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Current status of *trans*-platinum compounds in cancer therapy

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Abstract

Most of the structure/activity rules emerged from the initial studies by Rosenberg and Cleare and Hoeschele have now been questioned. Specifically: (i) *trans* compounds are inactive, (ii) charged compounds are inactive, (iii) complexes having only one leaving group are inactive, (iv) only complexes with two amine ligands, each carrying at least one H atom, are active. Exceptions to the first of these rules will be the subject of this article. These 'exceptions' which frequently show activity against cisplatin resistant tumour cells, fall in four classes: (1) *trans*-[$PtCl_2(L)(L')$] with L and/or L′ = pyridine-like ligands; (2) platinum(IV) complexes of formula *trans*-[$PtCl_2X_2(L)(L')$] with X = hydroxide or carboxylate, L = ammine, and L′ = amine; (3) *trans*-[$PtCl_2(L)(L')$] with L = alkyl-substituted amine and L′ = isopropylamine; and (4) *trans*-[$PtCl_2(L)(L')$] with L and/or L′ = iminoether. Greater inertness in biological medium appears to be a common feature of these compounds. Increased binding affinity for alternating purine–pyrimidine sites and enhanced interstrand cross-linking ability was found for the first and third class of compounds. Inter-strand cross-links and single-strand breaks were both proposed as cytotoxic lesions for the platinum(IV) species which presumably require reduction to platinum(II) prior to their interaction with DNA. Finally, stable monofunctional adducts with duplex DNA, causing unique local distortions in DNA which are able to inhibit in vitro DNA and RNA synthesis, were found for platinum–iminoether complexes. The recent development of new highly active platinum based drugs that do not fit the classical structure-activity rules indicates the need for a reappraisal of these rules. It is unlikely that any new widely applicable relationship will emerge. All of which goes to show that serendipity still contributes much to the study of Pt drugs, which is only appropriate given how they began.

Keywords: Platinum antitumour drugs; *Trans* configuration; Pyridine-like ligands; Iminoether ligands; Mixed ammine/amine ligands

1. Introduction

The anticancer drug cisplatin (and its close congener carboplatin) is one of the three most extensively used anticancer drugs in the world [1–3]. In combination with bleomycin and vinblastine, cisplatin provides the opportunity to cure over 80% of patients with testicular cancer and, in combination with paclitaxel, it greatly improves survival in patients with advanced ovarian cancer. In head and neck, and bladder cancers, as well as in cervical carcinoma, lymphoma, osteosarcoma, and melanoma, substantially improved outcomes result from treatment with platinum-containing protocols. Combination chemotherapy based upon platinum drugs with paclitaxel or vinorelbine (or, more recently, docetaxel) has also become the cornerstone of therapy for both non-small-cell and small-cell lung cancers (Scheme 1).

Improved treatment regimes including extensive hydration have ameliorated the nephrotoxicity, and the use of serotonin receptor antagonists has rendered tolerable the debilitating nausea and vomiting [3–6]. However, other side effects such as ototoxicity and peripheral neuropathy remain serious problems.

Substitution of the more stable cyclobutanedicarboxylate for the two chlorides led to carboplatin (**2**, *cis*-[Pt(1,1-cyclobutanedicarboxylate)$(NH_3)_2$] [7]. This drug diminishes renal effects [8] and produces substantially less nausea, vomiting, and neurotoxicity, and finds dose-limiting toxicity in myelosuppression [9].

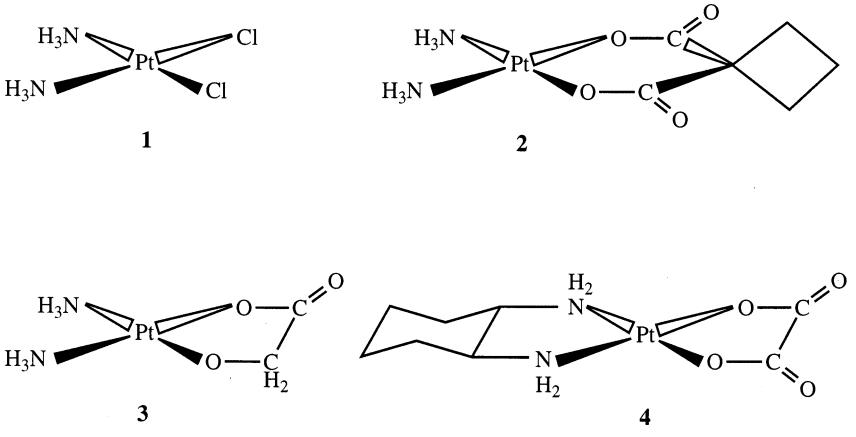
Nedaplatin (**3**, [Pt(glycolate)$(NH_3)_2$] contains a chelate glycolate in place of the two chlorides. Nedaplatin has been reported to be effective for both ovarian and cervical cancers, and it has been recently approved for clinical use in Japan [10].

Manipulation of the structure of the leaving group appears to influence tissue and intra-cellular distribution of the platinum coordination complexes, but it is unlikely to prevent cross-resistance. It was hypothesised that modification of the carrier ligands could lead to a different spectrum of DNA lesions and therefore might circumvent the problem of cross-resistance.

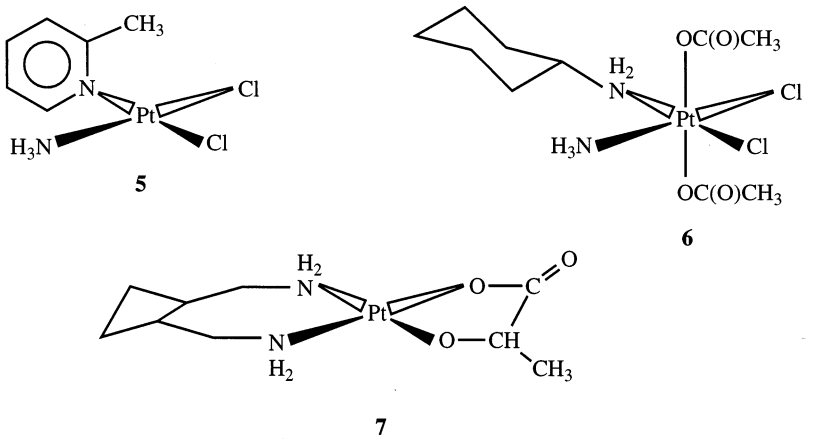
Among the most successful *cis*-DDP analogues containing chelate amine carrier ligands, oxaliplatin (**4**, *cis*-[Pt(oxalate)(*R*,*R*-1,2-diaminocyclohexane)]) has been developed successfully in France [11–13]. The DNA adducts of oxaliplatin are predominantly intra-strand cross-links as with *cis*-DDP and carboplatin [14,15]. Evidence for non-cross-resistance has been obtained in vivo [12] and its potential role in the initial treatment of colorectal cancer is being investigated [16–18].

The sterically-hindered complex ZD0473 (**5**, $[PtCl_2(NH_3)$(2-Me-py)], 2-Me-py = 2-methylpyridine) is active (by both intraperitoneal and oral administration) against acquired cisplatin-resistant human ovarian carcinoma xenografts [19], and entered clinical trials in 1997. It is less reactive than cisplatin, for example it is slower in inducing DNA interstrand cross-links in cells and in binding to plasma proteins (Scheme 2).

JM216 (**6**, *cis*,*trans*,*cis*-$[PtCl_2(acetate)_2(NH_3)$(cyclohexylamine)]), lead compound of a series of mixed ammine/amine dicarboxylates, has been specifically designed to circumvent the poor gastrointestinal absorption of cisplatin or carboplatin [7] and



Scheme 1.



Scheme 2.

it is currently in phase II clinical trials. In a panel of human ovarian carcinoma xenografts, JM216 was demonstrated to possess oral antitumour activity broadly equivalent to that of intravenously administered cisplatin or carboplatin [20]. In a panel of cisplatin-sensitive and cisplatin-resistant human tumour cell lines, JM216 showed a cytotoxic potency similar to that of cisplatin, along with the ability to overcome cisplatin resistance associated with reduced drug transport [21,22]. The toxicological profile of JM216 is similar to that of carboplatin, myelosuppression representing the dose-limiting toxicity [23].

