

The Discovery and Development of Cisplatin

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The discovery of the anticancer activity of cisplatin $\{cis-[PtCl_2(NH_3)_2]\}$ in the 1960s and its subsequent clinical success generated interest in the use of metal compounds in cancer treatment (1, 2). Thousands of platinum compounds have since been prepared and evaluated as potential chemotherapeutic agents, although few have entered clinical use (3, 4). Worldwide annual sales of platinum-based anticancer drugs are currently of the order of two billion U.S. dollars (5, 6). Several other metal compounds have been found to display promising anticancer activity including a variety of complexes of ruthenium and gold as well as metallocenes of Ti, Nb, Mo, and Re (2). The platinum(II) drugs, carboplatin $\{cis-[Pt(NH_3)_2(CBDCA)]\}$, CBDCA = 1,1-cyclobutanedicarboxylic acid and oxaliplatin $\{[Pt(oxalato)(1R,2R-chxn)]\}$, chxn = cyclohexane-1,2-diamine, have also entered the clinic, supplementing and complementing cisplatin. Cisplatin is responsible for the cure of over 90% of testicular cancer cases and it plays a vital role in the treatment of cancers such as ovarian, head and neck cancer, bladder cancer, cervical cancer, melanoma, lymphomas, as well as several others (3, 4, 7, 8).

Discovery

The discovery of cisplatin is well documented (9) and will only be covered briefly to explain the key observations that led to its discovery. Barnett Rosenberg was a biophysicist at the University of Michigan and had decided to examine whether electrical currents played a role in cellular division. This was prompted by Rosenberg's feeling that the mitotic spindles in a dividing cell appeared similar to the classic school science experiment, where magnetic field lines are formed by scattering iron filings on paper over a magnet. In order to examine this, *Escherichia coli* (*E. coli*) cells growing in ammonium chloride buffer had a current applied to them through "inert" platinum electrodes immersed in the buffer (9). After a period of time, the *E. coli* cells began appearing long and filamentous, much like spaghetti, instead of their classical sausage shape (10).

This effect was determined to be due to inhibition of cellular division; after much investigation, it was found that the phenomenon was not due to the electrical current, but platinum hydrolysis products formed from the "inert" platinum electrodes (11). A range of group 10 transition-metal compounds were then tested and found to also result in elongation of *E. coli* cells, and in fact the most effective platinum salt, $(NH_4)_2[PtCl_6]$ caused elongation of a range of gram negative bacilli (12). It was also reported by Rosenberg and co-workers that the cis form of the platinum(IV) complex, $[PtCl_4(NH_3)_2]$, was the agent responsible for inhibition; the trans complex was found to be ineffective (12).

Given these results, it was reasoned that the complexes may be interesting to test for their anticancer activity. To this end, the platinum(II) complex, $cis-[PtCl_2(NH_3)_2]$, and the platinum(IV) complex, $cis-[PtCl_4(NH_3)_2]$, were tested against Sarcoma 180 tumors in Swiss white mice. The complexes demonstrated "potent" activity, shrinking large solid tumors, and the mice survived and were healthy. In fact, after 6 months the cured mice did not show any signs of cancer (13, 14). Based on these results, cisplatin entered clinical trials and is now one of the most successful anticancer drugs in the clinic.

Synthesis

Cisplatin is a molecule with an interesting history. It was first synthesized by Michel Peyrone in 1845 (15) and subsequently became the subject of fervent debate over its structure (16). Some 50 years later it played a vital role in the establishment of Alfred Werner's theory of coordination chemistry when he correctly proposed its square planar configuration and distinguished between the cis and trans isomers: cisplatin and transplatin $\{trans-[PtCl_2(NH_3)_2]\}$ (16–18). Werner won the Nobel Prize for Chemistry for this work in 1913 (see <http://www.nobel.se>, accessed Jan 2006) (17).

The procedure for the synthesis of cisplatin has undergone several improvements. Early methods tended to be unreliable, often producing impure products, thus subsequent alterations aimed to improve upon the purity of the product, the overall yield of the reactions, and the reaction time. In 1970 Dhara reported on "A rapid method for the synthesis of $cis-[PtCl_2(NH_3)_2]$ " (19). The majority of subsequent cisplatin syntheses are based upon this method. In this procedure (Scheme 1) the starting material, $K_2[PtCl_4]$, is converted to the tetraiodo analogue, $K_2[PtI_4]$, by the addition of a saturated solution of KI. To this is added NH_3 , forming the yellow compound, $cis-[PtI_2(NH_3)_2]$, which is then collected and dried. Addition of an aqueous solution of $AgNO_3$ to $cis-[PtI_2(NH_3)_2]$ causes the precipitation of insoluble AgI that is filtered off and discarded. The filtrate containing $cis-[Pt(OH)_2(NH_3)_2]^{2+}$ is treated with KCl , resulting in the precipitation of the final product, $cis-[PtCl_2(NH_3)_2]$, as a yellow powder (19).

