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Interactions of organometallic anticancer agents with nucleotides and DNA

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Contents

Abstract

Three classes of organometallic complexes, metallocene diacidos Cp_2MX_2(M = Ti, V, Nb, Mo, Re), ferricenium salts and organotin compounds have been reported to exhibit impressive in vivo or in vitro antitumour activities against a range of tumour cell lines. Titanocene dichloride is currently on the phase II clinical trials. Biological studies suggest that DNA is one of the primary intracellular targets of the metallocene dihalides but their mechanism of action is poorly understood. By using various modern techniques, the binding modes of

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Cp_2TiCl_2, Cp_2VCl_2, Cp_2NbCl_2, Cp_2MoCl_2, Cp_2ZrCl_2, $Cp_2Fe^+X^-$, $(CH_3)_2SnCl_2$, $(C_2H_5)_2SnCl_2$, $(C_2H_5)_2SnCl_2$(phen) with DNA and nucleotides in aqueous solution have been investigated. The results show that all the above organometallic agents exhibit high affinity to phosphate group of nucleotides. In aqueous solution, 5′GMP(or 5′dGMP), 5′AMP, 5′CMP, 5′TMP with Cp_2TiCl_2 or Cp_2MoCl_2 form chelate complexes in which both base nitrogen atom and phosphate oxygen atom of nucleotides bind to the metal center; whereas the other organometallics may bind to dGMP via only the phosphate group. The interactions between Cp_2TiCl_2, Cp_2ZrCl_2, $(CH_3)_2SnCl_2$ or $(C_2H_5)_2SnCl_2$ with calf thymus DNA suggest that Cp_2TiCl_2(aq) may bind to both the base ring sites and the phosphate backbone of DNA; while other organometallics(aq) bind to DNA via only the phosphate group. In addition, the relationship between DNA binding property of metal anticancer complex and their anticancer activity is discussed and a hypothesis named 'Two-Pole Complementary Principle' is also put forward and some criteria are suggested as well.

Keywords: Organometallic complexes; Antitumour; DNA; Nucleotides

1. Introduction

1.1. Organometallic complexes as antitumour agents

During the past 40 years, organometallic chemistry has developed to a large and important branch of chemistry linking the fields of organic and inorganic chemistry and, organometallic compounds have been found practical applications as catalysts for industrial syntheses, as antiknock additives for gasoline, and as biocides. In addition to this, in the late 1970s, the discovery of the antitumour activities of titanocene dichloride [1–3] and certain diorganotin derivatives [4–6] stimulated much interest in the research of organometallic compounds as antitumour agents. Titanocene dichloride have been proved to be a potent agent against breast, lung and intestinal (colon) cancer tissues. In contrast to the serious nephrotoxicity, myelotoxicity, peripheral neurathy of the well known inorganic antitumour agent, diaminedichloroplatinum(II) (cisplatin), titanocene dichloride only exhibits slight side effects with regard to the liver when used in therapeutically necessary amounts. In addition, it has been shown recently that titanocene dichloride significantly overcomes platinum resistance in vitro and exhibits synergistic cytotoxicity with 5-fluorouracil (5-FU) [7]. The people are supported by encouraging results from two phase I clinical trials, and titanocene dichloride is currently in phase II clinical trials. Some di-*n*-butyltin(IV) derivatives of salicylic acids were found to be more active than cisplatin in vitro [8,9]. Thus, the organometallic compounds constitute a potent new class of antitumour agents.

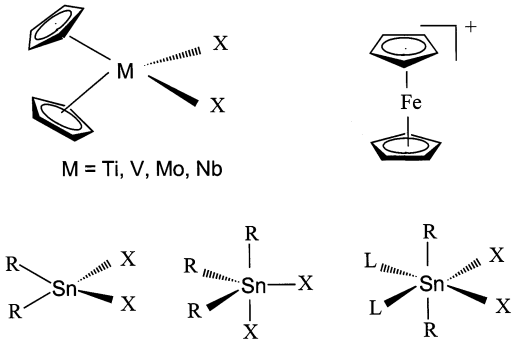
2. Antitumour activity of organometallic complexes

2.1. In vivo and in vitro activity

There are three basic classes of organometallic-based compounds that have been reported to exhibit antitumour activity, ferricenium salts, metallocene diacido compounds and diorganotin complexes. Scheme 1 shows the structures of the three kind of complexes.

The ferricenium salts are of the form $[Cp_2Fe]^+X^-$ where Cp=η^5-C_5H_5, X^-=$SbCl_6^-$, 2,4,6-$(NO_2)_3C_6H_2O^-$ [10], or $CCl_3CO_2\cdot CCl_3COOH^-$ [11]. Here the central iron(III) is coordinated to two cyclopentadienyl (Cp) ligands in the classic D_{5d}/D_{5h} 'sandwich' configuration. The coordinatively saturated ferricenium salts are water-soluble, hydrolytically stable, relatively strong oxidizing agents and are reported to inhibit the growth of Ehrlich ascites tumour, B16 melanoma, colon 38 carcinoma, Rauscher leukemia and other experimental solid tumour systems [12–15]. Some bi-nuclear ferricenium derivatives were also reported to inhibit the development of experimental tumours in mice [15]. The low toxicity and excellent water solubility also make them potentially valuable in clinical use [13].

The bis(cyclopentadienly) metal(IV) diacido complexes are 'bent sandwich' compounds. The central metal is in a distorted tetrahedral coordination geometry with two π-bonded Cp ligands and two other ligands bound in a *cis* configuration. In a molecular orbital description, the electronic structure of bent metallocene can be understood by a distorting D_{5h} molecular structure. The metallocene diacido complexes Cp_2MX_2 with M = titanium, vanadium, niobium, or molybdenum as central metal atom were first early transition metal compounds for which antitumour activity was detected. They had pronounced in vivo and vitro antineoplastic activity against various tumour cell lines [16–18]. At optimal dose, the metallocene diacido complexes show cytostatic activity against fluid and solid Ehrlich ascite tumour [19–22], fluid and solid sarcoma 180 [23,24], B16 melanoma [23,24], Lewis lung carcinoma [25], mouse mammary tumour TA3Ha [26] and colon 38 adenocarcinoma [24,27] and markedly inhibit the growth of xenografted human carcinomas



Scheme 1.

of the lung, breast, and gastrointestinal tract [28–31]. These results are noteworthy in view of previous clinical and experimental experience that human adenocarcinomas of the stomach and colon are generally rather insensitive to known cytostatic agents, and thus indicate possible clinical activity of metallocene complexes against human gastrointestinal carcinomas. In most experiments, titanocene dichloride Cp_2TiCl_2 was the most active metallocene complex, followed by vanadocene dichloride Cp_2VCl_2. Interestingly, however, titanocene dichloride was found to significantly overcome cisplatin resistance in ovarian carcinoma cell lines [7]. Moreover, titanocene complexes showed potent antiviral antiinflammatory activities which are comparable to phenylbutazone [17] while vanadocene complexes may be useful as contraceptive agents [32].

