Cisplatin is an effective anticancer drug widely used in the treatment of several human carcinomas (1–4). The mechanism of anticancer activity involves formation of platinum–DNA adducts that are capable of inhibiting DNA and RNA synthesis (5–16) and inducing programmed cell death (17,18). Cisplatin binds preferentially to the N7 position of purine residues. The mono-functional adduct subsequently closes to a bifunctional adduct by linking a second purine that can be either of the same strand or of the opposite strand (19). There is general consensus that the antitumor efficacy of cisplatin is associated with the formation of DNA 1,2-intrastrand (d(GpG)) or d(ApG) cross-links (5–16). The 1,2-intrastrand cross-links locally unwind and bend double-stranded DNA toward the major groove (14,20,21), and the disturbance of DNA secondary structure seems to be the ultimate reason for inhibition of DNA replication and/or transcription and for triggering apoptotic cell death (22,23).

While the anionic–leaving ligands are likely to play a role in determining the transport of the complex throughout the living organism, the nonexchangeable aminic ligands play an important role in the drug–DNA adduct formation and stereochemistry. Thus, it is of great interest to see how different configurations of these non-leaving ligands can influence the DNA-binding properties and consequently the biological activity of platinum complexes.

In this review we focus on platinum complexes with enantiomeric amine ligands. Because double-helical DNA has a chiral structure, complexes with enantiomeric ancillary ligands should form diastereomeric adducts with DNA.

**Platinum Complexes with Chiral Monoamines**

The activity of cis-PtA2X2 compounds (A = aminic ligand, X = anionic ligand) decreases in the order A = NH3 > RNH2 > RN2NH (24). Therefore, most investigations were restricted to platinum complexes with chiral primary amines. Platinum complexes with monodentate enantiomeric primary amines do not show significant differences in their biological activity (25). One compound of this class, the platinum complex with phenethylamine, is shown in Figure 1.

A possible explanation for this result is that the free rotations of the chiral substituent around the nitrogen–nitrogen (C—N) bond and of the amine around the platinum–nitrogen (Pt—N) bond average the steric effect due to the ligand asymmetry and offset any stereospecificity in the interaction with biological substrates.

**Platinum Complexes with Chiral C-Substituted Ethylenediamines**

The degree of rotational freedom in a complex of the type described in the previous section can be reduced by bridging together the two nitrogens of the cis amines. A ligand that fulfills these requisites is ethambutol. This molecule was already used in medicine as an anti-tuberculosis, and very interestingly, only the S,S isomer was found very active; the R,R enantiomer was completely inactive (26,27). Starting with an isomerically pure ligand, coordination to platinum leads to formation of different isomers. The reason for this is that, upon coordination to platinum, the nitrogens also become stable chiral centers and can have either R or S configuration. The two enantiomers shown in Figure 2 were isolated in the pure form, with biological activities that could be compared.

It is interesting to note that the bridging of the two nitrogens with the ethylene chain not only blocks the rotation around the Pt—N bonds but also hinders, to some extent, the rotation of the asymmetric 1-butanol-2-yl radical with respect to the C—N bond. This was revealed by the 1H nuclear magnetic resonance showing a remarkable diastereotopic splitting of the methylene protons of the CH3Me groups adjacent to the asymmetric carbons. Therefore, the average orientation of the 1-butanol-2-yl radicals is such that the ethyl residues are hindered in their rotation around the carbon–ethyl bond (28).

The much less rotational freedom of the asymmetric substituents in these complexes leads to a different biological activity for the two enantiomers. Indeed, enantiomer a is less mutagenic and less toxic than enantiomer b but, in contrast, exhibits good antitumor activity toward P388 sarcoma and Lewis lung carcinoma (29). Evidently, a can couple reduced mutagenic activity with good antitumor activity, and this appears to be a rather noteworthy result.

In the compound just described, the configuration at the nitrogen atoms was stable at neutral pH for days at room temperature; however, at higher temperatures and/or more basic pH, isomerization can take place. This phenomenon has prevented further studies on complexes of this class.

**Platinum Complexes with Chiral N-Substituted Ethylenediamines**

The complication arising from isomerization at the nitrogen atoms could be avoided by using chiral diamines in which the chiral carbon(s) are inserted in the organic chain bridging the two nitrogens. In this way the steric...
rigidity of the nonleaving ligands is further increased because the chiral groups are no longer free to rotate around the C—N bonds, as is the case of the compound considered in the preceding section.

Kidani and co-workers have reported that platinum complexes with 1,2-diaminocyclohexane, having different configurations at the two chiral carbons bridging the two nitrogens, had biological activities dependent on the chirality of the diamine ligand (30–33). Although the isomers with \( R,R \) and \( S,S \) configurations at the asymmetric carbons produce the same type of intra- and interstrand cross-links (34), the biological activity of the two enantiomers is different, and the \( R,R \) enantiomer exhibits higher antitumor activity and lower mutagenicity than the \( S,S \) isomer (35). Chiral diamines other than 1,2-diaminocyclohexane have also been investigated (36–40).

A comparative study of three strictly related platinum complexes with chiral diamines \([\text{PtCl}_2(\mathit{N—N})]_2\), where \( \mathit{N—N} = 1,2\text{-diaminopropane (1,2-DAP), 2,3-diaminobutane (2,3-DAB), or 1,2-diaminocyclohexane (1,2-DACH)}\) has been also carried out by some of us (Figure 3). The biological tests, in \textit{vitro}, have revealed a marked difference among isomers. For instance, the mutagenic activity, which is strictly related to the interaction of the drug with DNA, is even 10 times greater in one isomer relative to the corresponding enantiomer. In all cases examined the \( S,S \) isomer was by far the most mutagenic, indicating that the different isomers give adducts with DNA that can be discriminated by the enzymatic systems involved in mutagenesis (41).

The conclusions based on mutagenic data concerning the relevance of the configuration of nonleaving ligands in platinated DNA have been confirmed by the studies of inhibition of restriction enzyme activity. The extent of inhibition of the enzymes cutting at guanine (G)-rich sites is significantly different for the different isomers, the \( R,R \) form being more active than the others.

As a result of the markedly different behavior of the two enantiomeric forms, only the \( R,R \) enantiomer of \([\text{Pt(DACH)}(\text{oxalato})]\) (oxaliplatin) has been approved for clinical use (42). Hence, studies have mainly focused on DNA modifications and biological properties of enantiomeric DACH and closely related DAB complexes (43–47).

In the next section we concentrate mainly on a deeper insight into the biological behavior of the latter two types of complexes. However, before concluding this section, we consider nonleaving ligands of the type just described but having also an alkyl substituent on each coordinated nitrogen \( [\mathit{N,N’}-\text{Me}_2\text{DAB}] \) and bipiperidine; Figure 4). Although these compounds are less effective as antitumor drugs because the coordinated nitrogens are no longer primary amine groups (24), they are able to exert steric control on the coordination of nucleotides with platinum. This phenomenon has allowed us to unravel details of the dynamics and conformations of the 1,2-intrastrand cross-links that, as already pointed out, are the major lesions formed by cisplatin-type complexes on DNA (48–52).

### Biochemistry of Platinum Complexes with Enantiomeric DACH and DAB Ligands

The recently reported crystal structure of 1,2-GG intrastrand cross-link formed by oxaliplatin on a DNA dodecanucleotide duplex has shown that the overall geometry is similar to that of cisplatin. However, a novel feature of this structure is the presence of a hydrogen bond between the pseudoequatorial \( \mathit{N—H} \) hydrogen atom of the \( R,R\)-DACH ligand and the O6 atom of the cross-linked G in 3’ position (43,44). This finding has confirmed the importance of chirality in mediating the interaction between cisplatin analogs containing enantiomeric amine ligands and double-helical DNA.

We have shown in a recent work (46) that 1,2-GG intrastrand cross-links of \( R,R\)- and \( S,S\)-DAB platinum complexes (Figure 3) not only destabilize DNA differently but also bend and unwind DNA to a different extent.

DNA containing platinum adducts that induce stable directional bending and unwind attractive various damaged-DNA–binding proteins such as those containing the high-mobility group (HMG) domain (53–56). A recent report (45) has demonstrated that HMGB1 and TATA binding proteins recognize 1,2-GG intrastrand cross-links formed by

---

**Figure 1.** Example of the platinum complex with monodentate enantiomeric primary amines. Ph, phenyl; phetam, phenylamine.

**Figure 2.** Structures of enantiomeric forms of \([\text{PtCl}_2(\text{ethambutol})]\). Et, ethyl.

**Figure 3.** Structures of related platinum complexes with chiral diamines.
*R,R*-DACH–platinum(II) species. The affinity of these proteins to 1,2-GG intrastrand cross-links of cisplatin depends on several factors, and the efficiency with which the adducts thermodynami- cally destabilize DNA is among the most important. The binding of these pro- teins has been postulated to mediate the antitu- mor properties of the platinum drugs (55,56). In addition, several reports (57–59) have demonstrated that intrastrand cross-links of cis- platin and its direct analogs are removed from DNA during nucleotide excision repair (NER) reactions and that NER is also an important mechanism contributing to cisplatin resistance.

To shed light on how chirality at the carbon atoms of the carrier ligand in cisplatin analogs can affect processing its major adducts in cells, the studies have been performed to demonstrate how HMGB1 box proteins and the NER differentiate between major DNA adducts of cisplatin analogs having enan- tiomeric nonleaving ligands during *in vitro* reactions (47). For these studies the *R,R*- and *S,S*-DAB derivat- ives were chosen because the effect of chirality at the carbon atoms on the biological activity of these compounds was most pronounced (47). Electrophoretic mobility shift assays have shown that domains A and B of HMGB1 protein bind to the cross-links generated by *R,R*-DAB–platinum(II) with a higher affinity than to those generated by the *S,S*-DAB–platinum(II) enantiomer (Figure 5). The cross-links of both enantiomers are removed by NER with a similar efficiency; however, HMGB1 protein significantly inhibits removal of *R,R*-DAB–platinum(II) adducts, but not those of the *S,S*-DAB–platinum(II) enan- tiomer (Figure 6). Therefore, HMG domain proteins discriminate among different confor- mations of the 1,2-GG intrastrand cross-links of the two enantiomeric analogs of cisplatin, which results in different NER of these cross-links.

The results obtained with DAB–platinum(II) complexes apply also to the DACH–platinum(II) species (60). They imply that the higher affinity of HMGB1 proteins to an *R,R*-DACH–platinum(II) cross-link than to an *S,S*-DACH–platinum(II) cross-link coupled with a greater error-prone NER repair of the *S,S*-DACH–platinum(II) cross-links could explain both the better antitumor activity of the *R,R*-form of oxaliplatin and the greater mutagenic activity of the *S,S*-enantiomer.

**Conclusions**

Platinum complexes containing enantiomeric ligands pose an interesting theme to investigate structure–pharmacological activity relationship of platinum compounds. The pharmacologi- cally relevant target of platinum compounds is DNA. The major adducts are the 1,2-GG and 1,2-AG intrastrand cross-links. Recognition and repair of these lesions by DNA binding proteins are crucial steps in the cellular response to the drug treatment. The bulk of the results demonstrate that the different stereochemistry of these cross-links is responsible for their dif- ferent affinities for HMGB box proteins and, consequently, for the different NER of these lesions. It is possible to conclude that DNA cross-links of platinum complexes with enan- tiomeric carrier ligands not only can exhibit different conformational features but also can be processed differently by the cellular machinery as a consequence of these conformational differences. However, the conformational free- dom of the enantiomeric platinum compounds has to be limited, so a relevant chiral discrimi- nation might play a role in the biological activ- ity. This was the case for chiral centers inserted in the chelating chain of a diamine.

The results reviewed in this article expand the general knowledge of how the stereochem- istry of the carrier amine ligands of antitumor platinum compounds can influence some cru- cial processes underlying their toxicity toward...
Metal Toxicity • Benedetti et al.

12. Sherman SE, Gibson D, Wang AHJ, Lippard SJ. X-ray crystal structure of the anticancer drug cisplatin bound to duplex DNA. J Am Chem Soc 118:12309–12312 (1996).
18. Ormerod MG, O'Neill C, Robertson D, Kelland LR, Harrap KR. Cis-diaminedichloroplatinum(II)-induced cell death through apoptosis in sensitive and resistant human ovarian carcinoma cell lines. Cancer Chemother Pharmacol 37:462–467 (1996).
19. Sherman SE, Lippard SJ. Structural aspects of platinum anticancer drug interaction with DNA. Chem Rev 1977:153–168 (1977).
27. Kritsyn AM, Likhoshertov AM, Protopopova TV, Skoldinov AP. Ethambutol and related compounds. Synthesis and biological activity of platinum(II) complexes containing chiral amines. J Am Chem Soc 118:12309–12321 (1996).
28. Giannini G, Natile G. Steric constraints inside the metal coordination sphere as revealed by diastereotopic splitting of metal-centered NMR signals. Inorg Chem 35:32–35 (1996).
31. Kidani Y, Inagaki K, Saito R, Tsukagoshi S. Synthesis and antitumor activities of platinum(II) complexes of 1,2-diaminocyclohexane isomers and their related derivatives. Gann 67:921–922 (1996).
32. Kidani Y, Inagaki K, Saito R, Tsukagoshi K. Synthesis and antitumor activities of platinum(II) complexes of 1,2-diaminocyclohexane isomers and their related derivatives. J Hematol Oncol 7:197–208 (1997).
34. Boudny V, Vrana O, Gaucheron F, Kleinwächter V, Leng M, Keefe LJ. The crystal structures of four models for the binding of cisplatin to DNA. Biochemistry 40:2535–2538 (2001).
35. Noji M, Okamoto K, Kidani Y, Tashiro T. Relation of conformation, identification, and quantitation. Biochemistry 28:4142–4147 (1989).
36. Okamoto K, Noji M, Tashiro T, Kidani Y. Preparation of platinum(IV) aceto-ammine-dichloro-cyclohexylamine-platinum(IV) complexes with in-plane bulk to trap initial adducts. rele-tail atropisomers. J Am Chem Soc 112:8177–8179 (1990).
37. Pfenür R, Lippert B. Model of the second most abundant (1997) A,G cross-link in duplex DNA. Inorg Chem 36:490–493 (1997).
38. Kidani Y, Inagaki K, Iigo M, Hoshi A, Kuretani K. Antitumor activities of platinum(II) complexes of 1,2-diphenylethylenediamine isomers against leukemia P388. J Med Chem 31:9570–9571 (1988).