The success of this procedure is dependent upon the trans effect. The concept of the trans effect was introduced by Chernyaev in 1926 (20, 21) to explain the empirical observation that the rate of substitution of a ligand in a square planar or octahedral metal complex is dependent on the group opposite (or trans) to it (much more so than groups in cis positions). Studies have shown that the order of decreasing trans effect of ligands is approximately (22):

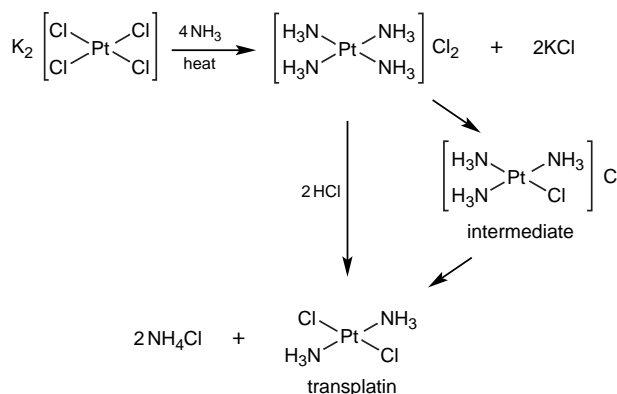
CO, CN⁻, C₂H₄ > PR₃, H⁻ > CH₃⁻, SC(NH₂)₂ > C₆H₅⁻, NO₂⁻, I⁻, SCN⁻ > Br⁻, Cl⁻ > py, NH₃, OH⁻, H₂O

When the intermediate triiodo species, K[PtI₃(NH₃)], reacts with the second ammonia group (Scheme I) there are two options; either displacement of the iodo ligand that is trans to an ammonia ligand, or displacement of the iodo ligand that is trans to another iodo ligand. The stronger trans-directing influence of the iodo ligand relative to the ammonia ligand means that the ligand trans to the iodide is more labile and hence is the one that is displaced, resulting in the desired cis configuration of the final product. Earlier procedures did not involve the initial step of converting K₂[PtCl₄] to K₂[PtI₄] that ensures the desired cis product is obtained with no contamination by Magnus' Green salt, [Pt(NH₃)₄][PtCl₄], owing to the stronger trans effect of iodo ligands as compared to chloro ligands.

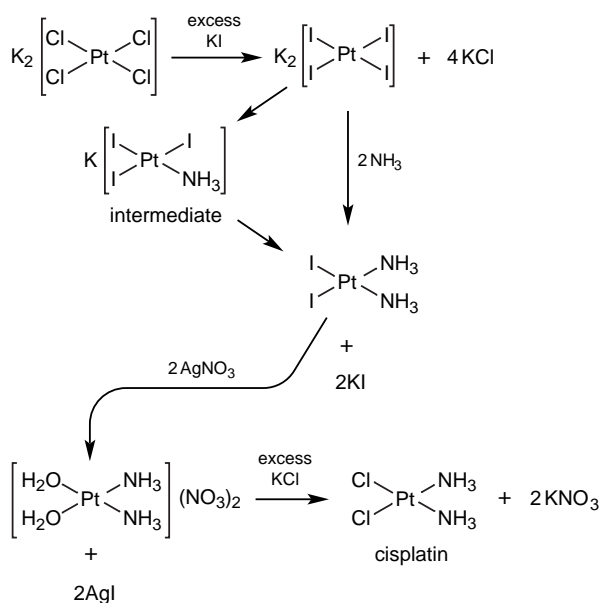
Transplatin was first synthesized by Reiset in 1844 (23, 24). Today the most commonly used procedure for the synthesis of transplatin (Scheme II) involves converting K₂[PtCl₄] to the [Pt(NH₃)₄]Cl₂ salt by the addition of ammonia while heating. After the volume has been reduced by evaporation, HCl is added and the volume is further reduced, causing the precipitation of the desired product as a yellow powder (25). It is interesting to note that both [Pt(NH₃)₄]²⁺ and [PtCl(NH₃)₃]⁺ are charged and hence quite water soluble, allowing them to remain in solution and undergo further reaction. However, transplatin is a neutral species and far less soluble; hence it precipitates out of solution, preventing it from undergoing subsequent reaction. This synthetic method also takes advantage of the trans effect to ensure the formation of the desired product. In the intermediate species, [PtCl(NH₃)₃]⁺, the most labile group is the ammonia group trans to the chloro ligand, owing to the greater trans effect of the chloro ligand compared to the ammonia ligand. Hence it is replaced in preference to the others, resulting in the formation of the desired trans product.

A method for distinguishing between the cis and trans isomers of square planar complexes was developed by Kurnakow in 1894 (16, 26). As shown in Scheme III, when reacted with thiourea, cisplatin produces a deep yellow solution of [Pt(Th)₄]²⁺ (Th = thiourea), whereas transplatin produces white insoluble *trans*-[Pt(NH₃)₂(Th)₂]Cl₂, thus providing a visual confirmation of the geometry of the complex. This test works on the basis of the greater trans-directing effect of thiourea (coordinated through the sulfur group) as compared to that of the amine or chloro ligands, which results in labilization and replacement of the ligands trans to the coordinated thiourea (21). The Kurnakow method has even been developed to separate a mixture of the two isomers and detect trace quantities of transplatin contaminant in samples of clinical cisplatin (27). Several other tests for distinguishing between cisplatin and transplatin also exist (25).

