV). Cancer Res 1994; 54: 4691-7.

[211] Schwartz, P., Gill, D.S. McGrath, K.J.: US4680308 (**1987**).

[212] Brown, D.B., Khokhar, A.R., Hacker, M.P., McCormack, J.J.: US4661516 (**1987**).

[213] Ali MS, Khan SR, Ojima H, Guzman IY, Whitmire KH, Siddik ZH, Khokhar AR. Model platinum nucleobase and nucleoside complexes and antitumor activity: X-ray crystal structure of [PtIV(*trans*-1*R*,2*R*-diaminocyclohexane)*trans*-(acetate)$_2$(9-ethylg-uanine)Cl]NO_3.H_2O. J Inorg Biochem 2005; 99: 795-804.

[214] Yamashita T, Hirose J, Noji M, Saito R, Tomida H, Kidani Y. Cytotoxicity of platinum(IV) and platinum(II) complexes containing 1*R*,2*R*-cyclohexanediamine as a ligand. Biol Pharm Bull 1993; 16: 1014-8.

[215] Khokhar AR, Al-Baker S, Siddik ZH. Synthesis and antitumor activity of 1,2-diaminocyclohexane platinum(IV) complexes. J Inorg Biochem 1994; 54: 39-47.

[216] Khokhar, A.R.: US5041578 (**1991**).

*[217] Khokhar, A.R., Siddik, Z.H., Newman, R.A.: US5318962 (**1994**).

[218] Ali Khan SR, Huang S, Shamsuddin S, *et al*. Synthesis, characterization and cytotoxicity of new platinum(IV) axial carboxylate complexes: crystal structure of potential antitumor agent [PtIV(*trans*-1*R*,2*R*-diaminocyclohexane)*trans*(acetate)$_2$ Cl_2]. Bioorg Med Chem 2000; 8: 515-21.

*[219] Kidani, Y., Noji, M.: US4845124 (**1989**).

[220] Vishnuvajjala, B.R.: US4658047 (**1987**).

[221] Narayanan, V.L., Wolpert-Defilippes, M.K., Haugwitz, R.D.: US4599352 (**1986**).

*[222] Khokhar, A.R., Siddik, Z.H.: US5434256 (**1995**).