Some ionic derivatives of metallocene compounds, given by ionic titanocene, niobocene, molybdenocene, and rhenocene complexes, have greater water solubility and also exhibit pronounced antitumour activity against Ehrlich ascites and several other experimental solid tumour systems in vivo [33–36].

In contrast, the Zr and Hf metallocene dichlorides have negligible tumour-inhibiting properties in term of prolonging the survival of mice infected with Ehrlich ascite tumours cells [19]. Furthermore, free cyclopentadiene and dicyclopentadiene do not affect the same systemic antitumour activity as the metallocene complexes. None of the hydrocarbons significantly inhibits tumour growth in mice bearing solid EAT even at ten times the metallocene dichloride dosages [37].

Tin(IV) prefers a tetrahedral, trigonal bipyramidal or octahedral geometry. The diorgontin(IV) antitumour complexes are given by tetra-coordinated (R_2SnX_2), penta-coordinated (R_2SnY (Y, tridentate ligand))and hexa-coordinated ($R_2SnX_2 \cdot L_2$) diethyl-, di-*n*-butyl- and diphenyl-tin derivatives containing Sn–O, Sn–N or Sn–S bonds. These complexes showed high in vitro activity against P388 leukaemia in mice as well as some human tumour cell lines [4–6,8,38–40]. Numerous diorganotin(IV) derivatives have been found to exhibit high in vivo cytotoxicity against P388 lymphocytic leukaemia but to exhibit less or no activity against other murine systems [41]. However, the new in vitro human tumour cell screening tests have once again demonstrated the potential of organotin complexes, some of which have exhibited high activity [42] and thus interest in them has been revitalized. It has also been demonstrated that certain triphenyltin benzoates exhibit exceptionally high in vitro activity against MCF-7 and WiDr cell lines [43]. Some novel triphenyltin carboxylates also reached interesting, low, ID values compared to clinically established antitumour drugs [44].

2.2. Cellular and in vivo mechanistic information

Most biological experiments performed so far suggest that nucleic acids, especially nuclear DNA as the probable primary intracellular target for metallocene complexes.

Incorporation studies [45,46] with tritium-labelled, specific precursors of the DNA, RNA and protein syntheses revealed pronounced and persistent inhibitions of nucleic acid synthesis activities following in vivo and in vitro application of

titanocene dichloride or vanadocene dichloride. Especially DNA synthesis was suppressed in a significant and long-lasting manner. While RNA and protein synthesis is only slightly and reversibly inhibited. Electron energy loss-spectroscopic studies revealed the central metal atoms titanium and vanadium to be mainly accumulated in those cellular regions where are rich in nucleic acids after treatment with titanocene dichloride in vivo [47–49]. In addition, titanium-DNA adducts were detected in A2780 cells treated with titanocene dichloride using atomic absorption spectrometry [7], suggesting that DNA may be a target for this drug.

Cytokinetic investigations revealed the appearance of a late S/early G_2 block [7] or G_2 block and the immigration of inflammatory cells belonging to the host defensive system in vivo, cell arrests at G_1/S boundary and G_2 were induced [50,51]. However, apoptotic cell death occurred from any phase of the cell cycle [7].

3. Organometallic-nucleic acids interactions

3.1. Aqueous chemistry of organometallic compounds

The information on aqueous chemistry of organometallics is very important to understand their mechanism of action. In aqueous solution, most of the metallocene and organotin complexes are not stable, and undergo dissociation, aquation and hydrolysis reactions. Both alkyl ligands and chloride ligands can be dissociated from the metal center, depending on pH and concentration [52–62]. Chloride hydrolysis for Cp_2MCl_2 can be modeled as shown in Scheme 2. Table 1 shows the pK_a values for aqua species of some organometallics and cisplatin.

$$\begin{aligned} Cp_2MCl_2 + 2H_2O &\rightleftharpoons [Cp_2MCl(OH_2)]^+ + Cl^- + H_2O \\ &\rightleftharpoons [Cp_2M(OH_2)_2]^{2+} + 2Cl^- \\ &\overset{pK_{a1}}{\rightleftharpoons} [Cp_2M(OH)(OH_2)]^+ + H^+ + 2Cl^- \\ &\overset{pK_{a2}}{\rightleftharpoons} Cp_2M(OH)_2 + 2H^+ + 2Cl^- \end{aligned}$$

Scheme 2.

Table 1
pK_a data for $Cp_2M(H_2O)_2^{2+}$, $Me_2Sn(H_2O)_2^{2+}$ and *cis*-$Pt(NH_3)_2(H_2O)_2^{2+}$ complexes

Complex	pK_{a_1}	pK_{a_2}	Reference
$Cp_2Ti(H_2O)_2^{2+}$	3.5	4.4	[54]
$Cp_2Mo(H_2O)_2^{2+}$	5.5	8.5	[56]
$Cp_2V(H_2O)_2^{2+}$	4.7	5.2	[54]
$Me_2Sn(H_2O)_2^{2+}$	3.2	5.2	[61]
cis-$Pt(NH_3)_2(H_2O)_2^{2+}$	5.6	7.3	[54]

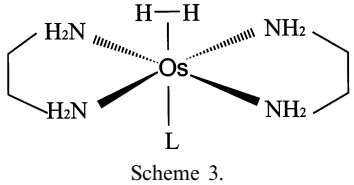
So the organometallic dichlorides are more stable in acidic or saline solutions than in water. For metallocenes, neutral oxo-bridged binuclear and by hydrolytic cleavage of cyclopentadienyl rings, oligonuclear species, e.g. $Cp_2ClTi–O–TiClCp_2$, $Cp_2(H_2O)Ti–O–Ti(H_2O)Cp_2$ and $[CpTiClO]_3$, are also formed in the course of hydrolysis reactions [55,63]. Detailed information of the aqueous chemistry of titanocene dichloride can be found from a review [63]. For diorganotin complexes, mono-, di- and poly-nuclear di-alkyltin(IV) species co-exist in the aqueous solution, depending on pH. Moreover, di-alkyl-dihydroxo-tin(IV) species prefer to be five- or six-coordinated by binding weakly to one or two water molecules [61]

In a detailed kinetic study [54,56] the order of decreasing hydrolytic stability of M–Cp bond of metallocene dichlorides in un-buffered aqueous solution was determined to be $Cp_2MoCl_2 > Cp_2VCl_2 > Cp_2TiCl_2 \gg Cp_2ZrCl_2 > Cp_2HfCl_2$. The antitumour-active compounds Cp_2MoCl_2, Cp_2VCl_2 and Cp_2TiCl_2 are expected to have stable M–Cp bonds over a period of days in unbuffered aqueous solutions, whereas Cp_2ZrCl_2 and Cp_2HfCl_2 undergo rapid ring protonolysis. At near physiological pH values, Cp_2TiCl_2 ring loss is also extensive with formation of a precipitate as '$Ti(C_5H_5)_{0.31}O_{0.30}(OH)$' [54]. However, some anions in blood plasma, such as phosphate and citric, can markedly retard the Cp ring dissociation from Ti(IV) at neutral pH [64].