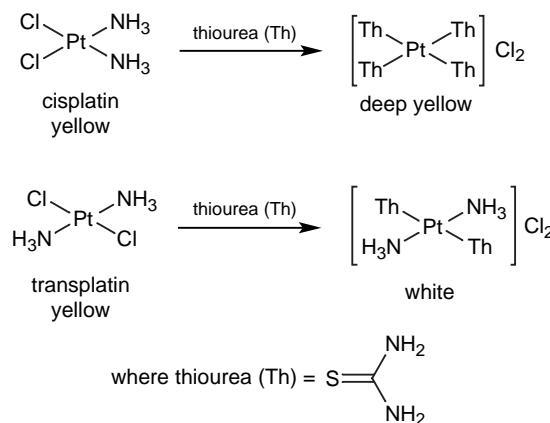
To date, three platinum(IV) complexes with three different types of axial ligands (chloro, hydroxo, and acetato) have entered clinical trials (28). The standard procedure for



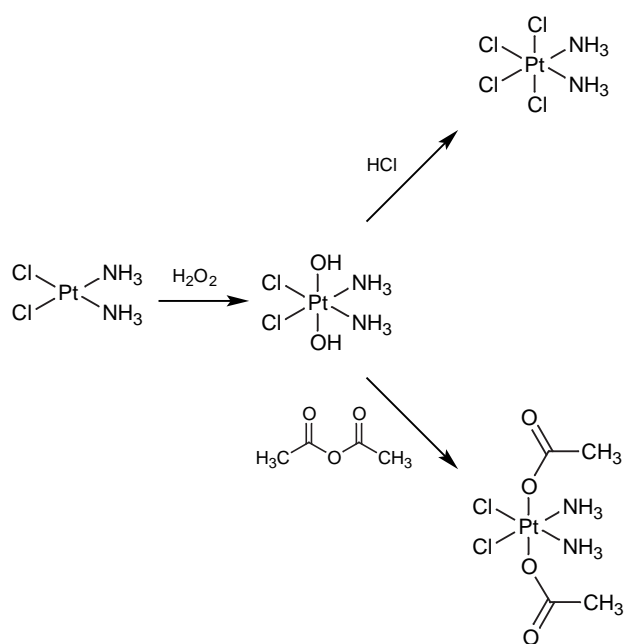
Scheme II. Synthetic scheme for the synthesis of transplatin (25).



Scheme I. Synthetic scheme for the Dhara synthesis of cisplatin (19).



Scheme III. The Kurnakow test. When coordination occurs with a cis complex, thiourea initially displaces the chloro ligands. The strong trans labilizing influence of the thiourea groups results in subsequent displacement of the ammonia ligands by two more thiourea groups, resulting in a soluble tetrathiourea complex. Conversely, the trans complex coordinates only through the two trans chloro groups, resulting in a white insoluble powder.



Scheme IV. Procedure for the synthesis of platinum(IV) trans-dihydroxo, dichloro, and diacetato complexes from cisplatin.

synthesizing such complexes (Scheme IV) initially involves the oxidation of a platinum(II) complex (such as cisplatin) to its trans-dihydroxo platinum(IV) analog by addition of hydrogen peroxide to an aqueous solution of the platinum(II) species (29). The trans-dichloro complex may be obtained directly by bubbling chlorine gas through an aqueous solu-

tion of the platinum(II) species (30). However, it is more commonly and cheaply synthesized by the addition of concentrated HCl to an aqueous solution of the dihydroxo platinum(IV) complex (31). In the low pH conditions of this reaction mixture, the hydroxo ligands become protonated and the resultant neutral aqua ligands are readily replaced by chloride ions (28). Platinum(IV) complexes with axial carboxylato ligands are usually produced by the reaction of the appropriate anhydride or acyl chloride with the dihydroxo platinum(IV) species. The success of this reaction is dependent upon the nucleophilic nature of the axial hydroxo ligands that attack the anhydride or acyl chloride, resulting in a platinum(IV) species with carboxylato ligands (32, 33).

How Cisplatin Works

Cisplatin is generally believed to exert its anticancer effects by interacting with DNA, inducing programmed cell death (apoptosis, Figure 1) (34). Following administration in the bloodstream of a patient, cisplatin encounters a relatively high chloride concentration in the blood plasma (approximately 100 mM) that limits replacement of its chloride ligands by water molecules (that is, the process of aquation is prevented). However, cisplatin is vulnerable to attack by proteins found in blood plasma, particularly those that contain thiol groups, such as human serum albumin and the amino acid cysteine. In fact, studies have shown that one day after cisplatin administration, 65–98% of the platinum in blood plasma is protein bound (35–37). This protein binding has been blamed for deactivation of the drug (36, 38–41) and some of the severe side effects of cisplatin treatment (35, 38–42).

