The Relation Between Stereochemistry and Biological Activity of Platinum(II) Complexes Chelated with Chiral Diamine Ligands: An Intricate Problem[+]

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Abstract: The origin of activity differences between stereoisomers of anticancer platinum(II) complexes chelated with chiral diamine ligands has been almost exclusively explained by diastereoselective interactions with DNA. Although this model has been widely accepted *in vitro* and *in vivo* experiments showed some conflicting results, leading to the conclusion that other biomolecules might be responsible for this stereoselectivity as well. These compounds, called bionucleophiles, are in most instances amino acids or proteins present in biological fluids. As these chiral molecules are very reactive towards the platinum complexes, they may contribute to stereoselectivity, but also to resistance and toxicity. This review gives a general survey of chiral platinum(II) complexes and their interactions with DNA. The bionucleophiles which have been identified and the consequences of their reaction with platinum(II) complexes are discussed. Analytical techniques used to investigate interactions between established and potential chiral platinum drugs and bionucleophiles are presented.

Key Words: Stereoisomers, cancer, platinum complexes, bionucleophiles, analysis, stereoselectivity, diaminocyclohexane.

[+] Dedicated to Dr. Michaël Gelbcke on the occasion of his retirement and to Prof. Ronald Gust.

1. INTRODUCTION

It has been established for about fifty years that stereochemistry is fundamental in pharmacology [1-6]. Indeed, the influence of relative and absolute configuration on absorption, distribution, target binding, and elimination of therapeutic molecules is a critical point to be studied before approval for medical applications. For this reason, public health organizations emphasize the analysis of each stereoisomer, to detect possible drawbacks of the drug candidates (for instance, see FDA's guidelines : « FDA's policy statement for the development of new stereoisomeric drugs », published in 1992).

Platinum anticancer drugs have been somewhat kept aside from this problem although three out of six complexes approved for therapy have a chiral structure (**3**, **5** and **6**) (Fig. **1**). The most well-known is oxaliplatin (**3**). This drug is the (1R,2R) enantiomer of the 1,2-diaminocyclohexane (DACH) oxalatoplatinum(II) complex and has been worldwide approved for the treatment of metastatic colorectal carcinoma [7, 8]. Both other complexes (**5** and **6**) have only limited approval. SKI 2053 R (or heptaplatin (**5**)) has been used in South-Korea since the middle 1990's for the treatment of gastric cancer but there is little information about its stereochemical related properties *in vitro* and *in vivo* [9]. Lobaplatin (**6**) is a mixture of trans-isomers. Some compounds are currently tested or have been used in clinical trials (Fig. **2**). They derive from oxaliplatin and the DACH group acts as chelating agent. Complex (**7**), called TRK-710, differs from oxaliplatin by its leaving group and is also accompagnied by a counter-ion since it bears a positive charge (Fig. **2**). Compound (**8**), called ormaplatin (or tetraplatin), which belongs to the platinum(IV) complex family, is a mixture of trans isomers of DACH and was a promising candidate for anticancer therapy but has been abandoned because of its high neurotoxicity in phase I clinical trials [10]. Some papers report cytotoxicity studies on tetraplatin stereoisomers, showing the same results obtained with oxaliplatin isomers: RR-**8** > SS-**8** and RS-**8** in the majority of the cell lines used [11]. Other studies have compared the racemate of **8** with SS-**8** [12] and RR-**8** [13]. However, curiously, most authors have not been interested in the use of pure optical isomers of compound (**8**) and analogues, although trans-R,R-diaminocyclohexane is easily accessible by commercial suppliers or can be isolated *via* the respective tartrate salt from the racemate.

The origin of the stereoselective activity of chiral platinum complexes has been attributed, as for « classical » drugs, to discriminations determined by the target, already identified as being the DNA. This was observed for the first time by Kidani for complexes with DACH ligands and their derivatives [14-16]. Indeed, as DNA has a chiral structure, this theory was the most relevant for the understanding of stereoselection. Many studies, using molecular modelling, spectroscopic techniques and crystallography, have shown energetically favourable interactions between chiral ligands of platinum complexes stereoisomers and DNA structure or small nucleotides [17-22]. The first class of compounds used to investigate the relations between spatial configuration of the ligand and the ability of the complexes to form efficient adducts with DNA was the DACH series [8, 23-25]. This special feature arises from the fact that each DACH stereoisomer has a particularly stable bulk structure with a nearly planar shape for (RR) and (SS)-(DACH) dichloroplatinum(II) (structure A, Scheme **1**) and a L-shaped molecule for the (RS) stereosiomer (structure B, Scheme **1**). As a consequence of these observations, when the RS stereoisomer forms an adduct with DNA, significant steric hindrance is expected, resulting in a slower binding of the complexes to DNA as well as a different recognition and processing of the formed adducts. This theory is confirmed by numerous cytotoxicity tests on DACH and their structural analogues which have, in most cases, shown the same order of activity (RR $\geq$ SS > RS).

Despite these evidences, also some controversial results were obtained with chiral platinum complexes. Sometimes, when small modifications are made in the structure of chemically related compounds (for instance: change in substitution pattern of an aromatic ring or an alkyl chain) or in the conditions used to test the complexes (for instance: change in cell lines, change from *in vitro* to *in vivo* test), a reversal of the stereoselectivity order can be detected. These observations have been made with diastereoisomers and even with enantiomers. It appears from these findings that other factors than DNA structure alone may influence stereoselectivity. Consequently, it is crucial to understand, how the biological components of the human body will interact with different isomers of platinum

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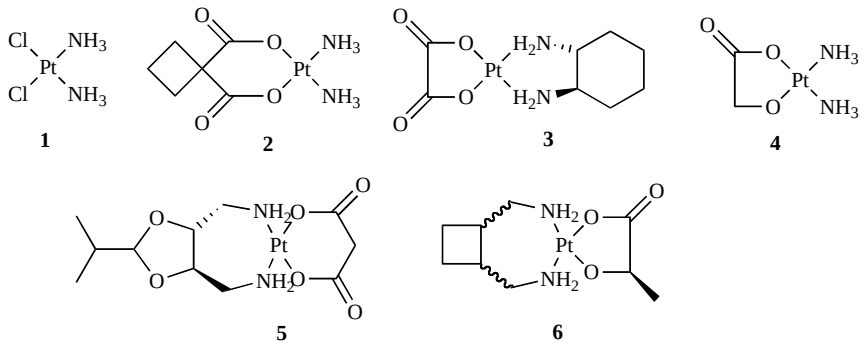


Fig. (1). Platinum complexes approved for antitumor therapy: cisplatin (**1**), carboplatin (**2**), oxaliplatin (**3**), nedaplatin (**4**), SKI 2053 R (**5**) and Lobaplatin (**6**).

anticancer drugs and to identify them. This analysis could shed more light on the mechanism of action of chiral platinum complexes and would help to design novel analogues with improved anticancer properties and a more favourable pharmacological profile.

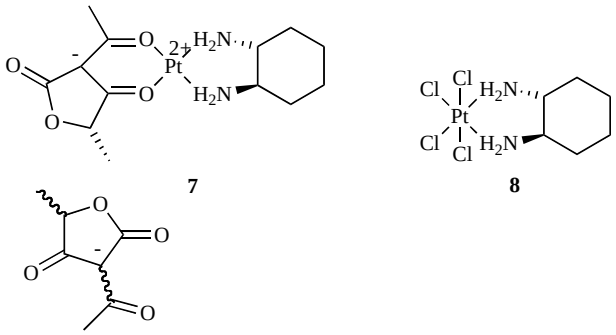
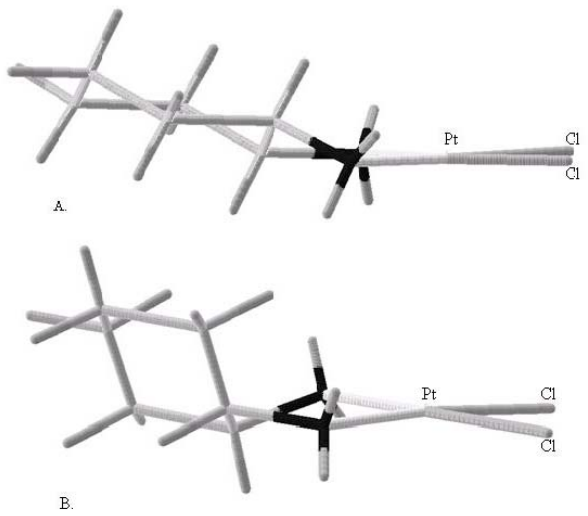


Fig. (2). Other important platinum complexes derived from oxaliplatin: TRK-710 (**7**) and ormaplatin (**8**).



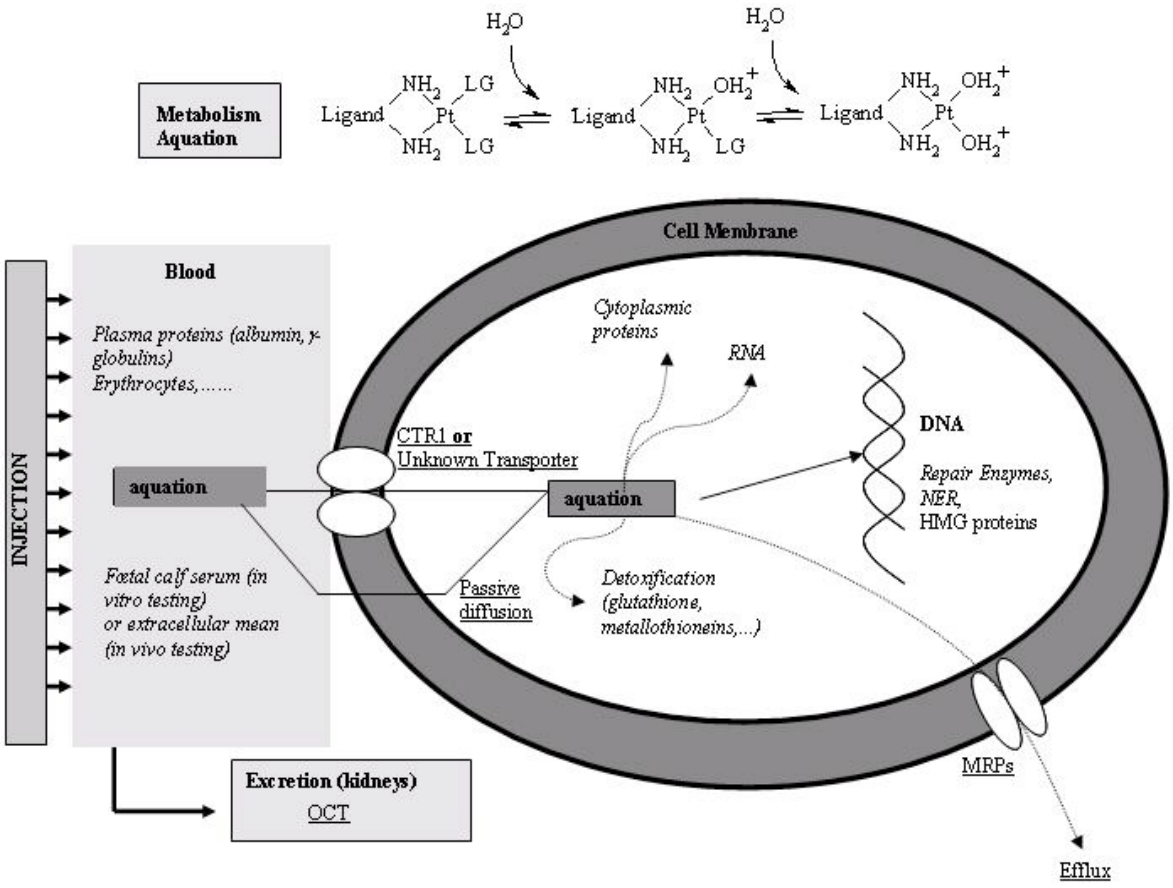
Scheme (1). Three-dimensional representations of (trans-1R,2R-diaminocyclohexane) dichloroplatinum(II) (A) and (cis-1R,2S-diaminocyclohexane) dichloroplatinum(II) (B) (3D-structures performed with PC-MODEL ® 7.0, Serena Software) .

