Platinum-Based Anticancer Agents: Innovative Design Strategies and Biological Perspectives

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Abstract: The impact of cisplatin on cancer chemotherapy cannot be denied. Over the past 20 years, much effort has been dedicated to discover new platinum-based anticancer agents that are superior to cisplatin or its analogue, carboplatin. Most structural modifications are based on changing one or both of the ligand types coordinated to platinum. Altering the leaving group can influence tissue and intracellular distribution of the drug, whereas the carrier ligand usually determines the structure of adducts formed with DNA. DNA–Pt adducts produced by cisplatin and many of its classical analogues are almost identical, and would explain their similar patterns of tumor sensitivity and susceptibility to resistance. Recently some highly innovative design strategies have emerged, aimed at overcoming platinum resistance and/or to introduce novel mechanisms of antitumor action. Platinum compounds bearing the 1,2-diaminocyclohexane carrier ligand; and those of multinuclear Pt complexes giving rise to radically different DNA–Pt adducts, have resulted in novel anticancer agents capable of circumventing cisplatin resistance. Other strategies have focused on integrating biologically active ligands with platinum moieties intended to selectively localizing the anticancer properties. With the rapid advance in molecular biology, combined with innovation, it is possible new Pt-based anticancer agents will materialize in the near future. Med Res Rev, 23, No. 5, 633–655, 2003

Key words: platinum complexes; anticancer agents; demethylcantharidin; protein phosphatase inhibition

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1. INTRODUCTION

Malignant disease is a major cause of mortality all over the world. Although great strides are currently being made in unraveling the molecular, cellular, and genetic processes that give rise to cancer, this knowledge has not been translated into effective new cures for the disease. Improvements in curability and survival are dependent on advances in early detection, surgery, radiotherapy, and chemotherapy; but once widespread metastatic disease has become established, chemotherapy is a central component of management. The era of chemotherapy commenced in the late 1940s and 1950s with the clinical introduction of the classical alkylating agents (e.g., nitrogen mustard, cyclophosphamide, and melphalan), antimetabolites (e.g., methotrexate and 5-fluorouracil), with a marked improvement particularly in the treatment of lymphomas and leukemias. Plant alkaloids (e.g., vinca alkaloids and taxoids) and hormonal agents (e.g., tamoxifen) are the more recent clinically effective antitumor cytotoxic drugs (Table I).

From the information presented in Table I, it is obvious that an article of this length cannot review the entire subject of drugs currently used in the treatment of cancer, because the types and properties of the different antitumor agents span a vast spectrum of chemistry and biochemistry. Therefore, we will restrict our discussion to one of the more serendipitous discoveries in cancer chemotherapy: that of cisplatin (*cis*-diamminedichloroplatinum (II), *cis*-[$PtCl_2(NH_3)_2$]) (**1**),[18,19] and its subsequent analogues. Cisplatin is a highly successful platinum-based antitumor agent that has been used clinically for over 30 years and continues to play a central role in cancer chemotherapy.[20–22]

This review also aims to highlight some of the more recent innovative strategies used in designing novel platinum-based anticancer agents with chemical and biological properties distinct from cisplatin and its classical Pt (II) analogues. For example, platinum derivatives bearing the 1,2-diaminocyclohexane (DACH) carrier ligand; the introduction of orally active Pt (IV) complexes; and multinuclear Pt complexes giving rise to radically different DNA–Pt adducts. Our own research integrating chemical components from traditional Chinese medicine (TCM) known to be biologically active against tumors, with a Pt-moiety will also be discussed.

2. DISCOVERY AND USE OF CISPLATIN

The biological activity of cisplatin was discovered fortuitously in 1965 during studies of the effect of an electric current on *Escherichia coli*.[18] Cell division was inhibited not by the electric current but by the production of *cis*-diamminedichloroplatinum (II) (cisplatin) from the platinum electrodes. Cisplatin was subsequently found to have potent antitumor activity. Nowadays, cisplatin is one of the most commonly used compounds for the treatment of a wide spectrum of human malignancies.[21] As a single agent or in combination, cisplatin is now the mainstay of treatment for testicular, ovarian, bladder, cervical, head and neck, and small-cell and non-small-cell lung cancers.[22]

Common problems associated with the clinical use of cisplatin include cumulative toxicities of nephrotoxicity, ototoxicity, and peripheral neuropathy.[23–25] Procedures such as forced diuresis[26–28] and pharmacological interventions with sulfhydryl chemoprotectants,[29] have helped to alleviate the dose-limiting nephrotoxicity. However, the most significant advance in obviating the side effects of cisplatin has come from the process of analogue development.

In addition to the serious side effects, the therapeutic efficacy of cisplatin is also limited by inherent or treatment-induced resistant tumor cell sub-populations.[30] Although the response and cure rates with cisplatin can be high (>90%), as in testicular cancer; in other disease types such as ovarian cancer the initial response rate can be up to 70%, but leading to a 5-year survival rate of only 15–20%.[31] Unfortunately, a majority of ovarian cancer patients (80–85%) do relapse and fail to respond to further cisplatin-based therapy as a result of acquired drug resistance. This limitation assumes even greater significance when it is realized that resistant tumors are frequently cross-resistant to diverse

Table I. Drugs of Choice for Different Types of Cancer

Drug Class	Principal mechanism of action	[@]Example(s) [references]	[#]Types of tumors commonly used for
Alkylating agents	Form covalent bonds with DNA and impede DNA replication	(1) Cyclophosphamide [1] (Nitrogen mustards)	Non-Hodgkin's lymphoma
		(2) Carmustine (BCNU) [2] (Nitrosoureas)	Brain glioblastoma
		(3) Busulphan [3] (Alkylsulphonate)	Chronic lymphocytic leukemia (CLL)
		(4) Cisplatin (Pt complex) [4]	Ovarian, head and neck, lung, testicular
Antimetabolites	Block or subvert pathways in DNA synthesis		
(a) Folate antagonists	Inhibit dihydrofolate reductase, preventing generation of tetrahydrofolate, thereby interfering with thymidylate synthesis	Methotrexate [5]	Acute lymphocytic leukemia (ALL)
(b) Pyrimidine analogues	Become converted into fraudulent nucleotides and inhibit thymidylate synthesis	Fluorouracil [6]	Colorectal, gastric cancers
(c) Purine analogues	6-Thiol analogues of the endogenous 6-OH purine bases that become converted into fraudulent nucleotides.	(1) Mercaptopurine [7] (2) Thioguanine [7]	Acute myelogenous leukemia (AML)
Antitumor antibiotics	Produce diverse effects which are cytotoxic:		
	(1) Interfere with topoisomerase II action, inhibiting DNA and RNA synthesis	Doxorubicin [8]	Osteogenic sarcoma, Hodgkin's disease, CML, soft tissue sarcoma
	(2) Causes fragmentation of DNA chains	Bleomycin [9]	Cervical cancer
	(3) Intercalates in DNA, interferes with RNA polymerase and inhibits transcription	Dactinomycin [10]	Wilms' tumor
	(4) Acts as an alkylating agent	Mitomycin [11]	Non-small cell lung cancer
Plant alkaloids			
(a) Vinca alkaloids	Inhibit mitosis at metaphase by binding to tubulin	Vincristine, vinblastine [12]	Small cell lung cancer, Non-Hodgkin's lymphoma
(b) Podophyllotoxins	Inhibit DNA synthesis by interfering with topoisomerase II, and also Inhibit mitochondrial function	Etoposide [13]	Lung cancer, Kaposi's sarcoma
(c) Taxoids	Promote the polymerization of tubulin and inhibit the disassembly of microtubules	Taxol (Paclitaxel) [14]	Ovarian carcinoma, breast cancer, small cell lung cancer
(d) Camptothecins	Inhibit topoisomerase I and DNA and RNA synthetases, and also inhibit microtubule formation	Irinotecan, topotecan [15]	Refractory colorectal cancer, advanced ovarian cancer
Hormones			
(a) Glucocorticoids	Inhibit lymphocyte proliferation		Leukemias, lymphomas
(b) Oestrogens	Block the effects of androgens in androgen-dependent prostatic tumors		Prostatic tumor
(c) Anti-oestrogens	Act on oestrogen receptors in mammary tumor	Tamoxifen, androgens [16][17]	Breast cancer
(d) GnRH analogues	Inhibit gonadotrophin release		Prostate, breast cancer

[@]Different classes of antiumor agents are usually used in combination chemotherapy.

[#]The list of diffenent tumor types is not exhaustive.

unrelated drugs, so that the benefits of second-line chemotherapy diminish substantially, and patients eventually succumb to their disease.[32] Cisplatin resistance remains a significant drawback, limiting its clinical utility and provided a strong impetus to develop new Pt-based drugs that can circumvent cisplatin resistance and reduce toxicity and thus may offer cancer patients greater benefits.

3. BIOLOGICAL TARGETS OF CISPLATIN

Cisplatin reacts with many cellular components that have nucleophilic sites such as DNA, RNA, proteins, membrane phospholipids, cytoskeletal microfilaments, and thiol-containing molecules.[33] Currently, the cellular target for platinum complexes is generally accepted to be DNA. Approximately 1% of the total cellular platinum binds to DNA, inducing various types of inter- and intra-strand cross-links.[34] The coordination of cisplatin to DNA occurs mainly through the N-7 atoms of purines, which are exposed in the major groove of the double helix and are not involved in base-pair hydrogen bonding. The resulting adducts can be grouped into six major categories: 1,2-intrastrand d(GpG) adducts between adjacent guanines; 1,2-intrastrand d(ApG) adducts between an adjacent adenine and guanine; intrastrand adducts between purines separated by one or more intervening bases; interstrand adducts linking the two strands of the DNA double helix; monofunctional adducts coordinated to a single purine; and protein–DNA cross-links, where cisplatin coordinates a protein molecule and a nucleobase (Fig. 1).[35–37]

The consequences of these cross-links to the cell and how they bring about cell death are unknown. However, results to date, in numerous cell lines suggest that cisplatin-damaged DNA causes cell-cycle perturbation, an arrest in the G2-phase to repair damage, and in the absence of adequate repair, the cells eventually undergo an abortive attempt at mitosis that results in cell death via an apoptotic mechanism (Fig. 2).[37–40]

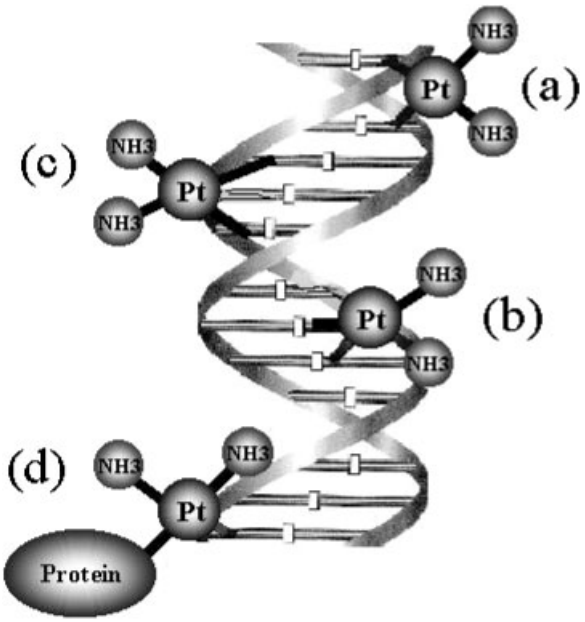


Figure 1. Main adducts formed in the interaction of cisplatin with DNA. (**a**) interstrand cross-link; (**b**) 1,2-intrastrand cross-link; (**c**) 1,3-intrastrand cross-link; (**d**) protein–DNA cross-link.