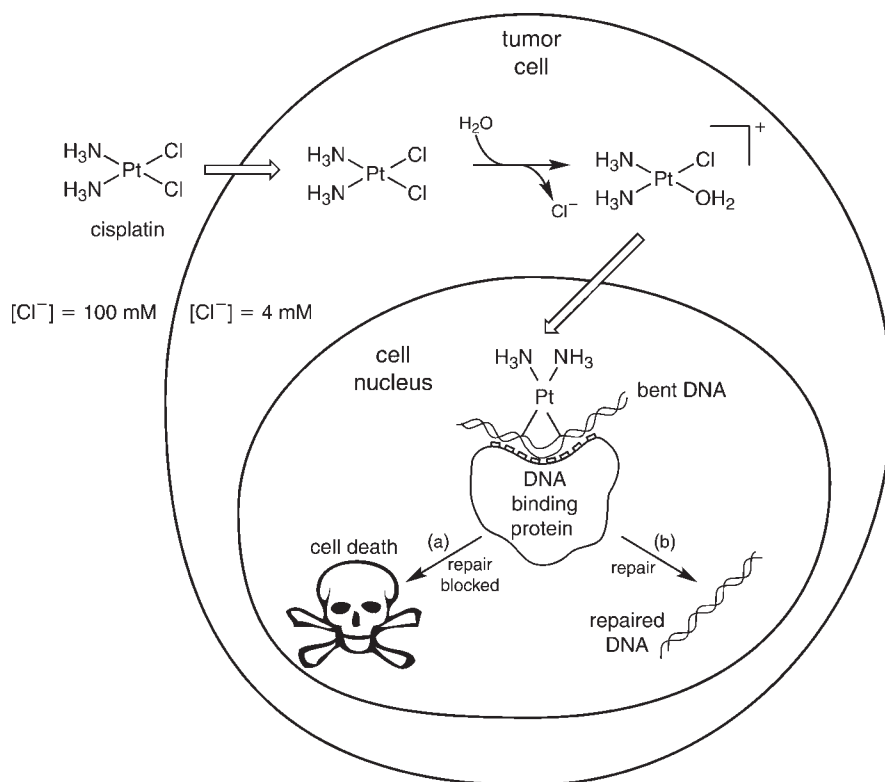


Figure 1. Schematic showing the cytotoxic pathway for cisplatin. After entering the cell, cisplatin is aquated, then binds to cellular DNA. If the DNA lesion is not repaired by the cell (path a), then cell death (apoptosis), can occur.

The cisplatin that remains intact can enter tumor cells, mainly by diffusing through the cell membrane (43), though recent results suggest that cisplatin can be actively transported across cell membranes by Cu-transporting proteins (44). The intracellular chloride concentration is relatively low (approximately 4–20 mM) and hence one of the chloro ligands of the intact cisplatin is replaced by water, forming a reactive, positively charged species that cannot readily leave the cell. In vitro studies have shown that this mono-aquated platinum species is responsible for at least 98% of the platinum binding to DNA within the cell nucleus (45). It reacts with one of the DNA bases, usually guanine, forming a monofunctional DNA adduct (46–48). The potential platinum binding sites on each of the DNA bases are shown in Figure 2. Ring closure to form a bifunctional adduct may occur either directly from the monofunctional adduct or may involve aquation of the second chloro ligand followed by rapid ring closure (49, 50). The primary bifunctional adducts are guanine–guanine and adenine–guanine (51), causing significant distortion of the DNA that can be recognized by one or more DNA binding proteins. These proteins can either initiate DNA damage repair (Figure 1, path b) or signal for apoptosis (cell death) to be initiated (Figure 1, path a) (2, 49, 50, 52, 53).

Platinum complexes with trans geometries were initially dismissed as being inactive. Rosenberg and coworkers were the first to report this, specifically for transplatin and *trans*-[PtCl₄(NH₃)₂] (11). Soon afterwards, this was confirmed to be the case for a much wider range of trans platinum complexes (54, 55). The vast difference in the anticancer activity of cisplatin and transplatin may seem counterintuitive at first, given their structural similarity. However, this fact has been rationalized in terms of the greater reactivity of transplatin compared to cisplatin; transplatin aquates approximately 4 times faster than cisplatin (56), reacts with ammonia approximately 30 times faster (57), and, following a 4-hour incubation with red blood cells, transplatin reacts with 70% of the glutathione, whereas cisplatin reacts with only 35% (58). This greater reactivity can be rationalized in terms of the trans effect. The high reactivity of transplatin results in rapid deactivation of the complex owing to side reactions on the way to its target that are likely to contribute to its lack of anticancer activity (3, 24, 54, 55). Recently, several trans compounds possessing significant anticancer activity have been reported that have bulky amine ligands that slow the substitution reactions (24). It has been suggested that the inability of transplatin to form 1,2-adducts, and the more rapid repair of the 1,3-adducts that it forms predominantly, contribute to its inactivity. However, more recent active trans complexes demonstrate that other modes of DNA binding can produce high anticancer activity.

The effectiveness of cisplatin treatment is limited by the phenomenon of tumor resistance. Several tumors are intrinsically resistant to cisplatin (e.g., colon cancer, non-small-cell lung cancer), while others acquire resistance after exposure to the drug over time (e.g., ovarian cancer, small-cell lung cancer) (59). The cellular mechanisms of cisplatin resistance have been identified as decreased cellular drug accumulation, an increased capability of cells to repair or tolerate the DNA damage caused by cisplatin, and increased levels of intracellular thiols that can bind to cisplatin and cause its deactivation (49, 52, 60, 61).

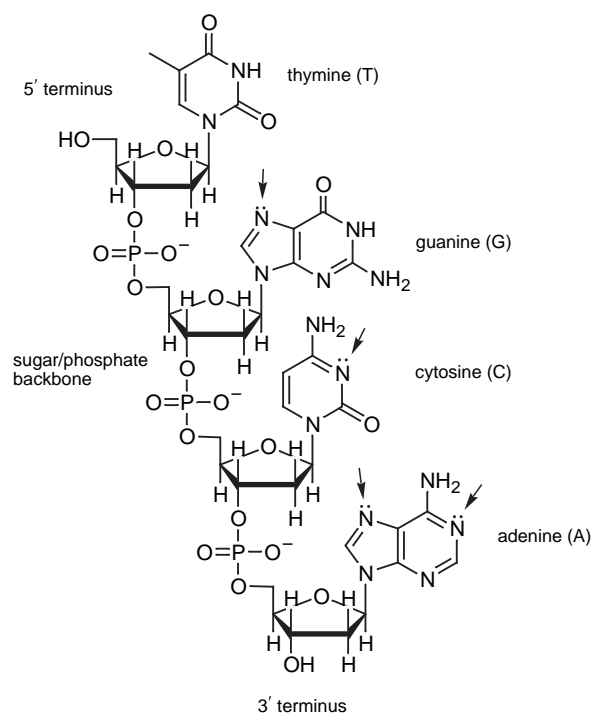


Figure 2. A schematic representation of a DNA strand showing the sites available for platinum binding. Guanine-N(7) is the preferred site for initial binding of the Pt(II) complex, since guanine is the most nucleophilic DNA base (48). Both in vitro and in vivo, the majority of bifunctional DNA adducts formed are intrastrand (that is, formed between bases on the same DNA strand). Approximately 65% are GG adducts, and 25% are AG adducts (34, 48, 74).