2. DISTRIBUTION AND MODE OF ACTION OF ANTICANCER PLATINUM(II) COMPLEXES

Metal complexes show a significantly different pharmacological behaviour due to the metal centre. Platinum compounds are in principle able to react with all nucleophiles present in blood and cells [26]. The different steps involved in the anticancer action of platinum(II) complexes are quite well understood and described (see Scheme **2**). All approved platinum complexes used in cancer chemotherapy are injected intravenously and, consequently, the behaviour of such complexes in the gastrointestinal tract is not well investigated. Recently, some attempts were made to develop orally active compounds. Good examples of these "per os" complexes are satraplatin (JM216, a platinum(IV) complex in phase III clinical trials), LA-12 (an aminoadamantane derivative of satraplatin, which has finished clinical phase I) [27], AMD473 (JM473) and some derivatives of oxaliplatin presenting more lipophilic leaving groups (C5-OHP-C1) [25,28]. In this context it has to be mentioned that oral absorption is based on crossing the intestinal wall by simple diffusion mechanisms which are mainly dependent on the partition coefficients of the compounds between water and cell membranes. In addition, such types of complexes have to be stable enough under the conditions in the gastro-intestinal tract.

When injected, the complexes can bind to circulating blood proteins, especially to albumin and, to a lesser extent, to haemoglobin and γ-globulins. For example, the fraction of cisplatin, oxaliplatin and carboplatin bound to plasma proteins is, respectively, 90 %, 85 % and ~ 37% [29]. These protein bound complexes are believed to be devoid of anticancer activity and lost for therapy. The proteins, which can react with platinum and other heavy metals, are essential to detoxify the organism. In cytosol, metallothioneins and glutathione have the same effect and « deactivate » metal drugs (biological cytoprotective agents). For this reason, interaction of platinum complexes with proteins and other sulfur-containing biomolecules is one of the critical points to be studied in conjunction with testing the platinum drug candidates in cellular or biological systems. Platinum complexes will be chemically modified in extra- and intra cellular body fluids but not in the same way as « organic drugs ». They are transformed by a non-enzymatic reaction, a stepwise hydrolysis (also called « aquation ») of the leaving groups ending up in an activated form in which the complex has one or two coordinated water molecules bound to the platinum core. Thanks to the positively charged hydrolysis products, these newly generated species are strong electrophiles which are now able to efficiently react with anions or electron-rich parts of biological components. If the complex can not reach the cancer cells, it is excreted mainly by the kidney and poorly in faeces. The renal clearance of cisplatin and oxaliplatin may be considered as slow in comparison to the large majority of drugs with 50 % of the dose eliminated by the kidney after only 5 days [30]. In the case of cisplatin, high platinum concentrations in the kidneys are accompanied by dose-limiting severe side-effects due to destruction of the renal tissues.

Only a small fraction of the drug reaches the cancer cells and has still to cross the cell and nucleus membranes to react with the final target: DNA. The precise mechanism of cell penetration has



Scheme (2). General pharmacokinetics of anticancer platinum(II) complexes (biological compounds which bind to platinum and reduce its activity are in italic or marked with dashed arrows, transport systems are underlined). Note that the aquation step may happen predominantly in cytoplasm, depending on the type of leaving group.

not yet been identified. It is generally well accepted that the complexes pass the cell wall by passive diffusion. Indeed the phenomenon can not be saturated or inhibited by structural analogues [26]. However, some clues for a transport suggest the implication of a metal transport protein: copper transporter 1 (CTR1) [31]. This protein seems to be involved in the uptake of the complexes as well as in their efflux [32-34]. Strictly speaking, this does not mean that CTR1 is the influx reporter for platinum complexes, but a reduction of cellular uptake in CTR1 knockout Saccharomyces cerevisiae strains of copper (16-fold) and cisplatin (8-fold) measured at 1 h suggests a certain role of CTR1 as one possible route of cisplatin influx.

When the activated platinum complex binds to DNA, several well identified phenomena take place which finally trigger apoptosis (programmed cell death). These include: i. modification (denaturation) of the DNA structure, ii. binding of specialized proteins (maybe the most important are High Mobility Group proteins or HMG) which recognize damaged DNA thereby shielding such regions from repair. Contrary, other proteins will restore the integrity of DNA by repair of the injured zones, leading in this way to resistance (Nucleoside Excision Repair or NER).

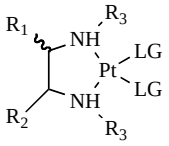
After analysis of the described mechanisms and reactions of the platinum complexes in blood and cells, it is obvious that, at each step, the stereochemistry of the diamine ligand has a critical role to play in anticancer activity. Stereoisomers may have different reaction rates with biological compounds (proteins, DNA, aminoacids,…) or the stability of these reaction products may be different according to their geometry.

3. INFLUENCE OF THE LIGAND'S STEREOCHEMISTRY ON PHARMACOKINETICS AND ACTIVITY OF PLATINUM(II) ANTICANCER COMPLEXES

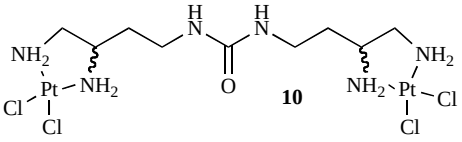
3.1. Platinum(II) Complexes Showing Stereoselective Biological Properties

Besides the above-mentioned chiral complexes, a large variety of such compounds has been synthesized and tested *in vitro* or in vivo. Some of them have shown significant stereoselectivity in their cytotoxicity and/or toxicity (Fig. **3** and Table **1**). It has been decided to analyze separately compounds with different size of diazaplatina cycles. Complexes have been divided into three groups: the compounds of which have, respectively, 5, 6 or 7 atoms in the chelate ring. Among them, only 5-membered cycle complexes provide useful results for analysis because of the variety of compounds tested. If structurally related complexes are compared to each other, shared features are seldom observed for the most active isomers. For instance, complexes R-(**9c**), S-(**15**), S-(**18a**) and S-(**18b**), which differ by their substituents in position 1 of the carbon chain, show the same absolute configuration and are also the most active compounds within their series. However, R-**9a** and R-**10** have an absolute configuration opposed to that of the above-mentioned molecules. In another class of compounds with two chiral centres and one or two phenyl ring(s) as substituents, surprising results have been obtained. Indeed, the SS enantiomers of diphenylethanediamine complexes (**19b-d**) are always the most cytotoxic, independently of the substitution pattern on the aromatic rings. Nevertheless, when one phenyl ring is replaced by an alkyl chain, the selectivity between enantiomers changes following the type of substituent on

5-Atoms cycles



LG = Cl^-, Br^-, sulfate
9a: R_1 = Me, R_2 = H, R_3 = H
9b: R_1 = R_2 = Me, R_3 = H
9c: R_1 = $HOCH_2$, R_2 = R_3 = H
9d: R_1 = R_2 = R_3 = Me



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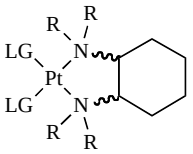
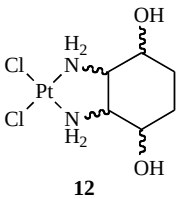


DACH

LG = Cl^-, sulfate
11a: R = H
11b: R = Me



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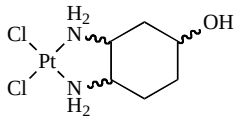

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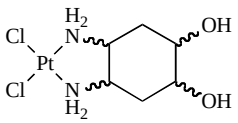

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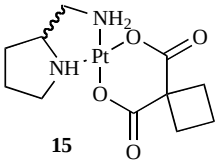

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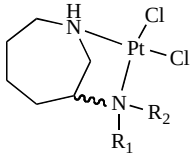
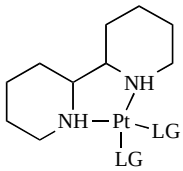
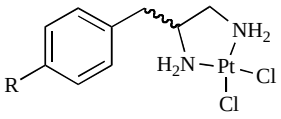


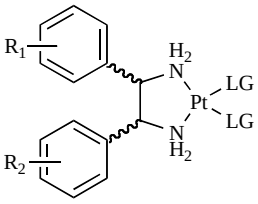
16a: R_1 = R_2 = H (ahaz)
16b: R_1 = H, R_2 = Me (meahaz)
16c: R_1 = H, R_2 = Et (etahaz)
16d: R_1 = R_2 = Me (dimeahaz)



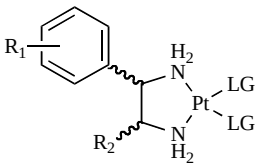
17: LG = nitrate, sulfate



18a: R = H
18b: R = Cl

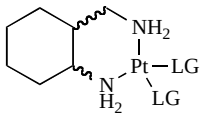


19a: R_1 = R_2 = 4-F, LG = Cl^-
19b: R_1 = R_2 = 4-OH, LG = Cl^-
19c: R_1 = R_2 = 2-OH, LG = Cl^-
19d: R_1 = R_2 = 3-OH, LG = Cl^-
19e: R_1 = R_2 = 2,6-Cl_2-4-OH, LG = Cl^-
19f: R_1 = R_2 = H, LG = sulfate
19g: R_1 = R_2 = 2,6-Cl_2-3-OH, LG = sulfate
19h: R_1 = R_2 = 2-Cl-6-F-4-OH, LG = sulfate
19i: R_1 = R_2 = 2,6-F_2-3-OH, LG = sulfate



20a: R_1 = H, R_2 = Me, LG = Cl^-
20b: R_1 = 4-F, R_2 = Me, LG = Cl^-
20c: R_1 = 4-F, R_2 = Et, LG = Cl^-, I^- or sulfate
20d: R_1 = 4-F, R_2 = iPr, LG = Cl^-, I^- or sulfate

6- Atoms cycles



AMCHA
LG = Cl^-, sulfate, nitrate, glucuronate, oxalate, gluconate
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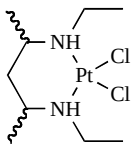

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7- Atoms cycles

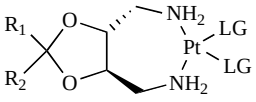

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Fig. (3). Platinum(II) complexes with chiral diamines as ligands, sorted following platinum-diamine cycle size (LG = Leaving Group).

the phenyl group and the type of alkyl chain, with a small advantage for RR enantiomers. These results definitively show that the relation between stereochemistry of the diamine ligand and the configuration of DNA is not the only way to explain differences in cytotoxicity of enantiomers.