In contrast to the Cp ring loss from metallocenes, the R_2–Sn bond seems rather stable in aqueous solution, but the triorganotin will release one alkyl group to give active R_2Sn species, the half-life of this process has been shown to be between 3 and 6 days [62]. The Cp_2M^{2+} and R_2Sn^{2+} have been suggested as the active species for the organometallic anticancer agents [41].

3.2. Aqueous organometallic-dGMP coordination investigated by using $[trans\text{-}en_2Os(\eta\text{-}H_2)]^{2+}$ as a ^1H-NMR probe

The mode of antitumour action of cisplatin appears to be fairly well established: the complex is believed to lose its chloride ligands and the metal subsequently coordinates with N7 atoms of two adjacent guanine bases on DNA, and this has been supported by the elucidation of the X-ray crystal structure of the covalent adduct of cisplatin with a 12-mer DNA duplex [65,66]. The hypothesis of DNA as the primary target for metallocenes along with the obvious structural similar *cis*-MX_2 moiety among cisplatin, metallocenes and diorganotin complexes requires an investigation of aqueous organometallic-nucleic acid coordination chemistry under near physiological conditions.



Scheme 3.

The osmium dihydrogen complex, [*trans*-$en_2Os(\eta\text{-}H_2)]^{2+}$ (Scheme 3) has been proven to be a versatile ^{1}H-NMR recognition probe for biomolecules in aqueous solution [67]. It binds readily to a variety of biomolecules such as nucleotides with ligand, L, substituted by these biomolecules, that results in the direct coordination of Os^{II} to the donor atoms(such as N, O, S) of the biomolecules. In each case the binding leads to characteristic ^{1}H-NMR properities (δ, T_1, J_{H-D}) for the dihydrogen unit that appears in a spectral window in the range $\delta = 0$ to -20 ppm, coordination structural difference in the binding molecules therefore can be distinguished [67].

Fig. 1 shows the reaction process of dGMP and the probe. The phosphate oxygen and N7 of the base ring both coordinate to the probe, the former being dynamically preferred ($K = 3 \times 10^2$) and the binding is complete after 20 min, while the latter is thermodynamically preferred ($K = 2.9 \times 10^3$) and binding is complete after 24 h. The peaks at $\delta = -13.16$ and -13.56 grow in 20 min, corresponding to D_2O binding and phosphate oxygen binding, respectively. Meanwhile, a peak at $\delta = -9.73$, already discernible after 20 min (Fig. 1(a)), continues to grow at the expense of the others. This peak is assigned to the N7 binding. There is a competitive reaction among D_2O binding, phosphate binding and N7 binding, which is not completed at equilibrium in 20 min. The affinity of Os^{II} for the D_2O and phosphate is not high and the conversion from D_2O binding and phosphate binding to N7 binding is almost complete after 24 h (Fig. 1(b)). Usually the binding of an antitumor metal agent to dGMP is via N7 or the phosphate group of dGMP, therefore, if an antitumor metal agent is added to the probe-dGMP binary system, it will compete for the binding sites of dGMP with the probe, and the characteristic peaks due to binding of the probe with phosphate or N_7 of dGMP will change. According to these changes, the binding sites of the antitumour metal complexes to dGMP can be determined.

When Cp_2TiCl_2, dGMP and the probe were mixed at 0.5:1:1 or 1:1:1 molar ratio, respectively, both of the peaks due to the phosphate binding and N7 binding diminished evidently in intensity after 20 min (Fig. 2(a, c)), in contrast to those of dGMP-probe binary system. Especially in the 1:1:1 system (Fig. 2(a)), the signal of N_7 binding diminished severely and the signal of phosphate binding disappeared completely. Meanwhile, a new peak at $\delta = -13.24$ ppm appeared after 20 min, which is assigned to the chloro binding with the probe, and this indicate Cl^- is dissociated from Cp_2TiCl_2. After 24 h, the intensity of the NMR signals of both N7 binding and phosphate binding in the ternary systems remained markedly weaker

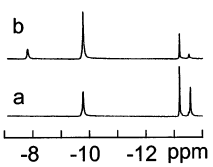


Fig. 1. Proton NMR spectra of the probe with dGMP in D_2O (pD = 7.20, both 0.010 mol l^{-1}) (a) after 20 min; (b) after 24 h.

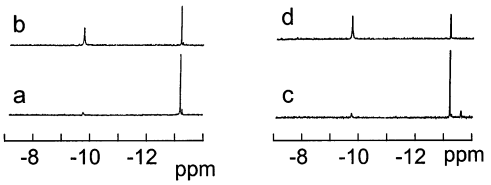


Fig. 2. Proton NMR spectra of the probe with dGMP and Cp_2TiCl_2 in D_2O. Left, drug:dGMP:probe = 1:1:1, dGMP, 0.001 mol l^{-1} (pD = 6.44) (a) after 20 min; (b) after 24 h; Right, drug:dGMP:probe = 0.5:1:1, dGMP, 0.001 mol l^{-1} (pD = 6.94); (c) after 20 min; (d) after 24 h.

than those of dGMP-probe binary system and the *cis* isomer of N7 binding didn't appear in the ternary systems. The above phenomenon shows that Cp_2TiCl_2 retards evidently both of the bindings between N7 and phosphate of dGMP with the probe, indicating that both sites (N7 and phosphate) have been partially occupied by Ti(IV) in the ternary systems, suggesting Ti(IV) being bound to both N_7 and phosphate of dGMP.

When Cp_2ZrCl_2 was in place of Cp_2TiCl_2 in the ternary systems, as shown in Fig. 3, only the signal of phosphate binding was affected and diminished in intensity. The peaks corresponding to the N7 binding remained generally undisturbed by Cp_2ZrCl_2 compared with those of dGMP-probe binary system. In addition, the peak due to chloride-binding also appeared. For the same reason, we conclude that the Cl^- in Cp_2ZrCl_2 was dissociated, and that Zr(IV) was coordinated to dGMP via only phosphate oxygen.

By means of the same method mentioned above, the dGMP binding sites of a wide range of antitumour active and inactive organometallic complexes, Cp_2MoCl_2 Cp_2NbCl_2 Cp_2VCl_2, Me_2SnCl_2, Et_2SnCl_2, Et_2SnCl_2(phen) have been determined, Cp_2MoCl_2 and Cp_2TiCl_2 form N7, phosphate chelate with dGMP while Cp_2VCl_2, Cp_2NbCl_2, Cp_2ZrCl_2, Me_2SnCl_2, Et_2SnCl_2 and Et_2SnCl_2(phen) only bind to the phosphate group of dGMP.

In all the above experiments, the peak corresponding to Cl^- binding to the probe appears after drug-dGMP interaction. This indicates that all the above organometallic complexes dissociate in water with the *cis*-chloro atoms as leaving group. It is worthwhile to mention that if the Et_2SnCl_2 solution is kept at pH 6.0 for a long time (three months), it cannot bind to dGMP at the same condition as fresh prepared solution. This suggests that the R_2Sn^{2+} is the active species while polynuclear tin species are not.