The cellular environment is rich in thiol-containing species, such as glutathione and metallothionein, that can bind to cisplatin and deactivate it (2, 52). This deactivation is due to the strong coordination of the soft sulfur donor coordinating to the soft platinum(II) in preference to harder ligands such as amine nitrogen donors; a concept known as Hard–Soft Acid–Base (HSAB) theory (62–64). HSAB theory was developed by Pearson to qualitatively predict the complexation preferences of metal ions and ligands; hard ligands (Lewis bases) prefer hard metals (Lewis acids) and soft prefers soft in a similar fashion (62). Hard–hard bonding occurs between weakly polarizable partners and has a strong ionic character, while soft–soft bonding occurs between easily polarizable partners and has a more covalent character.

Development

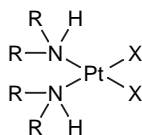
In spite of the widespread success of cisplatin, the search continues for new platinum drugs. This has been motivated by the desire to improve upon the clinical performance of cisplatin. The side effects associated with cisplatin treatment can be severe and may include toxicity to the kidney and nervous system, hearing difficulties, nausea, vomiting, and several others (4). In general, for a platinum drug to gain clinical approval, it must possess at least one distinct clinical advantage over cisplatin. Such advantages may include: activity against cancers with intrinsic or acquired resistance to

cisplatin treatment, reduced toxic side effects, or the ability to be administered orally (7).

Following the initial studies on cisplatin, a number of studies were performed to determine the structural features required to endow a platinum compound with antitumor activity. These “structure–activity relationships” were established by Cleare and Hoeschele following their examination of many platinum compounds (54, 55). Their discoveries indicated that the following structural criteria were necessary (3):

- The compound should have two amine groups with a cis geometry, as those with trans geometries are inactive.
- It is necessary, although not sufficient, for the compound to have two leaving groups that are cis with respect to one another. Leaving groups are those groups on the molecule that are the most easily lost.
- The ease with which the leaving groups are able to be lost affects the activity and the toxicity of the compound. It is preferable for the leaving groups to be only moderately easy to remove.
- The compound should be neutral.
- Compounds with fewer alkyl substituents on the amine ligands had greater activity. Each amine ligand should possess at least one proton.

The general structure of such complexes are



where X = leaving groups (e.g., two chloro groups or a bidentate malonate), ligand R = H or an alkyl substituent. The vast majority of platinum compounds tested have strictly adhered to these structure–activity criteria.

Rosenberg and his colleagues were pioneers in the investigation into cisplatin analogs with improved activity, and were responsible for the development of carboplatin (Figure 3, structure I) (7), which is the only platinum compound apart from cisplatin to have gained widespread clinical approval for treatment of a wide range of cancers (65). Carboplatin was invented by investigators whose goal was to obtain a compound with better activity and less toxicity than cisplatin (7, 8). It was found to be less toxic than cisplatin, and cause fewer and less severe side effects. The bidentate cyclobutanedicarboxylate leaving group of carboplatin is more stable than the chloride groups of cisplatin, resulting in a slower reaction in the body. This may be partly responsible for its reduced toxicity as compared to cisplatin (4, 7, 65). Oxaliplatin (Figure 3, structure II) was approved in France, the United Kingdom, and other European countries in 1996 and in the United States in 2002 for clinical use against advanced colorectal cancer (4, 65, 66) and is the only platinum compound to have displayed activity against colorectal cancer thus far (66).

AMD473, *cis*-[PtCl₂(NH₃)(2-methylpyridine)], Figure 3 structure III, was rationally designed to overcome resistance to cisplatin. It is believed that cisplatin becomes deactivated

when it reacts with cellular species that contain thiol groups. The bulky 2-methylpyridine group in AMD473 hinders the binding of thiols, as the 5-coordinate intermediate involved in associative ligand substitution mechanisms is destabilized by steric interactions. This is believed to be responsible for its good activity in several cisplatin resistant cell lines. Studies have also shown that it retains its activity when administered orally. AMD473 entered clinical trials in 1997 and has recently been licensed to NeorX for further development (<http://www.neorx.com>, accessed Jan 2006) (4, 59, 66).

Rosenberg's original experiment identified both platinum(II) and platinum(IV) species as possessing anticancer activity. In spite of this fact, the vast majority of research since then has focussed on platinum(II) compounds. Recognition of the enormous potential that platinum(IV) compounds possess as anticancer agents in terms of high activity, low toxicity, and perhaps the ability to be effective oral agents, has revived research in this area (28, 67).

It is generally accepted that inert platinum(IV) complexes are prodrugs that must be reduced to their more reactive platinum(II) analogs to exert anticancer activity, as the