In addition to these observations with parent complexes, particularly amazing results, which will be discussed in detail in section 3.3, have been obtained with compounds subjected to a small modification in substitution pattern or in leaving groups, or using different experimental conditions (Table **2** and Fig. **3**). The large majority of fundamental studies about mechanism of action of platinum complexes point out DNA as the most important target for these drugs [26,58], although interactions with other biological compounds, as proteins actin and filamin, have also been reported recently to be involved [59]. Since chiral structure and absolute configuration of DNA are basically the same in all living organism cells, including cancer cells, other factors must have a significant impact on the cytotoxicity of platinum complex stereoisomers.

3.2. Identified Factors Affecting Cytotoxic Activity of Anticancer Platinum(II) Complexes and their Relation to Stereochemistry

The most evident and most studied factor correlating the stereoselectivity with the cytotoxic properties of anticancer platinum

complexes is the target itself. Very soon, DNA has been identified as the origin of anticancer activity of platinum(II) complexes [60]. Particularly well adapted techniques have been used to demonstrate

Table 1. Stereochemical Discriminations in Cytotoxicity and Toxicity for Platinum(II) Complexes with Chiral Diamines As Ligands

Complexes	Experimental Model	Observations	Ref.
Cytotoxicity			
9a	Murine leukemia P-388 in vivo	R > S	[35]
9b	Murine leukemia P-388 in vivo	RR > SS	[35]
9c	Murine leukemia L1210 in vivo	R > S	[36]
10	Murine leukemia L1210 in vivo	RR > SS	[37]
11a	Murine leukemia L1210 *in vivo* [a] Murine leukemia P-388 *in vivo* [a]	trans > cis[b] RR > SS = Meso	[14] [35]
12	Murine leukemia P-388 in vivo	t,t,t > c,t,c[c]	[38]
15	Colon 26 carcinoma *in vivo* (mice)	S > R	[39]
16a	UCRU BL13/0 cell line in vitro	R > S	[40]
16b	PC9-cisR cell line in vitro	R > S	[41]
16c	PC9-cisR cell line in vitro UCRU BL13/0 cell line in vitro DU145 cell line in vitro	R > S R > S R > S	[41]
18a	MDA-MB 231 cell line in vitro Murine leukemia P-388 in vivo	R > S S > R	[42]
18b	Murine leukemia P-388 in vivo	S > R	[42]
19a	MCF-7 cell line in vitro	(RR,SS) > Meso	[43]
19b	MDA-MB 231 cell line in vitro	SS > RR > Meso	[44]
19c	NIH:OVCAR-3 cell line in vitro	SS > RR > Meso	[45]
19d	Murine leukemia P-388 in vivo Murine leukemia L1210 in vivo	SS > RR = Meso SS > RR = Meso	[46]
19f	Murine leukemia P-388 in vivo	RR > Meso > SS	[35]
20a	MCF-7 cell line in vitro	RR > SS > RS = SR	[47]
20b	MCF-7 cell line in vitro	SS > RR > RS = SR	[48]
20c	MCF-7 cell line in vitro MDA-MB 231 cell line in vitro LnCaP/FGC cell line in vitro	RR > SS > RS > SR SS > RR > RS = SR SS > RR > RS > SR	[49] - [d] - [d]
20d	MCF-7 cell line in vitro MDA-MB 231 cell line in vitro LnCaP/FGC cell line in vitro	RR > SS > SR > RS RR = SS > SR > RS RR > SS > SR > RS	- [e] - [e] - [e]
21	Murine leukemia L1210 in vivo	L-cis > D-cis > D-trans = L-trans [f]	[50]
22	Murine leukemia L1210 in vivo	RR > SS	[51]
23	Murine leukemia L1210 in vivo	RR > SS	[9]
Toxicity			
13	Murine leukemia P-388 in vivo	trans > cis [g]	[52]
14	Murine leukemia P-388 in vivo	trans > cis [g]	[53]
15	Renal toxicity in mice	S > R	[39]

[a] leaving group : Cl^-. [b] enantiomers not described. [c] defined according to hydroxy and amino groups relative positions, respectively OH vs. NH_2, NH_2 vs. NH_2 and NH_2 vs. OH (t= trans and c = cis). [d] unpublished results. [e] Publication accepted in Chem. Med. Chem.. [f] leaving group: Cl^-. [g] defined according to amino groups relative positions only (trans or cis).

these interactions and they also showed the way in which the structure of DNA could be modified by platinum coordination. X-ray crystal diffraction of complex-oligonucleotide adducts have highlighted large changes into nucleic acid conformation (see Scheme **3**). The Lippard's group from the MIT has shown very obviously on a double stranded DNA dodecamer that binding of cisplatin induced remarkable modifications of orientation and shape of DNA, with a bending of the structure by ± 20°. Other characteristics of the adducts led the authors to the conclusion that the DNA has shifted from a B-form into an A-form [61]. These findings were confirmed later by 2D NOESY ^{1}H-NMR experiments on the same type of adducts [62]. Nuclear magnetic resonance is especially well adapted to solution structures of platinum adducts with small DNA duplexes, giving an accurate representation under biologically relevant conditions. Such experiments were very useful in order to determine the mechanism by which enantio- or diastereoisomer differentiation occurs *in vitro* and vivo. Indeed, at this stage, two mechanisms could be involved into induction of stereoselectivity (see scheme **4**). The first of them occurs during the initial binding phase of the complex to the DNA and was directly referred to the diastereoisomeric complexes with DACH ligands [16]. It finds its origin in unfavourable bulky interactions between DNA and the ligands. In the case of cis-R,S-DACH platinum complexes, the cyclohexane ring adopts a nearly perpendicular arrangement with respect to the N_2PtO_2 plane. The second one takes place during the bending of the DNA and is a result of the later findings about twisting in DNA structure also leading to stereochemical discriminations. As a matter of fact, it has been largely demonstrated that this distorted shape of double stranded DNA is specifically recognized in the case of oxaliplatin type complexes by HMGB proteins, which shield this bended part of the DNA from repair and thereby trigger apoptosis [58].

To support these proposals, various experiments have been conducted with chiral platinum(II) complexes. The most frequently studied class of compounds consequently included the DACH complexes and close analogues thereof. Earlier studies investigating the reactions with dinucleotides (GpG) have confirmed that the activity order found *in vitro* is strongly dependent on the degree of the overall nucleic acid structural change: the most important differences are observed between RR-**11a**/SS-**11a** and RS-**11a**, the smallest between RR-**11a** and SS-**11a** [18]. Later, Boudny and coworkers have shown on larger nucleotide fragments that the critical structure modification which could explain enantiomeric differences is the bending of the structure [64]. Global structural changes obtained with RR-**11a** are practically the same as those obtained with cisplatin, while the torsion induced by SS-**11a** binding is less effective. On the other hand, the bending caused by RS-**11a**, the least active isomer, is larger than the one obtained with its diastereoisomeic counterparts. So, these results demonstrate the involvement of the ligand's stereochemistry into the extent of denaturation of DNA but fail to prove that the larger DNA distortions lead to the higher activities. Later, it has been demonstrated that this is the bending angle which is the critical factor explaining the cytotoxic power of these compounds. Other surprising observations have been made by calculating the ability of platinum complexes to damage DNA. In this experiment, Murray and coworkers have measured damaging efficacy of DNA in the following order: cisplatin > SS-**11a** > RR-**11a** [65]. On the other hand, in a merely theoretical model, molecular modelling studies have been carried out on adducts of RR-**11a**, SS-**11a** and RS-**11a** with two guanines [66]. Calculated energies of the products were consistent with stereoselective cytotoxicities as observed in some cancer cell lines. Another interesting experiment has been performed on R- and S-**15** by Morikawa and coworkers [67]. Using UV spectra and circular dichroism of DNA following exposition to each enantiomer, they revealed that the structural changes are larger for the most active enantiomer S-**15**. To be exhaustive, it must be noted that some works, which aimed to prove diastereoselective interactions of chiral platinum complexes deriving from DACH ligands with DNA dramatically failed although they used the same methods [68-69].

In addition to these findings on clinically important complexes, other chiral compounds have been studied from a fundamental point

Table 2. Conflicting Results in Cytotoxic Activity of Chiral Diamine Platinum(II) Complexes Isomers

Entries	Complexes	Observations (Cytotoxicity)	Ref.
1	**19a**	In MCF-7 cancer cell line in vitro, Rac-**19a** > Meso-**19a**[a] but in murine Leukemia P-388 *in vivo* : Meso-**19a** > Rac-**19a**	[54]
2	**11a**	In Murine leukemia L1210 *in vivo* : RR > SS In Murine leukemia P-388 *in vivo* : RR = SS In Murine leukemia B16 *in vivo* : RR = SS	[14] [55]
3	**11a**	In murine leukemia L1210 and P-388 in vivo: trans > cis[b] In sarcoma-180 in mice *in vivo* : cis > trans[c]	[15]
4	**16a**	In UCRU BL13/0 cancer cell line *in vitro* : R > S In PC9 cancer cell line : R = S	[40]
5	**18a**	In MDA-MB 231 cell line *in vitro* : R > S In Murine leukemia P-388 *in vivo* : S > R	[42]
6	**20c**	Different order of activity following cancer cell line[a]	[49]
7	**16a 16b, 16c**	The results predicted by molecular modelling were S > R but in most cases the order of activity is R > S	[40, 41]
8	**19c**	Following exposition time of RR- and SS-**19c** to the culture medium and use of buthionine sulfoximine (BSO) : SS = RR or SS > RR	[45]
9	**16b, 16c 16d**	In PC9 cancer cell lines in vitro: R > S R = S	[41]
10	**19g, 19h, 19i**	In hormone-dependent MXT ER^+ mammary tumour of the mouse : Meso-**19g** > Rac-**19g**, Meso-**19h** > Rac-**19h** but Meso-**19i** = Rac-**19i**	[56]
11	**20a, 20b**	Introduction of a fluorine atom in para position of the aromatic ring inverted the order of activity[a]	[47, 48]
12	**20b, 20c**	Lengthening of the diaminoalkyl chain with one carbon inverted the order of activity	[48, 49]
13	**21**	When LG is Cl^-, sulfate, glucuronate or gluconate: cis > trans but with LG = oxalate: trans > cis	[50]
14	**19e**	Rac-**19e** > Meso-**19e** but Meso-**19e** > Rac-**19e** in hormone-sensitive breast cancer	[57]