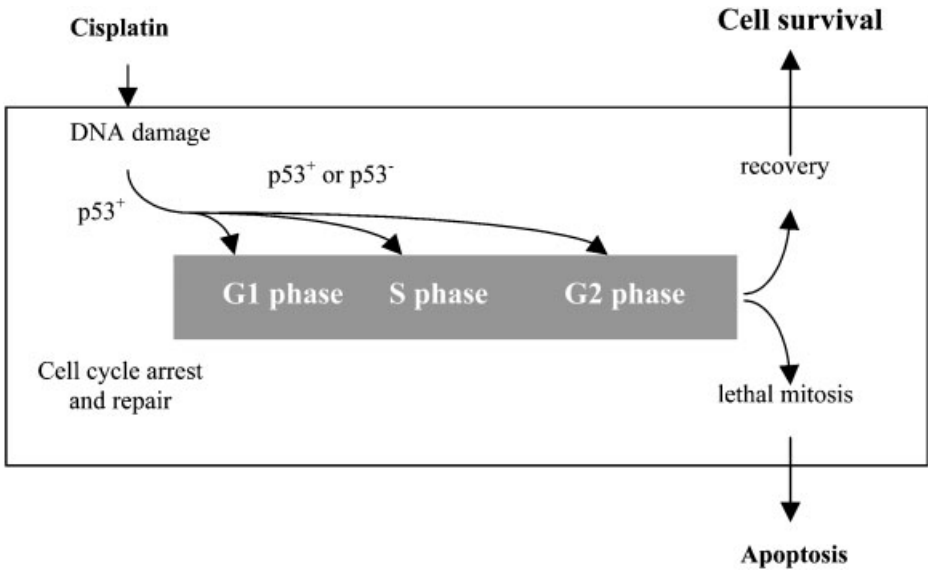


Figure 2. The cell-cycle perturbations that occur as a consequence of DNA damage induced by cisplatin. The gray box represents the time period during which cells arrest at various phases of the cell cycle with the intent to repair the damage.

A. DNA-Repair Mechanisms

The mechanism of cisplatin-induced DNA damage towards cell killing is beginning to be disentangled. In the past, it was thought that cisplatin cytotoxicity was the result of inhibition of DNA synthesis. However, DNA-repair deficient cells die at concentrations of cisplatin that do not inhibit DNA synthesis. Moreover, DNA repair-proficient cells survive at concentrations of cisplatin high enough to inhibit DNA synthesis and arrest the cells in the S phase.[38] Thus, cisplatin-induced cell death does not always correlate with inhibition of DNA synthesis. More recently, considerable evidence indicate that cisplatin can kill cells by apoptosis.[39,40] Apoptosis is a ubiquitous, genetically regulated mechanism of active cell death that is conserved in multicellular organisms.[41–43] It has unique morphological and biochemical features, including cell shrinkage, blebbing of the cell surface, loss of cell–cell contact, chromatin condensation and fragmentation, recognition by phagocytic cells, characteristic DNA degradation, and dependence on the energy supplied by ATP as well as an active protein synthesis.[44]

The specific mechanism(s) that trigger apoptosis in response to cisplatin have not yet been defined. Logically, such mechanisms must include ways to detect damage as well as to determine whether damage is sufficiently severe to be lethal. Much attention has therefore been focused on identification and characterization of proteins that recognize cisplatin-induced DNA damage.[21] At present, several families of proteins are implicated as being important: (1) nucleotide excision repair (NER) proteins; (2) mismatch repair (MMR) proteins; and (3) DNA-dependent protein kinase (DNA-PK); and (4) high-mobility group (HMG) proteins.

B. NER Proteins

It is becoming clear that the NER pathway is responsible for the repair of cisplatin–DNA adducts and appears to be one of the most important factors in cisplatin resistance.[45] It seems that only 16 genes are essential for the DNA damage recognition and excision functions of the intrastrand-adduct between two adjacent guanines.[46]

C. MMR Proteins

MMR is a post-replication repair system that corrects unpaired or mispaired nucleotides. The relationship between DNA damage recognition by MMR proteins and cytotoxicity remains incompletely defined. Human mismatch repair complex hMutS-α detects but does not remove cisplatin–DNA adducts. This protein has been shown to recognize specifically a single cisplatin intrastrand adduct between two adjacent guanines within a double-strand oligonucleotide.[47] As for the molecular pharmacology of cisplatin–DNA adduct repair, it is currently a matter of debate as to whether NER is more important than MMR in repairing the DNA. However, in ovarian cancer and colon cancer, at least, MMR is a comparatively small contributor to the cisplatin resistance phenotype, because an intact MMR system seems to be essential for linking DNA damage or repair with the initiation of apoptosis.[48]

D. DNA-Dependent Protein Kinase

DNA-PK also reportedly interacts with cisplatin–DNA lesions.[49] Binding to DNA of Ku subunits of DNA-PK is essential *in vitro* to activate the kinase activity of DNA-PK to phosphorylate itself or other transcription factors. It has been shown in apoptotic ovarian cancer cells that the presence of cisplatin–DNA adducts serves to inhibit the ability of the Ku subunits of DNA-PK to translocate onto a duplex DNA substrate. This is so that the kinase activity is abrogated and the ability of Ku subunits to bind DNA is decreased.[50]

E. HMG Proteins

The HMG proteins are a family of small, nonhistone chromatin-associated proteins involved in gene regulation and maintenance of chromatin structure. The HMG box proteins do have the common feature of binding to DNA involved in structural deformation and some of them also bind to cisplatin–DNA adduct.[51] HMG proteins recognize some structure distortions of DNA, and their interactions with distorted DNA regulating transcription either directly or by facilitating interactions with other transcription factors. Several HMG-family proteins specifically recognize cisplatin–DNA adducts. HMG1 and HMG2 proteins recognize intrastrand guanine–platinum–guanine diadducts.[52] An HMG family protein, called structure specific recognition protein (SSRP-1), specifically binds to cisplatin–DNA intrastrand adducts.[53] Finally, the ribosomal RNA (rRNA) transcription factor hUBF, another HMG-family protein, binds cisplatin G-G intrastrand adducts with equal affinity to its normal target, the rRNA promoter.[54]

F. Binding of Proteins to Cisplatin–DNA Adducts

At least two mechanisms involving HMG proteins have been proposed to describe how proteins that bind to cisplatin–DNA adducts might modulate the sensitivity of cells to the drug.[55] In the ''hijacking model,'' HMG proteins binding to cisplatin–DNA adducts could modulate cell cycle events after DNA damage and trigger apoptosis. In the ''repair shielding model,'' HMG proteins could protect cisplatin–DNA adducts from recognition by DNA repair enzymes. The adducts are shielded from incision by the NER machinery, which is likely to proceed via blocking of the initial recognition of the DNA damage (Fig. 3).[56] These hypotheses are not necessarily exclusive and could work in concert.

4. MECHANISMS OF CISPLATIN RESISTANCE

Cisplatin and carboplatin are components of standard treatment regimens for ovarian, bladder, cervical, head and neck, and small-cell and non-small-cell lung cancers.[32,57] Unfortunately, many patients with these malignancies eventually relapse and become refractory to chemotherapy.[58] The

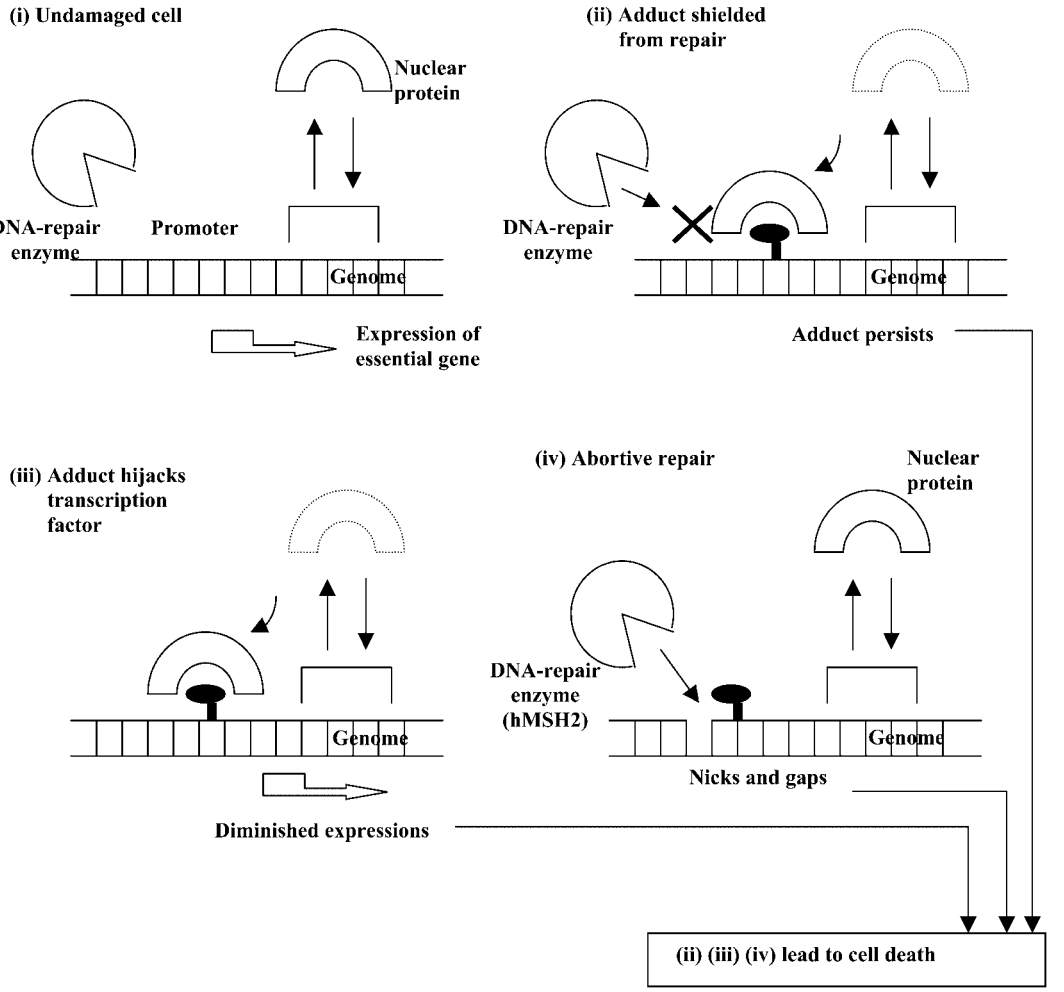


Figure 3. Models to explain how HMG domain and other nuclear proteins may enhance cisplatin cytotoxicity. (**i**) A normal cell with a nuclear protein interacting with the DNA. (**ii**) Repair-shielding model: Cisplatin–DNA adducts (lollipop symbol) attract the nuclear protein and are shielded from DNA-repair enzymes, promoting the persistence of the adducts and sensitizing the cell to cisplatin. (**iii**) Hijacking model: The adducts titrate the nuclear protein away from its normal site of binding, resulting in reduced expression of a critical gene. (**iv**) Abortive repair model (MMR): Misdirected repair attempts at sites of cisplatin damage by mismatch DNA-repair proteins (e.g., hMSH2) may generate DNA-strand breaks that signal for cell death.