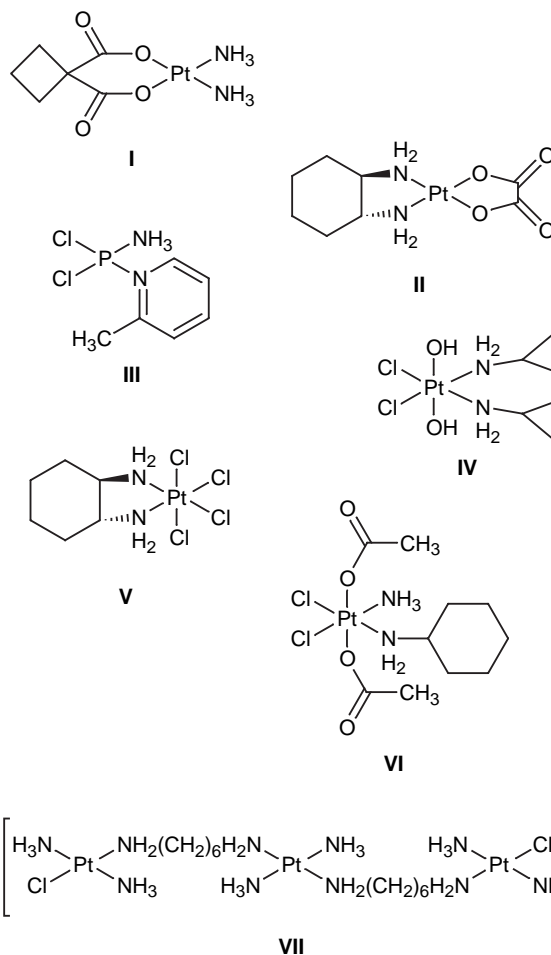
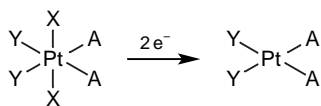


Figure 3. Structures of some platinum compounds that have entered clinical trials: I = carboplatin, II = oxaliplatin, III = AMD473, IV = iproplatin, V = tetraplatin, VI = JM216, and VII = BBR 3464. Compounds I and II are in widespread clinical use.

platinum(II) analog is the active species. The diagram shows a general octahedral Pt(IV) complex,



where: A = cis amine, Y = leaving groups in equatorial plane, and X = axial ligands. Two electron reduction to a square planar Pt(II) species involves the loss of the axial ligands. The ease with which the platinum(IV) complexes are reduced is related to their reduction potentials, which in turn are dependent on the nature of the axial ligands, X, that are lost as the octahedral platinum(IV) complex changes to a square planar platinum(II) complex.

A number of platinum(IV) compounds have entered clinical trials. The first to do so was iproplatin (*cis,trans,cis*-[PtCl₂(OH)₂(isopropylamine)₂], Figure 3, structure IV). It was initially selected for clinical development because of its unique structure, high solubility, broad spectrum of activity, and lower toxicity compared to cisplatin (65). However, during clinical trials it was found to display no significant advantages over carboplatin and was abandoned (68). Tetraplatin {ormaplatin, [PtCl₄(D,L-cyclohexane-1,2-diamine)], Figure 3, structure V} is another platinum(IV) drug that has entered clinical trials. Prior to entering the clinic, there was evidence to suggest that it could overcome cisplatin resistance and animal studies had shown it to be less nephrotoxic than cisplatin. However, in initial clinical trials it caused severe cumulative neurotoxicity, and was subsequently abandoned from the clinic (4, 7, 65).

In an effort to develop a drug that could be administered orally, JM216 {*cis,trans*-[PtCl₂(acetato)₂(NH₃)(cyclohexylamine)], Figure 3, structure VI} and several related analogues were synthesized (69). Prior to entering the clinic, JM216 displayed good activity in cisplatin-resistant cancer cell lines and minimal nephrotoxicity and neurotoxicity in animals. In addition, when administered orally, its activity was comparable to that of cisplatin and carboplatin administered by injection (7, 65). The drug (now known as satraplatin) is currently in Phase 3 trials for hormone-refractory prostate cancer (70).

Researchers are beginning to realize that compounds that conform to the original structure–activity relationships rarely offer substantial improvements upon the performance of cisplatin and are hence unlikely candidates for clinical approval. In recent years, the search has diversified to include platinum compounds that are in direct violation of the original structure–activity relationships. Many of them have shown promising activity (3).

A novel class of “polynuclear” compounds has emerged that are charged and possess more than one platinum center in the molecule (71–73), breaking two of the structure–activity rules. Several such compounds have been evaluated and one in particular, BBR 3464 (Figure 3, structure VII), was chosen for further testing. It was shown to be more potent than cisplatin and to have good activity in cisplatin resistant cell lines. It entered clinical trials in June 1998 and is progressing through Phase 2 trials (73). There is the hope that these multinuclear complexes will result in a breakthrough in platinum based chemotherapy.

Summary of Educational Advantages

The discovery of the anticancer activity of cisplatin is an important tale for chemical educators because it illustrates how dogged pursuit of an explanation for an unexpected and initially baffling result led to the identification of a new class of anticancer drug that is now arguably the world's most widely used (for further details, visit <http://chemcases.com/cisplat/index.htm>, accessed Jan 2006). Cisplatin itself has played an important role in the history of coordination chemistry, with Werner using the fact that he could obtain isomeric forms (*cis* and *trans*) of [PtCl₂(NH₃)₂] as evidence that platinum adopts a square-planar geometry. Thus, it is an interesting example to use when introducing both geometry and geometric isomerism in the context of metal complexes. The much lower biological activity of the *trans* isomer, which is due in part to more rapid aquation, more rapid reactions with proteins and consequently increased deactivation, is an excellent illustration of the *trans* effect, as are the procedures used to produce both *cis* and *trans* analogues in high purity. The difference between the interactions of the two isomers with DNA is also a good illustration of the dramatic effect that differences in geometry can have. The more rapid aquation of cisplatin inside cells than outside, because of the difference in chloride concentration, can be used to illustrate the effect of concentration on reaction rates and equilibria. The ability of cells to remove platinum using sulfur-containing peptides and proteins can be used to illustrate the hard–soft acid–base principle. The lower toxicity of complexes with bidentate dicarboxylate groups (e.g., carboplatin) illustrates the effect of using chelate ligands. The lower toxicity of platinum(IV) analogues illustrates the higher stability of platinum complexes in this oxidation state. The lower rate of deactivation of the platinum complex, AMD473, by sulfur-containing peptides shows the effect of bulky groups lying above the coordination plane on the accessibility of platinum to incoming nucleophiles.

Acknowledgments

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Literature Cited

- Rosenberg, B. *Plat. Met. Rev.* **1971**, *15*, 42–51.
- Guo, Z.; Sadler, P. J. *Adv. Inorg. Chem.* **2000**, *49*, 183–306.
- Hambley, T. W. *Coord. Chem. Rev.* **1997**, *166*, 181–223.
- Wong, E.; Giandomenico, C. M. *Chem. Rev.* **1999**, *99*, 2451–2466.
- Siafaca, K. *Future Oncol.* **1999**, *5*, 1045–1071.
- Farrell, N. P. Virginia Commonwealth University, Richmond, Virginia. Personal communication, 2004.
- Weiss, R. B.; Christian, M. C. *Drugs* **1993**, *46*, 360–377.
- O'Dwyer, P. J.; Stevenson, J. P.; Johnson, S. W. In *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*; Lippert, B., Ed.; Verlag Helvetica Chimica Acta: Zurich; Wiley-VCH: Weinheim, Germany, 1999; pp 31–69.
- Rosenberg, B. In *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*; Lippert, B., Ed.; Verlag Helvetica Chimica Acta: Zurich; Wiley-VCH: Weinheim, Germany, 1999; pp 3–27.

10. Rosenberg, B.; Van Camp, L.; Krigas, T. *Nature* **1965**, *205*, 698–699.
11. Rosenberg, B.; Van Camp, L.; Grimley, E. B.; Thomson, A. J. *J. Biol. Chem.*, **1967**, *242*, 1347–1352.
12. Rosenberg, B.; Renshaw, E.; Van Camp, L.; Hartwick, J.; Drobnik, J. *J. Bacteriol.* **1967**, *93*, 716–721.
13. Rosenberg, B.; Van Camp, L.; Trosko, J. E.; Mansour, V. H. *Nature* **1969**, *222*, 385–387.
14. Rosenberg, B.; Van Camp, L. *Cancer Res.* **1970**, *30*, 1799–1802.
15. Peyrone, M. *Ann. Rev.* **1845**, *51*, 15.
16. Mellor, D. P. *Chem. Rev.* **1943**, *33*, 137–183.
17. Kauffman, G. B. *Plat. Met. Rev.* **1997**, *41*, 34–40.
18. Werner, A. Z. *Anorg. Chem.* **1893**, *3*, 267.
19. Dhara, S. C. *Indian J. Chem.* **1970**, *8*, 193–134.
20. Chernyaev, I. I. *Ann. Inst. Platine USSR*, **1926**, *4*, 261.
21. Basolo, F.; Pearson, R. G. In *Mechanisms of Inorganic Reactions: A Study of Metal Complexes in Solution*; John Wiley and Sons, Inc.: New York, 1967; pp 351–453.
22. Nicholls, D. *Complexes and First-Row Transition Elements*; The Macmillan Press Ltd: London 1974.
23. Reiset, J. *Compt. Rend.* **1844**, *18*, 1103.
24. Natile, G.; Coluccia, M. *Coord. Chem. Rev.* **2001**, *216–217*, 383–410.
25. Kauffman, G. B.; Cowan, D. O. *Inorg. Synth.* **1963**, *7*, 239–245.
26. Kurnakow, N. S. *J. Prakt. Chem.*, **1894**, *50*, 483.
27. Woollins, J. D.; Woollins, A.; Rosenberg, B. *Polyhedron* **1983**, *2*, 175–178.
28. Hall, M. D.; Dolman, R. C.; Hambley, T. W. *Met. Ions Biol. Syst.* **2004**, *41*, 297–322.
29. Vollano, J. F.; Al-Baker, S.; Dabrowiak, J. C.; Schurig, J. E. *J. Med. Chem.* **1987**, *30*, 716–719.
30. Kauffman, G. B. *Inorg. Synth.* **1963**, *7*, 236–238.
31. Ellis, L. T.; Er, H. M.; Hambley, T. W. *Aust. J. Chem.* **1995**, *48*, 793–806.
32. Galanski, M.; Keppler, B. K. *Inorg. Chem.* **1996**, *35*, 1709–1711.
33. Giandomenico, C. M.; Abrams, M. J.; Murrer, B. A.; Vollano, J. F.; Rheinheimer, M. I.; Weyer, S. B.; Bossard, G. E.; Higgins, J. D. I. *Inorg. Chem.* **1995**, *34*, 1015–1021.
34. Eastman, A. In *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*; Lippert, B., Ed.; Verlag Helvetica Chimica Acta: Zurich; Wiley-VCH: Weinheim, Germany, 1999; pp 111–134.
35. Ivanov, A. I.; Christodoulou, J.; Parkinson, J. A.; Barnham, K. J.; Tucker, A.; Woodrow, J.; Sadler, P. J. *J. Biol. Chem.* **1998**, *273*, 14721–14730.
36. Kratz, F. In *Metal Complexes in Cancer Chemotherapy*; Keppler, B. K., Ed.; VCH: Weinheim, Germany, 1993; pp 391–429.
37. DeConti, R. C.; Toftness, B. R.; Lange, R. C.; Creasey, W. A. *Cancer Res.* **1973**, *33*, 1310–1315.
38. Barnham, K. J.; Djuran, M. I.; Murdoch, P. d. S.; Ranford, J. D.; Sadler, P. J. *Inorg. Chem.* **1996**, *35*, 1065–1072.