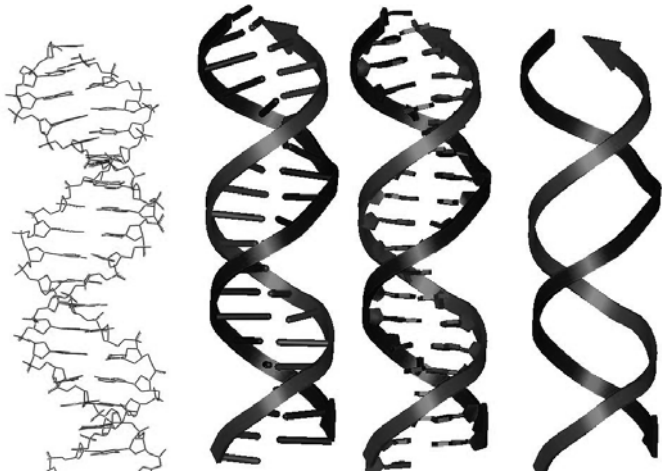
[a] See Table 1. [b] LG = Cl^-. [c] LG = oxalate.

of view. Complexes (**9d**) and (**17**) have been used to investigate the dynamic behaviour of guanine residues and derivatives after platinum binding [22,70,71]. In the first place, these compounds have been tested only with guanines or their derivatives and not with nucleotides, providing only few information about their real effect on DNA conformation. Nevertheless, these experiments clearly showed that chirality of the ligand may influence DNA bases orientation. Later on, adduct formation of SS- and RR-**9d** towards DNA fragments or dinucleotides has been evaluated [72]. The authors clearly demonstrate that a DNA destabilization is more important for SS-**9d** than in the case of the other enantiomer. Surprisingly, the bending angle after the binding of SS-**9d** was smaller in comparison to its enantiomeric counterpart. These observations are in agreement with cytotoxicity tests performed with structurally related complexes (**19b**-**19d**) (phenyl moieties instead of methyl groups), for which the most active isomer is always the SS one. Furthermore, recent investigations have been performed about the interactions between proteins which specifically recognize (HMG) and repair (NER) DNA sites bound to platinum complexes [73]. It has been shown that RR-**9d** causes lesions, which are more easily recognized by HMG proteins and, in addition, which are less sensitive to NER action. These observations pointed out the crucial role of protein interactions with binding sites of platinum complexes at DNA. To complete this overview of outstanding facts, a very recent report, using gel electrophoresis on small DNA fragments has demonstrated that HMG proteins bind differently to DNA after exposition to R- and S-**16a** [74]. This is explainable by different bending angles attributed to the respective configuration of the ligands.

3.3. Conflicting Results

As described above, numerous experimental proofs have been given to explain that DNA is the chiral selector yielding stereoselectivity in anticancer activity of stereoisomers of platinum complexes. Despite these observations, some results arise as exceptions to this rule (see Table **2**). They can be divided into six categories in which the order of activity varies significantly following: i. *in vitro* and *in vivo* testing (entry 1), ii. cancer cell lines (entries 2 to 6), iii. theoretical predictions and experimental results (entry 7), iv. experimental conditions and/or other compounds added during the cytotoxicity evaluation (entry 8), v. substitution pattern of the ligands or changes in leaving groups (entries 9 to 13), vi. biochemical cancer cell characteristics (entry 14).

The outstanding feature from these observations is that other factors must be taken into account to explain and/or to predict stereoselectivity before designing new chiral anticancer platinum complexes. It is obvious that these factors include biological molecules present in extracellular and/or intracellular fluids, but also proteins or receptors located on the cell membrane. Indications for this assertion consist in numerous differences observed when miscellaneous cancer cell lines have been used and, still more obviously, when *in vitro* or *in vivo* experiments are compared. It has already been mentioned that DNA is structurally equivalent in all cells, cancerous or not, even if the nucleoside composition is different. As platinum complexes bind preferably to GG and, to a lesser extent, to AG sequences, DNA sites particularly rich in guanine and adenine are more sensitive to reaction with platinum. Very few attempts have been made to design new chiral complexes with the expected enantiospecific activity on the basis of molecular modelling or docking experiments. In general, quantitative structure-activity relationship (QSAR) data are nearly not available in this field of research due to the problems described above. One step in this direction was presented by Hambley [20]; the group of Osella has more recently published a study about QSAR of platinum complexes with achiral ligands and (RR)-DACH [75]. Despite these examples, this theoretical approach fails with compounds (**16a**), (**16b**) and (**16c**) for which the opposite results were obtained, pointing out an intervention of other biological factors in stereoselective pharmacokinetics [40,41]. Another outstanding evidence of the influence of other bionucleophiles than DNA has been given by Bernhardt *et al*. on complex **19c** stereoisomers [45]. They clearly demonstrated in the case of both enantiomers of this compound that various preincubation times of the drug with the culture medium



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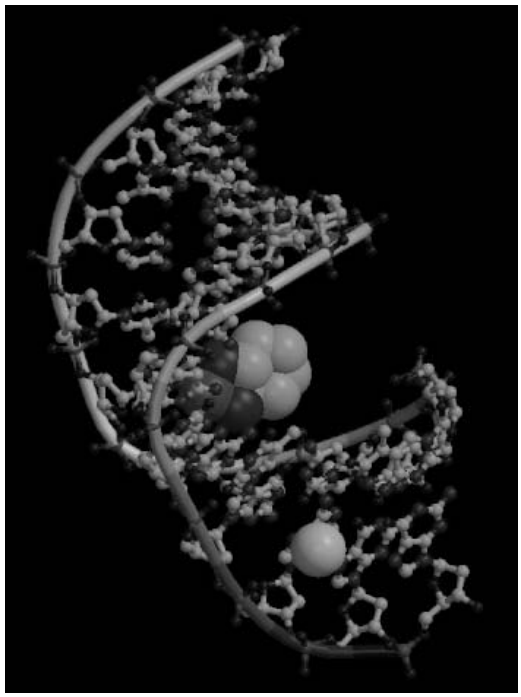

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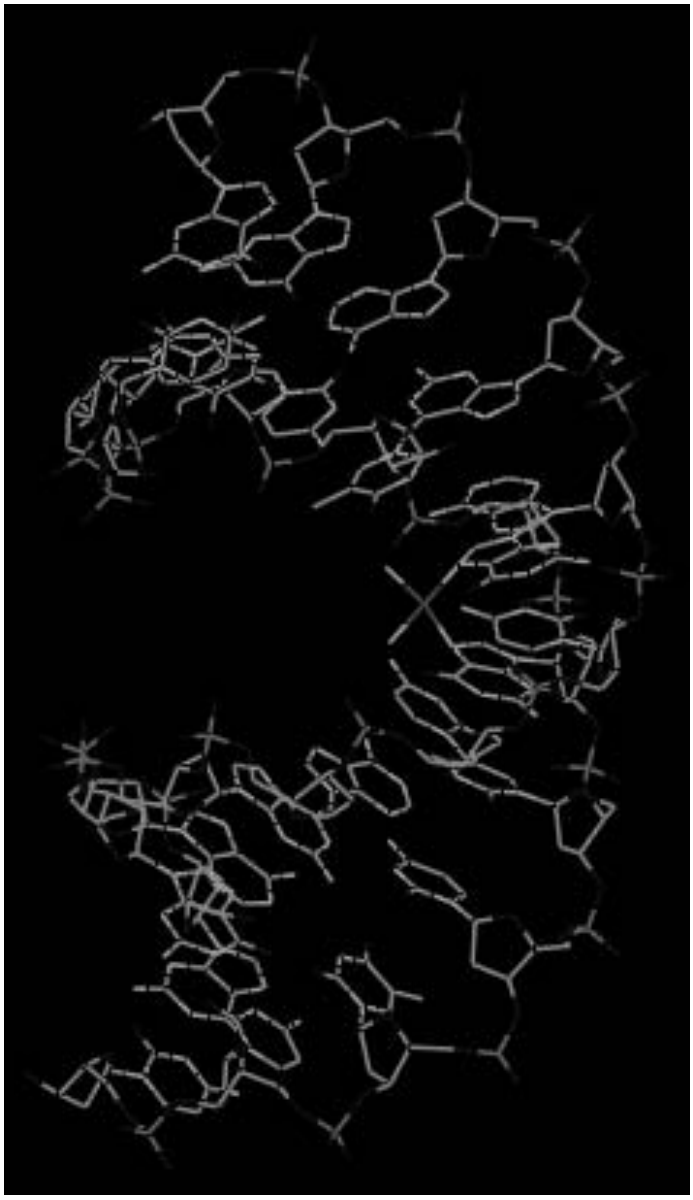


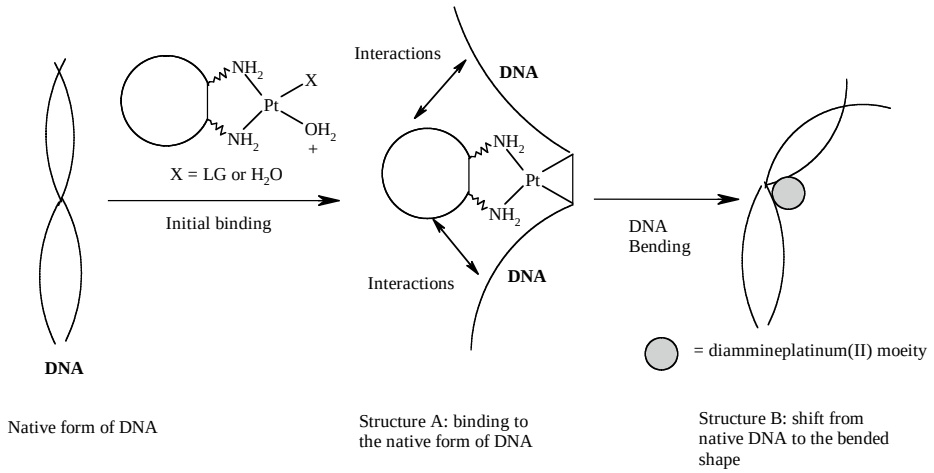
B

Scheme (3). Some representations of native DNA structure (A) and bended DNA structure after cisplatin (B) and oxaliplatin (C) attack (structures B and C from Protein Data Bank, respectively 1AIO [62] and 1PG9 [63].

strongly modified the activity profile of each enantiomer. So, the stereoselective cytotoxicity could be « revealed » after a long incubation time or using buthionine sulfoximine (BSO), an inhibitor of glutathione synthesis. They concluded that reactions with components of the cell culture medium took place before the complexes could bind to DNA.

Other interactions between platinum complexes and biological elements are conceivable, disrupting classical relations between structure and cytotoxic activity. For example, some ligands have been designed to interact with transport proteins, which could be involved in penetration of complexes into the cells or which bind to a specific receptor [76-78]. Furthermore, complexes have been prepared involving ligands known for their anticancer activity [79]. For instance platinum complexes, displaying a diphenylethanediamine ligand have shown activity in breast cancer cells. Diphenylethanediamine has a three-dimensional structure relatively close to that of diethylstilboestrol and derivatives, which has an oestrogenic activity and, therefore, a supplementary antiproliferative effect on a hormone dependant cancer cell line could be demonstrated [80].

Despite these well identified mechanisms, some aspects of stereoselectivity remain unclear and unexplained. This remark is almost exclusively related to the modification of the activity order when small structural changes are induced in the ligands (aromatic substituents, chain ramification,...). Although these experimental results do not give reasonable explanations, a logic answer might be found by taking into account interactions between the entire complex and other biological molecules than DNA. This conclusion is supported by the fact that small changes (see entries 11 and 12) in the structure, even at a significant distance of the platinum atom, caused inversion of the activity order in an enantiomeric series. Consequently, biomolecules being responsible for enantiomer differentiation have still to be unambiguously identified.



Scheme (4). Binding phases of platinum(II) complexes to DNA involved into stereoselectivity in cytotoxic activity.

4. OTHER FACTORS THAN DNA LEADING TO STEREOSELECTIVE CYTOTOXICITY OF PLATINUM(II) ANTICANCER COMPLEXES WITH CHIRAL DIAMINE LIGANDS

In part 2, it has already been mentioned that platinum(II) complexes especially in the activated form are able to react with all kinds of (bio)nucleophiles present in body fluids. Many of them are very abundant in both extra- and intracellular media and are devoted to transportation, protection and metabolic purposes. In particular sulfur-containing bionucleophiles (thiols or thioethers in amino acids, peptides and proteins) are equipped with a specific binding tendency towards heavy metals like platinum. But also nitrogen atoms and carboxylic acid groups show a high preference to coordinate to the platinum(II) center. Since the first studies on the mechanism of action of anticancer platinum complexes research has also focused on the interaction of platinum with biologically relevant nucleophiles. In this context mainly cysteine, methionine, glutathione and albumine were involved in these studies. Nevertheless, the importance of such species with respect to the anticancer properties or the pharmacokinetics of the platinum-based coordination compounds is still unclear and under debate. Two scenario are feasible: (i) such species are produced from the organism for the reason of detoxification (endogenous cytoprotective products) or (ii) they are further involved in the anticancer process (storage hypothesis) to a minor extend. Some of these biological compounds are listed in Table **3**. Since nearly all of these bionucleophiles are per se chiral, it is logical to expect that, just like with DNA, a platinum complex with an optically pure chiral ligand could interact differently with these bionucleophiles resulting in diastereoisomeric adducts with unequal affinity. Logically, stereoselection could also arise from that kind of interaction.

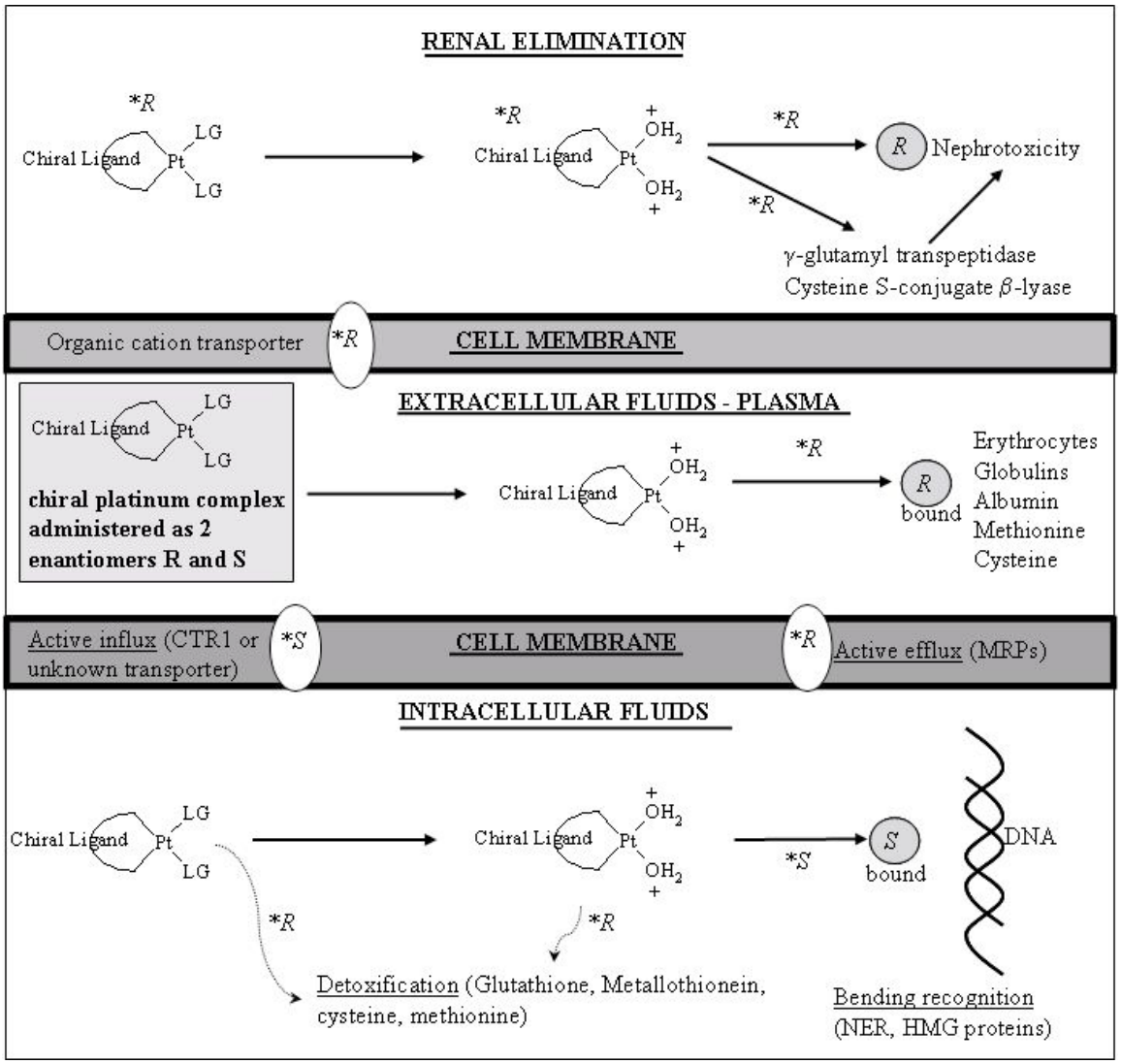
Table 3. Biological Compounds Which Interfere with Pharmacokinetics of Platinum(II) Complexes and are Potential Sources of Stereoselectivity

Entries	Bionubleophiles or Proteins and their Localization	Role in Platinum Complexes Pharmacokinetics[a]	Ref.
	EXTRACELLULAR FLUIDS		
1	Albumin	Irreversible binding	[81]
2	Gamma-globulins	Irreversible binding	[81]
3	Erythrocytes	Irreversible binding	[82]
	CELL MEMBRANE		
4	Multidrug Resistance Proteins (MRPs)	Efflux of platinum complexes, leading to resistance	[83-86]
5	Copper transporter 1 (CTR1)	Influx or efflux of platinum complexes	[34,87]
6	γ-glutamyl transpeptidase (GGT, kidney)	Detoxification of kidney from platinum derivatives AND involved in some toxic reactions (?)	[88,89]
7	Organic cation transporters (OCT, kidney)	Responsible for nephrotoxicity of platinum compounds (?)	[90]
	INTRACELLULAR FLUIDS		
8	Glutathione	Efflux of platinum complexes but not in all cancer cell lines OR conversion into more reactive species	[91-93]
9	Nucleoside excision repair (NER)	DNA repair after complex binding, leading to resistance	[94]
10	High-mobility group protein (HMG)	Recognition of platinum-DNA adduct, triggering apoptosis	[57]
11	Metallothionein	Detoxification of platinum complexes leading to resistance	[95-96]
12	Cysteine S-conjugate β-lyase	Responsible for nephrotoxicity of platinum compounds (?)	[97]
	MULTIPLE LOCALIZATIONS		
13	Methionine	Deactivates platinum complexes BUT may increase the rate of reaction with DNA	[98,99]
14	Cysteine	Deactivates platinum complexes and may be involved in toxicity	[99]

[a] entries annotated with (?) are considered as possibly involved into platinum complexes pharmacokinetics but with very few proofs

Important data have been collected with stereoisomers of oxaliplatin and derivatives with other leaving groups (3). The authors have obtained particularly intriguing results: i. the order of activity is dependent on the type of cancer cell line [14,15], ii. cis-**3** is more easily taken up by the cells although it is the less active isomer [100], iii. the leaving group influences the order of activity [25] and iv. trans-SS-**3** binds DNA at a lower level than cis-**3** (although trans-SS-**3** is more active than cis-RS-**3**) [100]. In conclusion to the experiments, some precious explanations have been given in relation with other biomolecules than DNA but without experimental demonstration. On the other hand, differences in cellular accumulation have been found with both diastereoisomers of **19a**, leading the authors to the conclusion that a stereoselective transport could transfer the respective isomer with unequal rates [101]. Since diastereoisomers have different physical properties and since simple diffusion through the phospholipid bilayer is esteemed as the major penetration pathway into the cell, different solubilities of rac- and meso-**19a** into the cell membrane might account for this phenomenon. Finally, it is noteworthy that several authors draw attention to interactions with bionucleophiles or unidentified biological components when unexpected results have been obtained [41,45,46,48,49, 53,63].

The mechanisms by which biological substances could affect chiral platinum complexes pharmacokinetics in a stereoselective way are summarized in scheme **5**. Each of them may endow one stereoisomer with stereoselectivity in several phases, i.e. distribution, sequestration by proteins, binding to targets and elimination. The different interactions involved may, of course, act on one or several steps, leading to a particular pharmacological profile for these potential anticancer drugs. Among all these elements, only very few have been studied or, even, simply suspected to explain results inconsistent with theoretically expected activity. This hypothesis could be applied to the case of compound **15**, exposed by Morikawa and coworkers, in which S-**15** is the most cytotoxic one but unfortunately also the most nephrotoxic, owing to a higher accumulation in the kidneys [39]. This might be caused by extensive transport *via* the organic cation transporter localized in the kidneys. Another good example is the stereoselective peripheral neurotoxicity observed with compounds **3** and **8** which may arise from a yet unidentified biological system [102]. In entries 1 to 3 blood proteins are listed which react easily with cisplatin and, to a lesser extent, with oxaliplatin, decreasing in this way the amount of biologically active platinum complex. It is important to note that albumin is also present in the *in vitro* cytotoxicity test when using fœtal calf serum.



Scheme (5). Summary of possible interactions between chiral platinum complexes with biological compounds. The case illustrated here is a stereoselection favouring the cytotoxicity of the S enantiomer and endowing with toxicity and resistance to R enantiomer. Interactions occurring preferably with R or S enantiomers are annotated respectively by *R and *S.

In entries 4 and 5 proteins localized in cell membrane are mentioned, being involved into complex influx (CTR1) or efflux (MRPs). The mechanism by which CTR1 captures cisplatin has been recently studied. It involves cysteine- and methionine-rich regions of the channel binding the complex with a high efficiency [103]. Some proteins found in kidney cells are able to transform platinum complexes into nephrotoxic compounds (entry 6) or concentrate them in the renal cells cytoplasm (entry 7). When the complexes enter the cell, they may be detoxified by some compounds (entries 8, 11 and 12). The real role of glutathione in cancer resistance remains controversial since some works clearly demonstrate that it participates to this phenomenon [104,105] while others fail to prove it [106]. After reaction with DNA, NER and HMG proteins interact with injured zones of the DNA, leading either to repair (NER) or apoptosis (entries 9 and 10). These DNA associated proteins are well identified and their interactions with platinum complexes binding zones have been extensively studied with cisplatin. In the case of oxaliplatin (**3**), Faivre and coworkers have brought important contributions to the understanding of relations between binding of the complex and HMG/NER action. They analyzed interactions between oxaliplatin DNA adducts and these proteins [107]. They came to the conclusion that the hydrophobic DACH ligand could interact directly with proteins, showing in this way that differences in DNA bending are not the only factors leading to different activities between cisplatin and oxaliplatin. Since amino acids are present in all human fluids, they are supposed to have some affinity for platinum complexes. Particularly, cysteine and methionine, containing respectively a thiol or thioether, are of high interest. These amino-acids react with platinum complexes and take part into their pharmacokinetics (entries 13 and 14). The extent of such interactions and their impact on the anticancer activity or detoxification of platinum anticancer drugs is yet unknown. Finally, it can be also mentioned that some other proteins may interact with platinum complexes and, by this way, take part to their cytotoxic mechanism of action or in resistance of the cancer cell lines to the complexes. Among these proteins, heat shock protein 90 (Hsp90) [108], telomerase [109-111] and survivin [112] have been identified but their precise role and their importance in the cytotoxic process has still to be determined.

5. ANALYTICAL TECHNIQUES AS BASIS FOR THE DRUG DEVELOPMENT PROCESS

As a consequence of the above-mentioned observations, interactions of bionucleophiles with platinum complexes are very important to be investigated during the development of new platinum anticancer drugs. The major pharmacological feature of chiral platinum complexes is that one stereoisomer may have excellent cytotoxic properties, while the other may be dangerous or have lower efficacy. Therefore, to optimize the design of novel platinum complexes with tumour inhibiting properties, it is essential to detect or to anticipate negative effects of bionucleophiles as soon as possible in the development process. Protection against nucleophilic attack can be achieved by using molecular modelling techniques to generate a shield effect around the platinum atom. This protection due to the steric hindrance of the ligand has been put forward to explain the lower reactivity of compound (**19a**) towards reactive biological compounds [113]. However, it has to be kept in mind that avoiding these undesirable reactions could also result in a negative effect on binding to DNA, leading to less active complexes. With the aim of overcoming these problems, a series of analytical techniques is available and extensively used in order to enhance our understanding of the mechanism of action of platinum-based drugs, thereby optimizing structure activity relationships and predicting the biological behaviour of novel drug candidates. The most widely applied methods in this field of research are listed in Table **4** and will briefly be mentioned. As far as biological efficiency is needed to design new drugs, emphasis has to be lain on methods which yield results in an acceptable time, with high throughput, and under standardized conditions. Among these analytical techniques (of course besides the biological evaluation), nuclear magnetic resonance (NMR), high-performance liquid chromatography (HPLC) and, probably with an increasing importance in the future, capillary electrophoresis (CE), are the most widely used analytical techniques for platinum drug development. The advantage of NMR is that experiments can be carried out directly in the NMR tube with a large variety of bionucleophiles or other nucleophiles in partly deuterated aqueous solution in order to mimic physiological conditions as much as possible. Major problems consist of the variation in acquisition times needed for the different nuclei which are short for ^{1}H, intermediate for ^{195}Pt and long for ^{13}C and ^{15}N. A major drawback is in some cases also the relatively high concentration needed for the NMR experiment. So, if the reaction speed is high, only ^{1}H-NMR can be used. Experiments may also be carried out with ^{15}N-enriched complexes using two-dimensional HMQC NMR techniques, providing information about the coordination sphere around the platinum center. Finally, another important point to note is that NMR allows, at the same time, structural analysis and quantification of the species formed during the reaction course. One problem is associated with the accuracy in comparison with other methods.

Table 4. Analytical Techniques Suitable to Study Interactions Between Chiral Platinum Complexes with Bionucleophiles

Name	Remarks	Ref.
Crystallography	- theoretically possible - a suitable crystal must be obtained - needs plenty of time to get results and is very difficult to use for screening purpose	-[a]
NMR	- for short acquisition time : ^{1}H-NMR, for longer acquisition time : ^{195}Pt-NMR. Also ^{15}N-NMR after enrichment in ^{15}N - reactions can be carried out directly in the NMR tube - provides information about conformation and relative configuration of the adducts - the type of experiment, in relation with the sensitivity of the nucleus, is strongly dependant on the reaction rate	[62-63,71 [b], 114-119]
HPLC	- maybe the most important technique - for separation of each stereoisomer of nucleophile-platinum adducts - allows metabolite quantification - results are only valid if the analysis is completed with structural determination of the adducts by NMR or MS (LC-NMR or LC-MS) or by correlations with experiments carried out with the same chromatographic system	[115[d],116-126]
ESI-MS	- structural characterisation of the adduct is possible - relative reaction rates may be deduced	[125,126[c]]
CE	- analysis of the interactions between bionucleophiles and complexes - determination of binding constants - allows high-throughput screening	[127[d],128[b], 129-130]

[a] No reference found. [b] these references do not deal with analysis of chiral complexes but are very useful for application of the methods. [c] not on chiral complexes. [d] review

Therefore, chromatographic techniques are very useful tools, particularly when hyphenated methods such as LC-NMR and LC-MS are utilized. These systems are especially efficient since they have a high resolution power and consequently all reaction products can be separated and identified. In this context, a highly promising method is capillary electrophoresis (CE) as well as CE-MS. One of the most important advantages of CE is that interactions between platinum complexes and bionucleophiles can be measured directly in aqueous solution under physiologically relevant conditions, allowing to determine the time-dependent binding of platinum complexes to *all* biologically occurring nucleophiles.

6. CONCLUSIONS

Platinum complexes still remain the only metal-based drugs in anticancer therapy and, as it has been noted with oxaliplatin, chiral complexes may, like other chiral drugs, lead to stereoselective activity or pharmacokinetics. However, it has to be recognized that the stereoselection, which has been extensively studied, was almost exclusively explained in terms of diastereoselective interactions with DNA. Although this approach has been supported by many experimental proofs and also by biological test results, the remarkably high reactivity of platinum complexes towards compounds, other than the targeted DNA, must be taken into account for a complete analysis of the pharmacokinetics. The predominant number of relevant bionucleophiles taking part in distribution and metabolism of platinum complexes has been identified. Therefore one eminent point within the drug development process is to focus on the above mentioned interactions before/or in conjunction with the evaluation of the biological activity. Indeed, these nucleophiles will be the first to react before the complexes reach DNA and, therefore, may induce resistance and side effects. The interest in chiral platinum complexes is logically the result of different interactions with bionucleophiles leading in last consequence to improved anticancer activity and maybe to less undesirable side effects. The best practice for a rational design of new chiral cytotoxic platinum complexes is therefore to systematically evaluate their behavior towards bionucleophiles at a very early stage during drug development in order to reduce development time and costs.

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ABBREVIATIONS

CE	=	Capillary electrophoresis
CTR1	=	Copper transporter 1
DACH	=	1,2-diaminocyclohexane
ESI-MS	=	Electrospray ionization mass spectrometry
GGT	=	γ-glutamyl transpeptidase
HMG	=	High mobility group proteins
HMQC	=	Heteronuclear multiple quantum coherence
HPLC	=	High performance liquid chromatography
Hsp90	=	Heat shock protein 90
LC	=	Liquid chromatography
LG	=	Leaving group
MRP	=	Multidrug resistance protein
MS	=	Mass spectrometry
NER	=	Nucleoside excision repair
NMR	=	Nuclear magnetic resonance
NOESY	=	Nuclear overhauser effect spectroscopy
OCT	=	Organic cation transporter
QSAR	=	Quantitative structure-activity relationship

REFERENCES

[1] Jenner P, Testa B. The influence of stereochemical factors on drug disposition. Drug Metab Rev 1973; 2: 117-84.

[2] Testa B. Chiral aspects of drug metabolism. Trends Pharmacol Sci 1986; 7: 60-4.

[3] Beckett AH. Chirality and its importance in drug development: what are the issues? Biochem Soc Tr 1991; 19: 443-6.

[4] Caldwell J. The importance of stereochemistry in drug action and disposition. J Clin Pharmacol 1992; 32: 925-9.

[5] Caner H, Groner E, Levy L. Trends in the development of chiral drugs. Drug Discovery Today 2004; 9: 105-10.

[6] Testa B, Mayer JM. In: Eichelbaum M, Testa B, Somogyi A Ed, Handbook of Experimental Pharmacology. Berlin Heidelberg, Springer. 2003; 153: 143-59.

[7] Culy CR, Clemett D, Wiseman LR. Oxaliplatin. Drugs 2000; 60: 895-924.

[8] Galanski M, Jakupec MA, Keppler BK. In: Pérez JM, Fuertes MA, Alonso C Ed, Metal Compounds in Cancer Chemotherapy. Trivandrum, Research Signpost. 2005; 155-85.

[9] Kim D-K, Kim G, Gam J, Cho Y-B, Kim H-T, Tai J-H, *et al*. Synthesis and antitumor activity of a series of [2-substituted-4,5-bis(aminomethyl)-1,3-dioxolane] Pt(II) complexes. J Med Chem 1994; 37: 1471-85.

[10] Jakupec MA, Galanski M, Keppler BK. Tumour-inhibiting platinum complexes-state of the art and future perspectives. Rev Physiol Bioch P 2003; 146: 1-53.

[11] Kido Y, Khokhar AR, Al-Baker S, Siddik ZH. Modulation of cytotoxicity and cellular pharmacology of 1,2-diaminocyclohexane platinum(IV) complexes mediated by axial and equatorial ligands. Cancer Res 1993; 53: 4567-72.

[12] Epelbaum R, Teicher BA, Holden SA, Ara G, Varshney A, Herman TS. Interaction of D, L- and D-tetraplatin with hyperthermia *in vitro* and in vivo. Eur J Canc Pt A 1992; 28: 794-800.

[13] Anderson WK, Quagliato DA, Haugwitz RD, Narayanan VL, Wolpert-DeFilippes MK. Synthesis, physical properties, and antitumor activity of tetraplatin and related tetrachloroplatinum(IV) stereoisomers of 1,2-diaminocyclohexane. Cancer Treat Rep 1986; 70: 997-1002.

[14] Kidani Y, Inagaki K, Tsukagoshi S. Examination of antitumor activities of platinum complexes of 1,2-diaminocyclohexane isomers and their related complexes. Gann 1976; 67: 921-2.

[15] Kidani Y, Inagaki K, Iigo M, Hoshi A, Kuretani K. Antitumor activity of 1,2-diaminocyclohexane-platinum complexes against sarcoma-180 ascites form. J Med Chem 1978; 21: 1315-8.

[16] Noji M, Okamoto K, Kidani Y, Tashiro T. Relation of conformation to antitumor activity of Pt (II) complexes of 1,2-cyclohexanediamine and 2-(aminomethyl)cyclohexylamine isomers against leukemia P388. J Med Chem 1981; 24: 508-15.

[17] Gullotti M, Pacchioni G, Pasini A, Ugo R. Complexes of Pt(II) with chiral diamines and guanosines. Stereochemical investigations related to the mechanism of the antitumor activity of cis-bis(amine)Pt(II) type complexes. Inorg Chem 1982; 21: 2006-14.

[18] Inagaki K, Kidani Y. Differences in binding of (1,2-cyclohexanediamine)Pt(II) isomers with d(GpG). Inorg Chem 1986; 25: 1-3.

[19] Xu Y , Natile G, Intini FP, Marzilli LG. Stereochemically controlled ligands influence atropisomerization of Pt(II) nucleotide complexes. Evidence for head-to-head and stable head-to-tail atropisomers. J Am Chem Soc 1990; 112: 8177-9.

[20] Hambley TW. The influence of structure on the activity and toxicity of Pt anti-cancer drugs. Coordin Chem Rev 1997; 166: 181-223.

[21] Benedetti M, Malina J, Kasparkova J, Brabec V, Natile G. Chiral discrimination in platinum anticancer drugs. Environ Health Persp 2002; 110: 779-82.

[22] Benedetti M, Saad JS, Marzilli LG, Natile G. Chiral discrimination in the formation reaction and at equilibrium for N,N,N',N'-tetramethyl-1,2-diaminocyclohexane-PtG_2 complexes. Dalton trans 2003; 872-9.

[23] Kidani Y, Noji M, Tashiro T. Antitumor activity of platinum(II) complexes of 1,2-diaminocyclohexane isomers. Gann 1980; 71: 637-43.

[24] Siddik ZH, Al-Baker S, Thai G, Khokhar AR. Antitumor activity of isomeric 1,2-diaminocyclohexane platinum(IV) complexes. J Cancer Res Clin Oncol 1994; 120: 409-14.

[25] Siddik ZH, Al-Baker S, Burditt TL, Khokhar AR. Differential antitumor activity and toxicity of isomeric 1,2-diaminocyclohexane platinum (II) complexes. J Cancer Res Clin Oncol 1993; 120: 12-6.

[26] Jamieson ER, Lippard SJ. Structure, recognition, and processing of cisplatin-DNA adducts. Chem Rev 1999; 99: 2467-98.

[27] Kozubik A, Horvath V, Svihalkova-Sindlerova L, Soucek K, Hofmanova J, Sova P, *et al.* High effectiveness of platinum(IV) complex with adamantylamine in overcoming resistance to cisplatin and suppressing proliferation of ovarian cancer cells in vitro. Biochem Pharmacol 2005; 69: 373-83.

[28] Kelland LR. In: Lippert B Ed, Cisplatin: chemistry and biochemistry of a leading anticancer drugs. Zürich, Wiley-VCH. 1999; 497-521.

[29] O'Dwyer PJ, Stevenson JP, Johnson SW. Clinical pharmacokinetics and administration of established platinum drugs. Drugs 2000; 59(S4): 19-27.

[30] Chabner BA, Ryan DP, Paz-Ares L, Garcia-Carbonero R, Calabresi P. In: Hardman JG, Limbird LE Ed, The pharmacological basis of therapeutics. New York, McGraw-Hill. 2001; 1432-5.

[31] Lin X, Okuda T, Holzer A, Howell SB. The copper transporter CTR1 regulates cisplatin uptake in Saccharomyces cerevisiae. Mol Pharmacol 2002; 62: 1154-9.

[32] Nitiss JL. A copper connection to the uptake of platinum anticancer drugs. Proc Natl Acad Sci USA 2002; 99: 13963-5.

[33] Ishida S, Lee J, Thiele DJ, Herskowitz I. Uptake of the anticancer drug cisplatin mediated by the copper transporter Ctr1 in yeast and mammals. Proc Natl Acad Sci USA 2002; 99: 14298-302.

[34] Katano K, Kondo A, Safaei R, Holzer A, Samimi G, Mishimaet M, *et al.* Acquisition of resistance to cisplatin is accompanied by changes in the cellular pharmacology of copper. Cancer Res 2002; 62: 6559-65.

[35] Gullotti M, Pasini A, Ugo R, Filippeschi S, Marmonti L, Spreafico F. Enantiomeric cisplatin analogues: an investigation on their activity towards tumors in mice. Inorg Chim Acta 1984; 91: 223-7.

[36] Noji M, Motoyama S, Tashiro T, Kidani Y. Synthesis and antitumor activity of platinum (II) complexes containing 2,3-diaminopropanol isomers. Chem Pharm Bull 1983; 31: 1469-73.

[37] Büning H, Altman J, Zorbas H, Beck W. Bis[Pt(II)] and bis [Pt(IV)] complexes with optically active bis (vicinal-1,2-diamines) and their interaction with DNA. J Inorg Biochem 1999; 75: 269-79.

[38] Hanessian S, Wang J. Hydrophilic analogs of (R,R)-diaminocyclohexane dichloroplatinum (DACH) and the influence of relative stereochemistry on antitumor activity. Can J Chem 1993; 71: 2102-8.

[39] Morikawa K, Honda M, Endoh K-I, Matsumoto T, Akamatsu K-I, Mitsui H, *et al.* Synthesis and antitumor activity, and nephrotoxicity of the optical isomers of 2-aminomethylpyrrolidine(1,1-cyclobutanedicarboxylato)platinum(II). J Pharm Sci 1991; 80: 837-42.

[40] Fenton RR, Easdale WJ, Er HM, O'Mara S, McKeage MJ, Russell PJ, *et al.* Preparation, DNA binding, and *in vitro* cytotoxicity of pair of enantiomeric Pt(II) complexes, [(R)- and (S)-3-aminohexahydroazepine]dichloro Pt(II). Crystal structure of the S enantiomer. J Med Chem 1997; 40: 1090-8.

[41] Rezler EM, Fenton RR, Easdale WJ, McKeage MJ, Russell PJ, Hambley TW. Preparation, characterization, DNA binding, and *in vitro* cytotoxicity of the enantiomers of the platinum(II) complexes N-methyl-, N-ethyl- and N,N-dimethyl-(R)- and -(S)-3-aminohexahydroazepinedichloroplatinum(II). J Med Chem 1997; 40: 3508-15.

[42] Brunner H, Hankofer P, Holzinger U, Treittinger B, Schönenberger H. Synthesis and antitumor activity of platinum (II) complexes containing substituted ethylenediamine ligands. Eur J Med Chem 1990; 25: 35-44.

[43] vom Orde HD, Reile H, Müller R, Gust R, Bernhardt G, Spruss T, *et al.* Tumor-inhibiting [1,2-bis(fluorophenyl)ethylenediamine]platinum(II) complexes. V. Synthesis and evaluation of enantiomeric [1,2-bis-(4-fluorophenyl)ethylenediamine]dichloroplatinum(II) complexes. J Cancer Res Clin Oncol 1990; 116: 434-8.

[44] Wappes B, Jennerwein M, von Angerer E, Schönenberger H, Engel J, Berger M, *et al.* Dichloro-[1,2-bis-(4-hydroxyphenyl)ethylenediamine]platinum(II) complexes: an approach to develop compounds with a specific effect on the hormone-dependent mammary carcinoma. J Med Chem 1984; 27: 1280-6.

[45] Bernhardt G, Gust R, Reile H, vom Orde HD, Müller R, Keller C, *et al.* [1,2-Bis(2-hydroxyphenyl)ethylenediamine]dichloroplatinum (II), a new compound for the therapy of ovarian cancer. J Cancer Res Clin Oncol 1992; 118: 209-15.

[46] Jennerwein M, Gust R, Müller R, Schönenberger H, Engel J, Berger MR, *et al.* Tumor inhibiting properties of stereoisomeric [1,2-Bis(3-hydroxyphenyl)ethylenediamine] dichloroplatinum (II)-complexes. Part II: biological properties. Arch Pharm 1989; 322: 67-73.

[47] Gust R, Gelbcke M, Angermaier B, Bachmann H, Krauser R, Schönenberger H. The stereoselectivity of antitumor active 1,2-diamino-1-phenylpropanedichloroplatinum (II) complexes. Inorg Chim Acta 1997; 264: 145-60.

[48] Dufrasne F, Gelbcke M, Schnurr B, Gust R. Synthesis and antitumor activity of enantiomerically pure [1,2-diamino-1-(4-fluorophenyl)propane]dichloroplatinum(II) complexes. Arch Pharm Pharm Med Chem 2002; 335: 229-39.

[49] Dullin A, Dufrasne F, Gelbcke M, Gust R. Enantiomerically pure [1,2-diamino-1-(4-fluorophenyl)butane]platinum(II) complexes: synthesis and antitumor activity against MCF-7 and MDA-MB-231 breast cancer and LnCaP/FGC prostate cancer cell lines. Arch Pharm Pharm Med Chem 2004; 337: 654-67.

[50] Okamoto K, Noji M, Tashiro T, Kidani Y. Preparation of platinum(II) complexes of diamine isomers [PtX(1,3-diamine)](X = dichloro, sulfato, dinitrato, oxalato, D-glucuronato, and D-gluconato) and determination of their antitumor activity against leukemia L1210. Chem Pharm Bull 1981; 29: 929-39.

[51] Vickery K, Bonin AM, Fenton RR, O'Mara S, Russell PJ, Webster LK, *et al.* Preparation, characterization, cytotoxicity, and mutagenicity of a pair of enantiomeric Pt(II) complexes with the potential to bind enantioselectively to DNA. J Med Chem 1993; 36: 3663-8.

[52] Witiak DT, Rotella DP, Wei Y, Filppi JA, Gallucci JC. Synthesis and P-388 antitumor properties of the four diastereoisomeric 1-hydroxy-3,4-dimainocyclohexane-Cl_2Pt(II) complexes. J Med Chem 1989; 32: 214-7.

[53] Witiak DT, Rotella DP, Wie Y, Filppi JA, Gallucci JC. Stereocontrolled syntheses for the six diastereoisomeric 1,2-dihydroxy-4,5-diaminocyclohexanes: Pt(II) complexes and P-388 antitumor properties. J Med Chem 1987; 30: 1327-36.

[54] Reile H, Spruss T, Bernhardt G, Müller R, Gust R, Schönenberger H. Tumor inhibiting [1,2-Bis(fluorophenyl)ethylenediamine]platinum(II) complexes:Part 4: biological evaluation - *in vivo* studies on the P 388 leukemia. Arch Pharm 1991; 324: 405-9.

[55] Vollano JF, Al-Baker S, Dabrowiak JC, Schurig JE. Comparative studies on platinum (II) and platinum (IV) complexes containing 1,2-diaminocyclohexane. J Med Chem 1987; 30: 716-9.

[56] Gust R, Schönenberger H. Synthesis an evaluation of the antimammary tumor activity and of the estrogenic side effects of [1,2-bis(2,6-dihalo-3-hydroxyphenyl)ethylenediamine] platinum (II) complexes. Eur J Med Chem 1993; 28: 103-15.

[57] Bernhardt G, Koch M, Spruss T, Gust R, Krauser R, Schlemmer R, *et al.* [Meso-1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine]sulfato platinum (II) – pharmacokinetic studies. Arch Pharm Pharm Med Chem 1999; 332: 195-200.

[58] Zlatanova J, Yaneva J, Leuba SH. Proteins that specifically recognize cisplatin-damaged DNA: a clue to anticancer activity of cisplatin. FASEB J 1998; 12: 791-9.

[59] Shen D-W, Liang X-J, Gawinowicz MA, Gottesman MM. Identifications of cytoskeletal [^{14}C]carboplatin-binding proteins reveals reduced expression and disorganization of actin and filamin in cisplatin-resistant cell lines. Mol Pharmacol 2004; 66: 789-93.

[60] Rosenberg B. In: Lippert B Ed, Cisplatin: chemistry and biochemistry of a leading anticancer drugs. Zürich, Wiley-VCH. 1999; 3-27.

[61] Takahara PM, Rosenzweig AC, Frederick CA, Lippard SJ. Crystal structure of double-stranded DNA containing the major adduct of the anticancer drug cisplatin. Nature 1995; 377: 649-52.

[62] Gelasco A, Lippard SJ. NMR Solution structure of a DNA dodecamer duplex containing a cis-diammineplatinum(II) D(GpG) intrastrand cross-link, the major adduct of the anticancer drug cisplatin. Biochemistry 1998; 37: 9230-9.

[63] Wu Y, Pradhan P, Havener J, Boysen G, Swenberg JA, Campbell SL, *et al.* NMR solution structure of an oxaliplatin 1,2-d(GG) in-

trastrand cross-link in a DNA dodecamer duplex. J Mol Biol 2004; 341: 1251-69.

[64] Boudny V, Vrana O, Gaucheron F, Kleinwachter V, Leng M, Brabec V. Biophysical analysis of DNA modified by 1,2-diaminocyclohexane platinum(II) complexes. Nucleic Acids Res 1992; 20: 267-72.

[65] Murray V, Whittaker J, Temple MD, McFadyen WD. Interaction of 11 cisplatin analogs with DNA: characteristic pattern of damage with monofunctional analogs. Biochim Biophys Acta 1997; 1354: 261-71.

[66] Miller KJ, McCarthy SL, Krauss M. Binding of cis-(1,2-diaminocyclohexane)platinum (II) and its derivatives to duplex DNA. J Med Chem 1990; 33: 1043-6.

[67] Morikawa K, Matsumoto T, Matsuoka Y, Koizumi M. The interactions of (-)-(R)-2-aminomethylpyrrolidine(1,1-cyclobutanedicarboxylato)Pt(II) monohydrate (DWA2114R) and related compounds with deoxyribonucleic acid. Chem Pharm Bull 1992; 40: 212-9.

[68] Pasini A, Velcich A, Mariani A. Absence of diastereoisomeric behaviour in the interaction of chiral platinum anticancer compounds with DNA. Chem-Biol Interact 1982; 42: 311-20.

[69] Jennerwein MM, Eastman A, Khokhar A. Characterization of adducts produced in DNA by isomeric 1,2-diaminocyclohexaneplatinum(II) complexes. Chem-Biol Interact 1989; 70: 39-49.

[70] Marzilli LG, Intini FP, Kiser D, Wong HC, Ano SO, Marzilli PA, *et al*. Chirality-Controlling Chelate (CCC) ligands in analogs of platinum anticancer agents. Influence of N9 substituents of guanine derivatives (G) on the distribution of chiral conformers of (CCC)PtG_2 with CCC = N,N'-dimethyl-2,3-diaminobutane. Inorg Chem 1998; 37: 6898-905.

[71] Ano SO, Intini FP, Natile G, Marzilli LG. Retro models of Pt anticancer drug DNA adducts: chirality-controlling chelate ligand restriction of guanine dynamic motion in (2,2'-bipiperidine)PtG_2 complexes. Inorg Chem 1999; 38: 2989-99.

[72] Malina J, Hofr C, Maresca L, Natile G, Brabec V. DNA interactions of antitumor cisplatin analogs containing enantiomeric amine ligands. Biophys J 2000; 78: 2008-21.

[73] Malina J, Kasparkova J, Natile G, Brabec V. Recognition of major DNA adducts of enantiomeric cisplatin analogs by HGM Box proteins and nucleotide excision repair of these adducts. Chem Biol 2002; 9: 629-38.

[74] Malina J, Vojtiskova M, Brabec V, Diakos CI, Hambley TW. DNA adducts of the enantiomers of the Pt(II) complexes of the ahaz ligand (ahaz = 3-aminohexahydroazepine) and recognition of these adducts by HMG domain proteins. Biochem Biophys Res Commun 2005; 332: 1034-41.

[75] Monti E, Gariboldi M, Maiocchi A, Marengo E, Cassino C, Gabano E, *et al*. Cytotoxicity of cis-platinum(II) conjugate models. The effect of chelating arms and leaving groups on cytotoxicity : a quantitative structure-activity relationship approach. J Med Chem 2005; 48: 857-66.

[76] Lin T-S, Zhou R-X, Scanlon KJ, Brubaker WF, Lee JJS, Woods K, *et al*. Synthesis and biological activity of several amino nucleosideplatinum (II) complexes. J Med Chem 1986; 29: 681-6.

[77] Ho Y-P, Au-Yeung SCF, To KKW. Platinum-based anticancer agents: innovative design strategies and biological perspectives. Med Res Rev 2003; 23: 633-55.

[78] Galanski M, Slaby S, Jakupec MA, Keppler BK. Synthesis, characterization, and *in vitro* antitumor activity of osteotropic diam(m) ineplatinum(II) complexes bearing a N,N-bis(phosphonomethyl) glycine ligand. J Med Chem 2003; 46: 4946-51.

[79] Paschke R, Paetz C, Mueller T, Schmoll HJ, Mueller H, Sorkau E, *et al*. Biomolecules linked to transition metal complexes - New chances for chemotherapy. Curr Med Chem 2003; 10: 2033-44.

[80] Gust R, Niebler K, Schönenberger H. Investigation of the configurational and conformational influences on the hormonal activity of 1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamines and of their platinum(II) complexes. 1. Synthesis, estradiol receptor affinity, and estrogenic activity of diastereomeric [N-alkyl- and N,N'-dialkyl-1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) complexes. J Med Chem 1995; 38: 2070-9.

[81] Graham MA, Lockwood GF, Greenslade D, Brienza S, Bayssas M, Gamelin E. Clinical pharmacokinetics of oxaliplatin: a critical review. Clin Cancer Res 2000; 6: 1205-18.

[82] Pendyala L, Creaven PJ. *In vitro* cytotoxicity, protein binding, red blood cell partitioning, and biotransformation of oxaliplatin. Cancer Res 1993; 53: 5970-6.

[83] Samimi G, Safaei R, Katano R, Holzer AK, Rochdi M, Tomioka M, *et al*. Increased expression of the copper efflux transporter ATP7A mediates resistance to cisplatin, carboplatin, and oxaliplatin in ovarian cancer cells. Clin Cancer Res 2004; 10: 4661-9.

[84] Safaei R, Katano K, Samimi G, Naerdemann W, Stevenson JL, Rochdi M, *et al*. Cross-resistance to cisplatin in cells with acquired resistance to copper. Cancer Chemother Pharmacol 2004; 53: 239-46.

[85] Kool M, de Haas M, Scheffer GL, Scheper RJ, van Eijk MJT, Juijn JA, *et al*. Analysis of expression of cMOAT (MRP2), MRP3, MRP4 and MRP5, homologues of the multidrug resistance-associated protein gene (MRP1), in human cancer cell lines. Cancer Res 1997; 57: 3537-47.

[86] Liedert B, Materna V, Schadendorf D, Thomale J, Lage H. Overexpression of cMOAT (MRP2/ABCC2) is associated with decreased formation of platinum-DNA adducts and decreased G2-arrest in melanoma cells resistant to cisplatin. J Investig Dermatol 2003; 121: 172-6.

[87] Song I-S, Savaraj N, Siddik ZH, Liu P, Wie Y, Wu CJ, *et al*. Role of human copper transporter Ctr1 in the transport of platinum-based antitumor agents in cisplatin-sensitive and cisplatin-resistant cells. Mol Cancer Ther 2004; 3: 1543-9.

[88] Paolicchi A, Sotiropuolou M, Perego P, Daubeuf S, Visvikis A, Lorenzini A, *et al*. γ-glutamyl transpeptidase catalyses the extracellular detoxification of cisplatin in human cell line derived from the proximal convoluted tubule of the kidney. Eur J Canc 2003; 39: 996-1003.

[89] Townsend DM, Deng M, Zhang L, Lapus MG, Hanigan MH. Metabolism of cisplatin to a nephrotoxin in proximal tubule cells. J Am Soc Nephrol 2003; 14: 1-10.

[90] Ludwig T, Riethmüller C, Gekle M, Schwerdt G, Oberleithner H. Nephrotoxicity of platinum complexes is related to basolateral organic cation transport. Kidney Int 2004; 66: 196-202.

[91] Fakih S, Munk VP, Shipman MA, Murdoch PDS, Parkinson PA, Sadler PJ. Novel adducts of the anticancer drug oxaliplatin with glutathione and redox reactions with glutathione disulfide. Eur J Inorg Biochem 2003; 1206-14.

[92] Jansen BAJ, Brouwer J, Reedijk J. Glutathione induces cellular resistance against cationic dinuclear platinum anticancer drugs. J Inorg Biochem 2002; 89: 197-202.

[93] Natarajan G, Malathi R, Holler E. Increased DNA-binding activity of cis-1,1-cyclobutanedicarboxylatodiammineplatinum(II) (carboplatin) in the presence of nucleophiles and human breast cancer MCF-7 cell cytoplasmic extracts: activation theory revisited. Biochem Pharmacol 1999; 58: 1625-29.

[94] Selvakumaran M, Pisarcik DA, Bao R, Yeung AT, Hamilton TC. Enhanced cisplatin cytotoxicity by disturbing the nucleotide excision repair pathway in ovarian cancer cell lines. Cancer Res 2003; 63: 1311-6.

[95] Kelley SL, