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The influence of structure on the activity and toxicity of Pt anti-cancer drugs

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Abstract

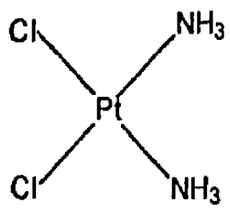
The development and current status of the relationships between the structure of platinum based anti-cancer drugs and their biological activities are reviewed. The early structure–activity relationships contributed to the development of many other active compounds and to the development of the understanding of the mechanism of activity of this class of drugs. However, they may also have contributed to the focusing on a group of compounds that has yet to produce a major advance over cisplatin or carboplatin. The recent development of new highly active platinum based drugs that do not fit the structure–activity rules indicates the need for a reappraisal of these rules. Some new structure–activity relationships are emerging but we conclude that in general it is unlikely that widely applicable rules will be sustained or be useful.

We also review the results of our recent work aimed at rationally probing the relationships between structure and activity. We describe studies aimed at determining why cisplatin does not bind to GpA sequences of duplex DNA and determining whether the GG interstrand adduct contributes to anti-cancer activity. Chiral probes of Pt/DNA interactions, cytotoxicity and other toxicities are also described. Stereoselective interactions between Pt complexes and DNA are described and the factors contributing to the stereoselectivity are discussed. © 1997 Elsevier Science S.A.

Keywords: Anti-cancer complexes; Cisplatin; Drug design; Structure–activity; Structure–toxicity

1. Introduction

The anti-cancer drug cisplatin (**1**, *cis*-$[Pt(NH_3)_2Cl_2]$) is one of the three most extensively used anti-cancer drugs in the world [1] and its use is continuing to increase with expenditure now approaching US$500 million per year [2]. It is an extraordinarily effective drug against some cancers, effecting cures in 70–90% of cases of testicular cancer, even when the disease has spread beyond the testes. It is also highly effective against ovarian cancers and contributes to the treatment of head and neck cancer, bladder cancer and lymphomas among others [1]. Recent studies have shown that in combination with new drugs, cisplatin may be effective against particularly refractive diseases including melanoma and breast cancer [1].



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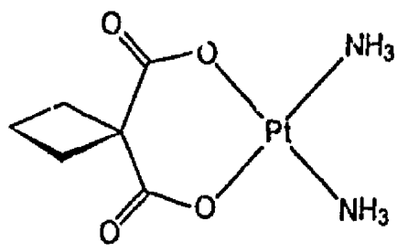
Rosenberg's discovery of the anti-cancer action of cisplatin in the 1960s [3–5] precipitated a widespread search for related complexes with similar or better activity. This search has continued largely unabated since then, motivated in part by the desire to find compounds that circumvent either or both of the natural resistance of many tumours to cisplatin and the acquired resistance of other tumours that arises following

initially successful treatment. An additional motivation has been the desirability of finding compounds that do not have the many toxic side effects that cisplatin exhibits. Improved treatment regimes including extensive hydration have overcome the nephrotoxicity (renal damage) and the use of serotonin (5-hyroxytryptamine) receptor antagonists such as ondansetron has substantially reduced the debilitating nausea and vomiting [6–9] that led some patients to refuse further treatment. However, other side effects such as neurotoxicity remain serious problems.

Somewhat disappointingly, the thousands of compounds estimated to have been prepared and tested have led to few new drugs or fundamental advances. More than 20 compounds have entered clinical trials [1] but at present only one other drug, carboplatin (**2**, *cis*-[Pt(NH_3)$_2$CBCDA], CBCDA = 1,1-cyclobutanedicarboxylic acid) is in widespread use. Carboplatin has fewer and less severe side effects than does cisplatin but both drugs probably produce the same active agent and DNA adduct profile and are cross resistant. In addition, the recent results that reveal that carboplatin may be marginally less effective against testicular cancer – in regards to long term cures – have the potential to lead to a decrease in its use against this disease. Approximately 10 compounds are currently at various stages of clinical testing and of these the orally active complex, JM216 (**3**), is particularly promising because of the new treatment regimes that it potentially allows [1]. JM216 has low nephrotoxicity [10], low neurotoxicity [11] and is not cross resistant with cisplatin or carboplatin [12] but variability in dosage associated with oral administration may limit its use [13]. Complexes of the cyclohexane-1,2-diamine ligand have also shown particular promise in pre-clinical trials but tetraplatin was rapidly withdrawn from a phase I trial because it caused severe neurotoxicity [14]. Another cyclohexane-1,2-diamine containing complex, oxaliplatin (**4**), has undergone phase II trials [15] and is reported to show some promise against colon cancer, but it too causes neurotoxicity at a severity level that might eventually limit its use [16].



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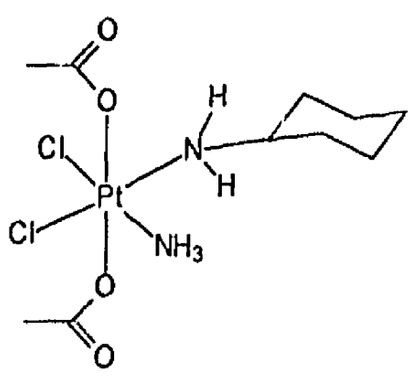

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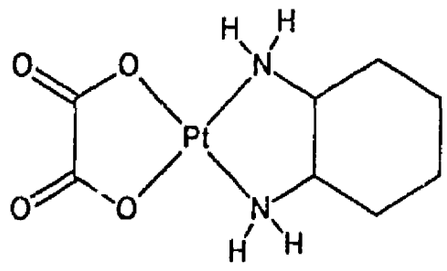


(4)

Thus, 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 might be viewed as a somewhat disappointing result given that more than 3000 compounds have been tested [1], but is not totally unexpected because in general a new drug only arises from every 10 000 compounds. It is widely hoped that rational drug design based on structural models of drug/target interactions will reduce to 1000 the number of compounds that need to be tested to yield a new drug. However, before rational drug design can be pursued, a detailed knowledge of the mechanism of action of the drug is required. Equally, if one is to "design out" toxic side effects, it is important to know what drug/target interactions are responsible for the toxicity. In the case of Pt drugs it is generally accepted that the binding of the metal to DNA is responsible for the anti-cancer activity [17] but which of the Pt/DNA adducts contribute to this activity has yet to be unequivocally established and little is known about the Pt/biomolecule interactions that are responsible for neurotoxicity for example. The approach we have taken in recent years is to use the principles of rational drug design to select compounds that can act as probes of the mechanisms of action and toxicity in order to contribute to the understanding of how activity might be increased and toxicity decreased. The primary purpose of this review is to summarise this work, however, as a prelude to this we consider it appropriate to give a brief overview of the vast body of information that has been collected over the last 25 years on the activity of Pt complexes and which potentially constitutes an ad hoc study of the relationship between structure and activity.

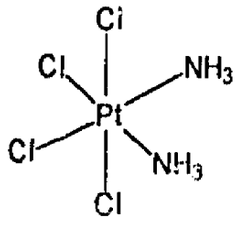
2. Structure–activity relationships

The great majority of the complexes tested have been structural analogues of cisplatin, having a *cis* geometry, two ammine or amine donor groups and two anionic leaving groups. Recently, there has been a levelling off or perhaps even a decrease in the appearance of new compounds of this type, possibly because it is beginning to appear that substantial advances are unlikely to made with these analogues. Also, it has become clear that these "me too" analogues will face difficulty in achieving widespread licensing or use unless they offer a very substantial clinical advantage. At the same time there has been an emergence of new structural types, often with promising activity and a promising lack of cross resistance with cisplatin. In this section we review the structure–activity relationships that were developed from the early studies, then go on to cover the types of exception to this relationship that have emerged in the intervening years and discuss the possibility of new structure–activity relationships being developed.

2.1. Development of the first structure–activity relationships

The first relationship between structure and activity emerged from the initial studies by Rosenberg and colleagues who found that the *cis* isomers of both $[Pt(NH_3)_2Cl_2]$ and $[Pt(NH_3)_2Cl_4]$ (**5**) interfered with the cell division in *E. coli* but

the equivalent *trans* isomers (**6,7**) were ineffective [18]. Soon after, Cleare and Hoeschele reported on the activity of a large number of Pt(II) complexes and a smaller number of Pd(II) complexes [19,20]. They confirmed that complexes with the *trans* geometry were inactive and added other criteria, stating (i) that a pair of *cis* leaving groups was necessary but not sufficient; (ii) that the complex should be uncharged; (iii) that the leaving groups should be moderately strongly bound because those with highly labile leaving groups are toxic and those with tightly bound groups were less active, though significantly the point was made that complexes with dicarboxylate ligands such as malonate were active; and (iv) that higher activity is found for those complexes where the amine groups have fewer alkyl substituents. Pd complexes were also tested and found to be inactive as were those with non-amine ligands such as pyridine or bipyridine, though the caveat was wisely added that other results might be seen with different tumour types.



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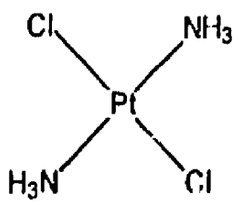

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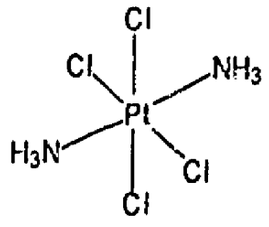
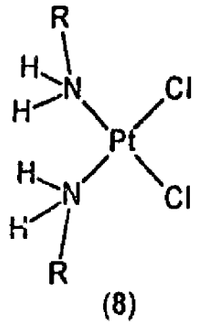


(7)

Tobe and colleagues studied a series of Pt(II) and Pt(IV) complexes with *cis* chloro and *cis* substituted amine ligands [21,22]. Large changes in activity and toxicity were observed when only small changes in structure were made. For example, in complexes of the type (**8**), on going from R=pentyl to R=hexyl the toxicity decreased by a factor of more than 6 and the activity decreased by a factor of more than 5. The role of solubility and lipophilicity was considered and it emerged for this series of complexes, that equal solubility in water and lipids correlated with minimum toxicity and maximum therapeutic index but maximum activity was observed with maximum solubility. A number of other factors were considered and apart from the confirmation of the inactivity of the *trans* geometry in bis(amine)dichloroplatinum(II) complexes, the authors concluded that "no systematic pattern of structure–activity relationships" was observed [21].



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Since then there has been little in the way of refinement or expansion of this set of structure–activity relationships. Studies on Pt(IV) complexes reveal that these are likely to be rapidly reduced in vivo [23–26] and are therefore a prodrug form

of the active Pt(II) complexes. Additionally, it has been suggested that it is essential for each of the amine ligands to carry at least one hydrogen atom for significant activity to be observed, a refinement of (iv) above [27].

The original structure–activity relationships remained valid until relatively recently because nearly all bis(amine)bis(aniono)platinum(II/IV) complexes, that have been reported to be active, conform to the "rules". Also, all of the compounds that have entered clinical trials have conformed to the rules. These early structure–activity relationships have served a purpose in that they probably facilitated the development of many active compounds and have helped in the development of an understanding of the mechanism of action of cisplatin and its analogues, but they may have also limited the focus to a set of analogues that have yet to yield a substantial improvement in clinical efficacy. Indeed it now seems likely that a quantum leap in the use of Pt based anti-cancer agents is more likely to emerge from compounds that deviate from these structure–activity relationships. Our reason for saying this is that in recent years a number of compounds have emerged that do not adhere to the structure/activity rules listed above and these compounds have activity profiles different to those of cisplatin and it close analogues.

The original structure–activity studies concluded with the suggestion that the empirical rules were "essential for the observance of anti-tumour activity" [19,20]. This conclusion, that was probably intended to apply only to the classes of complexes considered, has sometimes been invoked more prescriptively. Clearly, it would be wrong to expect that all Pt complexes should adhere to a single set of structure–activity rules any more than all sulphur containing drugs would and this has been confirmed by recent results that we now describe.

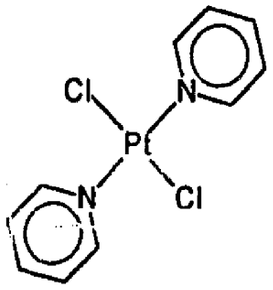
2.2. *Rule breakers*

Most of the structure/activity rules listed above, or corollaries of them, have now been broken. Specifically; (i) that *trans* compounds are inactive; (ii) that charged compounds are inactive; (iii) that complexes with Pt atoms having only one leaving group are inactive; (iv) that only complexes with two amine ligands each carrying at least one H atom are active; and (v) that Pd complexes are inactive. Each of these will now be discussed in turn.

2.2.1. *Active trans* compounds

At least three groups have independently reported active *trans* compounds. Farrell and colleagues have described three classes of complexes having the general form $[PtCl_2LL']$; (i) L=L′=pyridine or thiazole (e.g. (**9**,**10**)); (ii) L=quinoline, L′=substituted sulphoxide (R′R′SO) (e.g. (**11**)); and (iii) L=quinoline, L′=NH_3 (e.g. (**12**)) [28–30]. Examples of each of these groups have been shown to have high in vitro cytotoxicity [28–30] and some have modest but significant *in vivo* activity [31]. One of the most remarkable results to emerge is that replacement of a single NH_3 of *trans*-DDP by a quinoline, to give $[Pt(NH_3)(quinoline)Cl_2]$ (**12**), leads to a dramatic increase in activity and indeed a Pt(IV) variant of this complex (**13**) has in vivo activity approaching that of cisplatin [31]. It is well known that *trans*-DDP

is more reactive than cisplatin and deactivating side reactions on route to the target are likely to at least contribute to its lack of anti-cancer activity. Farrell has argued that the activation of the *trans* geometry can be effected by reducing the rate of replacement of the chloro ligands [31]. This, he suggests, can be achieved by blocking axial access to the Pt atom, inhibiting formation of the five coordinate intermediate that leads to ligand substitution. Indeed, the structure of the quinoline complex (**12**) reveals a close contact between the H8 and Pt atoms of 2.77 Å [32]. Substitution studies confirm the slower reaction kinetics in the case of the analogous quinoline complex (**14**), however, similar reaction kinetics are observed for the isoquinoline complex [33] where axial access to the Pt atom is less hindered. Taken with the high in vitro activity of *trans*-$[Pt(py)_2Cl_2]$, this suggests that differences in the bonding of aromatic ligands are at least equally important.



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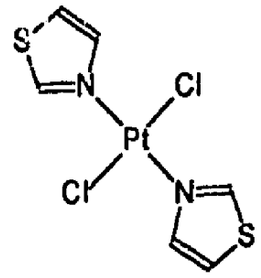

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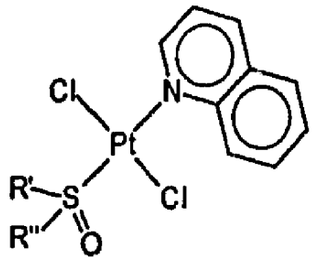

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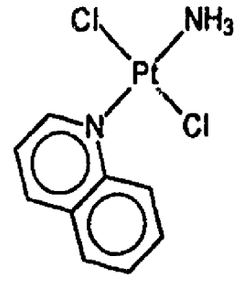

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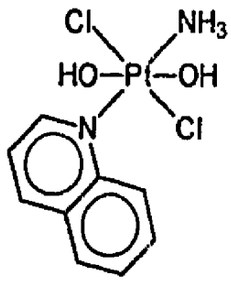

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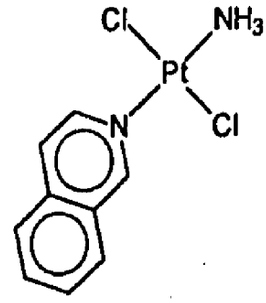

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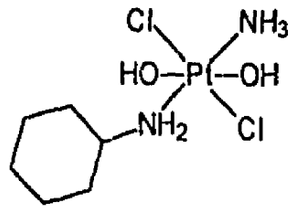
Kelland et al. have shown that a series of Pt(II) complexes of the types *trans*-$[Pt(NH_3)(RNH_2)Cl_2]$ and *trans, trans, trans*-$[Pt(NH_3)(RNH_2)(OH)_2Cl_2]$ are highly active in vitro, those with R = cyclohexyl being as active as cisplatin. Of these the Pt(IV) complexes where R = cyclohexyl (JM335 (**15**)), cycloheptyl and 1-adamantyl were also active in vivo [34]. In these cases, the differences in the bonding of the cyclohexylamine ligand compared to that of the ammine ligand

are unlikely to be significant and it may be that the steric bulk of the cyclohexylamine group plays a role in activating this compound. Indeed, we have found that in energy minimised models of cyclohexylamine complexes the shortest Pt···H contacts are in the range 2.8 to 2.9 Å. More curious though is the reported inactivity of the closely related *trans*-[$Pt(RNH_2)_2Cl_2$] including the complex with R = cyclohexyl [21].



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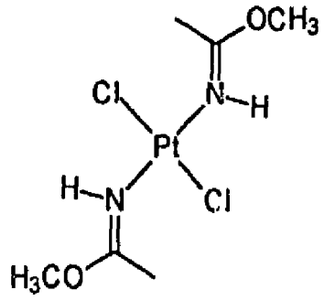
Coluccia et al. have reported an active *trans* complex with iminoether ligands, *trans*-[$Pt\{E\text{-}HN{=}C(OMe)Me\}_2Cl_2$] (16) [35,36] that is more active than its *cis* analogue. The methyl groups of the ligands lie over the coordination plane potentially restricting access to the Pt atom and reducing its reactivity but interestingly, in the *trans* isomer both methyl groups lie on the same side of the coordination plane but in the *cis* isomer they lie one on each side of the coordination plane [37]. Thus, there is not an obvious correlation between the ligands interfering with access to the Pt atom and the activity of the complex.



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2.2.2. Charged compounds

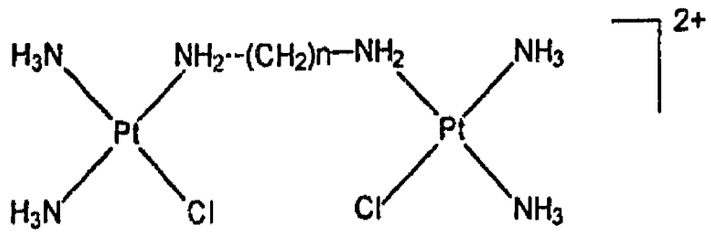
Early studies found that charged compounds were less active and it was proposed that neutrality was essential for high activity. This proposal accorded with the hypothesis that platinum compounds were taken up into cells by passive transport through the lipophilic cell wall since this pathway is less available to charged compounds. Also, charged compounds are generally eliminated from the body more rapidly. However, a small number of active charged complexes have now been reported. These include the positively charged bisplatinum complexes discussed below but also include negatively charged complexes. For example [PPh_3Me][$PtCl_3$(caffeine)] has been found to be active in an *in vivo* study of P388 leukaemia in mice [38]. Also, Cleare and Hoeschle noted that $[Pt(NH_3)Cl_3]^-$ was a possible exception to the requirement for neutrality but suggested that substitution of one Cl^- by a water molecule resulting in a neutral complex accounts for its anomalous activity [20] and the same argument would apply to the caffeine complex.

Hollis and colleagues prepared and investigated a series of 32 cationic complexes of the type *cis*-$[Pt(NH_3)_2(Am)Cl]^+$ where Am was a pyridine, a pyrimidine, a purine, a piperidine or a saturated amine (RNH_2) ligand [39]. A number of the complexes demonstrated activity similar to or higher than that of cisplatin in in vivo murine tumour models with the most active being those where Am was, pyridine, a 4-substituted pyridine, cytosine or 2′-deoxyguanosine. In discussing the unexpectedly high activity of these apparently monofunctional and charged agents the authors commented that the possibility of loss of one of the ammine ligands to give bifunctional agents needed to be considered especially since Lippert and colleagues had previously reported the loss of ammonia *trans* to 3-methylcytosine on heating in aqueous solution [40]. Since the ammine lost on heating was that *trans* to chloride, generating *trans*-$[Pt(NH_3)(Am)Cl(X)]$, at that time assumed to be inactive, it was concluded that this mechanism probably was probably not responsible for the antitumour activity of the $[Pt(NH_3)_2(Am)Cl]^+$ complexes [39]. Hollis et al. later showed that $[Pt(NH_3)_2(4\text{-Br-pyridine})Cl]^+$ also underwent hydrolysis yielding *trans*-$[Pt(NH_3)(4\text{-Br-pyridine})Cl_2]$ and reported that this complex was inactive against Sarcoma 180 in mice [41]. Consequently, they concluded that monofunctional adducts formed by these complexes were responsible for the anti-cancer activity. However, as described above, Farrell and colleagues have since demonstrated that closely related *trans* complexes such as *trans*-$[Pt(NH_3)(quinoline)Cl_2]$ are highly cytotoxic [28–30] and, thus, it seems plausible that loss of an ammine resulting in active bifunctional agents may indeed contribute to the activity of these compounds. It appears [31] that solubility may be an important factor in determining the activity of *trans*-$[Pt(NH_3)(Am)Cl_2]$ complexes and thus *trans*-$[Pt(NH_3)(4\text{-Br-pyridine})Cl_2]$ might be inactive when tested itself but still be produced in situ from $[Pt(NH_3)_2(4\text{-Br-pyridine})Cl]^+$ and be responsible for the activity of this latter complex. Gean et al. have reported active cationic complexes of the type $[Pt(Am)_2LCl]^+$ (where $Am = NH_3$ or Am_2 = ethane-1,2-diamine and L = anthraquinone-Y-$(CH_2)_n$–NH_2, Y = NH or O [42]) and these are discussed further below.

2.2.3. Complexes with monofunctional Pt atoms

The positively charged bisplatinum complexes (**17**, **18**) described by Farrell and colleagues [43] also break the rule that the Pt atoms should have two leaving groups. However, it has to be said that all of the compounds are potentially bifunctional in the sense that there are two platinum atoms, each of which can bind to DNA. The high activity of some of these charged bisplatinum complexes raises the question of how they enter the cell. They have a structural similarity to spermidine and spermine, both of which are charged at physiological pH and it is at least plausible that the bisplatinum complexes enter the cell by the spermidine/spermine pathways.

The apparent requirement for bifunctionality in Pt complexes remains an open question. Only the compounds reported by Hollis et al. seem to break this rule and even there, there is room for doubt.



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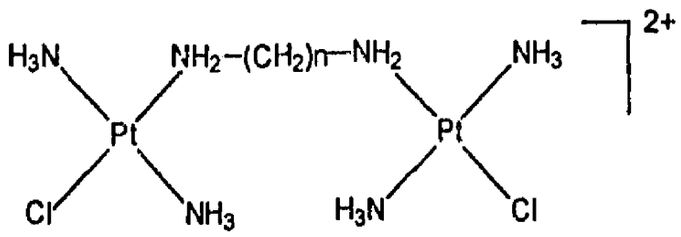

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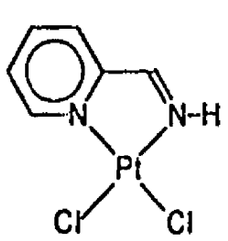
2.2.4. Complexes with non-amine neutral ligands

Cleare and Hoeschle noted a clear preference for amine ligands with higher activity being associated with lower levels of alkyl substitution and they also reported the low activity of complexes with pyridine like ligands [19,20]. Subsequently, the suggestion has been made that each of the N donor atoms should have at least one H atom to facilitate hydrogen bonding to DNA [27]. However, a number of active complexes with pyridine or imine like ligands have been reported. These include some of the *trans* compounds described above but also include a variety of complexes with *cis* geometries. For example a series of pyridyl- and quinoline-amines and -imines have been described by Brunner et al., some of which (e.g. **19**, **20**) show in vitro activity comparable with that of cisplatin [44]. Deacon and colleagues have described an even more unusual series of complexes that are active against Pt resistant and human tumour cell lines [45]. Some of these (e.g. **21**, **22**) not only have pyridine ligands but also have amide or amine/amide ligands with no H(amine) atoms. It appears from recent results that these compounds may lose the bidentate amide/amine on binding to nucleotides and hence act as agents for delivery of the $Pt(pyridine)_2^{2+}$ moiety [46]. Broomhead and Rendina have reported active bisplatinum complexes where the linking ligands are 4,4′-dipyrazolyl ligands (**23**) [47,48] and Reedijk et al. have recently described an active monomeric complex with a 2,2′-biimidazole ligand (**24**) [49].



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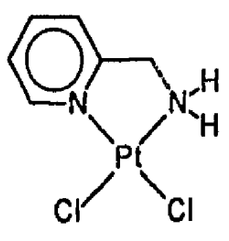

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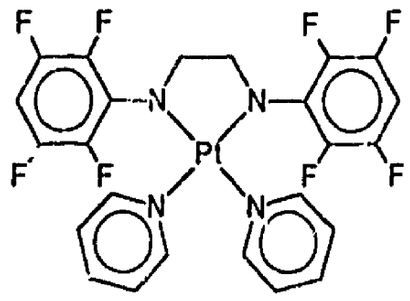
We have previously put forward the hypothesis that the H(amine) atoms are not essential for activity but rather it is their replacement with alkyl groups resulting in



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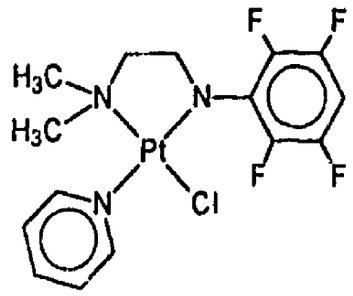

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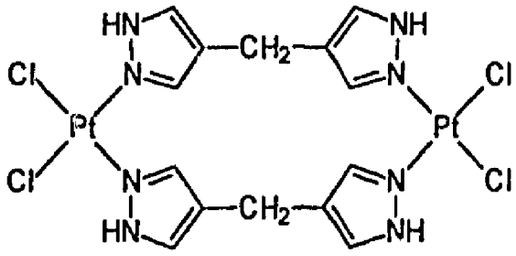

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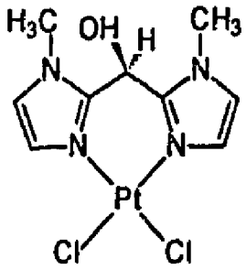

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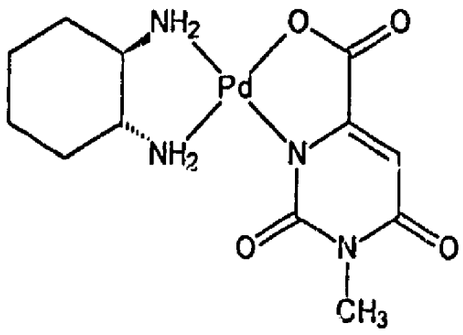
steric clashes with DNA that interferes with DNA binding and thus reduces anti-cancer activity [50]. The high activity of these complexes is in accord with this hypothesis

2.2.5. Active palladium complexes

A number of groups have reported moderately active Pd complexes but none has yet proven sufficiently active to warrant clinical trials. A feature common to all of these complexes is that only those with bidentate amine ligands were active, the suggestion being that complexes with monodentate ligands isomerised rapidly to the inactive *trans* geometry [51]. For example, $[Pd(en)(NO_3)_2]$, $[Pd(trans\text{-dach})(NO_3)_2]$ [51] and $[Pd(trans\text{-dach})(SCN)_2]$ [52] were found moderately active and more recently [Pd(*trans*-dach)(3-methylorotato)] (**25**) has been found to have an in vivo activity similar to that of cisplatin [53]. In this case too, an analogue with monodentate ligands, $[Pd(NH_3)_2$(3-methylorotato)], was found to be inactive confirming that the potential for isomerisation limits the activity of many Pd complexes. It has long been assumed that the inactivity of Pd(II) complexes is related to their more rapid reaction kinetics, when compared to Pt(II), leading to deactivation by reaction with biochemicals other than DNA and to a lower stability of the Pd/DNA adducts. In this context, the lower substitution rates for SCN^- compared with Cl^- might contribute to the stabilisation and activity of $[Pd(trans\text{-dach})(SCN)_2]$ [54]. In the case of the active $[Pd(en)(NO_3)_2]$ and $[Pd(trans\text{-dach})(NO_3)_2]$ complexes, the stability might arise from the rapid loss of the nitrato ligands and the formation of inert hydroxo bridged complexes [51].



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2.3. Corollaries of the structure–activity relationships

Less attention has been paid to the corollary of the structure–activity relationship, viz whether a complex that meets all of the criteria proposed by Cleare and Hoeschle can be expected to be active – probably because inactive compounds are less frequently reported on. That exceptions to the corollary of structure–activity rules have the potential to be informative is a paradigm of drug development, but almost no attention has been paid to Pt complexes that have all the structural features associated with high activity but are unexpectedly inactive and this is a point we come back to later in the review.

Braddock *et al.* found a number of bisaminedichloroplatinum(II) complexes that were hundreds of times less active than cisplatin and other closely related complexes [21]. For example, the compound of type (8) with R = octyl is nearly 20 times less active than the compound with R = hexyl and when R = 2-adamantane the activity drops a further factor of 4. Some of the complexes with branched aliphatic chains were also found to have low activity. It is possible that variations in lipophilicity contribute to this variation in activity but it was noted that there was no obvious correlation with solubility [21].

Cleare and Hoeschle noted that as substitution on the N(amine) atoms increased, activity tended to decrease [20]. Since then there has been frequent speculation as to whether this loss of activity was due to the additional substituents resulting in a lower solubility, interfering with DNA binding or some other factors. A detailed comparison of the behaviour of highly active and highly inactive complexes has the potential to clarify those factors that are most important in determining the anti-tumour activity of Pt(II) complexes.

2.4. New structure–activity relationships

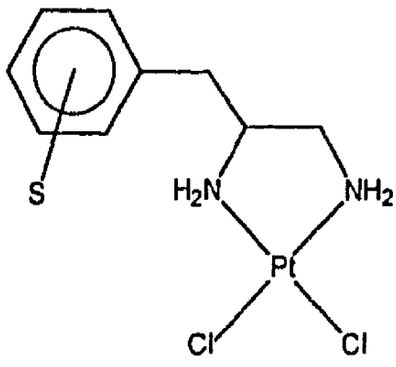
A systematic analysis of the activities of the thousands of Pt complexes tested might produce new structure–activity relationships and new insights into the mechanisms of action of Pt drugs. However, there are a number of complicating factors. Most of the early tests were carried out on mice implanted with animal tumours. More recently, in vitro tests (on cultured cells) have come to dominate. Initially mouse leukaemia cell lines such as L1210 and P388 were used but these have increasingly been replaced by a variety of human tumour cell lines. This approach

is exemplified by the US NCI change from animal based testing to testing against a panel of 60 human tumour cell lines. The use of in vitro testing has allowed a far greater number of compounds to be tested and more tumour types to be investigated allowing appropriate targets to identified. However, many cell types are in use and the activity of a given compound can vary dramatically against different cell lines making the development of simple structure/activity relationships more difficult. However, some groups have undertaken systematic studies of a series of compounds and some interesting results have emerged. We now discuss a selection of these results in order to highlight what we see as emerging points.

2.4.1. *Variations in the amine ligand*

Groups in the Institut für Pharmazie and the Institut für Anorganische Chemie at the Universität Regensberg have studied an extraordinarily large series of complexes in which the diamine ligand is derived from ethane-1,2-diamine. This is a particularly valuable set of results because most compounds have been tested in the same systems; in vitro cultures of the breast cancer cell line MDA-MB 231 and implants of the P388 leukaemia in mice.

For example, 30 compounds of types **26** and **27** were tested. The most active compounds in the *in vivo* model were those with no substituents on the phenyl ring. However, complexes with halo substituents were also found to be highly active with *ortho* substitution being more efficacious than either *meta* or *para* substitution. Higher activities were obtained by combining fluoro and chloro substituents. Increasing the length of the linker between the ethylene bridge and the phenyl group led to a decrease in activity. Addition of a methyl substituent to N_1 also decreased activity but addition to N_2 increased activity. These variations indicate very subtle dependency of activity on structure and it is clear that for a greater understanding to be developed, further information is required on cellular uptake, DNA binding and DNA repair as function of the structure of these complexes.



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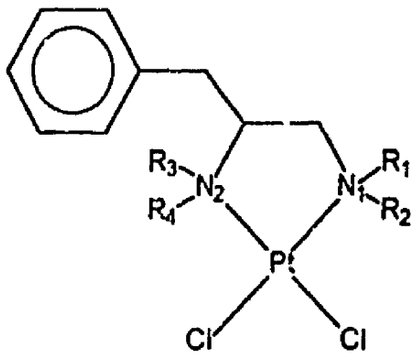

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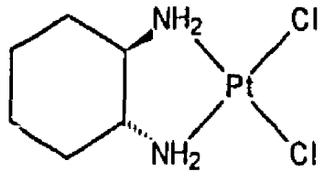
2.4.2. *Chiral compounds*

Differential activity or toxicity arising from the enantiomers of chiral compounds can be particularly informative with respect to the mechanisms involved because the differences are indicative of enantioselective interactions with chiral transport agents, deactivating agents or targets. A number of chiral Pt complexes have been resolved

and tested separately. The earliest of these were the cyclohexane-1,2-diamine (**28**) complexes reported by Kidani and colleagues [55,56]. Modest differences in activity were observed though it is now accepted that these were probably not significant. Significant differences in terms of mutagenicity of the same compounds have been reported. Since then other groups have reported very substantial differences in the mutagenicities of the *R,R*, *S,S* and *R,S* isomers of [Pt(2,3-diaminobutane)Cl_2] and [Pt(1,2-diaminopropane)Cl_2] [57] as outlined below.



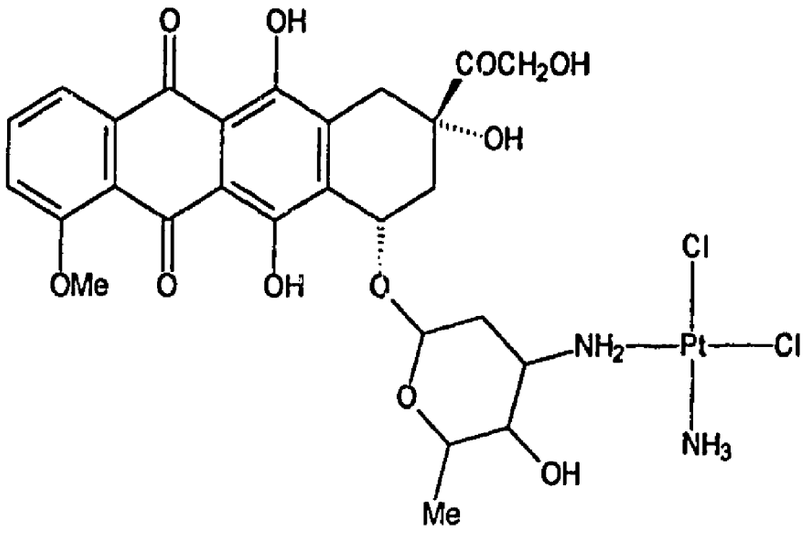
(**28**)

The Regensberg groups have also reported enantioselective differences in activity for complexes of a number of variants of the substituted ethane-1,2-diamine ligands referred to above. For instance, the racemic stereoisomers (*R,R* and *S,S*) of [Pt(bis{3-hydroxyphenyl}ethane-1,2-diamine)Cl_2] and [Pt(bis{4-hydroxyphenyl}ethane-1,2-diamine)Cl_2] were more active than the *meso* isomer (*R,S*) and of these the *S,S* enantiomers were the more active in terms of both in vitro activity against the breast cancer cell line MDA-MB 231 and *in vivo* activity against a number of leukaemias implanted in mice [58]. The ligands in these compounds are thought to mimic oestrogen receptor binding compounds such as hexestrol and the differences in activity may well reflect enantioselective interactions between the complexes and the oestrogen receptor [59]. Confirmation of a correlation of this type would give insight into the factors that can influence activity and aid in the development of more active compounds.

2.4.3. Compounds with bioactive carrier groups

The attachment of bioactive carrier groups represents a rational approach to developing Pt complexes with higher activity and/or lower toxicity. This approach has been trialed by a number of groups but to date it has not yielded significant clinical advances. For example, a number of groups have attached DNA intercalators with the expectation that this will increase the localisation of the drug in the vicinity of its ultimate target. Pasini attached doxorubicin via its amine group to give (**29**) [60,61] and Denny and colleagues attached anilinoacridine and acridinecarboxamide to ethane-1,2-diamine groups to give (**30**) and (**31**) [62,63]. A result common to these studies was improved activity against cisplatin resistant cells lines compared to that of the parent compound but no improvement relative to the ligand alone. Gibson and colleagues have investigated a series of complexes with anthraquinone intercalators (**32**, **33**) attached and have also reported promising activities [42]. Recently a common structure–activity relationship has emerged from studies on substituted anthraquinones and acridines by the Gibson and Denny groups, respectively. Both have compared the activity arising when the group that tethers the intercalator to the Pt moiety is attached to the 1 or 4 position (**31**, **32**) with that

arising when it is attached to the 2 position (**30**, **33**) and found that the former leads to high activity and the latter to low activity with differences of up to an order of magnitude [63,64]. This too is a relationship that deserves further attention.



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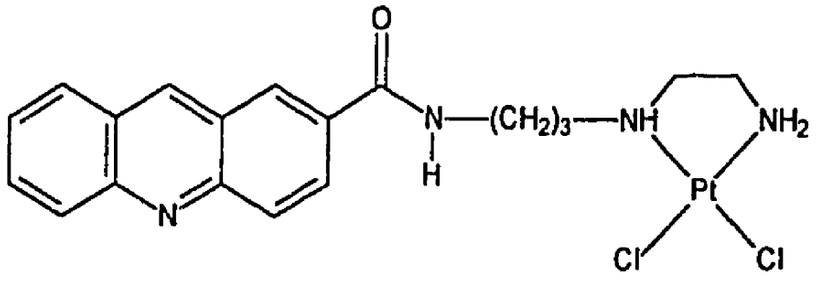

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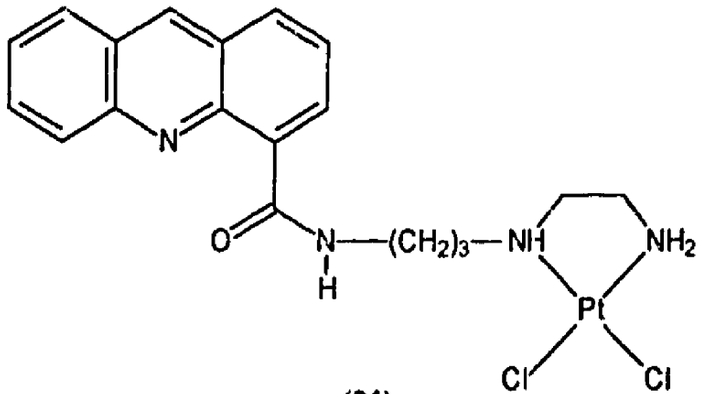

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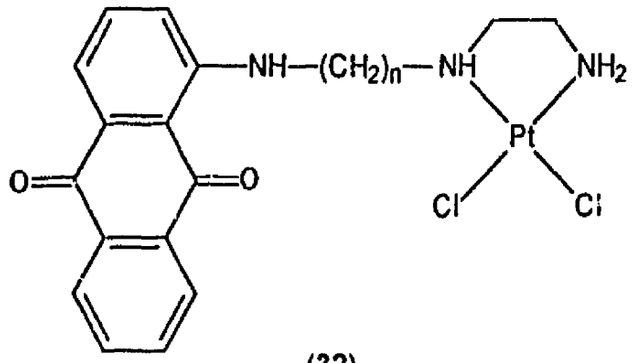
A number of groups have used carrier groups that bind to other targets such as oestrogen analogues that bind to oestrogen receptors [59,65,66]. The logic behind this approach is to target cells with high numbers of hormone receptors, a feature of some breast and prostate cancers for example. Others have attached amino acids, sugars and antitrypanosomatid drugs [67,68]. The results obtained are encouraging but insufficient to develop structure-activity relationships. However, there is the potential for variation in the structure of the carrier group to be correlated with activity of the complex as is shown by the work of the Regensberg groups. In



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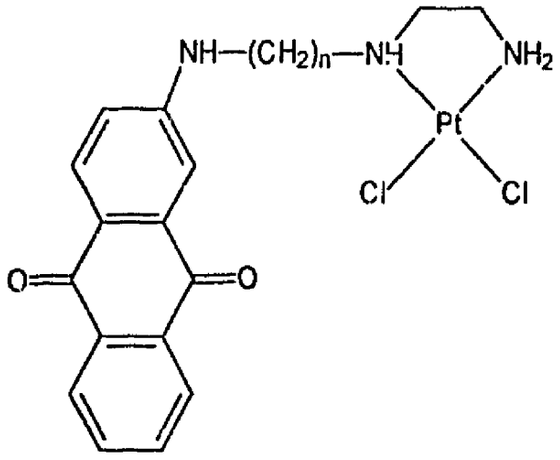

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addition to the possibility of new drugs arising in this way, valuable insights into factors determining activity can be expected.

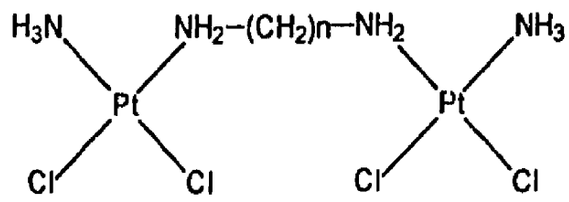
2.4.4. The relationship between lipophilicity and activity

Lipophilicity, commonly measured as solubility in or partitioning into non-polar solvents such as octanol, can be an important determinant of activity because passive uptake across the lipid bilayer making up the cell membrane, is facilitated by higher lipophilicity. Braddock et al. [21] reported a correlation between lipophilicity and toxicity with minimal toxicity being associated with moderate water and octanol solubility but activity was associated with maximal water solubility. Lipophilicity has also played a major role in the development of the new orally active Pt(IV) complexes where high lipophilicity was considered to be important for optimising uptake through the intestine. Pt(IV) complexes were chosen because they offered additional sites for altering lipophilicity and the effect of varying the axial carboxylate groups and the equatorial amine group has been investigated. More than 500 compounds were studied in the lead up to the development of these compounds [69]. Kelland et al. reported a steady increase in cytotoxicity with increasing length of the aliphatic chain attached to the axial carboxylate groups, up to C_{11} and a levelling off after this up to C_{20} [70]. Similarly, activity increased with the size of the alicyclic ring attached to the equatorial amine up to cyclohexane but decreased for larger rings. Increased intracellular accumulation was shown to be at least partially due to increased cellular uptake [70]. Siddik and colleagues reported similar results on similar compounds, observing that uptake correlated with partitioning

into octanol but they also noted that intracellular DNA binding was a better predictor of activity than was cell uptake [71,72]. Whether the results on cellular activity and uptake for the Pt(IV) complexes are biologically relevant is debatable because they are believed to be rapidly reduced in vivo and thus lose the axial ligands that appear to mediate in vitro uptake.

2.4.5. *Bisplatinum compounds*

Farrell and colleagues have now described and investigated a series of bisplatinum complexes [43,73,74]. More recently other workers have described similar bisplatinum compounds but with linking groups other than simple alkanediamines [75]. A number of these compounds have demonstrated high in vivo activity and there is good reason to hope that they represent a new class of platinum drugs. Clearly, there is the potential for structure–activity relationships to be developed for this new class. Some of these relationships such as those with reactivity and lipophilicity might be expected to correlate with those of cisplatin analogues but other quite different factors should emerge. Most obvious of these are the relationship between activity and the length and nature of the linking group. Indeed Farrell and colleagues have already reported a relationship between chain length and activity in compounds such as **34** and **35** [43,73]. In bisplatinum complexes such as **17** and **18**, with monofunctional Pt atoms, the activity has been found to depend on whether the leaving group lies *cis* or *trans* to the linking chain [74,76].



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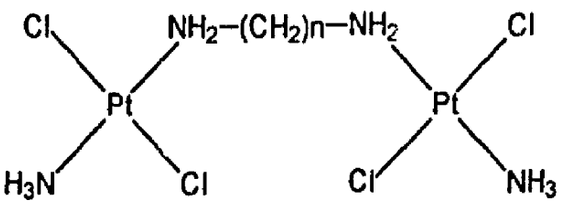

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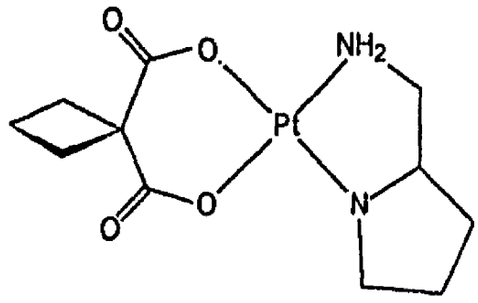
2.5. *Structure–toxicity relationships*

There have been substantially fewer studies directed toward establishing systematic relationships between structure and toxicity. This is perhaps surprising in that a decrease in some of the still problematic toxicities would be of value in the clinic. Most in vivo studies report toxicity values and structure–toxicity relationships have been drawn but these toxicities usually relate to animal morbidity rather than specific side-effects relevant to clinical application. An interesting enantioselective toxicity has emerged from preclinical studies leading to the development of DWA2114*R* (**36**)

where it was found that the *S* enantiomer (DWA2114*S*) exhibited higher nephrotoxicity in rats and this was associated with a five-fold higher concentration of platinum in the kidneys [77]. The anti-tumour activities of the two enantiomers were similar and therefore the *R* enantiomer was chosen for clinical trials where it has been found to have low nephrotoxicity [78].



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Another structure–toxicity relationship that has emerged is the, apparently general, high neurotoxicity of complexes of the *trans*-cyclohexane-1,2-diamine ligand. Thus, tetraplatin was withdrawn from clinical trials [14] and significant neurotoxicity has been reported in the clinical trials of oxaliplatin [16]. It seems at least plausible then, that compounds with lower neurotoxicity might be identified and be of clinical value and indeed the new, orally administrable Pt(IV) complex, JM216, does exhibit lower neurotoxicity in the rat [11]. Further progress toward the goal of finding less neurotoxic complexes is described below.

A significant reason for the lack of structure–toxicity studies is the lack of suitable in vitro or in vivo models for the various toxicities or the difficulty in carrying out such experiments. In the case of anti-tumour activity, there are numerous cultures of human tumours available and tumours can be readily implanted in rodents and these give data that is at least indicative of likely clinical utility. Recently, models that allow the measurement of neurotoxicity have become available and this has allowed for the studies described above and below.

One other toxicity that has received some attention is mutagenicity as this can be readily measured by the Ames test. Mutagenicity can be indicative of carcinogenicity, a not unexpected problem with drugs that bind to DNA. Cisplatin has been found to be highly mutagenic [79] and there is some evidence of increased numbers of cancers appearing in cisplatin treated patients [80,81]. If carcinogenicity proves to be a problem it will be of unusual significance because of the frequency with which cisplatin is used to treat young men for testicular cancer and the otherwise excellent long-term prognosis these patients have.

Coluccia et al. have reported that among a series of diam(*m*) inedichloroplatinum(II) complexes, those with mondentate amines such as NH_3 or α-methylbenzylamine were highly mutagenic but those with bidentate amines such as ethane-1,2-diamine and *N,N'*-bis(α-methylbenzyl)ethane-1,2-diamine were substantially less mutagenic [82]. In an extension of this work Fanizzi et al. reported a variation in mutagenicity with chirality with the *S* forms of the complexes of cyclohexane-1,2-diamine, butane-2,3-diamine and propane-1,2-diamine being more

mutagenic [57]. Similarly, Leopold et al. found that the *trans*(+) isomers of $[Pt(dach)Cl_2]$ and $[Pt(dach)(SO_4)]$ were substantially more mutagenic than the *trans*(−) or *cis* isomers [79]. They also found that these complexes were carcinogenic in mouse models but the only clear structure–activity relationships to emerge were the high carcinogenicity of cisplatin, the low carcinogenicity of *trans*-DDP and the intermediate carcinogenicity of the dach complexes [79]. It has been established that the GpG adduct formed by cisplatin is both mutagenic and genotoxic [83] so the possibility exists of establishing correlations between adduct levels and mutagenicity.

2.6. Summary

It is clear from the foregoing that the relationship between structure and activity is extremely complex and it seems unlikely that any new widely applicable relationships will emerge. This is not surprising when the many factors that can influence activity are taken into account. For instance, reactions prior to cell uptake, the rate and mechanism of cell uptake, deactivation prior to DNA binding, the rate of DNA binding, the adduct profile that results, and the repair and removal of the DNA adducts all have the potential to profoundly influence the activity of the complex. Each of these aspects can also differ substantially between one tumour type and another and the response of different tumours, even if all other factors remain the same, can be dramatically different. Therefore, simple unifying structure–activity rules, where they do emerge, are likely to be indicative of classes of compounds that act by similar mechanisms, but, in the absence of a knowledge of which of the foregoing factors are critical, such relationships are of little aid in moving rationally toward an improvement in activity.

If the primary long term goal is to improve activity in presently resistant disease then what is needed is a detailed knowledge of the role each of these activity determining factors plays and how they vary from one tumour to the next. For instance, if it can be established that a particular tumour is insensitive to Pt drugs because of reduced uptake, then a relationship between structure and uptake can be sought and used to rationally develop compounds that overcome the reduced uptake.

However, it is still generally true that compounds that adhere to the structure–activity rules can be expected to be active. It is likely that compounds that are active but do not adhere to these rules operate by different mechanisms. Thus, structure–activity relationships are potentially useful for categorising complexes into groups with common mechanisms of action. Also, compounds that do adhere to a given set of structure/activity rules but are inactive can be particularly useful in the gaining of an understanding of the factors that influence activity as we discuss further below.

3. Designing and evaluating probes of the relationship between structure and activity

Rational design and development of drugs can only be based on a knowledge of the drug/target interaction. However, it has yet to be unequivocally established which of the Pt/DNA adducts are responsible for cytotoxic activity and which are

responsible for mutagenicity. The approach we have adopted in recent years has been to use molecular modelling to investigate aspects of Pt/DNA interactions and to design new complexes to interact with DNA in a sequence specific or stereoselective way. We have then prepared these new complexes and undertaken extensive investigations into their chemical, biochemical and biological activities in order to develop a detailed understanding of the relationship between their structures and their biological activities. In the following sections, three aspects of this work are discussed in detail; (i) the reasons for the non-formation of the GpA adduct when cisplatin binds to duplex DNA, (ii) the role of interstrand adducts and (iii) chiral Pt complexes as probes of neurotoxicity, mutagenicity and the cytotoxic role of the intrastrand GpG and ApG adducts.

3.1. Modelling Pt/DNA interactions

We have previously reviewed the role modelling has played in the study of Pt/DNA interactions [84]. Here we briefly describe the features observed in our recently refined models of the GpG intrastrand adduct and discuss how these features correlate with the structure–activity relationships described above.

A major motivation behind the early modelling studies was the lack of an experimentally determined structure of a bifunctional Pt/duplex DNA adduct. This has recently been remedied by Takahara et al. [85] and some unexpected features in this structure have necessitated a revision of the force fields used to generate our earlier molecular models. One of the most surprising features of this structure is the geometry about the N7 atoms coordinated to the $Pt(NH_3)_2^{2+}$ moiety. Rather than the Pt atoms causing gross distortion of the DNA structure, the geometrical demands of the Pt atom are accommodated by it lying 1.2 and 0.8 Å out of the 5′ and 3′ guanine planes, respectively (Fig. 1(a)) [85]. The guanine planes are canted by 26° with respect to each other, substantially less than expected and less than predicted by molecular mechanics models. Thus, a reappraisal of the force field relating to the Pt/guanine interactions was required. By a fortunate coincidence, we have just completed the development of a molecular mechanics force field for other highly distorted Pt systems where the Pt lies similar distances out of the planes of aromatic ligands [86]. Transferral of this force field to that for Pt/DNA systems generated a model that reproduced very well the features of the crystal structure in that the Pt atom lies 1.04 and 0.78 Å out of the planes of the guanine bases and these bases are canted by 26.5° with respect to one another (Fig. 1(b)). In previous models, hydrogen bonds between the ammine ligands and O(phosphate) and O6(guanine) atoms were observed and it was postulated that it was these interactions that led to the requirement described above that there be at least one hydrogen atom on each amine group. In the crystal structure and our new model neither hydrogen bond is as evident (e.g. H···O6 2.54 Å). However, the DNA conformation is unusual, being A form on the 5′ side of the GpG adduct and B form on the 3′ side and in models of B-DNA generated using this revised force field the hydrogen bond to the O6(guanine) atom is again in evidence (H···O6 2.10 Å).

It appears from the crystal structure and these models that the hydrogen bonds

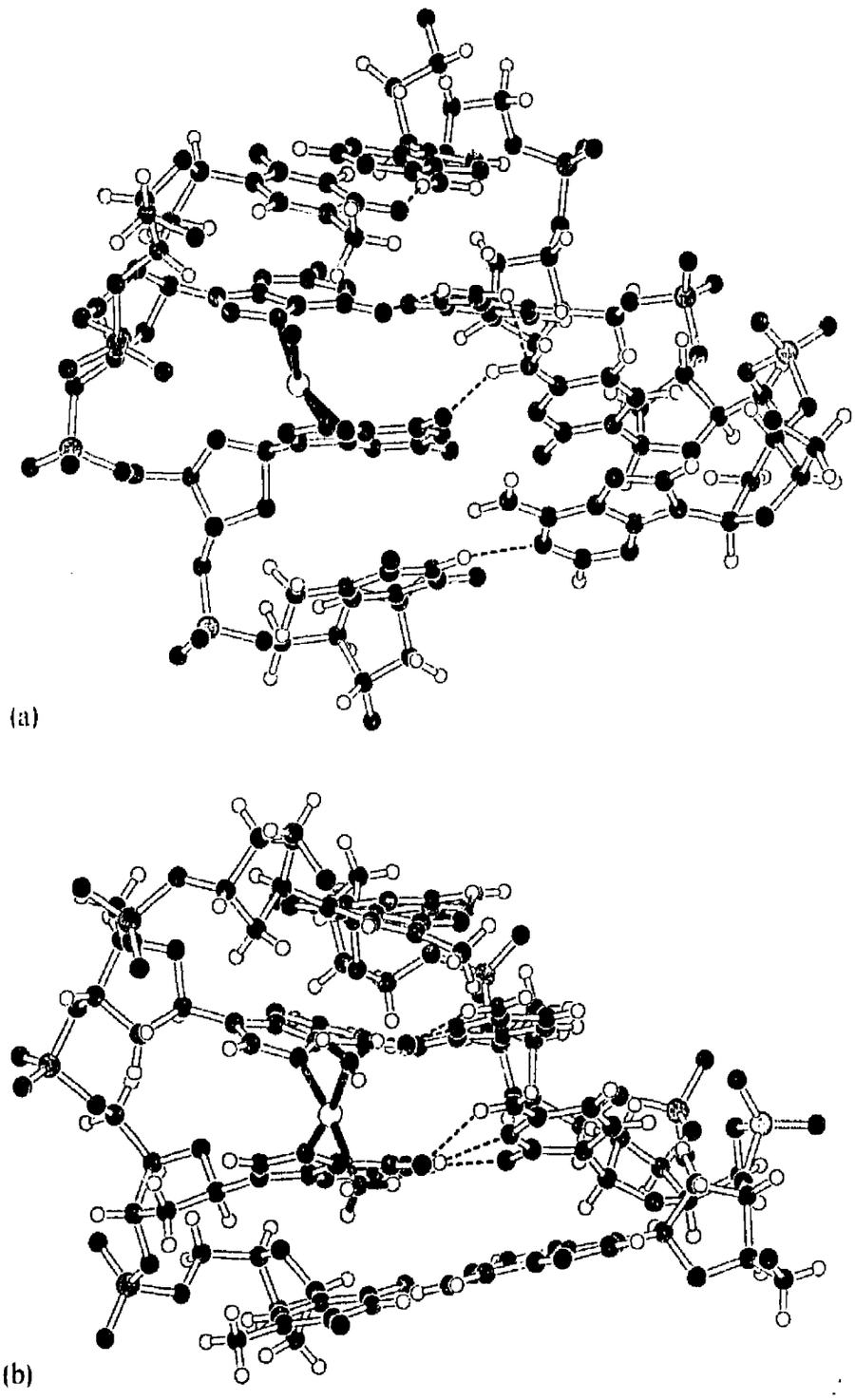


Fig. 1. (a) Central four base pairs from the crystal structure of $Pt(NH_3)_2$: (5′-d(CpCp(Br)UpCpTpG*pG*pTpCpTpCpC)-3′, 5′-d(GpGpApGpApCpCpApGpApGpG)-3′) [82]. (b) Central four base pairs from the molecular mechanics model of $Pt(NH_3)_2$: (5′-d(TpCpTpG*pG*pTpCpTp)-3′, 5′-d(ApGpApCpCpApGpAp)-3′).

are not important stabilising aspects of the GpG intrastrand adduct and we have argued previously that what is important is the avoidance of unfavourable clashes with the DNA and in particular with the exocyclic substituent in the 6-position of purines [50,84,87]. Replacement of all hydrogen atoms of the ammine ligands with methyl groups in the models described above results in contacts as short as 1.4 Å between the H(methyl) and O6(guanine) atoms. Also, this view is consistent with

the high activity of complexes with pyridine and related ligands because these cannot form hydrogen bonds but present no steric impediment to binding.

3.2. Why doesn't cisplatin form GpA adducts when it binds to DNA?

As mentioned previously, when cisplatin, or $[Pt(en)Cl_2]$ binds to DNA it forms two major bifunctional intrastrand adducts; one with GpG sequences that accounts for 60% or more of the Pt bound to DNA and one with ApG sequences that accounts for about 25% of the Pt [88–92]. Curiously, no measurable level of GpA adducts is observed. Chemically, the ApG and GpA sequences are identical in that each presents the Pt atom an N7(guanine) atom and an N7(adenine) atom for coordination. Indeed, when cisplatin is reacted with the trinucleotide ApGpA, both ApG and GpA adducts are observed. Thus, the non-formation of the GpA adducts when cisplatin is reacted with duplex DNA is evidently due to structural features associated with duplex DNA. Clearly, an understanding of how the structure of DNA can lead to sequence selectivity in its interactions with cisplatin would be of great value in developing an understanding of the factors that mediate Pt binding to DNA.

Dewan noted some time ago that when a Pt atom is bound to the N7(guanine) atom in an ApGpA sequence of idealised B conformation duplex DNA, that the distance from the Pt to the N7(adenine) in the 5′ direction is about 3 Å and that to the N7(adenine) in the 3′ direction is about 5 Å [93]. He proposed that the greater distance in the latter case could explain the non-formation of the GpA adduct. Although this difference might lead to a preference for the closure to an ApG adduct over closure to a GpA adduct in ApGpA sequences, it seems unlikely that the longer distance would completely preclude formation of GpA adducts in those cases where cisplatin was bound to the guanine of a GpA sequence flanked on the 5′-side by a base other than guanine or adenine. In addition, DNA is a highly flexible molecule and we have shown that the difference between these distances depends on the conformation adopted by the DNA [87].

In order to further investigate the reasons behind the non-formation of GpA adducts on duplex DNA, we generated models of both the ApG and GpA adducts [87]. This immediately demonstrates one of the advantages unique to computer based modelling – the ability to generate structures of adducts that are not observed experimentally. The model of the ApG adduct formed on B form duplex DNA (Fig. 2(a)) is similar to that of the GpG adduct described above. Hydrogen bonds are observed between the ammine group on the 5′ side of the adduct and a phosphate group and between the ammine group on the 3′ side and the exocyclic O6 atom of the guanine. In the case of the GpA adduct (Fig. 2(b)), the former hydrogen bond is observed but in the place of the latter is a highly unfavourable interaction between the ammine ligand on the 3′ side and the exocyclic NH_2 group of the adenine. The loss of the hydrogen bond will have contributed a few kJ to the destabilisation of the GpA adduct but more important we believe is the unfavourable interaction that takes its place. The model shown in Fig. 2(b) is that observed following energy minimisation and it can be seen that the adenine group has tilted to avoid the

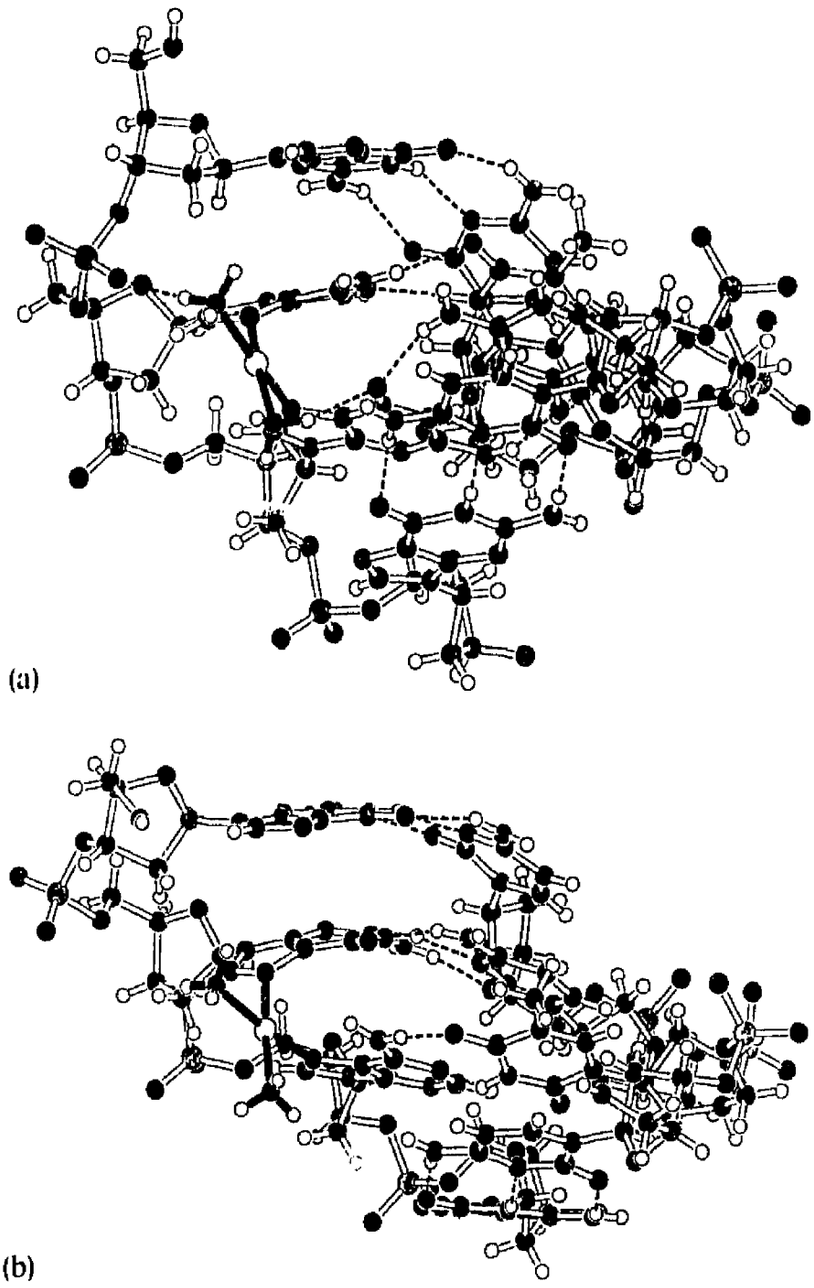


Fig. 2. (a) Molecular mechanics model of $Pt(NH_3)_2$: (5′-d(GpA*pG*pG)-3′, 5′-d(CpCpTpC)-3′). (b) Molecular mechanics model of $Pt(NH_3)_2$: (5′-d(GpG*pA*pG)-3′, 5′-d(CpTpCpC)-3′).

interaction with the ammine group. Despite this the interaction cannot be totally avoided and remains unfavourable (H···H 2.60 Å). It needs to be kept in mind that Pt binding to DNA is kinetically controlled and therefore models of the final adducts do not relate directly to the factors that control binding. The geometry of the transition state is unknown but probably involves a five-coordinate intermediate and at least one longer Pt–ligand bond length. On this basis, we produced models of such intermediates in the formation of the ApG and GpA adducts [87]. What is immediately apparent from such models is that all interactions observed in the models of the end products are also observed in the intermediate but, because of the additional steric crowding arising from the five coordinate geometry, these closer interactions become even more significant. Thus, the unfavourable $H(NH_3)\cdots H(NH_2)$ interactions shorten from ca. 2.60 Å to ca. 2.45 Å [87]. On this

basis, we proposed that the non-formation of the GpA adduct was due to a steric clash between one of the ammine ligands and the exocyclic NH_2 group of the adenine.

At this point, one might reasonably ask – so what? A model is just that and is of little use unless it can be tested. To us, the obvious test was to use the model to design compounds that would avoid the unfavourable interaction with the NH_2 group on the 3′ side of the GpA adduct, or better still, replace it with a favourable one such as a hydrogen bond. Three of the compounds designed or chosen on this basis are represented by structures **37**, **38** and **39**. All three retain one amine group, but in two the other amine group is replaced by a sulphoxide group and in the third by an oxime group. Each of these groups contains an O atom that has the potential to hydrogen bond to the NH_2 of the 3′-adenine in a GpA adduct. As an example, the energy minimised model of [Pt(enso)Cl_2] (**37**) bound to a GpA sequence is shown in Fig. 3. A range of experiments were undertaken to establish how these compounds bound to DNA. Unfortunately, the sulphoxide compounds bound weakly, probably as a consequence of the *trans* labilising effect of the sulphoxide group, and were not studied extensively. However, the amineoxime compound, [Pt(ambo)Cl_2], bound to DNA as rapidly and to a greater extent than cisplatin [94]. The rang, and amounts of adducts formed when it bound to calf-thymus DNA were determined by enzymatic digestion of the Pt treated DNA followed by HPLC analysis of the products [91]. Fractions were collected from the HPLC column and analysed for Pt content using graphite furnace atomic absorption spectroscopy (GFAAS) giving a profile of the Pt containing adducts as shown in Fig. 4(a). An analogous profile for [Pt(en)Cl_2] is shown in Fig. 4(b). [Pt(en)Cl_2], like cisplatin, forms about 60% GpG intrastrand adduct, 25% ApG intrastrand adducts and the remainder is made up of interstrand, GpNpG and monofunctional adduct. In comparison [Pt(ambo)Cl_2] forms approximately three fold fewer GpG and ApG adducts and a much greater proportion of monofunctional adducts were observed,

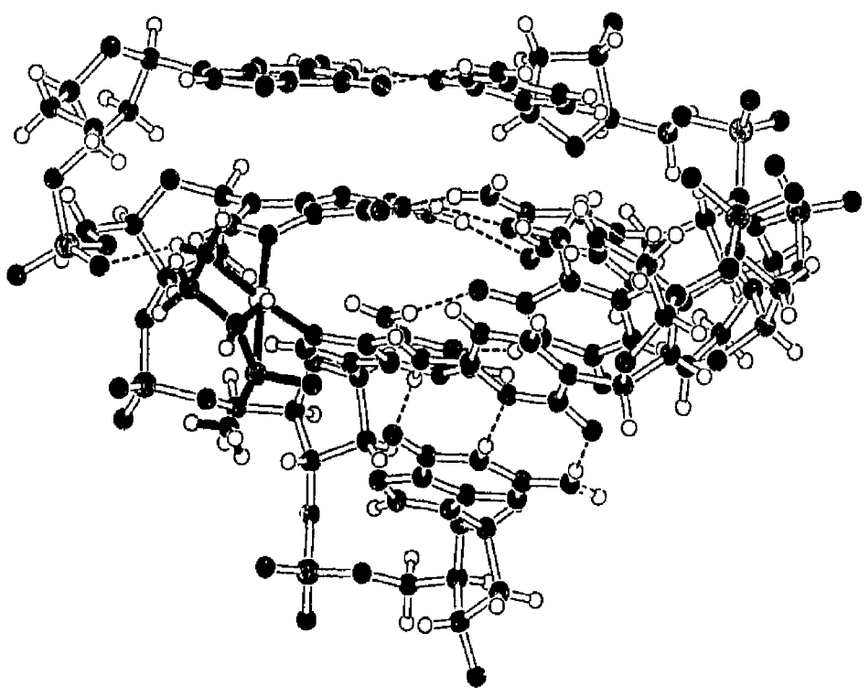


Fig. 3. Molecular mechanics model of Pt(enso): (5′-d(GpG*pA*pG)-3′, 5′-d(CpTpCpC)-3′).

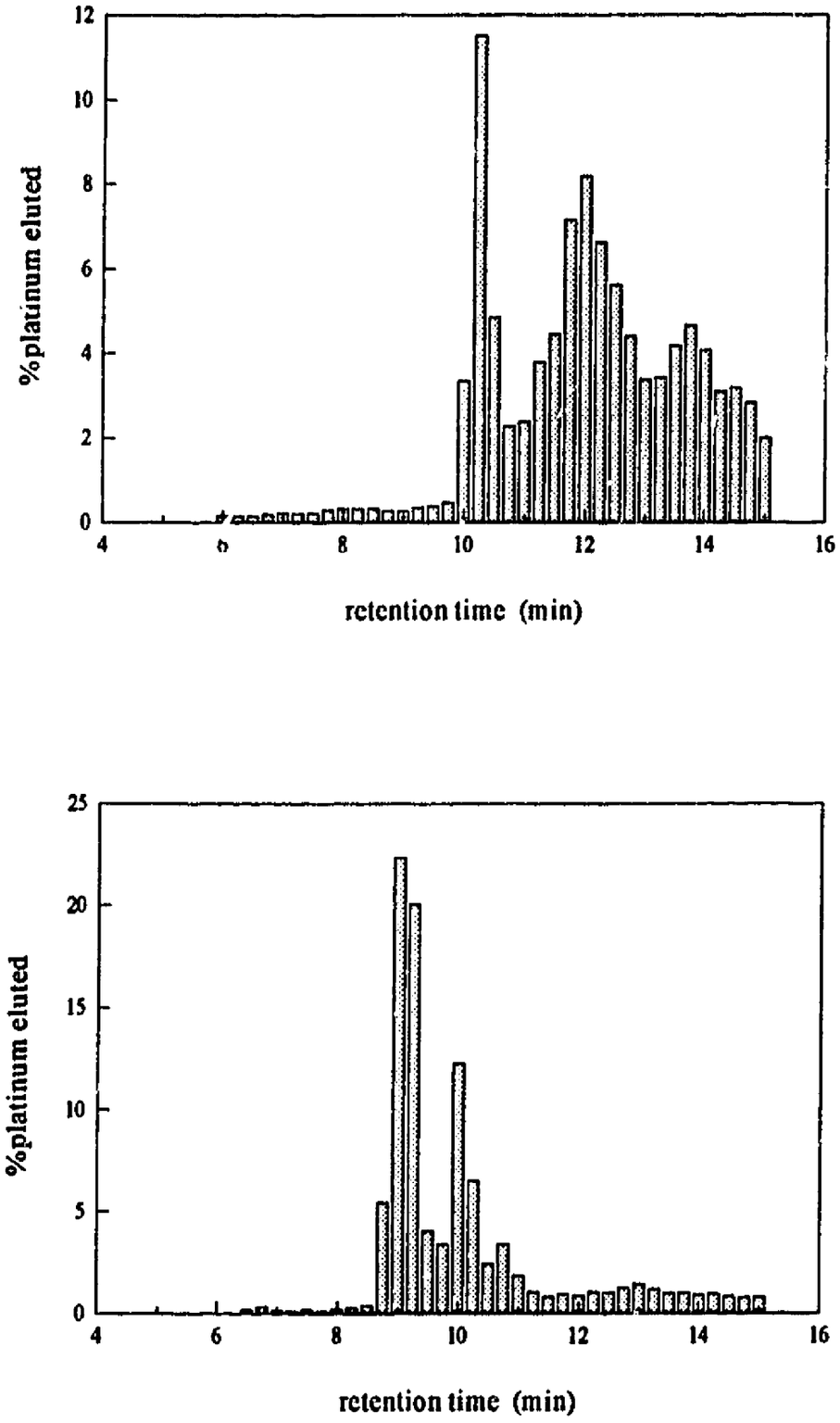
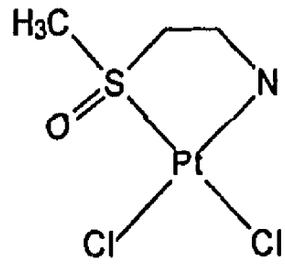


Fig. 4. Adduct profile for the binding of **39** (a) and $[Pt(en)Cl_2]$ (b) to salmon-sperm DNA.

a somewhat unexpected result given the similarity between the complexes. Disappointingly, no GpA adducts were observed. A possible explanation for both of these observations is that the oxime group stabilises one of the chloro groups, either the *trans* chloro through a *trans* effect, or the *cis* chloro through a favourable association with the hydroxyl group.

Since GpA adducts could not be observed directly we endeavoured to establish whether $[Pt(ambo)Cl_2]$ has an increased preference for binding to adenine rich sequences, relative to cisplatin. This was done by measuring inhibition of cutting of



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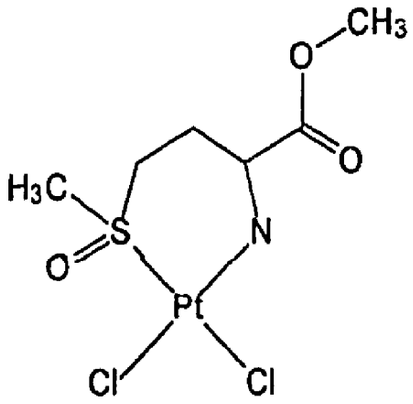

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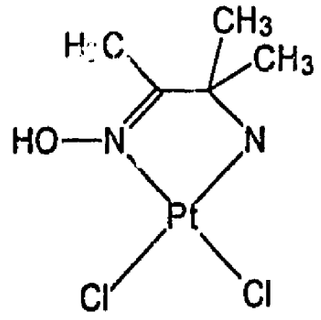


(39)

DNA by restriction enzymes, both on plasmid DNA and a synthetic oligonucleotide. The DraI restriction enzyme cuts at 5′–AAA↓TTT–3′ sequences and therefore, an oligonucleotide (shown below) with such a sequence flanked by additional thymines and adenines, to preclude effects of binding at adjacent guanines, was prepared with radioactive ends. Cutting this sequence with the DraI restriction enzyme produces two unequal strands and the uncut oligonucleotide and the two strands were easily identified and quantified using autoradiographed gel electrophoresis. Comparison of the inhibition of cutting by equal concentrations of cisplatin and [Pt(ambo)Cl_2] (Fig. 5) reveals that the latter complex is more effective [94]. A similar result was obtained using plasmid DNA and is in contrast to cutting of 5′–G↓GATCC–3′ sequences in plasmid DNA by the BamH1 restriction enzyme where cisplatin was substantially more effective at inhibiting cutting.

$^{5'}$CTAGATTGGATGCGACTTGATTGCGCATTTAAATTAGTTCGGTTGCGTCA$^{3'}$
$^{3'}$TAACCTACGCTGAACTAACGCGTAAATTTAATCAAGCCAACGCAGTGATC$^{5'}$

One of our goals is to correlate interactions with DNA with cytotoxic activity and for this reason **37**, **38** and **39** were tested against human bladder cancer cells. The results are shown in Table 1 and reveal that the three compounds are all extraordinarily inactive. In the case of the sulphoxide complexes, this correlates with the poor binding to DNA, believed to arise from *trans* effects. The result for [Pt(ambo)Cl_2] is more surprising however, particularly taking into account the high activity of other compounds with one sp^3 amine and one sp^2 amine such as the pyridylamine complexes (**20**) [44]. However, it does correlate with the formation of relatively fewer GpG and ApG intrastrand adducts than active compounds such as cisplatin and [Pt(en)Cl_2].

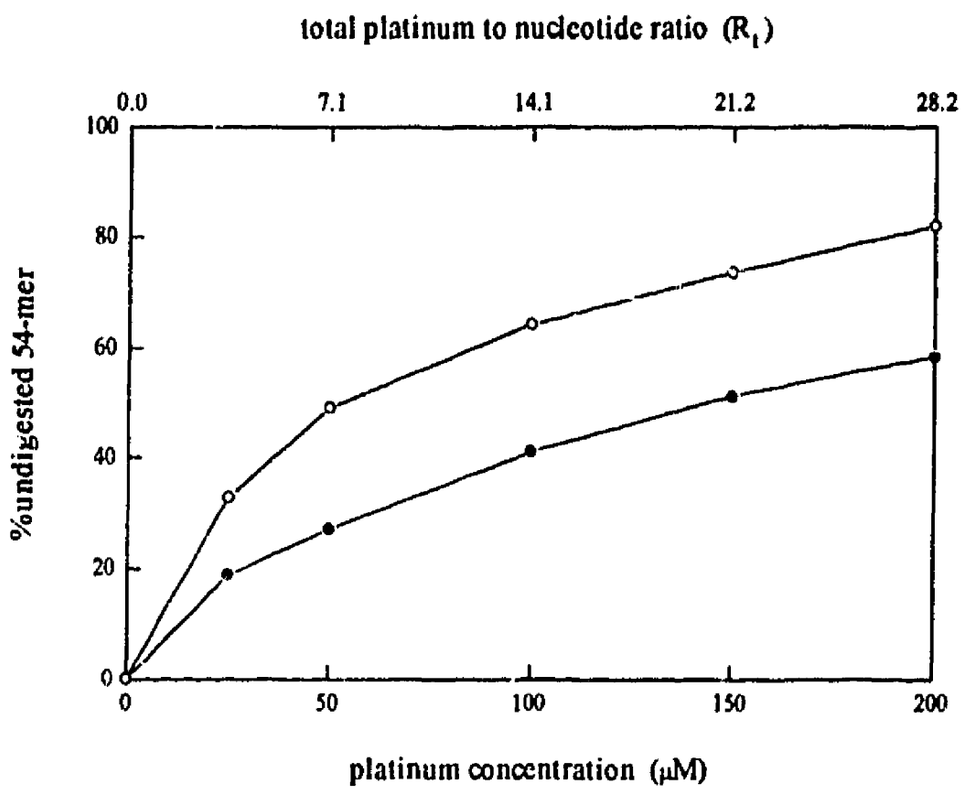


Fig. 5. Quantification of DraI digestion of a 50-mer oligonucleotide containing a single 5′-d(TpTpTpApApA)-3′ which has been treated with **39** (○) and cisplatin (●).

Table 1
In vitro activities of complexes against the BL13/0 bladder cancer cell line

Complex	IC_{50} (μm)
Cisplatin	4.5
(2*S*,S*S*)-[Pt(metso)Cl_2]	1000
(2*S*,S*R*)-[Pt(metso)Cl_2]	1300
[Pt(ambo)Cl_2]	1200

3.3. *How important are interstrand GG adducts?*

The debate over which of the bifunctional adducts – intrastrand or interstrand – are responsible for the anti-cancer activity of cisplatin and its analogues has gone on since the first studies into 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 linked the two strands of duplex DNA would interfere with replication. In the early 1980s, direct measurement of the adducts revealed that the intrastrand adducts accounted for 80–90% of the Pt bound to DNA and this led to a shift to the view that they must be the critical adducts. Since then, many techniques have been applied to the question providing evidence that is consistent with one or the other set of adducts being responsible for the activity and an unequivocal answer is still not available.

We began addressing this question by the comparing the computer generated models of the intrastrand GpG and interstrand GG adducts shown in Fig. 1(b) and

Fig. 6, respectively. Schematic views of these adducts are shown in Fig. 7. In the case of the intrastrand GpG adduct, as described above, there are hydrogen bonds between the amine ligands and O(phosphate) and O(guanine) atoms. Each of the ammine ligands is also involved in a hydrogen bond in the interstrand adduct, in this case both to O(phosphate) atoms. A view of the interstrand adduct, along the major groove (Fig. 6(a)) shows how the ammine ligands lie well out into the major groove making no other close contacts with the DNA. In contrast, in the intrastrand adduct, the ammine ligands, in particular the one that lies on the 3′ side of the adduct, lie close to the floor of the major groove. This difference led us to the design of a group of compounds in which the amine ligands are linked by two aliphatic chains (Fig. 7). When these compounds are bound in an interstrand fashion, the chains will lie in the major groove as shown in Fig. 8 and not interfere with binding, but when bound intrastrand, one of the chains will come up against the floor of the major groove and in particular can be expected to interact unfavourably with the O6(guanine) on the 3′ side of the adduct. As a consequence, we anticipated that such compounds would form interstrand adducts at least as readily as cisplatin but be relatively less inclined to form intrastrand adducts. Recently, we showed that the first compound in this series to be studied, $[Pt(hpip)Cl_2]$ (hpip = homopiperazine = 1,4-diazacycloheptane), fulfils these design goals. Measurements of cross-linking showed that $[Pt(hpip)Cl_2]$ forms interstrand adducts at approximately the same level as cisplatin and DNA digestion/HPLC analysis showed that it forms less than half the number of GpG and ApG intrastrand adducts [95]. Measurement of activity against two cancer cell lines, including human bladder cancer, revealed that $[Pt(hpip)Cl_2]$ is approximately 50 times less active than cisplatin meaning its activity is similar to that of *trans*-$[Pt(NH_3)_2Cl_2]$. Since $[Pt(hpip)Cl_2]$ forms interstrand adducts on DNA in the test tube as readily as cisplatin, but is inactive in vitro it is unlikely that the interstrand adducts are responsible for the in vitro cytotoxic activity of cisplatin. However, low activity might be due to any number of factors and these need to be investigated before such a conclusion can be confirmed. For instance we have ruled out inhibition of uptake because $[Pt(hpip)Cl_2]$ is taken up by cells more rapidly than cisplatin, and have ruled out more rapid repair of adducts formed by $[Pt(hpip)Cl_2]$ because it is no more active in repair deficient cell lines. Recent work by Brabec [96] suggests that a different profile of adducts is formed when cisplatin binds to supercoiled DNA and therefore it is necessary to establish whether the adducts formed by cisplatin and $[Pt(hpip)Cl_2]$ on DNA in the cell differ in other ways from what we have observed in the test tube and experiments are underway to do this.

3.4. How can we probe the role of the intrastrand adducts?

From the foregoing, and much other data, it seems that it is most likely to be the intrastrand adducts that are responsible for the cytotoxic activity of cisplatin and its analogues. We have therefore more recently turned our attention to testing this hypothesis and contributing to the understanding of the structural factors that mediate formation of the intrastrand adducts. The basis behind the design of our

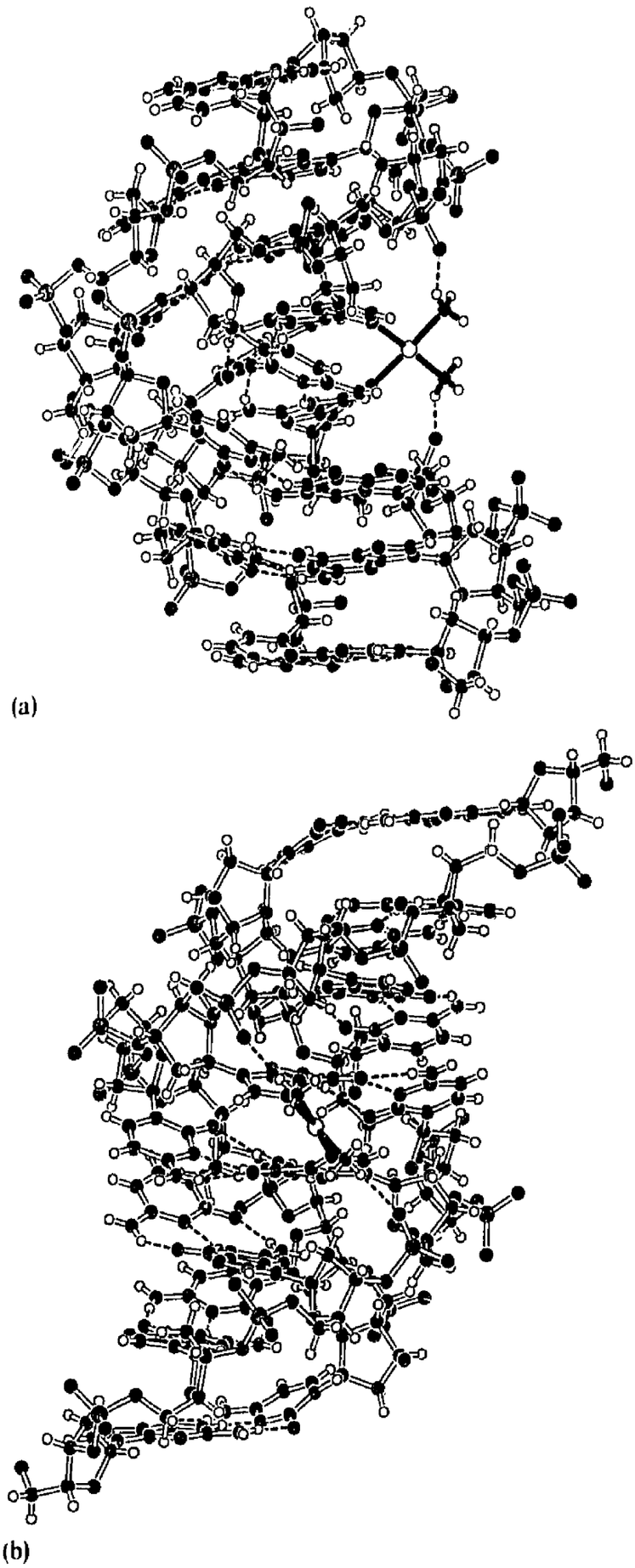


Fig. 6. Views along (a) and into (b) the major groove of a molecular mechanics model of the interstrand $Pt(NH_3)_2$: (5′-d(GpGpGpG*pCpCpCpC)-3′, 5′-d(GpGpGpG*pCpCpCpC)-3′) adduct.

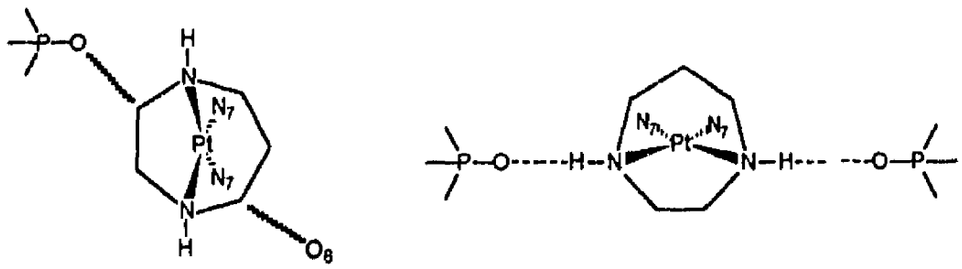


Fig. 7. Schematic diagrams of intrastrand and interstrand adducts demonstrating the logic behind the design of [Pt(hpip)Cl_2].

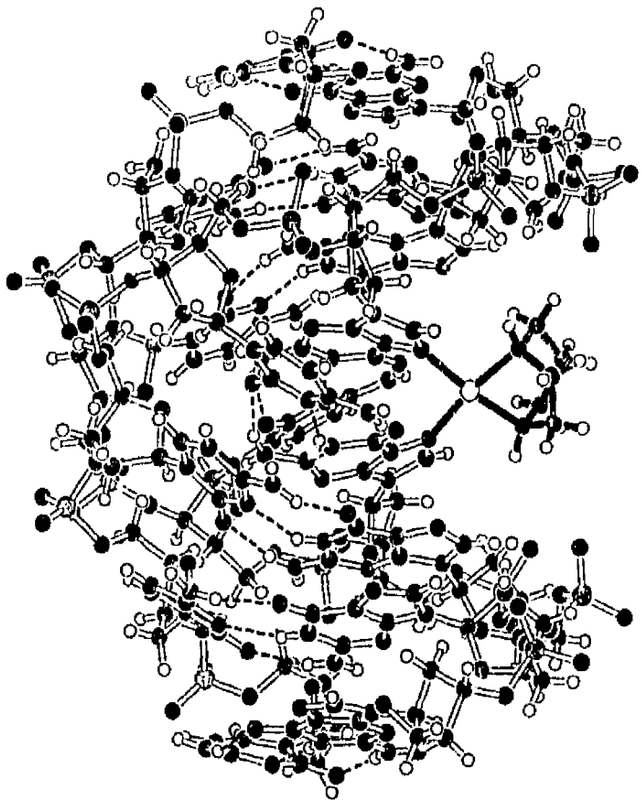


Fig. 8. Views along the major groove of a molecular mechanics model of the interstrand Pt(hpip): (5′-d(GpGpGpG*pCpCpCpC)-3′, 5′-d(GpGpGpG*pCpCpCpC)-3′) adduct.

probes is the chirality of the intrastrand adducts and the potential for enantioselective interaction between chiral Pt complexes and the GpG or ApG sequences as shown in Fig. 9. As mentioned above, in both GpG and ApG intrastrand adducts there are potential hydrogen bonds between the am(m)ine ligands and O(phosphate) and O6(guanine) atoms. Thus, a ligand with only one H(amine) atom on each N atom might have one enantiomer with both of the H atoms disposed appropriately for forming the hydrogen bonds and the other enantiomer would have the H atoms disposed incorrectly and, perhaps more importantly, have bulky substituents disposed toward the hydrogen bond acceptors. The loss of the hydrogen bond to the O(phosphate) would have a minor effect because the phosphate backbone can readily reorient itself to avoid the clash with the substituent. However, the clash with the O6(guanine) atom on the 3′ side is more significant, because as we have shown before, this interaction cannot be fully relieved and probably mediates binding. If this is the case then the enantiomer in Fig. 9(a) should readily form the

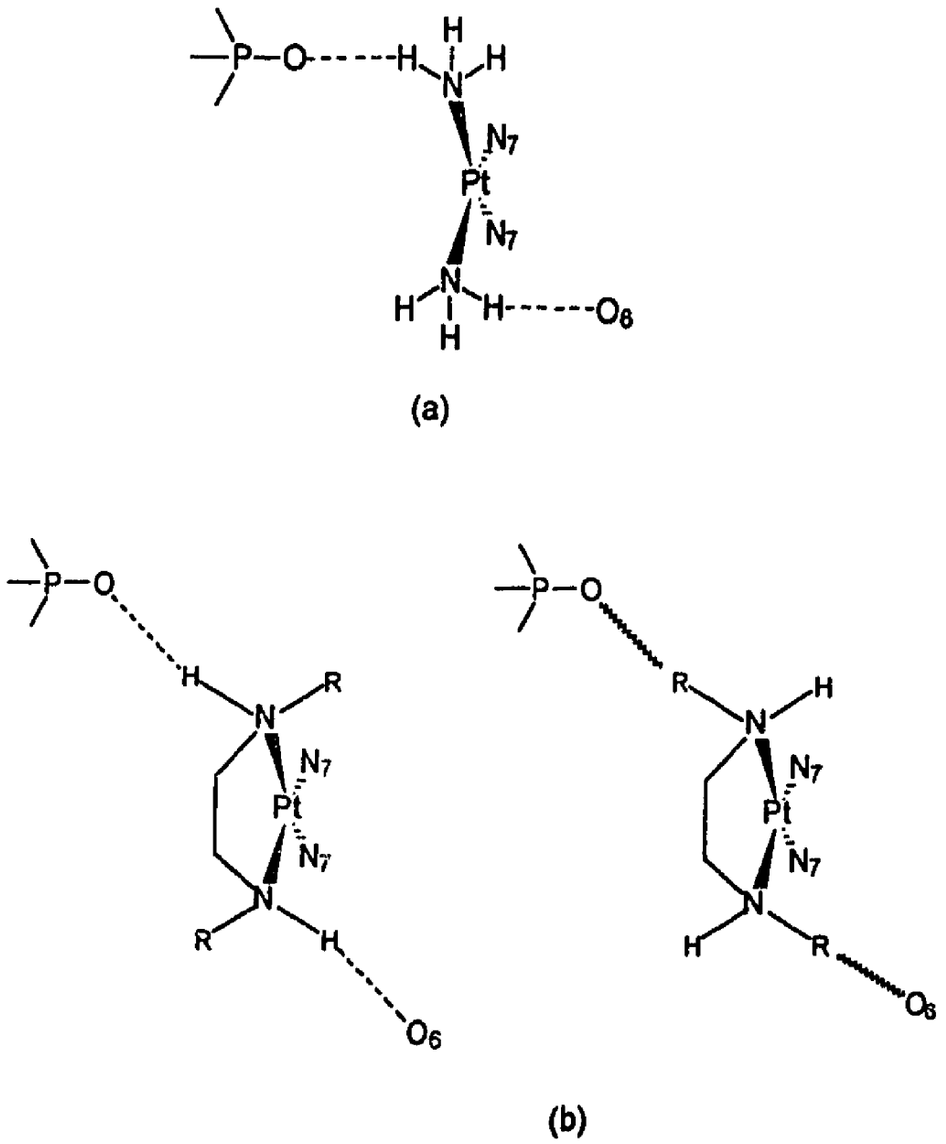


Fig. 9. Schematic diagrams of intrastrand and interstrand adducts demonstrating the logic behind the design of chiral Pt complexes.

intrastrand adducts, and if they are responsible for cytotoxic activity, have the potential to be highly active. Conversely, the enantiomer in Fig. 9(b) should bind less readily and be less active.

The ligands in these compounds are necessarily bidentate to prevent rotation about the Pt–N bond relieving the unfavourable interaction. It is possible to resolve Pt complexes in which the only chiral centres are on the amine atoms but given that the target complexes are neutral this is difficult and, in biological conditions, racemisation is likely. Therefore, the approach we have adopted is to use ligands with chiral carbon centres in the backbone that should impose the desired chirality at the N atom. In our first attempt at achieving this we used the ligand *N,N'*-diethyl-2,4-pentanediamine (eap) to produce the two enantiomeric complexes shown in structure **40**. Our expectation, based in part on molecular mechanics calculations, was that the ethyl substituents would adopt orientations *trans* to the methyl groups of the ligand. However, the crystal structure of the Pt complex (Fig. 10) revealed that the diastereomer adopted had one of the ethyl substituents *cis* to the methyl group [97]. NMR evidence showed that the desired configuration is also observed

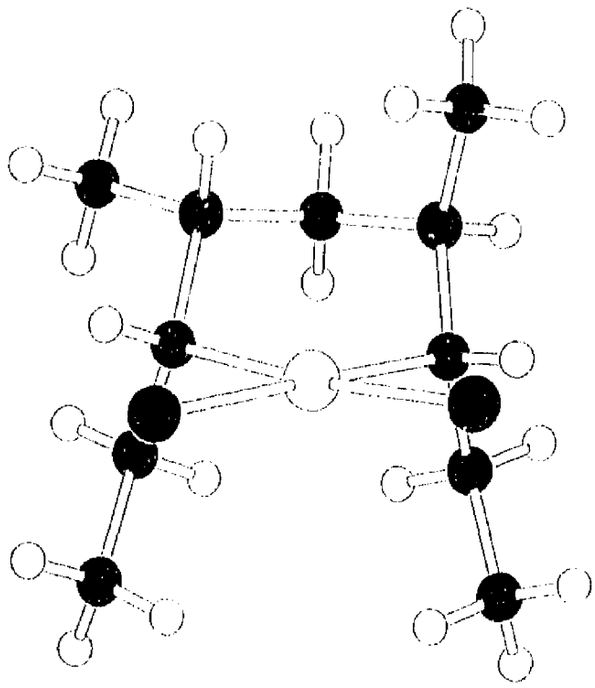
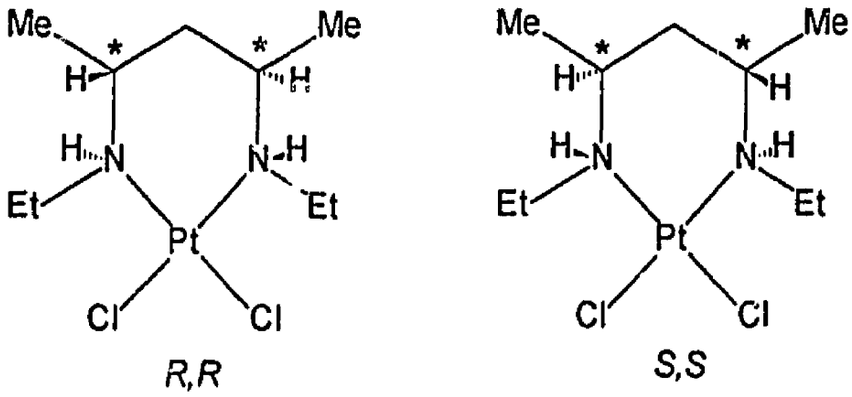


Fig. 10. Crystal structure of [Pt(*R,R*-eap)Cl_2].

but that observed in the solid state predominates by a factor of 7:3 [97]. The driving force behind the preference for this configuration appears to be a neutral or weakly attractive interaction between the chloro ligands and adjacent H(amine) atoms. In recent revisions of our force field for modelling such Pt complexes we have included a weak hydrogen bond between these atoms which results in better reproduction of the structures of these complexes and provides a rationale for the observed diastereomer distribution. A small enantioselective difference in cytotoxic activity was observed (Table 2) but was not considered to be significant enough to warrant further investigation.



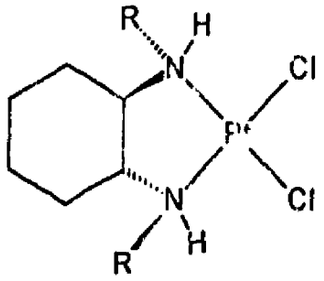
(40)

Given the problems encountered with the eap ligand, we next turned to more rigid chiral frameworks in the expectation that they would be more effective at imposing the desired chirality at the amine group. The first of these was cyclohexane-1,2-diamine (dach). Pt(II) and Pt(IV) complexes of dach have been extensively investigated in recent years because of their high activity and lack of cross resistance in cell lines resistant to cisplatin. The enantiomers of the unsubsti-

Table 2
In vitro activities of complexes against the BL13/0 bladder cancer cell line

Complex	IC_{50} (μm)
Cisplatin	1–1.5
$[Pt(en)Cl_2]$	5.2
$[Pt(R,R\text{-cap})Cl_2]$	14
$[Pt(S,S\text{-cap})Cl_2]$	20

tuted dach complexes $[Pt(R,R\text{-dach})Cl_2]$ and $[Pt(S,S\text{-dach})Cl_2]$ have been reported as having different activities but as described above the differences are modest and cell line dependent [55]. We have added a variety of substituents at both N atoms to produce a series of ligands and the cytotoxicities of their Pt(II) complexes (**41**) are listed in Table 3. In some cases, substantial enantioselective differences are observed and these accord with expectations based on the model described above.



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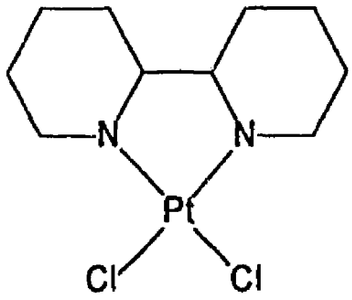
One means of achieving desired chirality at the amine groups is to use ligands such as that represented by structure **42** with endocyclic amine groups. Unfortunately, Pt(II) complexes of both the *meso* and racemic forms of this ligand have proven to be remarkably inactive against L1210 in cell culture [98]. The ligand ahaz (ahaz = 3-aminohexahydroazepine) is chiral, has one endocylic amine group and one enantiomer has been reported as having activity comparable to or better than that of cisplatin. Therefore, we have investigated the Pt(II) complexes (**43**) of the enantiomers of this ligand and modified forms of it in which a substituent is placed on the primary amine in order to produce the features outlined in Fig. 9.

Table 3
In vitro activities of complexes of variants of the dach ligand against the BL13/0 bladder cancer cell line

Complex	IC_{50} (μm)
Cisplatin	1.6 or 3.7[a]
$[Pt(R,R\text{-Chxn})Cl_2]$	0.9
$[Pt(S,S\text{-Chxn})Cl_2]$	1.5
$[Pt(R,R\text{-dimethylchxn})Cl_2]$	1.2
$[Pt(S,S\text{-dimethylchxn})Cl_2]$	2.4
$[Pt(R,R\text{-diethylchxn})Cl_2]$	6.6[a]
$[Pt(S,S\text{-diethylchxn})Cl_2]$	23.9[a]

[a] SRB assay (others by MTT assay).

The *R* and *S* enantiomers of [Pt(ahaz)Cl_2] are both highly active against the BL13 human bladder tumour cell line with the *R* enantiomer being more active (IC_{50}=1.3 μm) than cisplatin (IC_{50}=1.9 μm) and the *S* enantiomer being less active (IC_{50}=2.0 μm). This is one of the higher levels of enantioselectivity observed and therefore we have undertaken an investigation into what is responsible for it [99]. Uptake into the BL13 cell is similar for the two enantiomers. Molecular modelling studies suggest that the *S* enantiomer should bind more readily to DNA and this is supported by gross DNA binding studies which show that binding takes place more rapidly and to a greater extent for the *S* enantiomer than for the *R*. The difference is small and the enantioselectivity runs contrary to that observed in the cytotoxicity studies. The greatest difference in behaviour that we have observed is that the *R* enantiomer forms more monofunctional adducts initially and takes longer to convert these to bifunctional adducts. That this correlates with higher activity is not what might have been expected but is an observation that is clearly worthy of investigation in other compounds.



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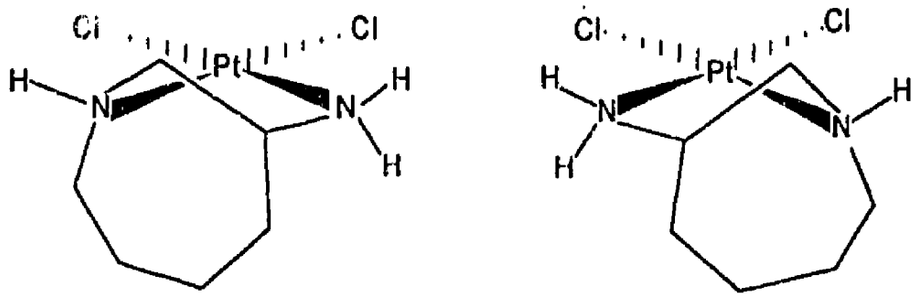
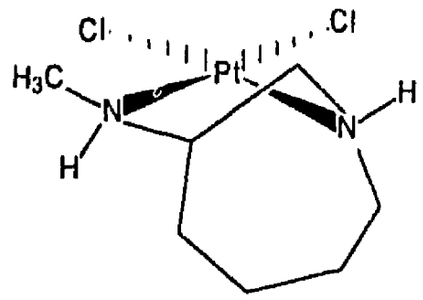


(43)

The complexes of variants of the ahaz ligand with substituents on the primary amine group (**44–46**) are all less active than the parent complex. This is not surprising since greater steric bulk generally leads to a reduction in activity, however, what is surprising is that the complex in which there are two methyl groups on what was the primary amine is no less active than those with a single methyl group or an ethyl group. In those cases where there is significant enantioselectivity, it is again the *R* enantiomer that is the more active. Studies into the binding of these complexes to DNA are currently underway.



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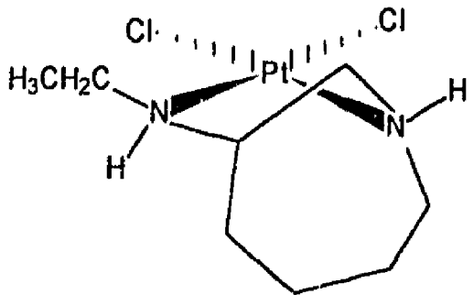

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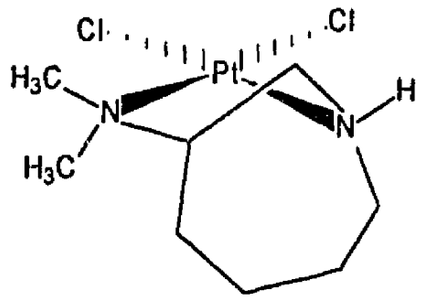

(46)

3.5. *The use of chiral complexes to probe toxicity*

For sometime it has been known that chiral platinum complexes exhibit greater enantioselectivity in terms of mutagenicity than they do in terms of cytotoxicity and that in some cases the less mutagenic enantiomer is the more active. This is an exciting observation since it suggests that different adducts or targets must be responsible for mutagenicity and cytotoxicity which in turn means it should be possible to design compounds with low mutagenicity and high activity. In order to do this it will be necessary to gather extensive information on the relationships between the adducts formed by complexes and their mutagenicities. For instance, it has recently been shown that the bifunctional ApG adduct formed by cisplatin is about five times more mutagenic than the GpG adduct [100,101]. We too have found enantioselectivity in studies of the mutagenicity of the complexes of cyclohexane-1,2-diamine derivatives (**41**) described above. For example, we found that the *S* enantiomer of [Pt(*N,N'*-dimethylchxn)Cl_2] is 1.5 times as mutagenic as the *R* enantiomer.

More recently McKeage et al. [102] have shown that the two enantiomers of dach complexes have different neurotoxicities. Thus, in a rat model, the *R,R* enantiomer induces significant neurotoxicity after 8 weeks but the *S,S* enantiomer does not have measurable effect until week 12. The difference is modest but would be significant if it translates to humans since the extent of treatment regimes falls within these times. Human trials have been carried out on oxaliplatin which is the *R,R* enantiomer and on tetraplatin (ormaplatin) which is a racemic mixture. In both cases neurotoxicity has been a problematic side effect and was found to be cumulative with onset after 8 weeks.

As mentioned above, enantioselective toxicities for the enantiomers of structure

36 (DWA2114*R* and DWA2114*S*) that emerged in preclinical studies were used to select the *R* enantiomer for clinical trials [78]. Thus, chiral compounds clearly have the potential to act as probes of the stereoselective factors that influence toxicity and correlations with drug/target interactions should reveal the biomolecules involved in the induction of toxicity.

3.6. Stereoselective interactions between Pt complexes and DNA

One of the interesting observations to emerge from our studies of the binding of the complexes described above to DNA has been stereoselectivity in the adduct profile. We believe that an understanding of the causes of this stereoselectivity and its extent has the potential to shed light on the factors that influence Pt/DNA interactions. Consequently we have carefully measured the degree of selectivity and examined its origins using molecular modelling techniques.

The complex [Pt(hpip)Cl_2] can bind to GpG sequences of DNA in two ways; one with the ethylene chain disposed toward the floor of the major groove and the other with the propylene chain disposed toward the floor. Two isomers were observed in the binding of [Pt(hpip)Cl_2] to GpG, to a synthetic dinucleotide and to calf-thymus DNA [103]. Using 1D and 2D NMR we identified the isomers formed with GpG as those corresponding to the two isomers expected from the interaction with duplex DNA [104]. This identification was achieved using cross peaks between the two H8(guanine) atoms of the nucleotide and the ethylene or propylene chains of the hpip ligand as indicated in Fig. 11. HPLC analysis of the adducts formed with duplex DNA showed that the isomer with the ethylene chain disposed toward the floor of the major groove predominated over the other isomer by a factor of 3:1. Molecular modelling studies showed that this stereoselectivity was consistent with the closer interactions that were observed in the case where the bulkier propylene chain made contact with the floor of the major groove as shown in Fig. 12 [103].

Stereoselectivity has also been observed in the DNA adduct profiles of the enantiomers of [Pt(ahaz)Cl_2] (**43**). In this case too, each enantiomer can bind to GpG sequences of DNA in two ways, one with the primary amine group *cis* to the 5′ guanine of the GpG pair and the other with it *cis* to the 3′ guanine. For the *R* enantiomer bound to salmon-sperm DNA the two isomers were observed in approximately equal amounts, accounting for 9% and 12% of the Pt bound to the DNA [105]. In contrast the two isomers formed by the *S* enantiomer accounted for 30% and 7% of the DNA bound Pt. Thus, not only does the *S* enantiomer exhibit substantial stereoselectivity in its interactions with DNA but there are also substantial differences between the behaviour of the two enantiomers. At this stage we have not identified the isomers and work is ongoing to do this. However, the DNA binding results are consistent with the molecular modelling studies we have carried out. In these models (Fig. 13), close and unfavourable contacts between the aliphatic chains of the ahaz ligand and the DNA are observed for both isomers formed by the *R* enantiomer (Figs. 13(a) and (b)) but for only one isomer formed by the *S* enantiomer (Fig. 13(c)). In the other isomer formed by *S* enantiomer (Fig. 13(d)) the ahaz ligand "fits" the shape of the major groove and makes no unfavourable

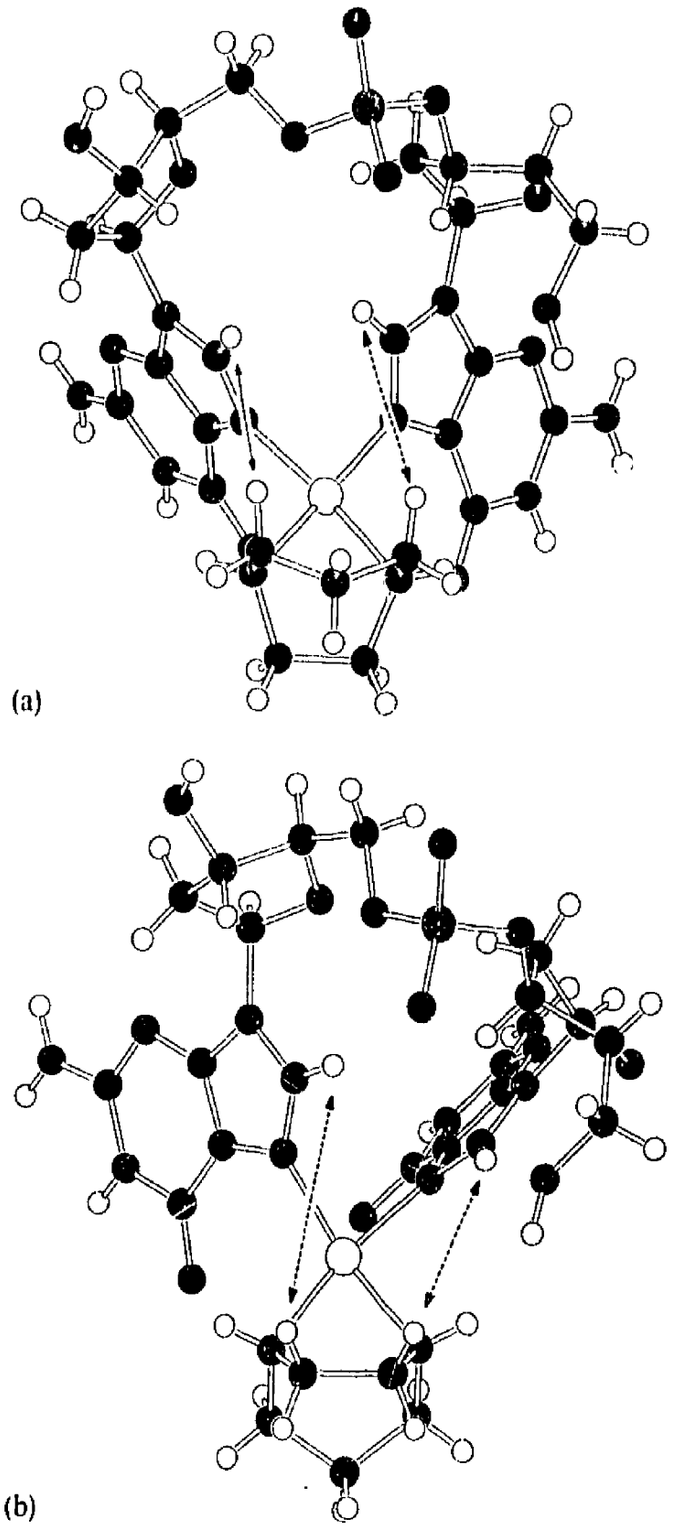

Fig. 11. Close contacts used to identify the isomers of [Pt(hpip)(GpG)] by NOESY correlations.

close contacts. Clearly, it is tempting to suggest that it is this isomer that accounts for 30% of the Pt(*S*-ahaz)$^{2+}$ bound to DNA. The remarkable difference in the proportions of monofunctional and bifunctional adducts formed by the two enantiomers of [Pt(ahaz)Cl_2] has been confirmed by measuring the proportions of monofunctional adducts and their rates of closure to bifunctional adducts using ^{14}C-thiourea labelling [99].

Our rationale for undertaking these studies was that if we could induce enantioselective interactions with DNA then we might expect to see corresponding enantiosel-

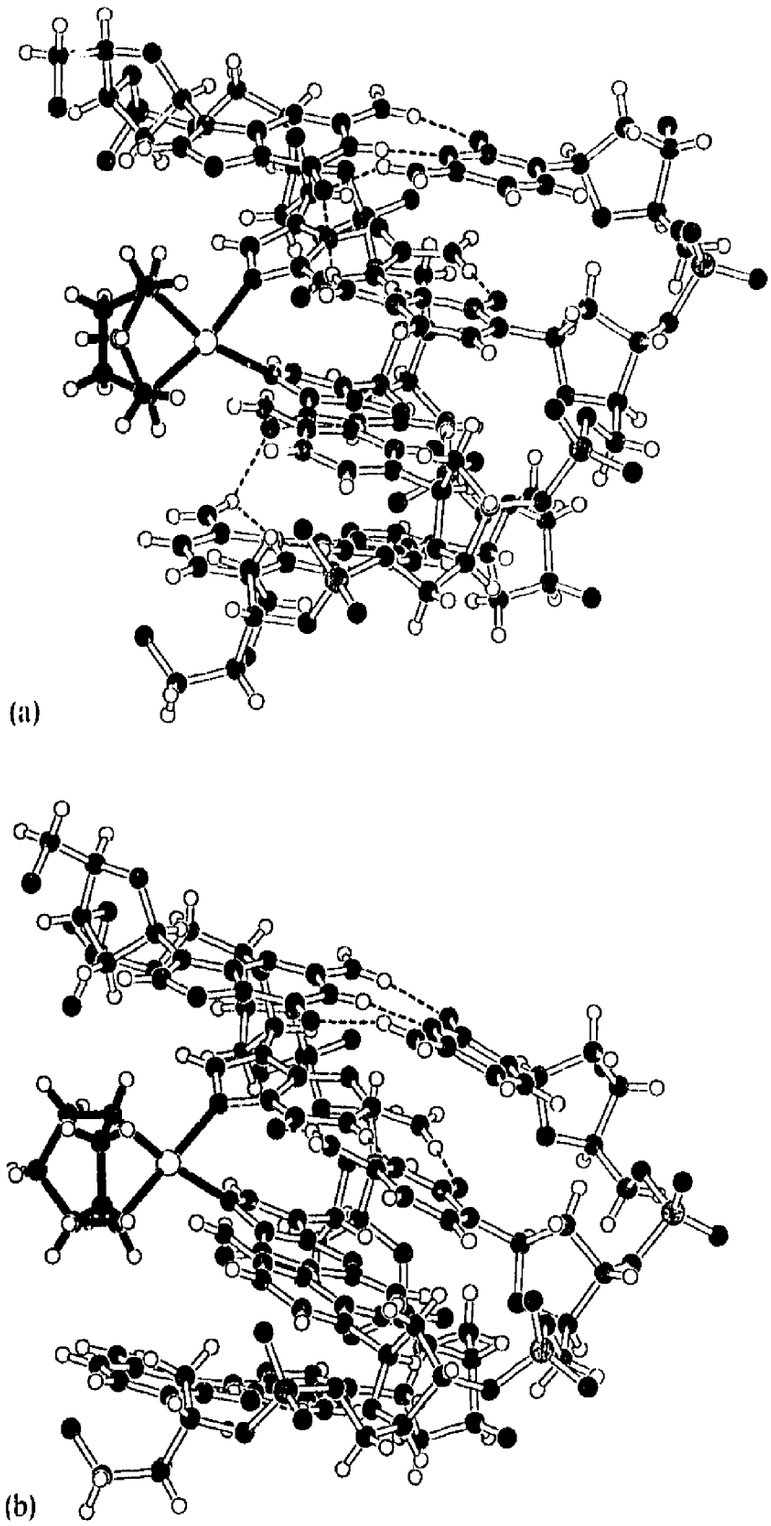

Fig. 12. Views of the molecular mechanics models of the isomers of the intrastrand adducts formed between $Pt(hpip)^{2+}$ and (5′-d(GpGpGpG*pG*pGpGpG)-3′, 5′-d(CpCpCpCpCpCpCpC)-3′).

ective differences in cytotoxic activity, particularly if the differences related to the putatively crucial GpG adducts. Having clearly achieved this with the $[Pt(ahaz)Cl_2]$ enantiomers, with the *S* enantiomer forming nearly twice as many bifunctional adducts we were surprised to find that where differences in in vitro activity were observed, it was the *R* enantiomer that is the more active as described above [99]. In most cell lines there is no significant difference but in the human bladder cancer cell line the *R* enantiomer reproducibly is the more active. There are

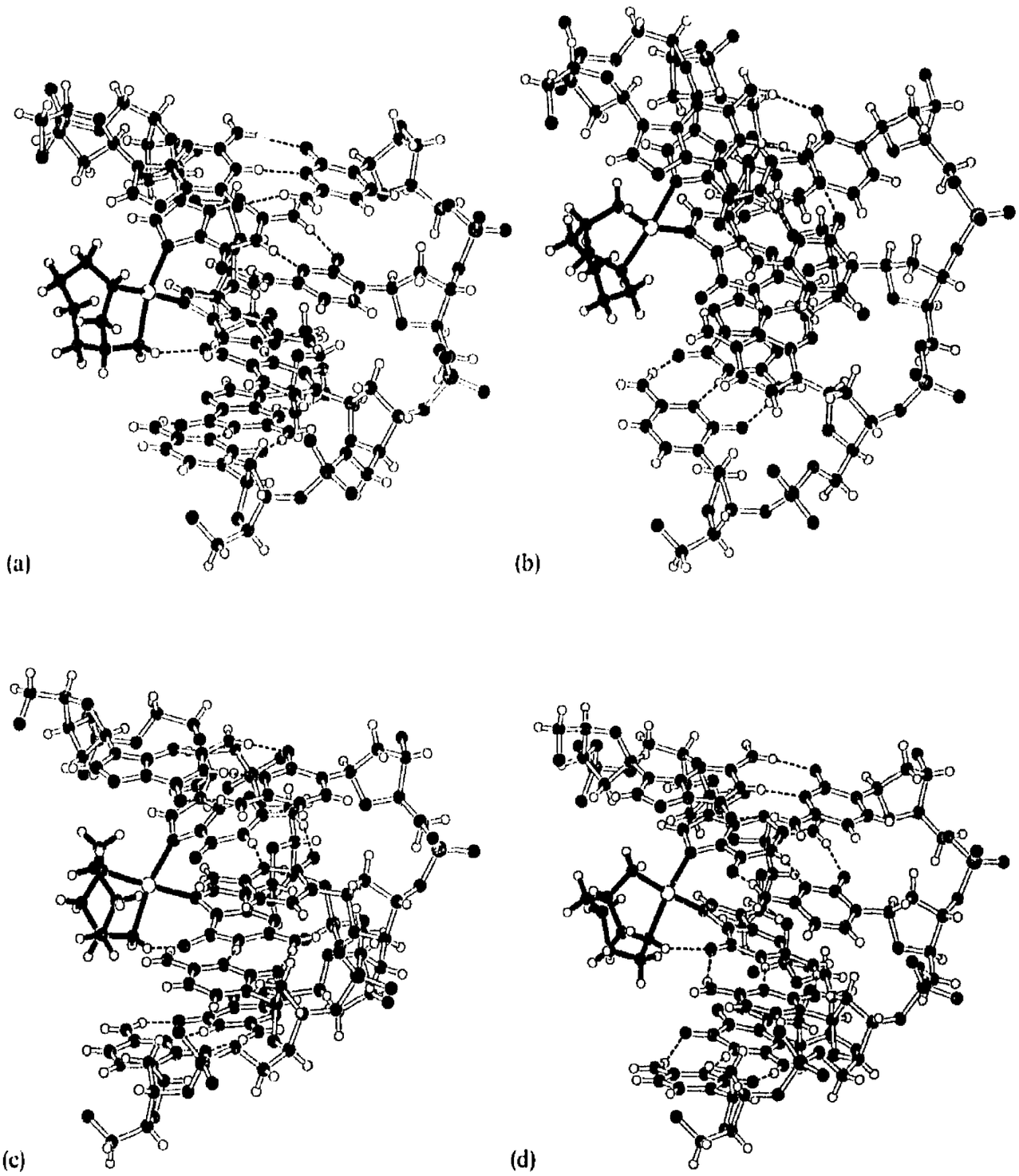

Fig 13. Views of the molecular mechanics models of the isomers of the intrastrand adducts formed between Pt(ahaz)$^{2+}$ and (5'-d(GpGpGpG*pG*pGpGpG)-3', 5'-d(CpCpCpCpCpCpCpC)-3').

many factors that might influence the relative activity of pairs of enantiomers including cellular uptake and adduct repair. The former we have now ruled out [99] and we plan to investigate the latter. However, it is still surprising that we do not see the DNA binding results reflected in the activities and this may call for a reappraisal of the role of the GpG adducts or of the importance of the rate and frequency at which they are formed.

The other rationale behind these studies was the design of pairs of enantiomeric complexes with different potentials for forming the hydrogen bonds with DNA that we believed to be important determinants of adduct formation. What is emerging

Table 4
In vitro activities of complexes of variants of the ahaz ligand against bladder (BL13/0), lung (PC9), resistant lung (PC9-cisR) and prostate (DU145) cancer cell lines

Drug	BL13/0 Mean IC_{50} (μm) (standard error)	PC9 Mean IC_{50} (μm) (standard error)	PC9-cisR Mean IC_{50} (μm) (standard error)	DU145 Mean IC_{50} (μm) (standard error)
Cisplatin	1.9(0.5)	1.4(0.2)	11.7(3.1)	1.8(0.8)
[Pt(*R*-ahaz)Cl_2]	1. 3(0.2)	5.9(0.1)	16.0(1.0)	2.9(0.1)
[Pt(*S*-ahaz)Cl_2]	2.0(0.2)	3.0(0.1)	15.3(1.3)	3.4(0.3)
[Pt(*R*-meahaz)Cl_2]	7.3(1.3)	13.3(1.9)	19.0(1.2)	6.7(1.3)
[Pt(*S*-meahaz)Cl_2]	11.7(1.6)	13.7(1.2)	38.3(8.0)	10.1(1.7)
[Pt(*R*-etahaz)Cl_2]	7.4(1.1)	20(3.5)	44(4.4)	9.5(0.9)
[Pt(*S*-etahaz)Cl_2]	19(2.1)	31(4)	79.7 (8.5)	21.3(2.4)
[Pt(*R*-dimeahaz)Cl_2]	7.7(2.1)	13.5(3.4)	29.3(1.5)	7.3(0.2)
[Pt(*S*-dimeahaz)Cl_2]	10.2(1.4)	11.8(2.6)	28.7(2.2)	11.5(0.8)

from the stereoselective interactions with DNA that we see for the [Pt(hpip)Cl_2] and [Pt(ahaz)Cl_2] complexes is that these hydrogen bonds are perhaps not as important as unfavourable hydrophobic interactions that occur when complexes with bulky ligands bind to DNA. 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.Table 4

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