39. Lempers, E. L. M.; Reedijk, J. *Adv. Inorg. Chem.* **1991**, *37*, 175–217.
40. Andrews, P. A.; Wung, W. E.; Howell, S. B. *Anal. Biochem.* **1984**, *143*, 46–56.
41. Dolman, R. C.; Deacon, G. B.; Hambley, T. W. *J. Inorg. Biochem.* **2002**, *88*, 260–267.
42. Borch, R. F.; Pleasants, M. E. *Proc. Natl. Acad. Sci. USA* **1979**, *76*, 6611–6614.
43. Gately, D. P.; Howell, S. B. *Br. J. Cancer* **1993**, *67*, 1171–1176.
44. Ishida, S.; Lee, J.; Thiele, D. J.; Herskowitz, I. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 14298–14302.
45. Davies, M. S.; Berners-Price, S. J.; Hambley, T. W. *Inorg. Chem.* **2000**, *39*, 5603–5613.
46. Martin, R. B. In *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*; Lippert, B., Ed.; Verlag Helvetica Chimica Acta: Zurich; Wiley-VCH: Weinheim, Germany, 1999; pp 183–206.
47. Arpalahiti, J. In *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*; Lippert, B., Ed.; Verlag Helvetica Chimica Acta: Zurich; Wiley-VCH: Weinheim, Germany, 1999; pp 207–222.
48. Legendre, F.; Chottard, J.-C. In *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*; Lippert, B., Ed.; Verlag Helvetica Chimica Acta: Zurich; Wiley-VCH: Weinheim, Germany, 1999; pp 223–246.
49. Jamieson, E. R.; Lippard, S. J. *Chem. Rev.* **1999**, *99*, 2467–2498.
50. Hambley, T. W. *Dalton Trans.* **2001**, 2711–2718.
51. Cohen, G. L.; Ledner, J. A.; Bauer, W. R.; Ushay, H. M.; Caravana, C.; Lippard, S. J. *J. Am. Chem. Soc.* **1980**, *102*, 2487–2488.
52. Kelland, L. R. *Drugs* **2000**, *59 Suppl.*, 1–8.
53. Fuertes, M. A.; Castilla, J.; Alonso, C.; Perez, J. M. *Curr. Med. Chem. Anti-Cancer Agents* **2002**, *2*, 539–551.
54. Cleare, M. J.; Hoeshcele, J. D. *Plat. Met. Rev.* **1973**, *17*, 2–13.
55. Cleare, M. J.; Hoeshcele, J. D. *Bioinorg. Chem.* **1973**, *2*, 187–210.
56. Tucker, M. A.; Colvin, C. B.; Martin, D. S. *Inorg. Chem.* **1964**, *3*, 1373–1383.
57. Colvin, C. B.; Gunther, R. G.; Hunter, L. D.; McLean, J. A.; Tucker, M. A.; Martin, D. S. *J. Inorg. Chim. Acta* **1968**, *2*, 487–489.
58. Berners-Price, S. J.; Kuchel, P. W. *J. Inorg. Biochem.* **1990**, *38*, 327–345.
59. Kelland, L. R. In *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*; Lippert, B., Ed.; Verlag Helvetica Chimica Acta: Zurich; Wiley-VCH: Weinheim, Germany, 1999; pp 497–521.
60. Chu, G. *J. Biol. Chem.* **1994**, *269*, 787–790.
61. Kartalou, M. *Mutat. Res.* **2001**, *478*, 23–43.
62. Pearson, R. G. *J. Am. Chem. Soc.* **1963**, *85*, 3533–3539.
63. Pearson, R. G. *J. Chem. Educ.* **1968**, *45*, 581–587.
64. Pearson, R. G. *J. Chem. Educ.* **1968**, *45*, 643–648.
65. Lebwohl, D.; Canetta, R. *Eur. J. Cancer* **1998**, *34*, 1522–1534.
66. Judson, I.; Kelland, L. R. *Drugs* **2000**, *59 Suppl.*, 29–36.
67. Hall, M. D.; Hambley, T. W. *Coord. Chem. Rev.* **2002**, *232*, 49–67.
68. Gordon, M.; Hollander, S. *J. Med.* **1993**, *24*, 209–265.
69. Giandomenico, C. M.; Abrams, M. J.; Murrer, B. A.; Vollano, J. F.; Harrap, K. R.; Goddard, P. M.; Kelland, L. R.; Morgan, S. E. In *Platinum and Other Metal Coordination Compounds in Cancer Chemotherapy*; Howell, S. B., Ed.; Plenum Press: New York, 1991; pp 93–100.
70. Sternberg, C. N.; Hetherington, J.; Paluchowska, P. H. T.; Slee, J.; Collette, L.; Debois, M.; Zurlo, A. *Proc. Am. Soc. Clin. Oncol.* **2003**, *22*, 395.
71. Farrell, N. P.; De Almeida, S. G.; Skov, K. A. *J. Am. Chem. Soc.* **1988**, *110*, 5018–5019.
72. Qu, Y.; Appleton, T. G.; Hoeshcele, J. D.; Farrell, N. P. *Inorg. Chem.* **1993**, *32*, 2591–2593.
73. Sharp, S. Y.; Kelland, L. R. *Curr. Opin. Oncol., Endocr. Metabol. Invest. Drugs* **2000**, *2*, 353–360.
74. Eastman, A. *Pharmacol. Ther.* **1987**, *34*, 155–166.