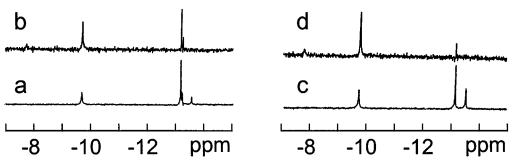
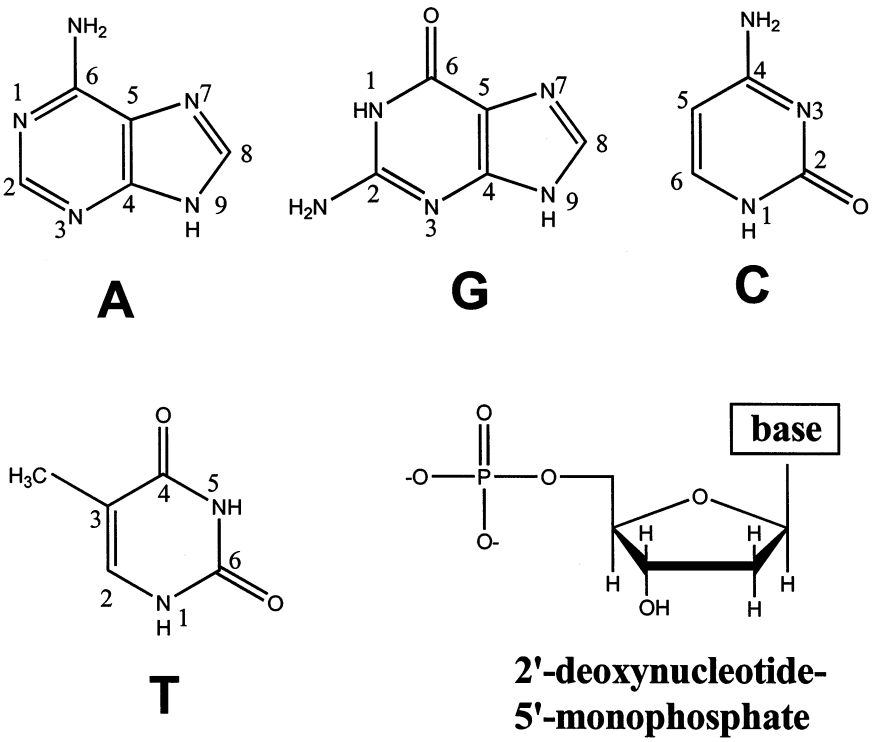


Fig. 3. Proton NMR spectra of the probe with dGMP and Cp_2ZrCl_2 in D_2O. Left, drug:dGMP:probe = 1:1:1, dGMP, 0.001 mol l^{-1} (pD = 6.74); (a) after 20 min; (b) after 24h; Left, drug:dGMP:probe = 0.5:1:1, dGMP, 0.001 mol l^{-1} (pD = 6.82); (c) after 20 min; (d) after 24 h.



Scheme 4.

In contrast to a recent NMR report [68] which failed to find any coordination between cytostatic $Cp_2NbCl_2(aq)$ with necleotides or amino acids in aqueous solution, the probe experiments confirm strong Cp_2Nb-phosphate(dGMP) binding in aqueous solution.

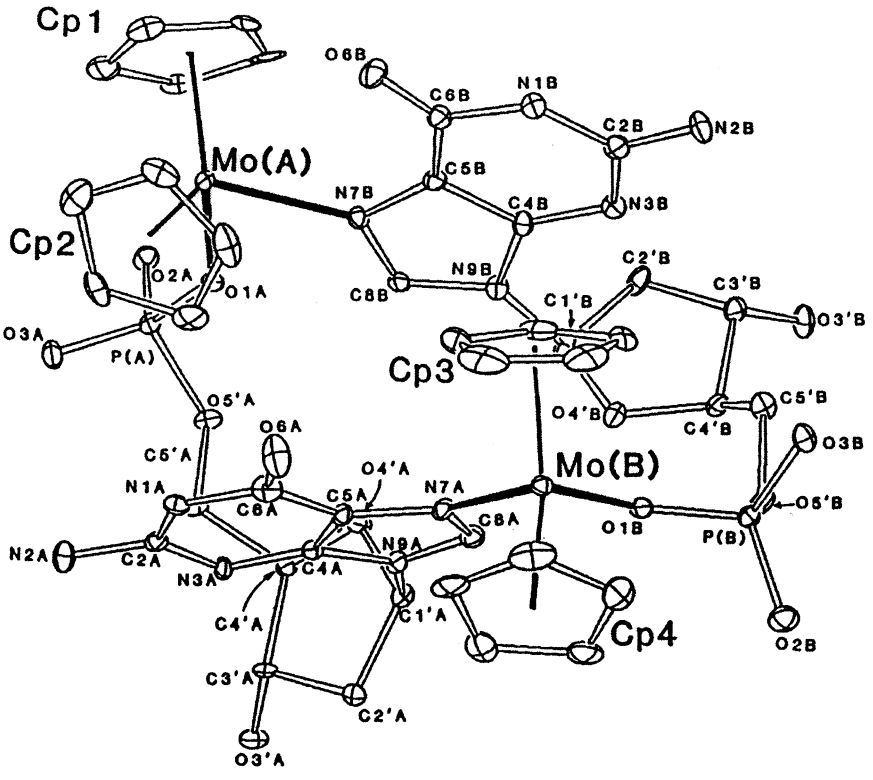
3.3. Aqueous and solid state organometallic-nucleotides coordination

Following the detection of antitumour properties of metallocene complexes, several groups have tried to synthesize model compounds of this species with nucleic acid components (see Scheme 4) to elucidate the molecular mode of action possibly involving direct coordination to the metal-containing moiety to DNA or RNA donor sites [56,69–74]. Unfortunately, the synthesis of model complexes of titanocene system with nucleic acid components as ligands has turned out to be difficult under physiological conditions, i.e. in aqueous solutions, because of hydrolytic side-reactions. Since 1984, a series of model compounds have been succeeded prepared in organic media using as starting materials not only titanocene dichloride but also the low-valent titanium(III) and titanium(II) species $[Cp_2TiCl]_2$ or $CpTi(CO)_2$. Monofunctional binding of a chlorobis(cyclopentadienyl)titanium(IV) unit to the N9 atom of the purinato ligand, as well as bifunc-

tional chelation of bis(cyclopentadienyl)titanium(III) units to the N7, O6 atoms of the theophyllinato ligand were observed [69–72]. However, the nonaqueous method of preparing these complexes and their hydrolytic instability leave in doubt whether they are accurate representations of titanocene nucleobase/nucleotide/DNA interactions under physiological conditions [57,73].

Quite another kind of binding was pointed out by Marks and coworkers [57,73] who investigated the interaction of Cp_2MoCl_2 and paramagnetic Cp_2VCl_2 with mononucleotides in aqueous solution near physiological pH under argon atmosphere by NMR and ESR methods as well as X-ray crystallographic techniques. They observed selective, labile outer-sphere complexation of vanadocene moiety to the nucleotide phosphate groups, and found that nucleotide-nucleotide Watson–Crick base-pairing was not disturbed by Cp_2VCl_2.