Lobaplatin (**7**, [Pt(lactate)(1,2-dimethylaminocyclobutane)]) was introduced in clinical trials in 1992 [24]. It is currently in phase II trials for treatment of cisplatin-resistant ovarian cancer [25], advanced head and neck cancers, [26] and small cell lung cancer [27].

Overall cisplatin remains the major front-line Pt containing drug with carboplatin used primarily where there is a need to minimise the toxic side effects. This result might be considered rather disappointing, given that more than 3000 compounds have been tested in preclinical evaluations and about 30 have entered human clinical trials [2], however, it is not totally unexpected since, in general, a new drug only arises from every 10 000 compounds tested.

1.1. Early structure/activity relationships

Much of the current understanding of the mechanism of action of platinum drugs comes from studies with *cis*-DDP. The initial study of Rosenberg and colleagues [28] demonstrated that *cis*-DDP induces filamentous growth in bacteria while inhibiting cell division. This suggests that *cis*-DDP interferes mainly with DNA replication rather than with RNA and protein synthesis [29]. Interaction with DNA is believed to occur after *cis*-DDP loses its chlorine ligands through aquation to

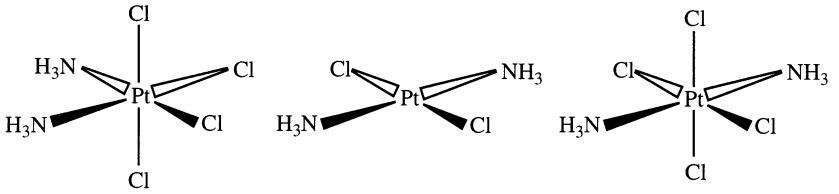
yield a reactive electrophile [30]. This situation occurs in biological systems when chloride ion concentration is low, as in the intracellular medium. Thus, the term of leaving group has been applied to the chlorine ligands or, in the case of analogues, to the moieties that replace the chlorine ligands and are displaced under physiologic conditions. In contrast the amino groups, or similar substituents not susceptible to displacement in physiologic conditions, are termed carrier ligands (Scheme 3).

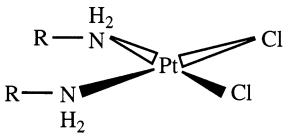
From the early beginning, the *cis* configuration was identified as potentially critical for activity because the *cis*-isomers of both $[PtCl_2(NH_3)_2]$ (**1**) and $[PtCl_4(NH_3)_2]$ (**8**) interfered with the cell division in *Escherichia coli* but the corresponding *trans* isomers (**9** and **10**, respectively) were ineffective [31].

Soon after, Cleare and Hoeschele reported on the activity of a large number of platinum(II) complexes [32,33]. They confirmed that complexes with the *trans* geometry were inactive and added other criteria to the first requirement that (i) a pair of *cis*-leaving groups is necessary. Specifically: (ii) the complex must be uncharged, (iii) the leaving groups should be adequately strongly bound since compounds with highly labile leaving groups are toxic and those with tightly bound leaving groups are less active (significantly, the point was made that complexes with dicarboxylate ligands could be active), and (iv) higher activity is to be expected for complexes having amine ligands with fewer alkyl substituents.

Tobe and co-workers studied a series of *cis* Pt(II) and Pt(IV) complexes with chloro and substituted amine ligands [34,35]. The role of solubility and lipophilicity was investigated and it was found that, in *cis*-$[PtCl_2(NH_2R)_2]$ complexes (**11**), comparable solubilities in water and lipids correlates with low toxicity and high therapeutic index, moreover the activity was higher for the more soluble compounds.

Finally studies on platinum(IV) complexes (all possessing *cis*-amine carrier ligands) revealed that also these compounds exert their antitumour effects through





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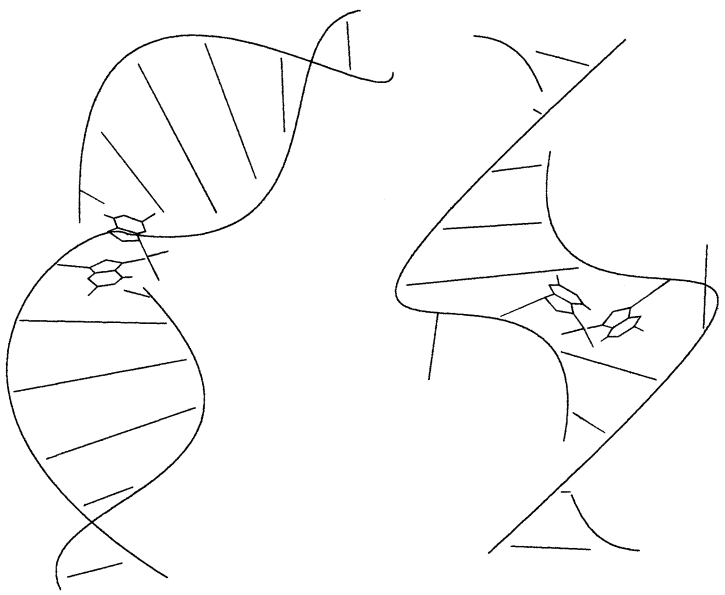
Scheme 3.

binding to DNA and formation of monofunctional and bifunctional adducts similar to those formed by their platinum(II) counterparts (both intra- and inter-strand) [36,37]. There is compelling evidence to suggest that these complexes are rapidly reduced in vivo [38–41] and are therefore a prodrug of the active Pt(II) complexes.

1.2. The platinum DNA interaction

It has yet to be established unequivocally which of the drug/DNA adduct(s) is responsible for the cytotoxic activity. The debate over which of the intrastrand or interstrand bifunctional adducts are responsible for anticancer activity of cisplatin and its analogues has gone on since the initial studies on their mechanism of action and continues today. In the early days, the interstrand adducts were favoured, partly because they were readily measured and partly because it is easy to imagine how an adduct that links the two strands of a DNA molecule will interfere with its replication. In the early 1980s, direct measurements of the adducts revealed that the intrastrand adducts account for 80–90% of the Pt bound to DNA and this led to a shift to the view that they must be the critical lesions (Scheme 4).

The *trans* isomer (**9**, *trans*-$[PtCl_2(NH_3)_2]$, *trans*-DDP or transplatin) which does not form 1,2-intrastrand lesions, exhibits in vitro cytotoxicity markedly lower than that of cisplatin and does not show in vivo antitumour activity [42]. Therefore, the significance of the *cis* configuration for anticancer activity nearly achieved the status of a dogma and has driven most of the subsequent work for the development



Scheme 4. Schematic drawing of intrastrand (left) and interstrand (right) cross-linking of two guanines by cisplatin.

of platinum analogues modified either in the leaving groups or in the carrier ligands.

Further support to the idea that intrastrand adducts are responsible for the cytotoxic activity of cisplatin came from the discovery that HMG proteins specifically recognise 1,2-intrastrand cross-links and therefore can regulate the processing of the main cisplatin lesion by altering the cellular sensitivity to the drug [43,44]. HMG proteins recognise the DNA damage induced by *cis*-DDP but not that of *trans*-DDP [45]. Recently a method for the screening of combinatorial mixtures of potential platinum antitumour drugs based on the ability of platinum compounds to bind to HMG proteins has been reported [46].