degree of resistance responsible for these relapses is usually only of the order of two- to four-fold, which may not appear to be very high. But for cisplatin, which is routinely used in dosages at the limit of its systemic toxicity, these levels of resistance can completely eliminate clinical effectiveness.[59] In addition to the development of acquired resistance, some tumors such as liver, colorectal or pancreatic carcinoma are intrinsically resistant in that they do not respond to chemotherapy including cisplatin from the very beginning of treatment.

With a better understanding of how cisplatin exerts its antitumor effects, much insight into mechanisms of resistance to cisplatin has been gained from preclinical laboratory-based investigations using different cancer cell lines. Cellular resistance to cisplatin is multifactorial and has been reviewed in detail.[60–62]

Two broad causes of resistance have been observed: (i) prevention of adequate amounts of drug from reaching and binding to the target DNA;[63,64] and (ii) a failure of cell death occurring

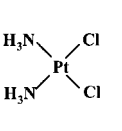
after binding of platinum to DNA.[65] More specifically, the mechanisms of resistance are believed to include one or more of the following: (i) reduced platinum accumulation; (ii) increased cytoplasmic detoxification by glutathione and/or metallothioneins; (iii) increased DNA repair (mainly through the NER machinery) and/or tolerance of platinum–DNA adducts; or (iv) overexpression or mutation of other genes, for example, *p53*, *bcl-2*, *c-myc*.[61,62]

Reduced Pt accumulation and increased cytoplasmic detoxification by glutathione and/or metallothioneins represent the major causes of inadequate drug concentrations reaching DNA. Once DNA binding has occurred, resistance mechanisms include increased DNA repair of adducts, and an ability to tolerate greater levels of DNA damage with concomitant failure to engage programmed cell death (apoptotic) pathways. The elucidation of these major biochemical mechanisms of resistance has been critical in providing a basis for the development of platinum-based compounds capable of circumventing cisplatin resistance.

5. DEVELOPMENT OF PLATINUM ANALOGUES

With a brief understanding of the importance of binding of various recognition proteins to the Pt–damaged DNA lesions and the proposed mechanism(s) of action of cisplatin, one can appreciate the challenge in designing new Pt compounds.

The first structure-activity relationship revealed that the *cis* isomers of both $Pt(NH_3)_2Cl_2$ (**1**) and $Pt(NH_3)_2Cl_4$ (**2**) uniquely interfered with the cell division in *E. coli*, but the equivalent *trans*-isomers (**3**), (**4**) were ineffective.[66] Subsequently, it was reported that a large number of Pt (II) complexes and



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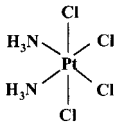

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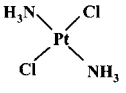

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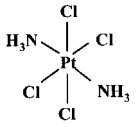
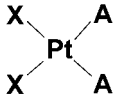


(4)

a small number of palladium (II) complexes with *trans* geometry were inactive and to demonstrate antitumor activity, such complexes would need to be neutral.[67] This information has led to intense activity in developing platinum complexes that are structural analogues of cisplatin, having a *cis* geometry, with two amine non-leaving groups "A" and two anionic leaving groups "X" as shown in Figure 4.[68,69]



where X = leaving group; A = amine carrier ligand

Figure 4. Empirical structure of a platinum complex.

Previous studies have established that X should be moderately bound functional groups (e.g., Cl^-, Br^-), since highly labile ligands such as NO_3^-, ClO_4^- would lead to toxicity; and that biological activity was reduced when X was selected from tightly bound ions such as N_3^-, SCN^-, NO_2^-, CN^-. Moreover, complexes where the amine groups have fewer alkyl substituents resulted in higher biological activity, and amine ligands carrying at least one hydrogen atom were also active.[67] To date, more than 28 platinum compounds have entered clinical trials with derivatives analogous to the classical *cis*-PtA_2X_2 structure often showing similar biological activity.[70]

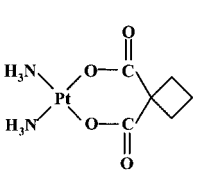
Currently, there are still a large number of Pt-analogues modifications still being pursued internationally, all of which are based on changing one or both of the ligand types coordinated to Pt, that is: [Amine Carrier Ligand]—[Pt]—[Leaving Group].

Altering the structure of the leaving group of cisplatin to less labile leaving groups appears to influence tissue and intracellular distribution of the platinum complexes and improve the drug's toxicity profile.[71,72] Whereas the more stable (carrier) amine group usually determines the structure of the adduct formed upon interacting with DNA, believed to be the ultimate target for antitumor activity.[35,36] Therefore, adducts produced by cisplatin and most of the classical platinum analogues are identical: which explains their very similar patterns of tumor sensitivity and susceptibility to resistance. An important question in platinum-based cancer chemotherapy and biology is whether the original structure-activity relationships adequately describe the range of complexes with useful antitumor activity. Complexes that are structurally different from cisplatin may be processed differently by cells. The determining factors of cytotoxicity may thus not follow the same patterns as found for cisplatin and its analogues. Therefore, a further unique class of platinum antitumor agents with distinct chemical and biological properties distinct from the classical cisplatin analogues is badly needed.

A. Carboplatin and its Analogues

To date, only one of the second generation platinum compounds, carboplatin (*cis*-diammine-1,1′-cyclobutanedicarboxylatoplatinum (II), $[Pt(C_6H_6O_4)(NH_3)_2]$, CBDCA) (**5**), has received worldwide clinical acceptance. Carboplatin exhibits reduced side effects, with myelosuppression being dose-limiting.[73,74] However, randomized trials of cisplatin versus carboplatin in advanced ovarian cancer have shown comparable response and recurrence rates. Therefore, it may be deduced that effective platinum-based chemotherapy has been made somewhat safer through the discovery of carboplatin, but its spectrum of clinical activity is similar to that of cisplatin.[75] More importantly, the problems of intrinsic and acquired cisplatin resistance persist.[76,77]

Since the introduction of carboplatin in the early 1980s, several other analogues designed mainly to reduce the toxicity of cisplatin-based chemotherapy have entered clinical trials. Many of the complexes such as zeniplatin (**6**),[68,78] enloplatin (**7**),[68,78] and miboplatin (**8**)[68] possess identical



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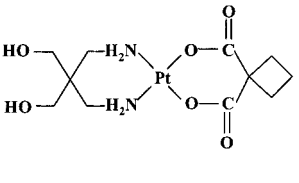

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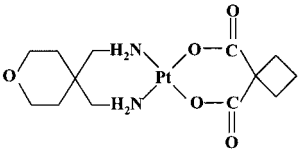

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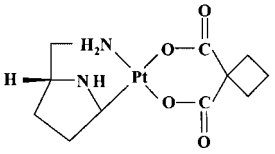

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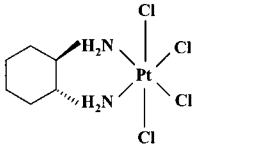
leaving group chemistry to carboplatin, where the oxygenated leaving groups conferred good aqueous solubility and greater stability. However, both zeniplatin and enloplatin caused some nephrotoxicity in patients undergoing phase I clinical trials and were subsequently abandoned. Other compounds introduced through this modification also did not show clear advantages over carboplatin.

B. Diaminocyclohexane PT Complexes

Newer cisplatin analogues have been actively synthesized and tested to combat the problem of platinum resistance. Of special interest is the so-called DACH family of platinum compounds. The substitution of the amine radicals in cisplatin by a DACH radical resulted in a stable complex with good antitumor activity.[79] Interest in this class of compounds was increased further when other DACH complexes demonstrated activity against cisplatin-resistant L1210 leukemia.[80] The lack of cross-resistance was attributed to the 1,2-DACH ligand.[81,82] Other research groups have also reported the cytotoxic activity of DACH complexes in other cisplatin-resistant tumor cells.[83,84] Among the platinum derivatives bearing the DACH carrier ligand, compounds such as tetraplatin (**9**) reached the early clinical trial stage; however adverse therapeutic index and toxicity compromised its further development.



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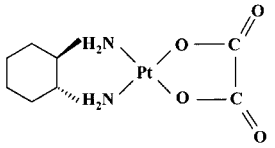

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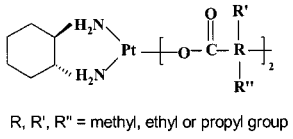

R, R', R'' = methyl, ethyl or propyl group

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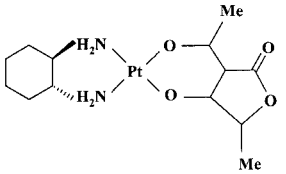

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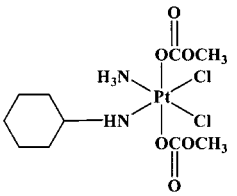
Another significant DACH Pt analogue was oxaliplatin (**10**), which demonstrated antitumor activity in cisplatin-resistant murine L1210 leukemia cells[85] and various human cancer cell lines.[77] It has since been successfully developed, and is approved for the treatment of patients with advanced colorectal cancer mainly in France and Argentina. The bulky DACH carrier ligand of oxaliplatin is thought to contribute to the enhanced activity as well as to the lack of cross-resistance between oxaliplatin and cisplatin.[86–88] The DACH ligand may hinder DNA repair by preventing or reducing the binding of specific damage repair proteins such as the mismatch repair enzyme complex, thereby decreasing the replicative bypass of platinum–DNA adducts.[65,86,88] Defects in mismatch repair and enhanced replicative bypass have also been reported as mechanisms of resistance to cisplatin or carboplatin but do not appear to contribute to oxaliplatin resistance.[89–91]

Many other DACH complexes have been evaluated and a total of twelve DACH analogues have entered human trials.[92] Two compounds are still currently in human trials: L-NDDP (**11**), and TRK-710 (**12**).[68,69] However, it has become clear that Pt-DACH complexes are not effective in all cisplatin-resistant tumors. For example, Pt-DACH complexes in small cell lung[93,94] and cervical squamous cell lines[95] still exhibit cross-resistance.