In contrast to the labile interaction between Cp_2VCl_2(aq) and nucleotides, the 'softer' Mo(IV) center of Cp_2MoCl_2 forms relatively stable bonds with donor sites of nucleotides and nucleobases [56]. ^{1}H- and ^{31}P-NMR studies of Cp_2MoCl_2-nucleotide interactions at physiological pH (pH 7.4) show that Cp_2Mo^{2+} coordinates in a covalent fashion to the base nitrogen sites and phosphate oxygen sites of mono-nucleotides. This kind of binding was further confirmed by the X-ray crystal



Scheme 5. Crystal structure of $[Cp_2Mo(dGMP)]_2$ (from Kuo et al. [56]).

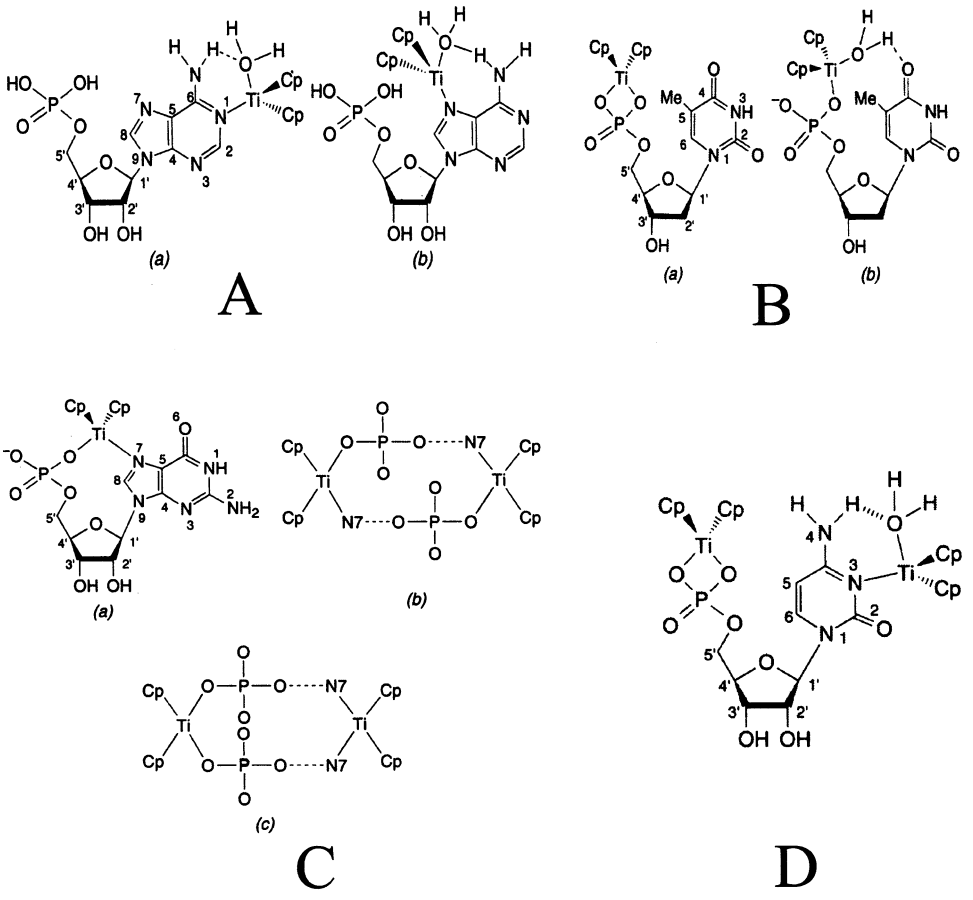
structure of Cp_2Mo-dGMP dimmer [56] (see Scheme 5) which was isolated from aqueous media at pH 7.4 under argon atmosphere. In the crystal state, Cp_2Mo^{2+} moiety is simultaneous covalent bound to N7 of the guanine base and phosphate oxygen of different nucleotides. ^{1}H-NMR studies show that Cp_2MoCl_2(aq) exhibits little or no coordinative nucleotide selectivity and cause, as in the case of Cp_2VCl_2(aq), little disruption in the Watson–Crick base pairing. Furthermore, the covalent bonding in the Cp_2Mo-nucleotide complex is weak as it can be disrupted by the presence of other free nucleotides.

These results seem to be the most detailed information so far on metallocene-nucleotide interation in aqueous solution near physiological pH under argon atmosphere, however, in the presence of oxygen, the reactivity of metallocene may be different from that under argon atmosphere [75]. A recent NMR study [76] showed that stable molybdocene-oligonucleotide (d(ATGGTA) or dCpG) complex or complexes were formed in 50 mM salt solution at low pD (ca. 3.0), but stable molybdocene-oligonucleotide adducts were not formed at pD 7.0.

There is relatively less known about the aqueous coordination between nucleotides and Cp_2TiCl_2, the most promising organometallic antitumour agent. Preliminary NMR studies [68,77] with Cp_2TiCl_2 and Cp_2NbCl_2 suggest that complexation of Cp_2TiCl_2 to nucleotide is weak in water and the binding sites might be similar to those of with Cp_2MoCl_2 but the binding sites were not determined; while no interaction between Cp_2NbCl_2 and nucleotides or amino acids was observed. Moreover, at pH 6, little or no complexation of Cp_2TiCl_2(aq) to nucleotides or nucleobases is observed [68,125]. However, at pH 5, the interaction between the dimethylsubstituted deritives $(MeCp)_2TiX_2$ ($X = Cl$, $O_2CCH_2NH_3Cl$) with purine nucleotides formed complexes which were stable for 24 h [125]. This suggest that formation of stable chelates between $(MeCp)_2TiX_2$ and nucleic acid constituents in vivo is possible [125].

Solid state FT-IR and high-resolution ^{1}H and ^{31}P-NMR studies in wet DMSO [78–80] suggest Cp_2Ti^{2+} moiety binds to N1 and N7 of AMP; N3 and phosphate of CMP; N7 and phosphate of GMP; but only to phosphate of TMP, Scheme 6, shows the possible coordination structures. Moreover, the A–T Watson–Crick base pairing was disturbed by Cp_2TiCl_2 in DMSO [79], this is quite different from Cp_2VCl_2 and Cp_2MoCl_2 which have no effect on the A–T or G–C Waston–Crick base pairing [56,73].

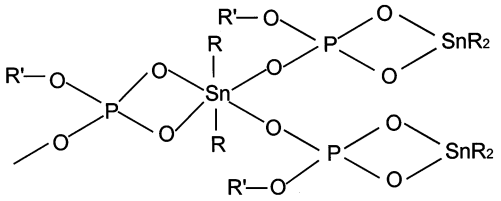
In contrast to Cp_2TiCl_2 and Cp_2MoCl_2, the interaction between diorganotin(IV) compounds R_2SnCl_2 and R_2SnCl_2(phen) ($R = Et$, *n*-Bu, Ph) with nucleotide monophosphates in neutral aqueous solution give polymeric species in which Sn(IV) is directly coordinated to the phosphate group of nucleotides [81,96–98]. No evidence (^{1}H-, ^{13}C-NMR, FT-IR) was found for coordination through donor atoms of base rings or sugar rings in either solution or solid state [81,94–99]. The possible coordination mode for organotin-nucleotide polymers is given in Scheme 7.