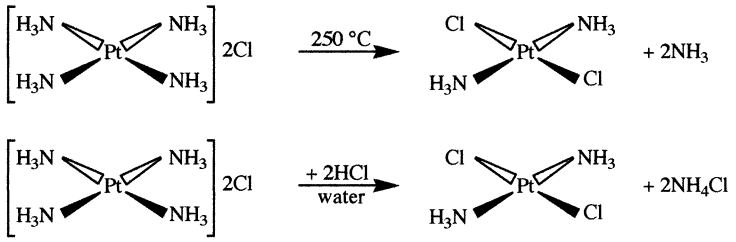
2. Basic chemistry of *cis*- and *trans*-DDP: what makes the difference

Some exceptions to the rule that two good leaving groups in *cis* position are necessary for antitumour-active platinum complexes have been reported in recent years. These 'exceptions', which frequently show activity against *cis*-DDP-resistant tumour cells, fall in four classes: (1) *trans*-$[PtCl_2(L)(L')]$ with L and/or L′ = pyridine-like ligands [49–53]; (2) platinum(IV) complexes of formula *trans*-$[PtCl_2X_2(L)(L')]$ with X = hydroxide or carboxylate ligands, L = ammine, and L′ = amine [47,48]; (3) *trans*-$[PtCl_2(L)(L')]$ with L = alkyl-substituted amine and L′ = isopropylamine [54,55]; and (4) *trans*-$[PtCl_2(L)(L')]$ with L and/or L′ = iminoether ligand [56–58]. Poly-platinum(II) complexes with bridging diamine ligands of the type *trans*-$[\{PtCl(NH_3)_2\}_2(\mu\text{-}NH_2(CH_2)xNH_2)]Cl_2$ [59,60] have not been included in this review since they have leaving groups on two distinct platinum centres (linked together by a bridging ligand) and cannot be considered *trans*-complexes in the classical sense being this notation used to indicate compounds with two leaving groups bound to the same metal centre from opposite sides.

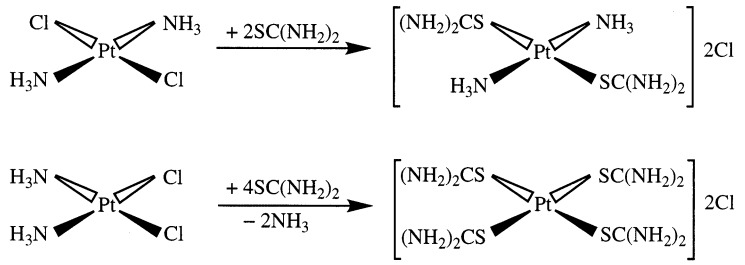
In this section the attempt is made to survey the basic chemistry of *trans*-platinum complexes and to compare their chemistry with that of the corresponding *cis*-isomers.

The synthesis of *trans*-DDP was simultaneous to that of the *cis*-isomer; it was obtained by thermal decomposition of tetrammineplatinum(II) chloride and reported as 'Reiset's Second Chloride' [61]. The yield was rather low due to extensive decomposition to Pt(0) but it could be increased by performing the decomposition at lower temperature (190–195°C) under reduced pressure [62]. A second and still the most common procedure for the preparation of *trans*-DDP is the action of HCl on an aqueous solution of $[Pt(NH_3)_4]Cl_2$, this method originally proposed by Peyrone [63] was later modified by Kauffman and Cowan [64] (Scheme 5).

A summary of the physico-chemical properties of *trans*-DDP as determined prior to 1973 can be found in the book of Hartley [65]. They comprise also an X-ray crystallographic investigation (Pt–N = 2.05(4) Å, Pt–Cl = 2.32(1) Å) [66,67]. Thiourea derivatization ('Kurnakow test' [68]) is used to differentiate between the two isomers: it leads to complete ligand substitution in the case of *cis*-DDP and to only chloride substitution in the case of *trans*-DDP (Scheme 6). The result of the



Scheme 5.



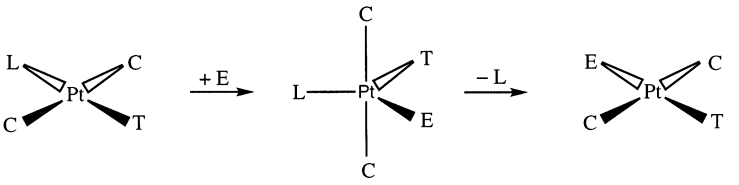
Scheme 6.

Kurnakow test could lead to the wrong conclusion that the ammine ligands are more tightly bound in the *trans* than in the *cis* complex, but this is not the case. ^{15}N-NMR experiments have shown that the J(Pt–N) coupling constant is greater in the *cis* than in the *trans* isomer (303 and 278 Hz for *cis* and *trans* isomers, respectively) [69]. Furthermore the exchange of the aminic protons in the methylamine derivative is faster in the *cis* than in the *trans* isomer, thus indicating a more acidic NH (and therefore a greater electron donation from nitrogen to platinum) in the former case [70].

Therefore, why in the 'Kurnakow test' the ammines are lost in the *cis* complex but not in the *trans* complex, is a question of a low energy pathway available for the ongoing reaction.

Ligand substitution in square-planar complexes usually takes place through an associative mechanism leading to a five-coordinate trigonal bipyramidal transition state in which the entering group, the leaving group, and the ligand *trans* to the leaving group occupy the trigonal plane (Scheme 7). Some ligands (usually characterised by readily accessible empty orbitals) have higher propensity to occupy the trigonal plane in the five-coordinate transition state and therefore to undergo substitution themselves or to direct substitution on the *trans* ligand [71].

This simple fact explains several things such as: the 'Kurnakow test', the greater stability to the treatment with TU of the monofunctional adducts with DNA given by *cis*-DDP if compared to those given by *trans*-DDP, the general greater inertness to isomerization of *trans*-compounds with respect to *cis*-compounds, the solvolysis pattern, etc.



Ligands: C = *cis*, E = *entering*, L = *leaving*, T = *trans*

Scheme 7.

The solvolysis pattern is shown in Scheme 8. Both k_1 and k_{-1} are faster for *trans*-DDP as compared to *cis*-DDP. In contrast k_2 and k_{-2} are smaller for the *trans* over the *cis* isomer (this is a consequence of the trend in *trans*-effect Cl > N > O).

Some general conclusions can be drawn for the chemical behaviour of *trans*-platinum complexes as compared to *cis*-platinum complexes: *Trans*-platinum complexes will react faster with nucleophiles in the dichloro and chloro-aqua forms, yet not in the diaqua form. Monoadducts with DNA will be more easily displaced by the action of *trans*-labilizing nucleophiles (such as thiourea and glutatione, Scheme 9) therefore repair of *trans*-DDP monoadducts could occur simply by a chemical pathway whereas repair of *cis*-DDP monoadducts might require an enzymatic pathway. Finally, because of the *trans* disposition of the leaving ligands, *trans*-platinum complexes will give chelation reaction less readily than *cis*-platinum complexes (1,2-intrastrand cross-links are totally forbidden while there is the possibility of 1,3 and larger intrastrand cross-link formation).

3. Activation of the *trans* geometry by pyridine-like carrier ligands

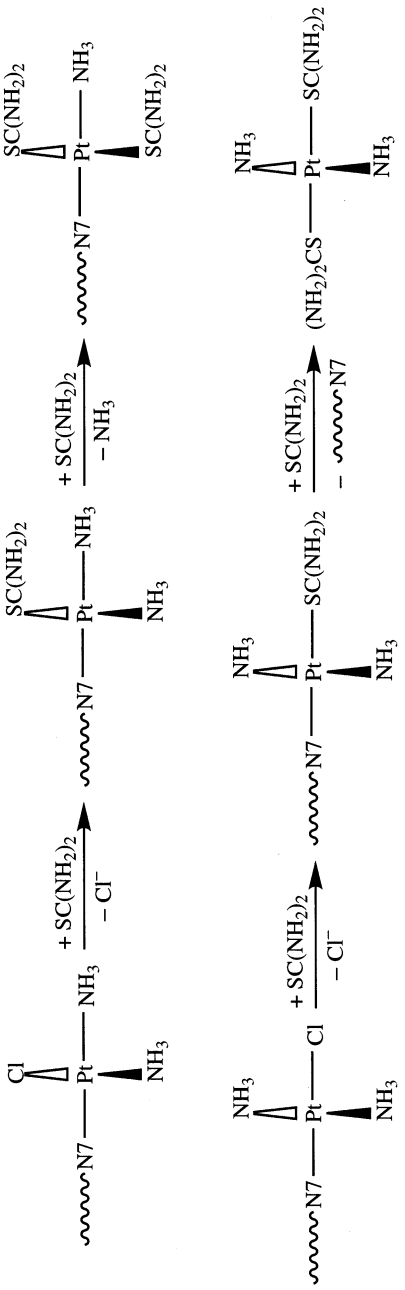
It is clear from the previous section that *trans*-DDP is more reactive than cisplatin, therefore undesired reactions on its way to the pharmacological target are likely to contribute, at least in part, to the lack of anticancer activity. Farrell argued that the antitumour activity of *trans* complexes could be increased by using sterically demanding carrier ligands which reduce the rate of replacement of the chloro ligands [49]. Bulky ligands would limit axial access to the Pt atom, and therefore inhibit the formation of the five-coordinate intermediate that leads to the ligand substitution.

Three distinct series of *trans*-[$PtCl_2$(L)(L′)] [50] complexes were examined: (a) L = L′ = pyridine (**12**), *N*-methylimidazole (**13**), or thiazole (**14**), (b) L = quinoline and L′ = RR′SO (R = Me, R′ = Me, Bz, or Ph) (**15**), and (c) L = NH_3 and L′ = quinoline (**16**) or thiazole (Scheme 10).

The method of Kauffman and Cowan [64] exemplified in Scheme 6 was used for the preparation of the *trans*-[$PtCl_2$(L)(L′)] complexes with pyridine or *N*-methylimidazole [72]. In the case of thiazole the conversion of [Pt(thiazole)$_4$]Cl_2 to *trans*-

Cl
H3N — Pt — NH3
Cl
k1, s-1
1.9x10-5
k-1, s-1M-1
3.0x10-2
Cl
H3N — Pt — NH3
OH2
+ Cl−
k2, s-1
4.0x10-6
k-2, s-1M-1
0.2
OH2
H3N — Pt — NH3
OH2
2+
+ 2Cl−

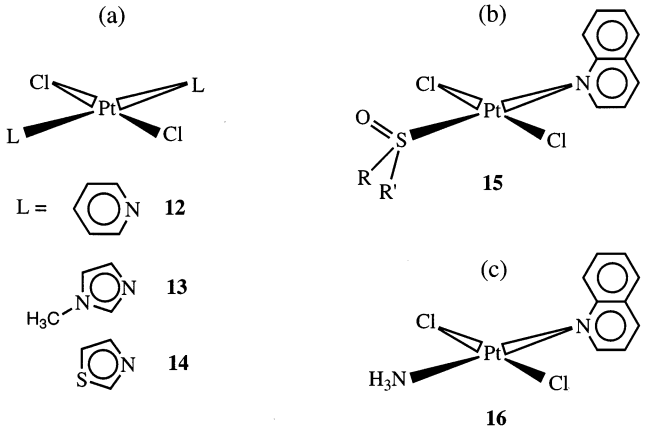
Scheme 8.



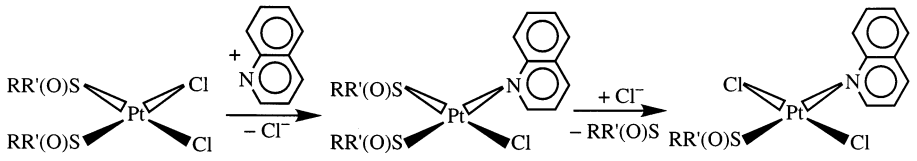
Scheme 9.

$[PtCl_2(thiazole)_2]$ was accomplished by heating the tetrathiazoleplatinum(II) dichloride at 100°C instead of treating the salt with HCl [50]. The *trans*-$[PtCl_2(RR'SO)(quinoline)]$ complexes were prepared by direct reaction of a suspension of *cis*-$[PtCl_2(RR'SO)_2]$ with quinoline [50]. The reaction course is likely to be that depicted in Scheme 11 [73].

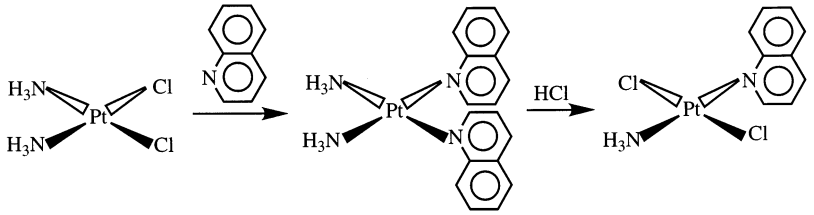
The *trans*-$[PtCl_2(NH_3)(quinoline)]$ complex was prepared according to the method of Kauffman and Cowan [64] as summarised in Scheme 12.



Scheme 10.



Scheme 11.



Scheme 12.

3.1. Cytotoxicity of the trans complexes compared to those of the cis isomers and cis-DDP

In general, the cytotoxicity of all *trans* complexes examined was approximately one-order of magnitude greater than that of *trans*-DDP and, furthermore, the *trans* complexes were at least as cytotoxic as their direct *cis*-analogues [51]. Despite the apparent structural dissimilarities among the three series of complexes, a similar cytotoxicity profile towards tumour cells was found using the COMPARE program [74], and this was different from that of *cis*-DDP but similar to that of rifamycin antibiotic (cellular action through inhibition of DNA-dependent RNA polymerases) [75]. Finally, the range of cytotoxicity [defined as IC_{50} (least sensitive)/IC_{50} (most sensitive), IC_{50} = drug concentration causing 50% inhibition of cell growth] was found significantly smaller for all *trans*-platinum complexes than for *cis*-DDP [51].

As a general feature *trans* complexes with bulky planar ligands were not cross-resistant to *cis*-DDP (resistance factor ≤ 1) in both murine L1210 leukaemia and human ovarian tumour cells resistant to *cis*-DDP.

The enhanced cytotoxic potency of *trans*-platinum complexes with pyridine-like ligands along with their ability to overcome cisplatin resistance has important mechanistic implications, suggesting that complexes structurally distinct from cisplatin may be characterised by distinct cellular pharmacological properties.

3.2. The trans-bispyridine complex

The cellular uptake appeared to be greater for the pyridine as compared to the ammine complexes (L1210 cells) and was greater for the *trans*- over the *cis*-isomer (factor of ca. 4). Binding of the pyridine complexes to calf thymus DNA was almost equivalent for the *cis*- and *trans*-isomers but significantly less than for the analogous ammine complexes (order of binding affinity *trans*-DDP $\gg$ *cis*-DDP $\gg$ *cis*-$[PtCl_2(py)_2]$ > *trans*-$[PtCl_2(py)_2]$) [51].

The planar ligands appear to sterically hinder approach of incoming nucleophiles to the axial positions of the platinum centre. This steric feature may have some consequences upon the reactivity of the platinum substrate not only towards DNA but also towards glutathione (γ-L-glutamyl-L-cysteinylglycine, GSH) and other sulphur-containing biomolecules. *Trans*-DDP is more reactive than *cis*-DDP towards GSH, however, the presence of pyridine ligands can significantly slow down the reaction.

The sequence specificity of *trans*-$[PtCl_2(py)_2]$ includes alternating purine-pyrimidine sequences [52], while the major stop sites for the *cis*-isomer correspond to those of *cis*-DDP [76]. The alternating purine–pyrimidine sequences are expected to be a good source of interstrand cross-links and, as matter of fact, *trans*-$[PtCl_2(py)_2]$ gives many more interstrand cross-links than *cis*-$[PtCl_2(py)_2]$ at the same r_b values (between 14 and 23% cross-linked material [52] compared to $< 5\%$ for *cis*-DDP [77], r_b = drug to nucleotide ratio). A greater interstrand cross-linking ability or formation of cross-links structurally different from those of *cis*-DDP could be responsible for the toxicity of *trans*-$[PtCl_2(py)_2]$ towards *cis*-DDP resistant cells.

Pyridine substitution for ammine also results in inversion of the *cis*/*trans* structure–activity relationship with respect to DNA-unwinding [78], the unwinding angle (Φ, °) being in the order *trans*-[$PtCl_2(py)_2$] (17°) > *cis*-DDP (13°) > *trans*-DDP (9°) > *cis*-[$PtCl_2(py)_2$] (4°) [52].

3.3. The trans-amminequinoline and trans-amminethiazole complexes

Even the replacement of a single NH_3 of *trans*-DDP by an aromatic amine such as quinoline or thiazole, to give *trans*-[$PtCl_2(NH_3)$(quinoline)] (**16**) or *trans*-[$PtCl_2(NH_3)$(thiazole)], dramatically increases the cytotoxicity of the *trans* isomer. Importantly, the thiazole complex shows also in vivo antileukaemic activity, thus at least in part overcoming the main caveat of *trans*-bispyridine complexes, i.e. the lack of in vivo efficacy.

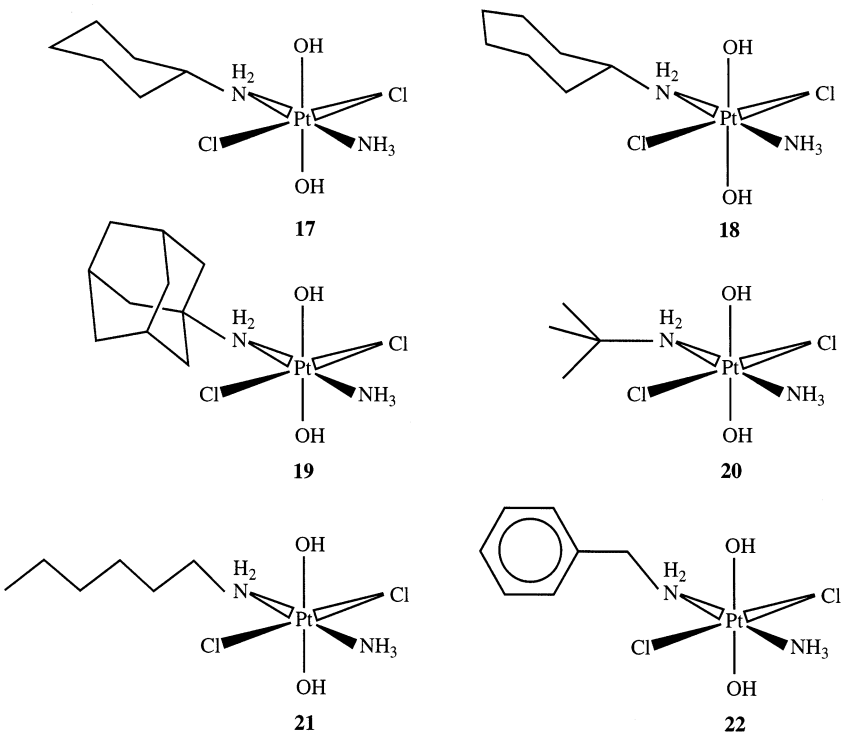
The biological activity of such 'non classical' *trans*-platinum complexes has been discussed in terms of both the overall altered affinity towards biologically relevant nucleophiles and unique DNA binding modes [79,80]. Classical dichloroplatinum(II) species form monofunctional adducts on DNA which subsequently transform into bifunctional DNA cross-links. Incubation of *trans*-[$PtCl_2(NH_3)$(quinoline or thiazole)] with calf thymus DNA for 48 h leaves ca. 30% of the total covalent DNA-platinum adducts monofunctional. The conformational changes caused by these compounds in globally platinated DNA are strikingly different from those produced by transplatin and are reminiscent of the 1,2-intrastrand (GG) cross-links formed by cisplatin. Molecular mechanic calculations suggest that the structural alterations in double-stranded DNA may be ultimately produced by the monofunctional adduct, with quinoline partially intercalating into the base stack on the 5′ face of the platinated guanine. DNA unwinding caused by ethidium linked platinum complexes [78] has also been related to this type of 'pseudobifunctional' binding mode. Both the quinoline (**16**) and thiazole complexes form a considerable amount of interstrand cross-links (up to 30%, $t_{1/2} = 5$ h) with a rate markedly higher than that of transplatin (ca. 12%, $t_{1/2} = 11$ h). In addition the replacement of an amine ligand in *trans*-[$PtCl_2(NH_3)_2$] by quinoline or thiazole changes the cross-link specificity of the *trans*-platinum geometry from purine-pyrimidine (GC, transplatin-like) to purine–purine (GG, cisplatin-like). Both the efficiency of formation and the specificity of interstrand adducts may contribute to the cytotoxic properties of these complexes.

4. Active *trans*-platinum(IV) species with aliphatic amines

The increased inertness of the platinum(IV) species (as compared to the platinum(II) species for which an associative mechanism for ligand substitution is available) could be the key factor for the antitumour activity of several platinum(IV) complexes reported by Kelland et al. in which the carrier ligands have *trans* geometry [47].

Within a platinum drug discovery program performed over many years to identify more effective platinum-based anticancer drugs particularly targeted towards the circumvention of resistance to *cis*-DDP, a series of over forty compounds, half platinum(II) and half platinum(IV) counterpart with *trans* geometry of the carrier ligands were investigated [81] (Scheme 13).

The synthesis of the *trans* isomers of platinum(II) was usually accomplished by the method of Kauffman and Cowan [64], that is by action of concentrated HCl on the aqueous solution of the $[Pt(amine)_4]Cl_2$ species. The method utilises the difference in the *trans* effect of halide and amine ligands in platinum(II) complexes to achieve selective substitution and thus control sterochemistry. The oxidation of platinum(II) complexes with hydrogen peroxide yields platinum(IV) complexes in which the sterochemistry of the platinum(II) complex is retained and hydroxide ligands are added in axial positions [82–84]; analogous reaction is given by chlorine (Scheme 15). Dicarboxylate and dicarbamate complexes can be obtained from the dihydroxo species by reaction with carboxylic anhydride and alkylisocyanate, respectively [85] (Scheme 14).



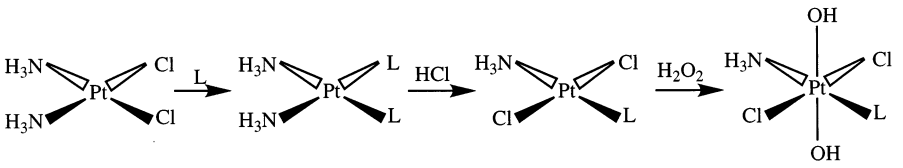
Scheme 13.

4.1. Cytotoxicity and antitumour activity

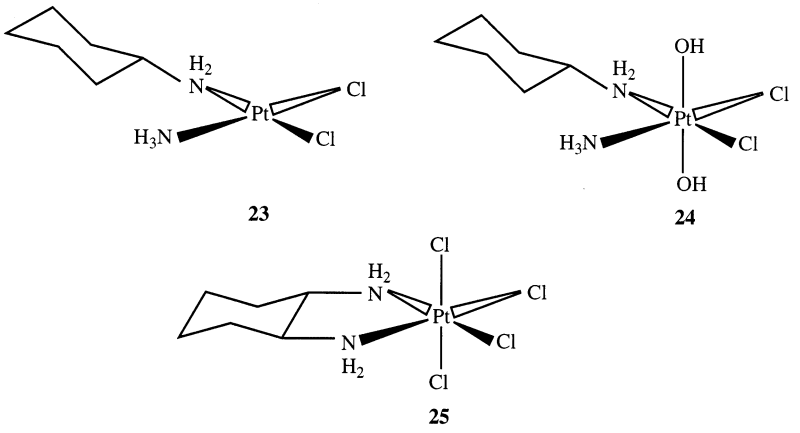
Many of the *trans* complexes studied in vitro against a panel of human cell lines exhibited a potency comparable to that of *cis*-DDP and also overcame acquired cisplatin resistance (stemming from either reduced uptake or enhanced platinum–DNA adduct removal) [81]. Fourteen *trans* complexes showed significant in vivo antitumour activity against the subcutaneous murine ADJ/PC6 plasmacytoma model. All of them were platinum(IV) complexes possessing axial hydroxide ligands, only one had axial ethylcarbamate ligands. When tested, all of their dichloroplatinum(II) or tetrachloroplatinum(IV) counterparts were inactive. Three out of the 14 complexes (**17**–**19**) retained some efficacy against a *cis*-DDP resistant variant of the ADJ/PC6 plasmacytoma and five (**17**, the same as **17** but with Br axial ligands, **20**, **21** and **22**) exhibited antitumour activity against subcutaneously grown advanced-stage human ovarian carcinoma xenografts.

4.2. Cellular pharmacological properties

In a comparative study of the initial binding properties of five *cis*-oriented compounds (*cis*-DDP (**1**), JM216 (**6**), JM118 (**23**), JM149 (**24**), and tetraplatin or ormaplatin (**25**), Scheme 15) and two *trans*-oriented compounds (*trans*-DDP (**9**)



Scheme 14.



Scheme 15.

and JM335 (**17**)), no correlation between levels of total platinum bound to DNA and cytotoxicity was found in two human ovarian carcinoma cells (the intrinsically cisplatin resistant SKOV-3 line and the relatively cisplatin sensitive CH1 line) [48]. For the *cis*-oriented compounds a strong positive correlation was found between cytotoxicity and recognition by ICR4 (monoclonal antibody raised again cisplatin treated DNA). JM335 (**17**) was capable of forming interstrand cross-links in SKOV-3 cells but, notably, none was observed in CH1 cells. In contrast, unusually for platinum complexes, JM335 induced the formation of single-strand breaks in CH1 cells. It appears that also these platinum(IV) complexes require reduction prior to DNA binding [86], however a direct Pt(IV)–DNA interaction cannot be excluded [87].

The reason for JM335 cytotoxicity and ability to overcome cisplatin resistance has been addressed by investigating the cellular pharmacological properties of this drug in some ovarian carcinoma cell lines. The selective activity of JM335 (and of its platinum(II) dichloro homologous JM334) against the intrinsically cisplatin-resistant SKOV-3 cells could be connected with the level of DNA binding and repair. SKOV-3 cells are not able to repair DNA adducts formed by *trans* compounds within the *N*-ras gene whereas DNA damage induced by *cis* counterparts (or by cisplatin) is repaired.

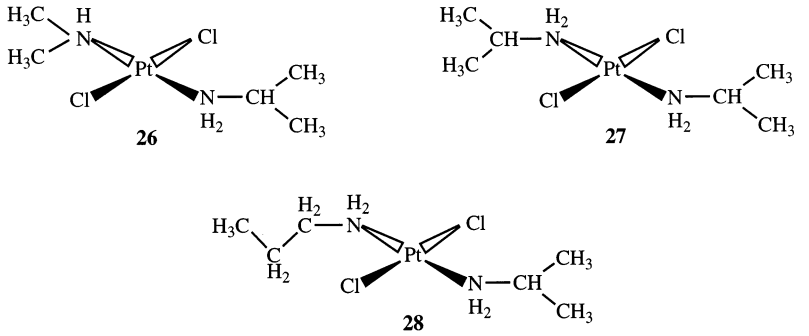
Interestingly, JM335 and JM334, as well as their *cis* counterparts, induce a slowdown of *S*-phase and apoptosis in treated cells, the induction of apoptosis being markedly faster for the *trans* than for the *cis* compounds [88].

JM335 (17), the most widely studied *trans*-oriented platinum(IV) species, having cytotoxicity comparable to that of *cis*-DDP and over 50-fold greater than that of *trans*-DDP (IC_{50}: JM335, 3.1; *cis*-DDP, 4.1; *trans*-DDP, 162 mM. IC_{50} = drug concentration which inhibits 50% cell growth) and showing non-cross resistance (where resistance stems from either reduced platinum accumulation, or enhanced removal of Pt–DNA adducts, or increased tolerance to Pt–DNA lesions [47]) represents a structural lead to the development of platinum complexes exhibiting non-cross-resistance to *cis*-DDP [89].

5. Active *trans*-platinum(II) species with aliphatic amines

Very recently it has been reported that *trans*-[$PtCl_2$(amine)(isopropylamine)] complexes (**26**–**28**, Scheme 16) exhibit a cytotoxic activity in cisplatin sensitive cells comparable to that of *cis*-DDP [54]. These compounds have IC_{50} values lower than that of *cis*-DDP in HL-60 leukaemic cells (tumour cells overexpressing *c-myc* and *N-ras* oncogenes) [90].

The same complexes exhibit IC_{50} values significantly lower than those of *cis*-DDP against *cis*-DDP resistant murine keratinocytes transformed by *H-ras* oncogene (Pam 212-ras cells). Contrary to cisplatin, these complexes display a much lower cytotoxicity in normal Pam 212 murine keratinocytes than in Pam 212-ras transformed murine keratinocytes.



Scheme 16.

Trans-[$PtCl_2$(dimethylamine)(isopropylamine)] (**26**) readily forms interstrand cross links in double-strand DNA [55]. At a similar level of DNA platination, the *trans*-[$PtCl_2$(dimethylamine)(isopropylamine)] complex produces many more stop sites of T4 DNA polymerase than does *cis*-DDP, moreover most frequent stop sites are at alternating purine–pyrimidine sequences which have been reported as a good source of DNA interstrand cross-links [91].

6. Activation of the *trans*-geometry by iminoether carrier ligands

A fourth class of active *trans*-platinum complexes has been reported by the group of Coluccia and Natile and have general formula *trans*-[$PtCl_2$(iminoether)$_2$] [iminoether = HN=C(OR)R′] [56–58].

Iminoethers are *N*-donor ligands which share some features with both the aliphatic and the aromatic amines. Like aromatic amines, iminoether ligands are planar (sp^2 hybridisation of the nitrogen atom as compared to the sp^3 hybridisation in aliphatic amines), however, like aliphatic amines, they have one hydrogen atom linked to the nitrogen and suitable for hydrogen-bond formation. Iminoether ligands are also nonsymmetric having the steric hindrance concentrated on the side of the nitrogen atom opposite to that of the proton. Another peculiarity of iminoethers is the possibility of isomerism within the ligand moiety. Depending upon the relative position of the substituents with respect to the C=N double bond, they can have *E* or *Z* configurations (*E* and *Z* correspond to having the platinum and the alkoxy group *trans* and *cis* with respect to the C=N double bond, respectively) [92].

Iminoether ligands are generated in situ by addition of an alcohol to a nitrile already coordinated to platinum. The addition reaction takes place readily in the presence of a base and leads to the formation of the *Z*-isomer (the alkoxide and the hydrogen add to the triple bond from opposite sides). Subsequently $Z \rightarrow E$ isomerization can take place under basic conditions. The relative thermodynamic stability of the *Z* and *E* isomers depends upon the size of the alkoxide (R′) and nitrile (R)

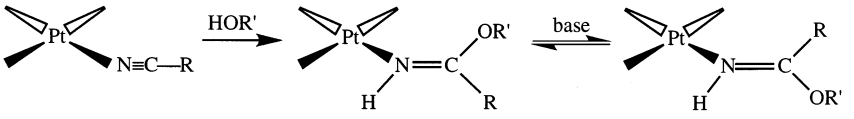
organic groups. The *Z* isomer is thermodynamically favoured for small R′ and large R groups, vice versa for the *E* isomer (Scheme 17).

6.1. Cytotoxicity and antitumour activity

The in vitro cell growth inhibitory activity of *trans*-iminoether compounds (such as **29**) was evaluated in comparison to those of *cis*-DDP in a panel of human tumour cell lines containing examples of ovary, colon, lung, and breast cancers, as well as a subline of ovarian cancer cells with acquired resistance to *cis*-DDP (A2780/cp8) [93]. The *trans*-iminoether compounds showed a growth inhibitory potency similar to that of *cis*-DDP (mean $IC_{50} = 8$ μM). Moreover, *trans*-iminoether compounds were able to circumvent the *cis*-DDP resistance of A2780/cp8 cells, which are characterised by having a reduced cisplatin accumulation and a greater intracellular glutathione content with respect to the parental line (the resistance factor, defined as IC_{50} (acquired − resistance cells)/IC_{50} (sensitive cells), was ca. 1).

The antitumour activity (P388 leukaemia-bearing mice) of **29** depends upon the treatment schedule. The compound was most active when administered daily for 7 consecutive days ($\%T/C = 196$, where $T/C =$ life span of treated animals over that of control animals) and also had a significant effect on an in vivo selected *cis*-DDP resistant P388 subline (P388/DDP) ($\%TC = 133$). In contrast, the antitumour activity of the *cis*-iminoether complexes did not depend upon the treatment schedule ($\%T/C = 145$), and a cross-resistance of the same compound with *cis*-DDP was observed on the P388/DDP subline.

Therefore substitution of iminoethers for ammines brings only slight changes in the activity of the *cis* complexes, while it has a dramatic effect on the behaviour of the *trans* species [56]. The same *trans*-iminoether complex showed an activity comparable to that of *cis*-DDP in reducing the primary tumour mass and lung metastases in mice bearing Lewis lung carcinoma. Therefore, **29** represents the first example of a *trans*-platinum complex active on both limphoproliferative and solid metastasising murine tumours [57], and its favourable pharmacokinetic properties were confirmed by using a subcutaneously growing tumour and a drug administration site (i.p.) distant from that of the tumour. Importantly, *trans*-platinum iminoether complexes are well tolerated by tumour bearing mice, which show, at the end of treatment, a loss of body weight lower than that induced by cisplatin.



Scheme 17.

6.2. Cellular pharmacological properties

The cellular accumulation of *trans*-platinum iminoether complexes is greatly enhanced (> 50-fold) with respect to that of cisplatin in both A2780 and A2780/Cp8 ovarian cancer cells. A possible explanation for the marked accumulation of these compounds can be found in the enhanced lipophilicity of the iminoether compounds. Substitution of iminoethers for amines induces a normal-octanol/saline partition ratio markedly greater than that of cisplatin [94,95] and promising for a possible oral administration of this drug.

In accord with the greater intracellular platinum accumulation, also the degree of platination of cellular DNA is greater for *trans*-platinum iminoether complexes than for cisplatin, thus indicating that DNA is an intracellular target for platinum–iminoether complexes and providing a foundation for their mutagenetic and DNA-synthesis inhibitory activity [57].

A major difference between **29** and cisplatin is also observed when their effects upon tumour cell growth and cell-cycle progression are evaluated. Both complexes induce apoptosis in murine P388 leukaemia [96] or human ovarian AL780 cells [94]. The apoptosis induction, as well as the cell-cycle modifications (*S*-phase accumulation followed by G2M accumulation) associated to treatment with equitoxic doses of **29** and cisplatin indicate a faster response of tumour cells to the platinum–iminoether complex than to cisplatin.

6.3. DNA binding properties

Importantly, excision-repair-deficient eukaryotic cells were four times more sensitive to *trans*-iminoether complexes than normal cells, similarly the drug was much more toxic towards a strain of drosophila defective in excision repair systems, thus implicating cellular DNA as cytotoxic target.

As for *trans*-$[PtCl_2(py)_2]$ [51], also *trans*-iminoether complexes exhibit slower reactivity towards DNA (in cell-free medium) than *trans*-DDP. However, while in the case of *trans*-$[PtCl_2(py)_2]$ DNA reaches saturation at much lower level of platination, in the case of *trans*-iminoether complexes the level of binding after 14 h reaction at 37°C is comparable to that of the ammine species [57]. Therefore it appears that the iminoether ligands, like pyridine, probably because of their steric hindrance, slow down the reaction with a sterically demanding nucleophile such as a nucleobase inserted in a DNA duplex, however, differently from pyridine, iminoether ligands do not prevent the reaching of the same level of platination as for ammine compounds.

Trans-iminoether complexes interact preferentially with guanine residues, but, unlike *cis*-DDP which interacts selectively at multiple guanine sites, the iminoether complexes show affinity also for isolated guanines in py–G–py sequences [56,93].

In double helical DNA (calf thymus DNA) monofunctional adducts at guanine residues are preferentially formed even when DNA is incubated with the platinum complex for a relatively long time (48 h at 37°C in 10 mM $NaClO_4$). Under similar conditions *trans*-DDP forms prevalent amount of bifunctional DNA adducts [97].

This appears to be another striking difference between *trans*-iminoether complexes and *trans*-[$PtCl_2(py)_2$], the latter species manifesting an unusually high ability to form interstrand cross-links (up to 14–23% of total platinum bound).

The above observations, initially made on calf thymus DNA, were also confirmed on plasmid DNA (pGEM-4Z [93], pBR322 [56], and pSP73 [98]). In agreement with the observation that *trans*-iminoether complexes form in double-helical DNA kinetically stable monofunctional adducts [93,97], the measured unwinding angle was only 6°, a value which is coincident with unwinding angles induced by monofunctional Pt(dien) (dien = diethylenetriamine) and $Pt(NH_3)_3$. Greater unwinding angles are induced by bifunctional *cis*-DDP (13°) [99,100] and *trans*-DDP (9°) [78] and also by *trans*-[$PtCl_2(py)_2$] (17°) [52]. The work on plasmid DNA also confirmed the low tendency of platinum–iminoether complexes to form interstrand cross-links ($< 3\%$ as compared to ca. 6 and 12% for *cis*- and *trans*-DDP, respectively) and the low rate of formation of such cross-links ($t_{1/2} > 18$ h as compared to ca. 4 and 11 h for *cis*- and *trans*-DDP, respectively) [91].

The chemical reactivity of monofunctional adducts was investigated in single- and double-strand oligonucleotides specifically modified by *trans*-iminoether complexes. Monofunctional adducts in single-strand oligonucleotides rearrange to several distinct bifunctional adducts by a rather slow reaction. In contrast, monofunctional adducts are stable in double-strand DNA and do not form interstrand cross-links, clearly confirming the stability of monofunctional adducts of *trans*-iminoether complexes in double-strand DNA. Moreover the distortions induced by monofunctional adducts in double helical oligonucleotides are selectively located at the 5′-site with respect to the adduct and involve the two complementary residues within the adjacent base-pair.

The monofunctional adduct formed by reaction of **29** with a double-strand decanucleotide was investigated by NMR (Fig. 1) and shown to undergo a bending towards the minor groove of ca. 45° [101].

6.4. Monofunctional adducts appear to inhibit transcription and do not induce denaturational conformational distortions

DNA globally modified by *trans*-iminoether complexes prematurely terminate RNA synthesis with a similar efficiency as DNA adducts of *cis*-DDP. The prevalent lesions formed on DNA by *trans*-iminoether complexes are monofunctional adducts [93,97] and it has been shown that monofunctional DNA adducts of *cis*-DDP, *trans*-DDP, and Pt(dien) do not terminate RNA synthesis [91,102,103]. Thus, the monofunctional adducts of *trans*-iminoether complexes on one hand, and those of Pt(dien), *cis*-DDP, and *trans*-DDP on the other hand, appear to modify the DNA conformation in a fundamentally different manner.

Double strand DNA modified by *trans*-iminoether complexes did not inhibit the binding to their immunogens of antibodies which either specifically recognise 1,2-intrastrand cross-links formed by *cis*-$Pt(amine)_2$ moieties (AB_{cis}) [104,105], or those that specifically recognise short single strand segments in a double strand DNA containing a *trans*-$Pt(amine)_2$ moiety (AB_{trans}). Thus, *trans*-iminoether com-

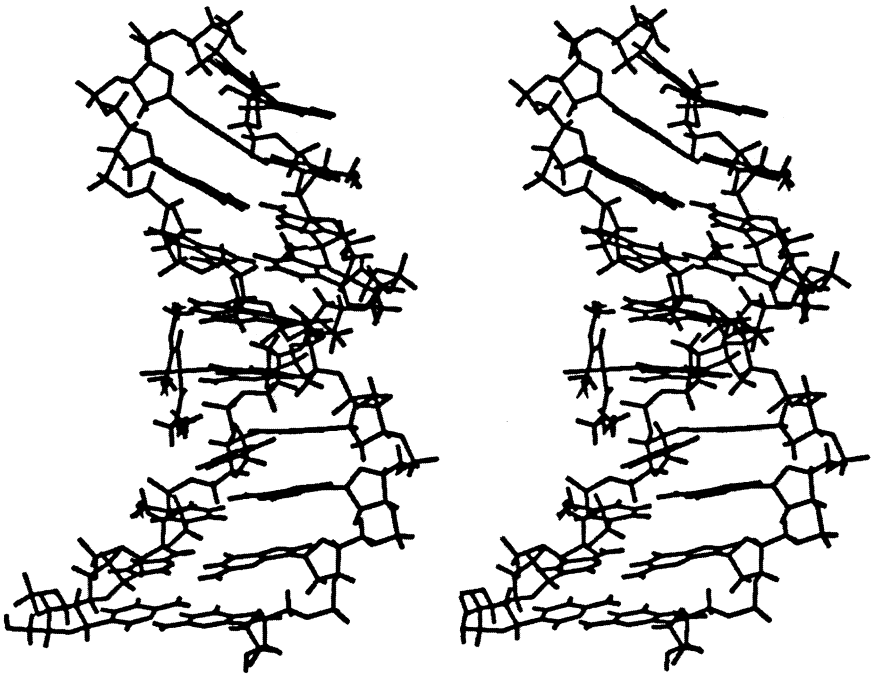


Fig. 1. Stereoview of the molecular model for the refined monofunctional adduct of **29** with the duplex d(CCTCG*CTCTC) · d(GAGAGCGAGG).

plexes induce conformational changes of non-denaturating character in DNA, this is in clear contrast with modification of DNA by clinically ineffective *trans*-DDP or monofunctional Pt(dien), both of which induce denaturational conformational alterations in DNA [103,106,107]. In this respect, *trans*-iminoether complexes resemble the antitumour *cis*-DDP which induces in DNA local conformational changes of non-denaturational character.

These results were fully confirmed by differential pulse polarography analysis which readily and with a great sensitivity distinguishes between non-denaturational and denaturational conformational alterations induced in DNA by various physical or chemical agents. At relatively low level of platination ($r_b < 0.05$) *trans*-iminoether complexes, like *cis*-DDP and other antitumour analogues, induce in DNA non-denaturational conformational distortions, in contrast *trans*-DDP, monofunctional Pt(dien), and other inactive platinum(II) complexes induce in DNA denaturational conformational alterations [106,108].

6.5. Active trans-complexes with a single iminoether ligand

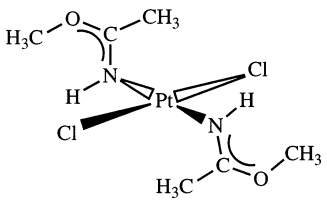
One of the most remarkable results emerging from this series of active *trans*-platinum compounds is that replacement of a single NH_3 of *trans*-DDP by an

iminoether, to give *trans*-[$PtCl_2(NH_3)$(iminoether)], leads to a dramatic increase in activity. In a panel of human tumour cell lines the two isomers with *Z* and *E* configurations of the iminoethers (**30** and **31**, Scheme 18), both showed a cytotoxic potency higher than that of *trans*-DDP, the mean IC_{50} values being 36, 102, and 217 M, respectively. In vivo, compound **30** is more active and less toxic than **31** in the murine P388 leukaemia system (%*T*/*C* values of 152 and 190 for *E* and *Z* isomers, respectively). Compound **30** is also able to cure ca. 30% of the leukaemia-bearing mice, and retains its efficacy against SKOV-3 human cancer cell xenografts in nude mice [109].

Similarly to transplatin, also compound **30** reacts with naked DNA to form monofunctional adducts that do not evolve into intrastrand cross-links, but close slowly into interstrand cross-links between complementary guanine and cytosine residues. These interstrand cross-links behave as hinge joints increasing the flexibility of DNA double helix. As for *cis*-DDP and *trans*-DDP also interstrand cross-links formed by **30** are not recognised by HMG1. The major conclusion from this study was that the interaction properties of **30** are very similar to those of *trans*-DDP, therefore the antitumour activity of **30** probably stems from different pharmacokinetic properties which allow the drug to reach, with therapeutic efficacy, the tumour site.

7. Conclusions and perspectives

In the search for new platinum drugs there has been, in recent years, an increasing interest in *trans*-platinum compounds, as witnessed by the discovery of active *trans*-platinum complexes by at least four independent groups. Unlike *trans*-DDP, which is less toxic to tumour cells than *cis*-DDP by a factor of 5–20, the cytotoxic potency of *trans*-platinum complexes with bulky carrier ligands (such as aromatic amines, iminoethers, cyclohexylamine, ramified aliphatic amines) is dramatically increased. More importantly, some of the novel *trans*-platinum derivatives are endowed with in vivo antitumour selectivity, thus showing that even platinum complexes with *trans* geometry may have a favourable toxicological and/or pharmacokinetic profile. Novel *trans*-platinum complexes are characterised by having a spectrum of activity different from that of cisplatin and are often active



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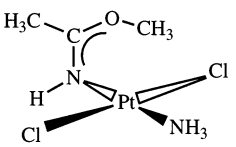

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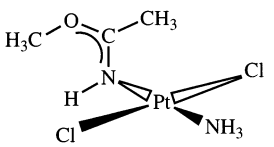

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Scheme 18.

towards cisplatin-resistant tumour cells, thus satisfying a major requirement of platinum drug discovery programs.

Since one of the most striking differences between cisplatin and its clinically ineffective isomer *trans*-DDP is the ability to form different types of DNA adducts, the DNA binding mode of antitumour-active *trans*-platinum complexes has received considerable attention. As expected, DNA adducts formed by *trans*-platinum complexes are qualitatively and/or quantitatively different from those of cisplatin, thus indicating that cisplatin-like DNA adducts are not unique determinants for cytotoxicity (and antitumour activity) of platinum drugs. According to their unique DNA binding mode (monofunctional adducts and/or interstrand cross-links between complementary bases) it is tempting to speculate that the processing of DNA structural distortions induced by active *trans*-platinum complexes by tumour cells is fundamentally different from that of distortions induced by cisplatin. In favour of this hypothesis is the observation that the damage induced by *trans* complexes with quinoline or iminoethers is not recognised by HMG proteins. Similarly, JM335 DNA adducts are not recognised by monoclonal antibodies specific of cisplatin-adducts.

Cellular pharmacology investigations provide further evidence that *trans*-platinum compounds behave differently from cisplatin. The precocious cell-cycle modification and the faster kinetic of cell death indicate that the cellular response to damages induced by active *trans*-platinum complexes is different from that induced by cisplatin and classical analogues.

The past 10 years have witnessed the successful identification of numerous determinants of cellular response to cisplatin [110,111]. It has been demonstrated that after DNA platination the final outcome of sensitive tumour cells depends upon DNA repair, cell-cycle modifications, activation of signal transduction pathways, and induction of apoptosis. However, still many aspects of cisplatin mechanism are not clear, and most likely other cellular determinants remain to be identified. From this point of view, the studies of cellular response to active *trans*-platinum complexes are still in their infancy, and they need to be continued in order to understand the reasons for tumour cell sensitivity.

In summary, the track to the discovery of antitumour platinum complexes with *trans* geometry is now disclosed. In the initial studies the main emphasis was placed on the break of classical *cis*/*trans* structure–activity relationship and its mechanistic implications. Whether the promising preclinical behaviour of *trans*-platinum complexes will lead to new platinum drugs for cancer patients will become clearer in the near future, after performing more detailed pharmacological and toxicological investigations.

Acknowledgements

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