C. Orally Active PT (IV) Complexes

For over 20 years following the introduction of cisplatin, further development of Pt-based drugs had focused primarily on Pt (II) complexes. In clinical practice, cisplatin, carboplatin, and oxaliplatin are administered by intravenous infusion. A drug that could be effectively delivered orally is strongly desirable as it would allow substantial flexibility in dosing and increase the potential for the use of platinum drugs. However, an effective oral formulation of cisplatin is not achievable at the clinical level because of its poor water solubility and low level of bioavailability.[96] Although carboplatin has greater water solubility its very low organic/aqueous partition coefficient resulted in low absorption through the gastrointestinal tract. Studies in mice revealed an oral bioavailability of only 11–15% for carboplatin, with a major proportion of the dose (60–80%) excreted in the faceces.[97] The very low bioavailablity together with the observed gastrointestinal side effects made further oral administration of carboplatin unwarrantable.

To achieve clinically useful oral bioavailability with a Pt complex, novel chemistry is required. This quest led to the identification of a new class of Pt (IV) compounds such as JM216 (satraplatin) (**13**) and its derivative JM221 (**14**). JM216 is the first Pt-containing anticancer agent expressly developed for oral administration, and has antitumor activity comparable to those of parentally administered cisplatin or carboplatin.[98,99] Orally administered JM216 was found to be devoid of nephrotoxicity and neurotoxicity in Phase I clinical trials,[100] and myelosuppression was found to be the dose-limiting toxicity.[101] JM216 is currently undergoing Phase III clinical trials in Europe and the US for ovarian, non-small-cell lung and small cell lung cancers.[102]



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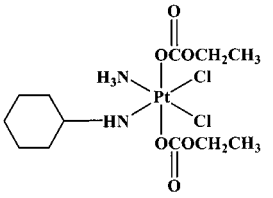

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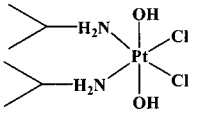


(15)

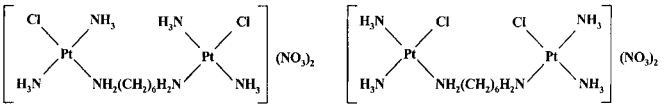
JM216 and JM221 have been evaluated in a number of intrinsic and acquired cisplatin-resistant cell lines and showed a lack of cross-resistance with cisplatin, particularly in those where reduced platinum accumulation played a dominant role.[103,104] These results suggested that the greater lipophilic nature of Pt (IV) compounds enabled the circumvention of cisplatin-resistance because of decreased Pt accumulation. Two other Pt (IV) compounds, iproplatin (**15**) and ormaplatin (also known as tetraplatin) (**9**), have undergone clinical trials. However, both were subsequently abandoned: ormaplatin for unpredictable peripheral neurotoxicity, and iproplatin because of the lack of superior performance.[69]

D. Multinuclear PT Complexes

As discussed earlier, the mechanism of tumor resistance to cisplatin is believed to be multifactorial. Therefore, this warranted a new approach to the design of Pt drugs that can circumvent cisplatin resistance by developing compounds that form radically different Pt–DNA adducts to current

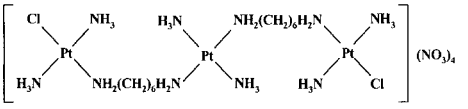
platinum drugs. To this endeavor, Farrell and co-workers have successfully synthesized multinuclear platinum complexes with bridging linkers, particularly binuclear platinum complexes with the general formula $[\{PtCl_m(NH_3)_{3-m}\}\mu\text{-}H_2N\text{-}R\text{-}NH_2\text{-}\{PtCl_n(NH_3)_{3-n}\}]^{[(2-m)+(2-n)]+}$ (m or n = 0–3 and R is a linear or substituted aliphatic linker) exhibiting activity in both cisplatin-sensitive and cisplatin-resistant cells.[105–107]

The 1, 1/*trans*, *trans* series, for example BBR3005 (1, 1/t, t) (**16**), was identified as having the most promising pattern of antitumor activity and DNA-binding. Complexes with improved DNA affinity for long range cross-linking were also accomplished through the incorporation of a third platinum center with the alkane diamine chain such as BBR3464 (**17**). The 4^+ charges, the presence of at least two Pt coordination units capable of binding to DNA, and the consequences of such DNA binding is a remarkable departure from the cisplatin structural prototype. BBR3464 represents a highly promising agent, where in preclinical studies, it exhibited a complete lack of cross-resistance to cisplatin-resistant cell lines.[108,109] It is also much more potent than cisplatin *in vitro* in an osteosarcoma cell line.[110]



(1,1/t,t)-isomer (16)

(1,1/c,c)-isomer (18)



(1,0,1/t,t,t) (17)

The DNA-binding profiles of the multinuclear compounds differ significantly from cisplatin.[111] A comparison shows that multinuclear compounds bind rapidly to DNA but steric effects caused diminished binding to calf thymus DNA for 1, 1/c, c isomer (**18**) of BBR3005 relative to the 1, 1/t, t isomer (**16**). Multinuclear complexes also induced irreversible B → Z form transition in poly(dG-dC) · poly(dC-dG),[107,112] implying serious consequences could occur during transcription and DNA replication. Although DNA–DNA interstrand cross-linking is highly efficient for both agents, only the 1, 1/t, t derivative (**16**) forms a (Pt, Pt) intrastrand cross-link to adjacent guanines of a d(GpG) sequence (Fig. 5).[113] Whereas these lesions have profound effects upon DNA replication and gene

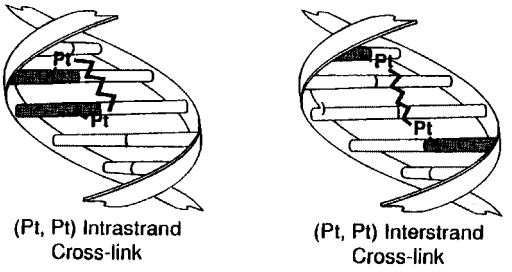


Figure 5. Schematic binding modes for dinuclear bifunctional DNA-binding compounds. The 1, 1/t, t geometry forms both types of adducts; the 1, 1/c, c forms only interstrand cross-links.

transcription, its detection by DNA damage-recognition proteins is less efficient because of the conformational changes they exert on DNA. Moreover, some of the multinuclear complexes may also bind to and inactivate repair proteins and hence repair of the lesions may be less readily accomplished.[107] Increased cellular Pt uptake of BBR3464 relative to cisplatin has been observed and this drug is currently undergoing phase I trials; there is high expectation that these multinuclear Pt complexes may become a new class of Pt-based antitumor drugs.

E. Sterically Hindered PT Complexes

Glutathione (GSH) has been implicated in tumor resistance by reducing drug accumulation;[114] by reacting with drugs to form inactive species;[115] and by enhancing DNA repair.[116] *cis*-Amminedichloro(2-methylpyridine)platinum (II) (ZD0473) (**19**) is a sterically hindered Pt complex that was rationally designed to circumvent resistance by blocking cellular detoxification by GSH and other cellular thiols whilst the ability to form cytotoxic lesions with DNA remained. The reported crystal structure of ZD0473 and its analogue *cis*-ammine-dichloro(3-methylpyridine)platinum (II) (**20**) revealed that the pyridine ring in the former is tilted by 102.7° with respect to the PtN_2Cl_2 square plane when compared with the latter which is tilted by only 48.9°.[117] Corresponding slower rates of hydrolysis and reduced reactivity towards guanosine 5′-monophosphate (5′-GMP) and GSH were reported for ZD0473 compared to the 3-methylpyridine complex.[117,118]

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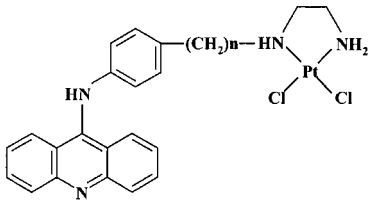

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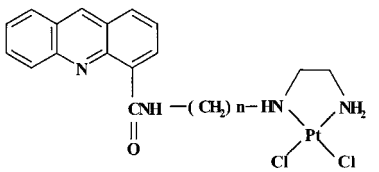

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In vitro studies in human tumor cell lines including human ovarian carcinoma, which have developed acquired resistance to cisplatin and carboplatin, with ZD0473 demonstrated that it was able to significantly overcome platinum resistance.[119] Also, in *in vivo* studies using human ovarian carcinoma xenografts, ZD0473 has demonstrated significantly greater growth delays compared to cisplatin and carboplatin.[120] This drug is currently undergoing the early stages of clinical trials.

F. Novel Design Strategies Using Bioactive Ligands

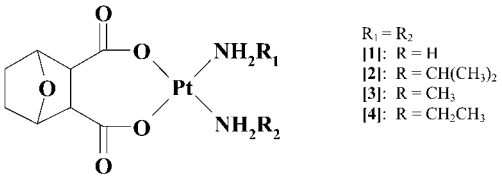
New platinum compounds have been developed whereby a selection of biologically active carrier ligands are attached to Pt coordination moieties. For example, novel Pt complexes were created utilizing DNA intercalators such as anilinoacridine (**21**) and acridinecarboxamide (**22**) as the carrier ligands, with the anticipation that the compounds would localize in the vicinity of DNA and exert superior cytotoxic effects.[121–123] Although these complexes exhibited improved activity in cisplatin-resistant cell lines compared to cisplatin, there was no improvement relative to the use of the carrier ligands alone.

Other examples include the attachment of a Pt moiety to bioactive carrier ligands such as doxorubicin;[124] oestrogen analogues;[125] amino acids;[126] and sugars.[127] Although this design approach has produced interesting results, there is still potential for optimization of the structure of the bioactive carrier ligands, and additional clinical benefits have yet to emerge.

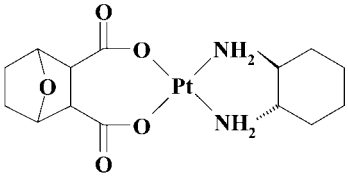
6. NOVEL TCM-PLATINUM COMPLEXES

Expanding on the conceptual framework of the use of bioactive ligands, and based on an ''East meets West'' philosophy, our research team has designed a novel series of platinum compounds with anticancer activity. The objective was to exploit the benefits of TCM in creating novel TCM-Pt complexes with specific biological activity, and complementary to that of a conventional western cancer chemotherapeutic agent. Integration of a modified TCM component serving as a bioactive leaving group, with a Pt moiety gave rise to TCM-Pt compounds (**1**–**5**) (Fig. 6).[128]

In China and other Chinese communities worldwide, TCM is commonly used as an adjunct to conventional western medicine, including cancer chemotherapy. Each form of medicine has its own intrinsic strengths and weaknesses and they can be complementary. There are some evidence that TCM may help to 'boost the immune system,' thus enhancing the tolerability of the patients undergoing conventional cancer therapy.[129,130]



TCM-Pt [**1-4**]

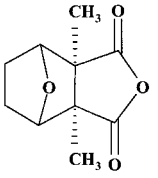


TCM-Pt [**5**]

Figure 6. Novel TCM-Pt compounds (**1**–**5**).

A. Cantharidin and its Derivatives

Cantharidin (exo-2,3-dimethyl-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid anhydride) (**23**), the active principle of *Epicanta gorhami* or *Mylabris* (''blister beetles'') was recognized in ancient Chinese and Roman medicine for its aphrodisiac, strong flogistic, and vesicant activities. It has long been used as a TCM for the treatment of liver, lung, intestinal and digestive tract tumors and clinical trials have indicated that cantharidin had therapeutic effects on patients with primary hepatoma, but unfortunately, can result in severe side effects such as dysphagia, hematemesis, and dysuria.[131] It has been reported that cantharidin and its derivatives have strong affinity and specificity for a ''cantharidin-binding'' protein, which has been isolated and identified as protein phosphatase 2A (PP2A).[132] It is also known that the liver cytosol is one site that is rich in PP2A and from *in vitro* experiments, the level of PP2A inhibition parallels cytotoxicity.[133,134]



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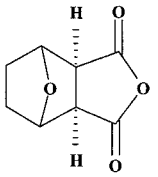

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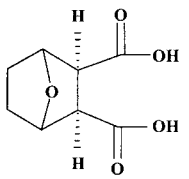

(25)

Demethylcantharidin (DMC) or norcantharidin (**24**) and endothall (**25**) are the demethylated analogue of cantharidin and its diacid, respectively. DMC has also been used to treat cancers in China since 1984, and did not present the adverse effects of cantharidin.[131,135] It has also been shown to have an inhibitory effect on the proliferation of several tumor cell lines including HeLa (human cervical carcinoma), CaEs-17 (human esophageal carcinoma),[132] and human epidermoid laryngeal carcinoma.[136]

Endothall (1-oxabicyclo-[2,2,1]-heptane-2,3-dicarboxylic acid), the diacid form of DMC is the least toxic structural analogue of cantharidin that still inhibits PP2A. Endothall is readily soluble in water, and its potassium salt is currently used as a herbicide, especially in aquatic ecosystems.[137] The potential antitumor properties of endothall has only been described by Blazsek[138] and Thiery[139] and its mitotic defects can be understood from its inhibition of PP2A. Significantly, *in vitro*, endothall inhibited the growth of primary hepatocellular carcinoma cell lines more than that of normal hepatocytes or colon carcinomas.[139] This implied a tumor selectivity of the cantharidin-class of PPI towards liver cancer.

Demethylcantharidin, without the toxicity of cantharidin, was therefore selected as the bioactive leaving ligand to be integrated with a Pt moiety in our design of a single new chemical entity (NCE). The novel compounds should demonstrate antitumor properties and more significantly, selectivity towards liver cancer.

B. Synthesis

Demethylcantharidin was readily prepared by a first step Diels–Alder reaction between furan and maleic anhydride, followed by Pd-C catalyzed hydrogenation. TCM-Pt compounds (**1**–**5**) were synthesized by reacting DMC with a series of $(NH_2R)_2Pt(NO_3)_2$ (Fig. 7).[128]

TCM-Pt (**1**–**4**) consist of the classical homologous series of amine platinum (II) complexes whereas TCM-Pt-**5** is a 1,2-DACH platinum (II) compound. Platinum compounds with the DACH carrier ligand are of particular interest in the studies of resistance mechanisms since they have been effective in treatment of cells resistant to cisplatin.[86,140,141] Therefore TCM-Pt-**5** was rationally designed to impart potent antitumor activity, circumvention of resistance, and selectivity towards liver cancer.

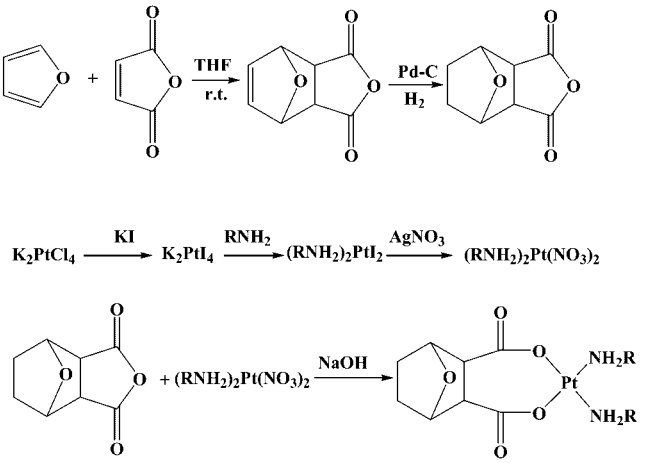

Figure 7. Synthetic pathway of the novel TCM-Pt complexes.

C. Biological Activity

The novel TCM-Pt compounds were found to exhibit potent *in vitro* antitumor activity against L1210 mouse leukemia and a range of human cancer cell lines; activity that was at least comparable to cisplatin in some cell lines, and vastly superior to carboplatin in most.[128] TCM-Pt (**1**–**5**) also demonstrated selective antitumor activity towards the SK-Hep-1 human liver cancer cell line, to which cisplatin and carboplatin responded poorly. Potent antitumor activity was verified *in vivo* by using a human liver tumor xenograft that was performed in nude mice.[128]

Significantly, the novel compounds were generally found to be devoid of cross-resistance in cisplatin-resistant L1210 (14-fold) and human non-small cell (NSC) lung cancer (eightfold) cell lines.[128] In the latter cell line, we were able to establish that at least two mechanisms appear to contribute to the acquired cisplatin resistance: a decreased total cellular platinum accumulation,[142] and increased glutathione and glutathione S-transferase levels.[143] The dissimilar patterns obtained for cisplatin or carboplatin and the novel TCM-Pt compounds suggest that the latter may operate under different mechanisms of cytotoxic action. From among the series of TCM-Pt complexes, the biological profile of TCM-Pt-**5** was the most impressive, in terms of potency, liver selectivity and ability to overcome cisplatin resistance.

As discussed earlier, cantharidin and its derivatives are inhibitors of protein phosphatase 2A (PP2A). It was therefore not surprising that the novel TCM-Pt compounds also exhibited PP2A inhibitory activity.[128] Recent literature findings suggest that PP2A activity is essential for the NER mechanism, which repairs DNA lesions induced by cisplatin and as a consequence, tumor resistance may develop.[144] Inhibition of PP2A can usurp DNA repair by inhibiting the removal of DNA–Pt adducts from damaged DNA. Hence it is feasible that the TCM-Pt (**1**–**5**) circumvented cisplatin resistance by inhibiting PP2A and disrupting the NER repair mechanism.

D. Protein Phosphatase 2A

PP2A is an abundantly expressed enzyme that targets mainly phosphoseryl and phosphothreonyl residues in its substrates.[145] It has been implicated in the regulation of different cell cycle events, because of its multiplicity and different substrate specificity.[146] In cell culture studies, PP2A has been shown to have a role in control of the cell cycle,[147] growth and proliferation,[148] and cell fate determination.[149] More importantly, recent literature findings suggest that PP2A may play a role in the process of tumorigenic transformation, which subsequently has brought it to the forefront of cancer research.[150–152]

E. Novel Dual Mechanism of Antitumor Action

By inclusion of demethylcantharidin in the design strategy, the TCM-Pt compounds were postulated to have a novel dual mechanism of antitumor action: inhibition of PP2A by DMC and DNA binding by the Pt moiety. Based on our research findings, inhibition of PP2A by the novel compounds is also likely to have played a significant role in overcoming Pt resistance.

On close examination, novel TCM-Pt compounds, freshly prepared in normal saline, did not exhibit PP2A inhibitory (PPI) action, but inhibition increased progressively with prolonged incubation over 24 hr. This was in total contrast to cisplatin and carboplatin, which showed no PP2A inhibitory activity.[128] We have since successfully developed a gas chromatography with flame ionization detection (GC-FID) method, that confirmed the release of demethylcantharidin, as the diacid, from TCM-Pt (**1**–**5**) over a period of 24 hr, after which the level of PPI activity approached that of the control DMC.[153]

Thus it appears that DMC, as a PP2A inhibitor, has introduced a new mechanism of action in Pt-based anticancer drugs. It naturally follows that the next phase of research will be to elucidate fully the role of DMC in eliciting this apparent new mechanism of antitumor action, and to provide evidence that the inhibition of PP2A by the TCM-Pt compounds directly influences circumvention of cisplatin resistance and liver selectivity.

7. CONCLUSION

This review has tried to put into perspective the different design strategies that have been employed by various research groups, in arriving at useful platinum-based complexes for the treatment of tumors.

A common feature among the different approaches lies with the use of a bioactive component as either the amine carrier ligand or the leaving group. To these endeavors, inclusion of a bioactive carrier ligand such as the DACH has resulted in circumvention of platinum resistance. The use of a bioactive leaving group such as demethylcantharidin, a modified TCM component, has apparently introduced selective antitumor activity towards the SK-1 human liver cancer cell line. In addition, demethylcantharidin-based platinum complexes were found to be devoid of cross-resistance with cisplatin.

Whilst qualitative structure-activity relationships have been established for cisplatin and its classical analogues, as well as for carboplatin as its analogues, quantitative SAR relationships have yet to emerge for platinum complexes. Recent exploitation using Density Functional Theoretical method has shown promise in obtaining QSAR relationship for platinum complexes. Preliminary results from QSAR suggest that the optimum platinum-based anticancer agent has not yet been uncovered and thus provides a platform for further investigation.

REFERENCES

1. Coggins PR, Ravdin RG, Eisman SH. Clinical Pharmacology and preliminary evaluation of cytoxan (cyclophosphamide). Cancer Chemother Rep 1959;3:9–11.
2. Rege VB, Owens AH, Jr. BCNU (NSC-409962) in the treatment of advanced Hodgkin's disease, lymphosarcoma, and reticulum cell sarcoma. Cancer Chemother Rep 1974;58:383–392.
3. Haddow A, Timmis GM. Myleran in chronic myeloid leukemia: Chemical constitution and biological action. Lancet 1953;264:207–208.
4. Einhorn LH, Donohue JP. Cis-diamminedichloroplatinum, vinblastine, and bleomycin combination chemotherapy in disseminated testicular cancer. Ann Intern Med 1977;87:293–298.
5. Wang JJ, Freeman AI, Sinks LF. Treatment of acute lymphocytic leukemia by high-dose intravenous methotrexate. Cancer Res 1976;36:1441–1444.

6. Gupta S. Treatment of advanced gastric cancer with 5-fluorouracil versus mitomycin C. J Surg Oncol 1982;21:94–96.
7. Lyman MS, Burchenal JH. Acute leukemia. Am J Nurs 1963;63:82–86.
8. Booser DJ, Hortobagyi GN. Anthracyclin antibiotics in cancer therapy. Drugs 1994;47:223–258.
9. Miyamoto T. Antitumor effects and pulmonary toxicity of bleomycin administered by continuous subcutaneous infusion in patients with advanced cervical cancer. Jpn J Cancer Chemother 1987;14:1830–1835.
10. De Camargo B, Franco EL. Single-dose versus fractionated-dose dactinomycin in the treatment of Wilms' tumor: Preliminary results of a clinical trial. The Brazilian Wilms' Tumor Study Group. Cancer 1991;67:2990–2996.
11. Arcangeli G, Zaniboni A, Milano S, Meriggi F, Simoncini E, Marpicati P, Marini G. MICE: A new active combination for non-small cell lung cancer. Eur J Cancer 1993;29A:1848–1850.
12. Morgan DA, Gilson D, Fletcher J. Vincristine and etoposide: An effective chemotherapy regimen with reduced toxicity in extensive small-cell lung cancer. Eur J Cancer Clin Oncol 1987;23:619–621.
13. Belani CP, Doyle LA, Aisner J. Etoposide: Current status and future perspectives in the management of malignant neoplasms. Cancer Chemother Pharmacol 1994;34:118–126.
14. Rowinsky EK, Cazenave LA, Donehower RC. Taxol: A novel investigational antimicrotubule agent. J Natl Cancer Inst 1990;82:1247–1259.
15. Burris H, Irvin R, Kuhn JG, Von Hoff DD. Clinical trials with the topoisomerase I inhibitors. Semin Oncol 1992;19:663–669.
16. Fisher B. The evolution of paradigms for the management of breast cancer: A personal perspective. Cancer Res 1992;52:2371–2383.
17. Bergan RC, Reed E, Myers CE, Headlee D, Brawley O, Cho H-K, Figg WD, Tompkins A, Linehan WM, Kohler D, Steinberg SM, Blagosklonny MV. A phase II study of high-dose tamoxifen in patients with hormone-refractory prostate cancer. Clin Cancer Res 1999;5:2366–2373.
18. Rosenberg B, Van Camp L, Krigas T. Inhibition of cell division in Escherichia coli by electrolysis products from a platinum electrode. Nature 1965;205:698–699.
19. Rosenberg B, Van Camp L, Trosko JE, Mansour VH. Platinum compounds: A new class of potent antitumor agents. Nature 1969;222:385–386.
20. Ash DC. Cis-platinum (II) diamminedichloride: Clinical review of a new cancer chemotherapeutic agent. J Clin Hemat Oncol 1980;10:55–62.
21. Chu G. Cellular responses to cisplatin: The roles of DNA binding proteins and DNA repair. J Biol Chem 1994;269:787–790.
22. Rosenberg B. Platinum complexes for the treatment of cancer: Why the search goes on. In: Lippert B, editor. Chemistry and biochemistry of a leading anticancer drug. New York: Wiley-VCH; 1999. p 1–27.
23. Schaefer SD, Post JD, Close LG, Wright CG. Ototoxicity of low- and moderate-dose cisplatin. Cancer 1985;56:1934–1939.
24. Goren MP, Wright RK, Horowitz ME. Cumulative renal tubular damage associated with cisplatin nephrotoxicity. Cancer Chemother Pharmacol 1986;18:69–73.
25. Alberts DS, Noel JK. Cisplatin-associated neurotoxicity: Can it be prevented? Anticancer Drugs 1995;6:369–383.
26. Pera MF, Zook BC, Harder HC. Effects of mannitol or furosemide diuresis on the nephrotoxicity and physiological disposition of cis-dichlorodiammineplatinum(II) in rats. Cancer Res 1979;39:1269–1278.
27. Ozols RF, Corden BJ, Collins J, Young RC. In: Hacker PM, Douple E, Krakoff HI, editors. Platinum coordination complexes in cancer chemotherapy. Boston: Martinus-Nijhoff Publishers; 1984. p 321–329.
28. Ozols RF, Corden RJ, Isash J, Wesley MN, Otschaga Y, Young RC. High-dose cisplatin in hypertonic saline. Ann Intern Med 1984;100:19–24.
29. Borch RF, Bedon PC, Gringeri A, Montine TJ. In: Nicolini M, editor. Platinum and other metal coordination compounds in cancer chemotherapy. Boston: Martinus-Nijhoff; 1988. p 207–215.
30. Perez RP, Hamilton TC, Ozols RF, Young RC. Mechanisms and modulation of resistance to chemotherapy in ovarian cancer. Cancer 1993;71:1571–1580.
31. Ozols RF. Ovarian cancer: New clinical approaches. Cancer Treat Rev 1991;18:77–83.
32. Ozols RF. Chemotherapy for advanced epithelial ovarian cancer. Hematol Oncol Clin North Am 1992;6:879–894.
33. Douple EB. Cis-diamminedichloroplatinum(II): Effects of a representative metal coordination complex on mammalian cells. Pharmac Ther 1984;25:297–326.
34. Eastman A, Schulte N, Sheibani N, Sorenson CM. Mechanisms of resistance to platinum drugs. In: Nicolini M, editor. Platinum and other metal coordination compounds in cancer chemotherapy. Boston: Martinus Nijhoff Publishing; 1988. p 178–196.

35. Reedijk J, Fichtinger-Schepmann AMJ, van Oosterom AT, van de Putte P. Platinum amine coordination compounds as anti-tumor drugs: Molecular aspects of the mechanism of action. Structure Bonding 1987;67:53–89.
36. Sherman SE, Lippard SJ. Stuctural aspects of platinum anticancer drug interactions with DNA. Chem Rev 1987;87:1153–1181.
37. Hambley TW. Platinum binding to DNA: Structural controls and consequences. J Chem Soc Dalton Trans 2001:2711–2718.
38. Sorenson CM, Eastman A. Mechanism of cis-diammunedichloroplatinum(II)-induced cytotoxicity: Role of G2 arrest and DNA double-strand breaks. Cancer Res 1988;48:4484–4488.
39. Simonian PL, Grillot DAM, Nunez G. Bcl-2 and BcL-X_L can differentially block chemotherapy-induced cell death. Blood 1997;90:1208–1216.
40. Eastman A. The mechanism of action of cisplatin: From adducts to apoptosis. In: Lippert B, editor. Cisplatin, chemistry and biochemistry of a leading anticancer drug. Basel: Wiley-VCH; 1999. p 111–134.
41. Wyllie AH, Kerr JFR, Currie AR. Cell death: The significance of apoptosis. Int Rev Cytol 1980;68:251–307.
42. Searle J, Kerr JFR, Bishop CL. Necrosis and apoptosis: Distinct modes of cell death with fundamentally different significance. Pathol Annu 1982;17:229–259.
43. Hickman JA. Apoptosis induced by anticancer agents. Cancer Metastasis Rev 1992;11:121–139.
44. Dive C, Wyllie AH. Apoptosis and cancer chemotherapy. In: Hickman JA, Tritton TR, editors. Frontiers in pharmacology. Oxford: Blackwell Scientific Press; 1993. p 21–56.
45. Reed E. Platinum-DNA adduct, nucleotide excision repair and platinum based anti-cancer chemotherapy. Cancer Treat Rev 1998;24:331–344.
46. Mu D, Shu DS, Sancar A. Reaction mechanism of human DNA repair exision nuclease. J Biol Chem 1996;271:8285–8294.
47. Yamada M, O'Regan E, Brown R, Karrap P. Selective recognition of a cisplatin-DNA adduct by human mismatch repair proteins. Nucleic Acids Res 1997;25:491–495.
48. Reed E. Cisplatin. In: Pinedo HM, Longo DL, Chabner BA, editors. Cancer chemotherapy and biological response modifiers. BV Amsterdam: Elseiver Science; 1999. p 144–151.
49. Turchi JJ, Henkels K. Human aKU autoantigen binds cisplatin-damaged DNA but fails to stimulate human DNA-activated protein kinase. J Biol Chem 1996;271:13861–13867.
50. Henkels KM, Turchi JJ. Induction of apoptosis in cisplatin-sensitive and -resistant human ovarian cancer cell lines. Cancer Res 1997;57:4488–4492.
51. Zamble DB, Lippard SJ. The response of cellular proteins to cisplatin-damaged DNA. In: Lippert B, editor. Cisplatin, chemistry and biochemistry of a leading anticancer drug. Basel: Wiley-VCH; 1999. p 74–110.
52. Hughes EN, Engelsberg BN, Billings PC. Purification of nuclear proteins that bind to cisplatin-damaged DNA: Identity with high mobility group proteins 1 and 2. J Biol Chem 1992;267:13520–13527.
53. Slamon DJ, Godolphin W, Jones LA. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 1989;244:707–712.
54. Treiber DK, Zhai X, Jantzen H.-M, Essigmann JM. Cisplatin-DNA adducts are molecular decoys for the ribosomal RNA transcription factor hUBF (human Upstream Binding Factor). Proc Natl Acad Sci USA 1994;91:5672–5676.
55. Jamieson ER, Lippard SJ. Structure, recognition and processing of cisplatin-DNA adducts. Chem Rev 1999;99:2467–2498.
56. Huang JC, Zamble DB, Reardon JT, Lippard SJ, Sancar A. HMG-domain proteins specifically inhibit the repair of the major DNA adduct of the anticancer drug cisplatin by human excision nuclease. Proc Natl Acad Sci USA 1994;91:10394–10398.
57. Loehrer PJ, Einhorn LH. Cisplatin. Annu Intern Med 1984;100:704–713.
58. Giaccone G. Clinical perspectives on platinum resistance. Drugs 2000;59:9–17.
59. Wolf CR, Hayward IP, Lawrie SS, Buckton K, Mclntyre MA, Adams DJ, Lewis AD, Scott ARR, Smyth JF. Cellular heterogeneity and drug resistance in two ovarian adenocarcinoma cell lines derived from a single patient. Int J Cancer 1987;39:696–701.
60. Johnson SW, Shen D-W, Pastan I, Gottesman MM, Hamilton TC. Cross-resistance, cisplatin accumulation, and platinum-DNA adduct formation and removal in cisplatin-sensitive and -resistant human hepatoma cell lines. Exp Cell Res 1996;226:133–239.
61. Akiyama S-I, Chen Z-S, Sumizawa T, Furukawa T. Resistance to cisplatin. Anticancer Drug Des 1999;14:143–151.
62. Kelland LR. Preclinical perspectives on platinum resistance. Drugs 2000;59:1–8.
63. Andrews PA, Howell SB. Cellular pharmacology of cisplatin: Perspectives on mechanisms of acquired resistance. Cancer Cells 1990;2:35–43.

64. Mistry P, Kelland LR, Abel G. The relationships between glutathione, glutathione-S-transferase and cytotoxicity of platinum drugs and melphalan in eight human ovarian carcinoma cell lines. Br J Cancer 1991;64:215–220.
65. Chaney SG, Vaisman A. Specificity of platinum-DNA adduct repair. J Inorg Biochem 1999;77:71–81.
66. Rosenberg B, Van Camp L, Grimley EB, Thomas AJ. The inhibition of growth or cell division in Escherichia coli by different ionic species of platinum (IV) complexes. J Biol Chem 1967;242:1347–1352.
67. Cleare MJ, Hoeschele JD. Studies on the antitumor activity of Group VIII transition metal complexes. Part 1. Platinum II complexes. Bioinorg Chem 1973;77:187–195.
68. Weiss RB, Christian MC. New cisplatin analogues in development. A review. Drugs 1993;46:360–377.
69. Lebwohl D, Canetta R. Clinical development of platinum complexes in cancer therapy: A historical perspective and an update. Eur J Cancer 1998;34:1522–1534.
70. Wong E, Giandomenico CM. Current status of platinum-based antitumor drugs. Chem Rev 1999;99:2451–2466.
71. Macquet JP, Butour JL. Platinum-amine compounds: Importance of the labile and inert ligands for their pharmacological activities toward L1210 leukemia cells. J Natl Cancer Inst 1983;70:899–905.
72. Knox RJ, Friedlos F, Lydall DA, Roberts JJ. Mechanism of cytotoxicity of anticancer platinum drugs: Evidence that cis-diamminedichloroplatinum(II) and cis-diammine(1,1-cyclobutanedicarboxylato)platinum(II) differ only in the kinetics of their interaction with DNA. Cancer Res 1986;46:1972–1979.
73. Calvert AH, Harland SJ, Newell DR, Siddik ZH, Jones AC, NcElwain TJ, Raju S, Wiltshaw E, Smith IE, Baker JM, Peckham MJ, Harrap KP. Early clinical studies with cis-diammine-1,1-cyclobutane dicarboxylate platinum(II). Cancer Chemother Pharmacol 1982;9:140–147.
74. Evans BD, Raju KS, Calvert AH, Harland SJ, Wiltshaw E. JM8 (cis-diammine-1,1-cyclobutane dicarboxylate platinum II), a new platinum analogue active in the treatment of advanced ovarian carcinoma. Cancer Treat Rep 1983;67:997–1000.
75. Ruckdeschel JC. The future role of carboplatin. Semin Oncol 1994;21:114–118.
76. Harrap KR. Preclinical studies identifying carboplatin as a viable cisplatin alternative. Cancer Treat Rev 1985;12:21–33.
77. Rixe O, Ortuzar W, Alvarez M. Oxaliplatin, Tetraplatin, Cisplatin and Carboplatin: Spectrum of activity in drug-resistant cell lines and in the cell lines of the National Cancer Institute's anticancer drug screen panel. Biochem Pharmacol 1996;52:1855–1865.
78. Bitha P, Carvajal SG, Citarella RV, Child RG, Delos Santos EF, Dunne TS, Durr FE, Hlavka JJ, Lang SA, Jr. Lindsay HL, et al. Water-soluble third generation antitumor platinum complexes, [2,2-bis(aminomethyl)-1,2-propanediol-N,N′]-[1,1-cyclobutane-dicarboxylato(2-)-O,O′]platinum(II) and [1,1-cyclobutanedicarboxylato(2-)-O,O′]-[tetrahydro-4H-pyran-4,4-dimethanamine-N,N′]platinum(II). J Med Chem 1989;32(8):2015–2020.
79. Connors TA, Jones M, Ross WC, Braddock PD, Khokhar AR, Lobe ML. New platinum complexes with anti-tumor activity. Chem Biol Interact 1972;5:415–424.
80. Burchenal JH, Kalaher K, O'Toole T, Chisholm J. Lack of cross-resistance between certain platinum coordination compounds in mouse leukaemia. Cancer Res 1977;37:2455–2457.
81. Burchenal JH, Kalaher K, Lokys L. Studies of cross-resistance, synergistic combinations and blocking of activity of platinum derivatives. Biochimie 1978;60:961–965.
82. Burchenal JH, Kalaher K, Dew K, Lokys L. Rationale for development of platinum analogs. Cancer Treat Rep 1979;63:1493–1498.
83. Perez RP, Perez KM, Handel LM, Hamilton TC. In vitro interactions between platinum analogues in human ovarian-carcinoma cell lines. Cancer Chemother Pharmacol 1992;29:430–434.
84. Jones M, Siracky J, Kelland LR, Harrap KR. Acquisition of platinum drug resistance and platinum cross resistance patterns in a panel of human ovarian carcinoma xenografts. Br J Cancer 1993;67:24–29.
85. Tashiro T, Kawada Y, Sakurai Y. Antitumor activity of a new platinum complex, oxalato(trans-L-1,2-diaminocyclohexane)platinum(II): New experimental data. Biomed Pharmacother 1989;43:251–260.
86. Raymond E, Faivre S, Woynarowski JM. Oxaliplatin: Mechanism of action and antineoplastic activity. Semin Oncol 1998;25:4–12.
87. Raymond E, Chaney SG, Taamma A. Oxaliplatin: A review of preclinical and clinical studies. Ann Oncol 1998;9:1053–1071.
88. Raymond E, Faivre S, Chaney S, Woynarowski J, Cvitkovic E. Cellular and molecular pharmacology of oxaliplatin. Mol Cancer Ther 2002;1:227–235.
89. Fink D, Nebel S, Aebi S. The role of DNA mismatch repair in platinum drug resistance. Cancer Res 1996;56:4881–4886.
90. Fink D, Zheng H, Nebel S. In vitro and in vivo resistance to cisplatin in cells that have lost DNA mismatch repair. Cancer Res 1997;57:1841–1845.

91. Vaisman A, Varchenko M, Umar A. The role of hMLH1, hMSH3, and hMSH6 defects in cisplatin and oxaliplatin resistance: Correlation with replicative bypass of platinum-DNA adducts. Cancer Res 1998;58: 3579–3585.
92. Hoeschele JD, Showalter HDH, Kraker AJ, Elliott WL, Roberts BJ, Kampf JW. Synthesis, structural characterization, and antitumor properties of a novel class of large-ring platinum(II) chelate complexes incorporating the cis-1,4-diaminocyclohexane ligand in a unique locked boat conformation. J Med Chem 1994;37:2630–2636.
93. Hospers GAP, Mulder NH, de Jong B, de Ley L, Uges DRA, Fichtinger-Schepmann AMJ, Scheper RJ, de Vries EGE. Characterization of a human small cell lung carcinoma cell line with acquired resistance to cis-diamminedichloro platinum(II) in vitro. Cancer Res 1988;48:6803–6807.
94. Teicher BA, Holden SA, Herman TS, Stomayor BA, Khandekar V, Rosbe KW, Brann TW, Korbutt TT, Frei E, III. Characteristics of five human tumor cell lines and sublines resistant to cis-diamminedichloroplatinum(II). Int J Cancer 1991;47:252–260.
95. Mellish KJ, Kelland LR, Harrap KR. In vitro platinum drug chemosensitivity of human cervical squamous cell carcinoma cell lines with intrinsic and acquired resistance to cisplatin. Br J Cancer 1993;68: 240–250.
96. Hasegawa Y, Morita M. Antitumor effect of oral cisplatin on certain murine tumors. Chem Pharm Bull 1985;33:5511–5514.
97. Van Hennik MB, Van der Vijgh WJF, Klein I, Vermoken JB, Pinedo HM. Human pharmacokinetics or carboplatin after oral administration. Cancer Chemother Pharmacol 1989;23:126–127.
98. Kelland LR, Abel G, McKeage MJ, Jones M, Goddard PM, Valenti M, Murrer BA, Harrap KR. Preclinical antitumor evaluation of bis-acetatoamminedichloro-cyclohexylamine Platinum(IV): An orally active platinum drug. Cancer Res 1993;53:2581–2586.
99. Rose WC, Crosswell AR, Schruig JE, Casazza AM. Preclinical antitumor activity of orally administered platinum (IV) complexes. Cancer Chemother Pharmacol 1993;32:197–203.
100. McKeage MJ, Raynaud F, Ward J, Berry C, O'Dell D, Kelland LR, Murrer B, Santabarabara P, Harrap KR, Judson IR. Phase I and pharmacokinetic study of an oral pltinum complex given daily for 5 days in patients with cancer. J Clin Oncol 1997;15:2691–2700.
101. Judson I, Cerny T, Epelbaum R, Dunlop D, Smyth J, Schaefer B, Roelvink M, Kaplan S, Hanauske A. Phase II trial of the oral platinum complex JM216 in non-small-cell lung cancer: An EORTC early clinical studies group investigation. Ann Oncol 1997;8:604–606.
102. Anon X. Satraplatin. Drugs Res Dev 2002;3(1):67–71.
103. Kelland LR, Murrer BA, Abei G, Giandomenico CM, Mistry P, Harrap KR. Ammine/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;52:822–828.
104. Harrap KR. Initiatives with platinum and quinazoline-based antitumor molecules. Cancer Res 1995;55: 2761–2768.
105. Farrell N, Qu Y, Hacker MP. Cytotoxicity and antitumor activity of bis(platinum) complexes. A novel class of platinum complexes active in cell lines resistant to both cisplatin and 1,2-diaminocyclohexane complexes. J Med Chem 1990;33:2179–2184.
106. Farrell N. Nonclassical platinum antitumour agents: Perspectives for design and development of new drugs complementary to cisplatin. Cancer Invest 1993;11:578–589.
107. Farrell N, Appleton TG, Au Y, Roberts JD, Soares Fontes AP, Skov KA, Wu P, Zou Y. Effects of geometric isomerism and ligand substitution in bifunctional dinuclear platinum complexes on binding properties and conformational changes in DNA. Biochemistry 1995;34:15480–15486.
108. Di Blasi P, Bernareggi A, Beggiolin G, Piazzoni L, Menta E, Formento ML. Cytotoxicity, cellular uptake and DNA binding of the novel trinuclear platinum complex BBR 3464 in sensitive and cisplatin resistant murine leukemia cells. Anticancer Res 1998;18:3113–3118.
109. Perego P, Gatti L, Caserini C, Supino R, Colangelo D, Leon R, Spinelli S, Farrell N, Zunino F. The cellular basis of the efficacy of the trinuclear platinum complex BBR 3464 against cisplatin-resistant cells. J Inorg Biochem 1999;77:59–64.
110. Perego P, Caserini C, Gatti L, Carenini N, Romanelli S, Supino R, Colangelo D, Viano L, Leone R, Spinelli S, Pezzoni G, Mazotti C, Farrell N, Znino F. A novel trinuclear platinum complex overcomes cisplatin resistance in an osteosarcoma cell system. Mol Pharm 1999;55:528–534.
111. Farrell N, Spindelli S. In: Farrell N, editor. Uses of inorganic chemistry in medicine. Cambridge: The Royal Society of Chemistry; 1999. p 124–134.
112. Wu PK, Kharatishvili M, Qu Y, Farrell N. A circular dichroism study of ethidium bromide binding to Z-DNA induced by dinuclear platinum complexes. J Inorg Biochem 1996;63:9–18.
113. Zou Y, Van Houten YB, Farrell N. Sequence specificity of DNA-DNA interstrand cross-link formation by cisplatin and dinuclear platinum complexes. Biochemistry 1994;33:5404–5410.

114. Versantvoort CHM, Broxterman HJ, Bagrij TR, Scheper J, Twentyman PR. Regulation by glutathione of drug transport in multidrug-resistant human lung tumor cell lines overexpressing multidrug resistance-associated protein. Br J Cancer 1995;72:82–89.
115. Pendyala L, Creaven PJ, Perez R, Zdanowicz JR, Raghavan D. Intracellular glutathione and cytotoxicity of platinum complexes. Cancer Chemother Pharmacol 1995;36:271–278.
116. Lai GM, Ozols RF, Young RC, Hamilton TC. Effect of glutathione on DNA repair in cisplatin-resistant human ovarian cancer cell lines. J Natl Cancer Inst 1989;81:535–539.
117. Chen Y, Guo Z, Parsons S, Sadler PJ. Stereospecific and kinetic control over the hydrolysis of a sterically hindered platinum picoline anticancer complexes. Chem Eur J 1998;4:672–676.
118. Chen Y, Guo Z, Parkinson JA, Sadler PJ. Kinetic control of reactions of a sterically hindered platinum picoline anticancer complex with guanosine 5′-monophosphate and glutathione. J Chem Soc Dalton Trans 1998;21:3577–3585.
119. Holford J, Sharp SY, Murrer BA, Abrams M, Kelland LR. In vitro circumvention of cisplatin-resistance by the novel sterically hindered platinum complex AMD473. Br J Cancer 1998;77:366–373.
120. Raynaud FI, Boxall FE, Goddard PM. cis-Amminedichloro(2-methylpyridine)platinum(II) (AMD473), a novel sterically hindered platinum complex: In vivo activity, toxicology, and pharmacokinetics in mice. Clin Cancer Res 1997;3:2063–2074.
121. Palmer BD, Lee HH, Johnson P, Baguley BC, Wicham G, Wakelin LPG, McFadyen WD, Denny WA. DNA-directed alkylating agents. 2. Synthesis and biological activity of platinum complexes linked to 9-anilinoacridine. J Med Chem 1990;22:3008–3014.
122. Lee HH, Palmer BD, Baguley BC, Chin M, McFadyen WD, Wickham G, Denny WA. DNA-directed alkylating agents. 5. Acridinecarboxamide derivatives of (1,2-diaminoethane)dichloroplatinum(II). J Med Chem 1992;35:2983–2987.
123. Murray V, Motyka H, England PR, Wickham G, lee HH, Denny WA, McFadyen WD. An investigation of the sequence-specific interaction of cis-diamminedichloroplatinum(II) and four analogues, including two acridine-tethered complexse, with DNA inside human cells. Biochemistry 1992;31:11812–11817.
124. Zunino F, Savi G, Pasini A. Synthesis and antitumor activity of a platinum(II)-doxorubicin complex. Cancer Chemother Pharmacol 1986;18:180–182.
125. Gust R, Niebler K, Schonenberger H. Investigation of the configurational and conformational influences on the hormonal activity of 1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamines and of their platinum(II) complexes. 1. Synthesis, estradiol receptor affinity, and estrogenic activity of diastereomeric [N-alkyl- and N,N′-dialkyl-1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine]dichloro-platinum (II) complexes. J Med Chem 1995;38:2070–2079.
126. Apfelbaum HC, Blum J, Mandelbaumshavit F. Preparation and anticancer activity of two tryptamine derived platinum complexes. Inorg Chim Acta 1991;186:243–246.
127. Sachinvala ND, Chen H, Niemezura WP, Furusawa E, Cramer RE, Rupp JJ, Ganjan I. Synthesis, characterization, and anticancer activities of the first platinum complexes from sucrose. J Med Chem 1993;36: 1791–1795.
128. Ho Y-P, To KKW, Au-Yeung SCF, Wang X, Lin G, Han X. Potential new antitumor agents from an innovative combination of demethylcantharidin, a modified traditional Chinese medicine, with a platinum moiety. J Med Chem 2001;44:2065–2068.
129. Cassileth BR. Alternative and complementary cancer treatments. Oncologists 1996;1:173–179.
130. Borchers AT, Hackman RM, Keen CL, Stern JS, Gershwin ME. Complementary medicine: A review of immunomodulatory effects of Chinese herbal medicines. Am J Clin Nutr 1997;66:1303–1312.
131. Wang GS. Medical uses of Mylabris in ancient China and recent studies. J Ethnopharm 1989;26:147–162.
132. Li YM, Casida JE. Cantharidin-binding protein: Identification as protein phosphatase 2A. Proc Natl Acad Sci USA 1992;89:11867–11870.
133. Graziano MJ, Waterhouse AL, Casida JE. Cantharidin poisoning associated with specific binding site in liver. Biochem Biophys Res Commun 1987;149:79–85.
134. Graziano MJ, Pessah IN, Matsuzawa M, Casida JE. Partial characterization of specific cantharidin binding sites in mouse tissues. Mol Pharmacol 1988;33:706–712.
135. Yi SN, Wass J, Vincent P, Iland H. Inhibitory effect of norcantharidn on K562 human myeloid leukemia cells in vitro. Leuk Res 1992;15:883–886.
136. Liu XH, Blazsek I, Quittet P, Comisso M, Anjo A, Wang GS, Misset JL. Effects of norcantharidin, a potent phosphatase type 2A inhibitor, on normal and malignant hematopoietic cell growth. Eur J Cancer 1995;31: 953–963.
137. Simsiman GV, Daniel TC, Chesters G. Diquat and endothall: Their fates in the environment. Residue Rev 1976;62:131–174.

138. Blazsek I, Thiery JP, Anjo A, Reynes M, Legras S, Marion S, Jasmin C. Inhibitors of protein phosphatase type 2A are novel potential anticancer agents. Cancer Detection Prevention [Abstract] 1996;20:332A.
139. Thiery JP, Blazsek I, Legras S, Marion S, Reynes M, Anjo A, Adam R, Misset JL. Hepatocellular carcinoma cell lines from diethylnitrosamine phenobarbital-treated rats. Characterization and sensitivity to endothall, a protein serine/threonine phosphatase-2A inhibitor. Hepatology 1999;29:1406–1417.
140. Page JD, Husain I, Sancar A, Chaney SG. Effect of the diaminocyclohexane carrier ligand on platinum adduct formation, repair, and lethality. Biochemistry 1990;29:1016–1024.
141. Schmidt W, Chaney SG. Role of carrier ligand in platinum resistance of human carcinoma cell lines. Cancer Res 1993;53:799–805.
142. Bungo M, Fujiwara Y, Kasahara K, Nakagawa K, Ohe Y, Sasaki Y, Irino S, Saijo N. Decreased accumulation as a mechanism of resistance to cis-diamminedichloroplatinum(II) in human nonsmall cell lung cancer cell line. Br J Cancer 1990;62:72–77.
143. O'Dwyer PJ, Hamilton TC, Young RC, LaCreta FP, Carp N, Tew KD, Padavic K, Comis RL, Ozols RF. Depletion of glutathione in normal and malignant human cells in vivo by buthionine sulfoimine: Clinical and biochemical results. J Natl Cancer Inst 1992;84:264–267.
144. Ariza RR, Keyse SM, Moggs JG, Wood RD. Reversible protein phosphorylation modulates nucleotide excision repair of damaged DNA by human cell extracts. Nucleic Acids Res 1996;24:433–440.
145. Cohen P, Klumpp S, Schelling DL. An improved procedure for identifying and quantitating protein phosphatases in mammalian tissues. FEBS Lett 1989;250:596–600.
146. Zolnierowicz S. Type 2A protein phosphatase, the complex regulator of numerous signaling pathways. Biochem Pharmacol 2000;60:1225–1235.
147. Clarke PR, Hoffman I, Draetta G, Karsenti E. Dephosphorylation of cdc25-C by a type-2A protein phosphatase: Specific regulation during the cell cycle in Xenopus extracts. Mol Biol Cell 1993;4:397–411.
148. Sontag E, Fedorov S, Kambayashi C, Robbins D, Cobb M, Mumby M. The interaction of SV40 small tumor antigen with protein phosphatase 2A stimulates the map kinase pathway and induces cell proliferation. Cell 1993;75:887–897.
149. Mayer-Jaekel RE, Ohkura H, Gomes R, Sunkel CE, Baumgartner S, Hemmings BA, Glover DM. The 55 kD regulatory subunit of Drosophila protein phosphatase 2A is required for anaphase. Cell 1993;72:621–633.
150. Lee TH. The role of protein phosphatase type-2A in the Xenopus cell cycle: Initiation of the G2/M transition. Semin Cancer Biol 1995;6:203–209.
151. Schonthal AH. Regulation of gene expression by serine/threonine protein phosphatases. Semin Cancer Biol 1995;6:239–248.
152. Wera S, Hemmings BA. Serine/threonine protein phosphatases. Biochem J 1995;311:17–29.
153. To KKW, Ho Y-P, Au-Yeung SCF. Determination of the release of hydrolyzed demethylcantharidin from novel traditional Chinese medicine-platinum compounds with anticancer activity by gas chromatography. J Chromatogr A 2002;947:319–326.

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