Scheme 6. Schematic illustration of the Cp_2Ti-nucleotide complexes: (A) with AMP; (B) with CMP; (C) with GMP; (D) with TMP.

4. Organometallic-DNA interactions

The aqueous interactions between organometallic agents and native DNA have also been investigated. In a study using inductively coupled plasma (ICP) spectroscopy, DNA binding to metallocene dichlorides was examined [82]. It was shown that Cp_2Ti-DNA adducts were formed at pH 5.3, whereas, at pH 7.0, CpTi-DNA adducts were formed but the binding sites on DNA were not determined. Similar finding was observed with niobocene dichloride, zicronocene dichloride, although the latter has no antitumour activity; whereas vanadocene dichloride, exhibiting similar antiproliferative properties as titanocene dichloride, failed to bind DNA covalently. A ^{31}P-NMR study [83] also implies binding between calf thymus DNA with Cp_2MoCl_2 in aqueous solution.



Scheme 7. Proposed structure for the diorganotin–nucleotide complexes, R = Et, n-Bu; R′ = AMP, CMP, and GMP.

The aqueous interactions of solvated di- and tri-organotin(IV) species $R_2Sn(IV)$ and $R_3Sn(IV)$ (R = Me, Et, n-Bu, n-Oct, Ph in ethanol solution) with native DNA (calf thymus) have been investigated by ^{119}Sn Mössbauer spectroscopy at pH between 5 and 7.4 [84–87]. The addition of ethanolic organotins [$R_2SnCl_2(EtOH)_2$ or $R_3SnCl(EtOH)$] to DNA yield solid products, the ^{119}Sn Mössbauer spectra suggest that the tin is coordinated by phosphodiester groups of the nucleotides. While the water-soluble hydrolyzed dimethyltin(IV) species do not show any interaction with native DNA [84,87].

By means of UV, CD, fluorescence spectroscopy, cyclic voltammetry and electrophoresis, the interactions between the three type of organometallic agents, Cp_2TiCl_2, Cp_2ZrCl_2, $[Cp_2Fe]^+$, Et_2SnCl_2 and Et_2SnCl_2(phen), with calf thymus DNA and salmon DNA as well as plasmid pBR322 DNA, were investigated in detailed manner [81,88–100]. The results show that there are two types of binding sites between DNA and Cp_2TiCl_2, one is the phosphate group of DNA and the other is the base nitrogen rings of DNA; while only phosphate group on DNA can bind to Cp_2ZrCl_2, Et_2SnCl_2, Et_2SnCl_2(phen); however, $[Cp_2Fe]^+$ can bind to the sugar-phosphate backbone and cleave the DNA chain.

The UV studies reveal that Cp_2TiCl_2 causes 'hypochromic effect 'of DNA at low R_t ($= C_{TDC}/C_{DNA(P)}$) but 'hyperchromic effect' at high R_t (see Fig. 4(A)) Hypochromism results from the contraction of DNA in the helix axis, as well as

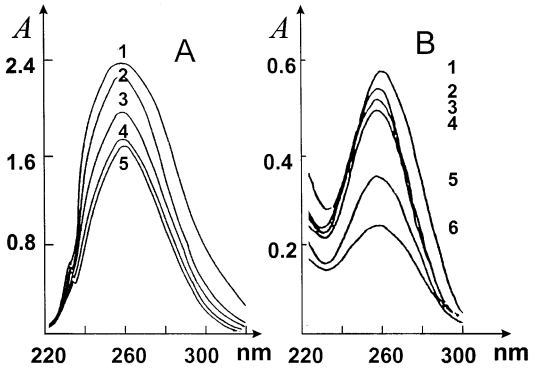


Fig. 4. The effects of Cp_2TiCl_2 and Et_2SnCl_2 on the UV spectra of calf thymus DNA. (A) Cp_2TiCl_2, 1–5, R_t: 1.66, 0.83, 0.42, 0, 0.21; (B) Et_2SnCl_2, 1–6, R_t: 0, 0.2, 0.5, 1.0, 2.0, 4.0, respectively.

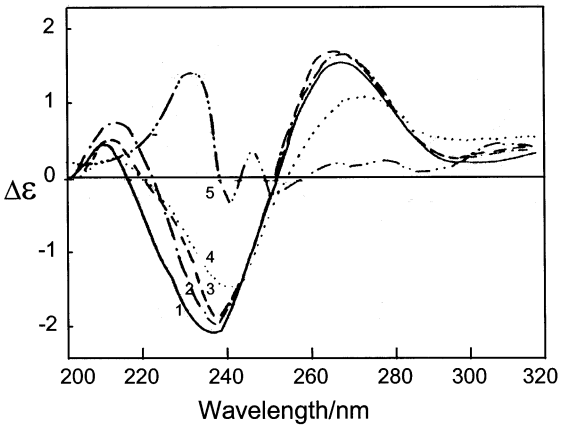


Fig. 5. The effects of Cp_2TiCl_2 on the CD spectra of calf thymus DNA, 1–5, R_t: 0, 0.25, 0.50, 1.0, 2.0.

from the change in conformation on DNA, while hyperchromism results from the damage of DNA double-helix structure. This indicates that Cp_2TiCl_2 binds to phosphate group of DNA backbone at low R_t and to the base nitrogen rings at high R_ts. Et_2SnCl_2 can only cause DNA hypochromism but not hyperchromism(see Fig. 4(B)), indicating binding of Et_2SnCl_2 with DNA only through its phosphate group, the same conclusion as the ^{119}Sn Mössbauer studies [87]. In addition, the phosphate anions inhibition on the Et_2Sn-DNA interaction also supports Et_2Sn-phosphate(DNA) binding [81,96].

CD spectroscopic studies using calf thymus DNA show that Cp_2TiCl_2 causes small decrease in intensity of negative CD band of DNA as well as a little increase and blue shift of the positive CD band at low R_t (<1) (see Fig. 5), suggesting the conformational transition B-DNA to A-DNA. At high R_t (≥ 1), both the negative and positive CD bands of DNA lose intensity markedly and show red shift evidently, suggesting a decrease in helix and denaturation of DNA, which is consistent with the binding of Cp_2TiCl_2 with DNA base rings.

Ethidium bromide(EB) has long been used as a probe of DNA secondary structure as it can be intercalated into the double-stranded DNA and enhance the sensitivity of fluorescence [100–102]. If the duplex of DNA decreases, the fluorescence of DNA-EB complex is quenched evidently. The fluorescence emission spectra of Cp_2TiCl_2 or Cp_2ZrCl_2 interacts with calf thymus DNA-EB with various R_t are shown in Fig. 6. Cp_2TiCl_2 (Fig. 6(A)) causes weak fluorescence quenching at $R_t = 0.25$, this may be due to the binding of $[Cp_2Ti(H_2O)_2]^{2+}$ to the phosphate group of DNA causes a contraction of DNA which drives a few EB molecules out of DNA duplex. Fluorescence quenching becomes gradually intensive when R_t rise and at $R_t > 1$, EB was completely set free from DNA duplex, and there are significant red shifts of the fluorescence emission peaks with the increase of R_t. The red shifts are due to the EB transferring from hydrophobic environment to hydrophilic environment, which indicates that EB is free from DNA after the drug

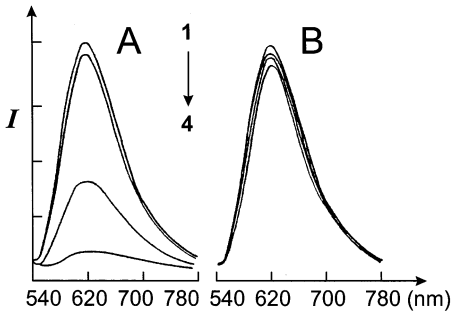


Fig. 6. Fluorescence spectra of calf thymus DNA-EB in the presence of varying concentration of Cp_2TiCl_2 or Cp_2ZrCl_2. (A) Cp_2TiCl_2; (B) Cp_2ZrCl_2; 1–4 R_t: 0, 0.5, 1, 2, respectively.

reacting with DNA. These evidences indicate that the double-helix structure of calf thymus DNA is damaged by Cp_2TiCl_2, which implies that the drug binds to the bases of DNA. While in the presence of Cp_2ZrCl_2 (Fig. 6B), the fluorescence quenching of DNA-EB complex was very weak at all R_t values. This indicate that Cp_2ZrCl_2 cannot damage the secondary structure of DNA at the same condition, suggesting it only bind to the phosphate group of DNA.

A further fluorescent analysis [99] using Scatchard methods (Fig. 7) reveals Cp_2TiCl_2 inhibit EB-DNA binding in a uncompetitive manner at low R_ts, further confirms binding of the drug to the phosphate group of DNA. While both competitive and uncompetitive modes exist at high R_t. This indicates that Cp_2TiCl_2 has two types of binding sites on DNA, i.e. besides binding to the phosphate, it may bind to base nitrogen rings of DNA.

CV method is one of the powerful methods that elucidate the interaction modes between drugs and DNA [103]. Native DNA is not reducible at the mercury electrode because the stability of the intact double helix makes the reducible bases inaccessible to the electrode. In Hepes buffer, pH 7.0, the addition of DNA cause the peak potentials of Et_2SnCl_2, E_{pc} and E_{pa} both to be shifted to more positive

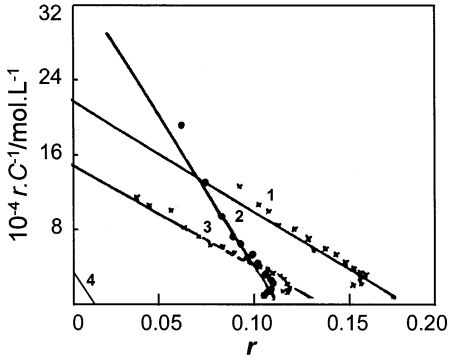


Fig. 7. Scatchard plots. 1–4, R_t: 0, 0.25, 0.5, 1.0, respectively.

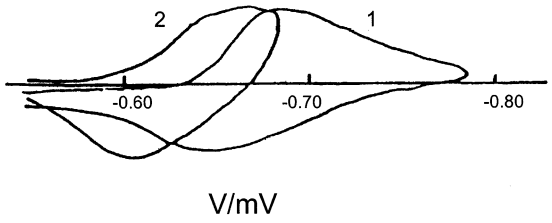


Fig. 8. CV spectra of Et_2SnCl_2(4.5 mM) in the absence (1) and in the presence of 1 equiv. of DNA(2), supporting electrolyte, 50 mM KNO_3, 5 mM Hepes, pH 7.0, Sweep rate, 200 mV s^{-1}.

values, while the peak currents keep the same, as shown in Fig. 8. The obvious positive shift of peak potentials indicates the binding between drug and the phosphate of DNA [103,104].

5. DNA binding mode-activity relationship

The antitumour properties of the organometallics have been well established, while the mechanism of action of this class of compounds is still poorly understood. Among these compounds, titanocene dichloride is known to have the highest in vivo anticancer activity and lowest toxicity; Cp_2VCl_2, the organotin dihalides are highly active in vitro but less active in vivo [41], while Cp_2ZrCl_2 is inactive even in vitro. The DNA binding properties of organometallic complexes seem to play a vital role in their anticancer activity. Cp_2TiCl_2 and Cp_2MoCl_2 can bind to dGMP(or DNA) via both N_7 and phosphate and this binding may disturb the DNA base pairing and damage the secondary structure of DNA while Cp_2VCl_2, Cp_2ZrCl_2, $(C_2H_5)_2SnCl_2$, $(C_2H_5)_2SnCl_2$(phen) bind to dGMP and DNA only via phosphate group and do not affect DNA base pairing and cannot damage the secondary structure of DNA. Other metal ions, such as Cu^{2+} have also been reported to bind to both N7 and phosphate of DNA, this kind of binding reduces the H-bonds between base-pairs in DNA and weaken the secondary structure of DNA [105], it is not surprise that certain Cu(II) complexes also have been reported to be antitumour active agents [106]. So binding to DNA via both N7 and phosphate might be very important for metal anticancer complexes. This is also supported by the case of antitumour ruthenium complexes, *cis*- and *trans*-$Ru(DMSO)_4Cl_2$, have also been revealed binding to both phosphate group and base N7 of dGMP [107,108]. Even for cisplatin, has also been found to coordinated to GMP (or dCMP) and DNA *via* both N7 (or N3) and phosphate [109–114]. Therefore, N7-metal binding may be a key factor to the anticancer property of metal complexes. The role of phosphate binding under physiological conditions seems to be only secondary for coordination, nevertheless, it is quite important for hydrogen binding, and the binding between metal ion and phosphate might be the first step of metal agents-DNA (or nucleotides) interactions, just as in the case of Cp_2TiCl_2(aq)-DNA interaction. This is also supported by Kozelka and Barre's

recent work [115] which shows that Pt(II) coordinates to the nonbridging phosphate oxygen atom of d(TpT) and forms macrochelates (N7 of G and the nonbridging phosphate oxygen) with d(TpG) in *N*,*N*-dimethylformamide(DMF). Therefore the binding modes of a metal complex with DNA play a key role in their anticancer activity. It seems all highly active anticancer metal drugs have the ability to bind with both phosphate groups and nitrogen sites on bases of DNA.

DNA is believed to be the main cellular target for most of the metal anticancer agents. The anticancer nature is the coordination of metal ions with DNA molecules, causing DNA damage in cancer cells, blocking the division of the cancer cells and resulting in cell death. When drug molecules (pre-anticancer molecules) enter into an organism, they will first undergo a series of processes including hydrolysis, transport and membrane-crossing, and then reach the nearby of target DNA molecule and form active intermediates $[cis\text{-}R_nM(H_2O)_2]^{m+}$ which will interact with DNA molecules directly.

Metal anticancer complexes are often electrophilic and may react with many cellular components, such as simple ions and molecules like Cl^-, $(HPO_4)^{2-}$, OH^- and H_2O; amino acids, peptides, proteins and polyphosphates like His, Met, Cys, glutathione, metallothionein, transferrin and ATP. Viewing from the coordination chemistry, metal complexes (including those of Pt) can bind to several types of possible biomolecules in the cell. But only the binding on DNA which lead to cell death is considered the most important. In the case of platinum complexes, it is quite clear that in the cells, after the relatively slow hydrolysis, *cis*-Pt have a preference for DNA over proteins and other molecules. Sadler and co-workers have shown that L-methionine increases the rate of reaction of 5′-GMP with cisplatin and that *S*-bound L-HMet in the adduct $[Pt(dien)(L\text{-}HMet\text{-}S)]^{2+}$ (dien = 1,5-diamino-3-aazapentane) can be replaced by N7 of 5′-GMP [116–118]. These work, together with the results of van Boom and Reedijk [119], who reported the intramolecular displacement of a Pt-bound thioether by a guanine nucleobase, suggest that novel routes to DNA platination from anticancer drugs may exit. Therefore it is conceivable that a methionine-containing protein or peptide could transport and transfer some platinum to DNA [118]. A very recent paper by Sadler and co-workers has suggest that transferrin, a human-blood plasma protein, could serve as a delivery vehicle for titanocene antitumour drugs [120].

DNA molecule is a two-pole molecule. Its surface is a negatively-charged backbone of phosphatepentose chains, inside of double helix are hydrophobic bases stacking layer by layer. For exerting its potency, the drug molecules may bind with the phosphate groups of DNA at first, and then, with the help of DNA's conformational dynamic changes (say, partial unwinding of the double helix), the lipophilic groups of the drug molecule may be drawn by DNA's hydrophobic sections, the nitrogen sites on DNA molecules may be exposed, the metal atom could invade into the internal part of DNA and coordinate with the bases. Oxygen site of the phosphate group has a higher negative charge relatively, it is a good donor with high electrostatics; so its action with the metal atom is caused mainly by static electricity, forming electrovalency, belonging to charge-controlling reaction. Nitrogen atoms on the bases are donors with lower electronegativity, they react

with the metal atom to form covalent bonds, belonging to orbital-controlling reactions. For some 'soft' metals such as Pt(II), binding to the heterocyclic nitrogen atoms of DNA is always detected in aqueous solution. However, decreasing solvent polarity considerably enhances the affinity of metal ions for negatively charged moieties such as phosphodiester groups [121]. It is therefore conceivable that in the densely packed cell nucleus, where the dielectric screening is expected to deviate from that in bulk water, interactions with the phosphodiester groups could play a significant role in the formation and/or processing of metal-DNA adducts. Actually, as mentioned above, Pt(II)-phosphodiester binding and Pt(II)-N7(G), O(phosphodiester) chelate binding have been detected from the interactions between $[(NH_3)_3Pt(NO_3)]NO_3$ or *cis*-$(NH_3)_2Pt(NO_3)_2$ with $d(TpT)^-$ and $d(TpG)^-$ in DMF [115]. It has also been suggested that guanine-phosphoryl macrochelate complexes play a role as intermediates in rearrangements of platinum monoadducts formed with DNA [115].

It appears that these two interactions happen one after another and coexist. This interaction mode is in conformity with the principle of multiple binding by flexible fitting and maximum binding, such a principle is generally accepted when the biochemical reactions are considered.

The antitumour activity of metallo drugs such as cisplatin is believed to be a consequence of the inhibition of DNA replication through the formation of adducts having covalent intrastrand cross-link with DNA. It has been found that the covalent binding of chiral complexes to DNA occurs enantioselectively [122–124]. The left hand isomers (Λ) of similar complexes, such as *cis*-$Ru(phen)_2Cl_2$, $Ru(phen)_2(py)OH_2^{2+}$ and $[cis\text{-}Rh(phen)_2Cl_2]^+$, have been found to preferentially bind to right hand B-DNA helix covalently. This suggests a potential chemotherapeutic application of chiral metal complexes.

By a detailed analysis of the hydrolysis mechanism of various inorganic and organometallic antitumour agents and their structure-activity relationship, the following three points seem to be vital for highly anticancer active complexes: (1) appropriate hydrolysis rates; (2) active intermediate $[cis\text{-}R_nM(H_2O)_2]^{m+}$ can be formed after hydrolysis, (3) coordination with both the phosphate and nitrogen of bases of DNA can be achieved.

6. Two-pole complementary principle (TPCP)

The similarities in chemical features of high active metal complexes infer that there should be some structural similarities in their molecular level. Some common molecular structural features and acting laws for metal anticancer agents have been generalized into a rule named two-pole complementary principle (TPCP) [99], which includes three aspects.

1. Two-pole complement in molecular structures. The molecular structure of anticancer metallic drugs normally show two polar parts, namely, hydrophilicity and hydrophobicity or positive and negative in charge. Correspondingly, they will present easily-leaving groups and stable keeping groups in aqueous solution.

Such two-pole structures enable the drug molecules not only to be dissolved in water and transported to the surface of the cell membranes, but also to cross the membranes by passing through the lipid bilayers and arrive at the nearby of the target molecules.

2. Two-pole complement in the receptor-substrate action mode. The interaction between the drug molecule and its target molecule is normally executed by forming an active intermediate which binds with phosphate oxygen sites (electrovalently) and purine or pyrimidine nitrogen sites (covalently) of DNA backbone through charge-controlling and orbital-controlling. This is a two-pole complement of electrovalent and covalent action mode.
3. Two-pole complement in the symmetry of the receptor-substrate system. The interaction characteristics of chiral drug molecules with B-DNA behave as using the left hand enantiomers of the drug molecule covalently binding with the right hand DNA, forming a two-pole chiral complementary complex.

From the above principles, some criteria which can be used to assess activity of metal anticancer agents are also suggested [99].

1. The dipole moment of the active intermediate species of an anticancer complex cannot be zero.
2. The drug molecule should possess both hydrophilic and hyrdophobic groups simultaneously. In an homologue of drugs, proper oil/water partition coefficients are needed and the hydrophobic groups should not be too bulky.
3. In the physiological medium the drug molecule should have appropriate hydrolysis rate to give active intermediates combining at least two water molecules in *cis*-configuration.
4. The metal ion should have appropriate Pearson 'hard-soft' degree (i.e. proper valence and radius) as well as abundant valence shell orbitals so that it may have an affinity not only for DNA's oxygen sites to form electrovalent bonds but also for nitrogen sites on purine, pyrimidine to form covalent bonds.
5. The chiral drug molecule should be the left hand enantiomer so that it can form a covalent complementary structure with DNA molecule which has right hand chirality.

The above TPCP has generalized the molecular structure, action modes and steric selectivity for metal anticancer agents. This principle could provide some guidance for the design and synthesis of new metal antitumor drugs. However, for vast number of metal antitumor complexes, the action mechanisms may differ dramatically, and there are always some complexes cannot be accorded with the TPC Principle. And the TPC Principle itself may still has some incomplete aspects, and need to be further studied to make it more complete.

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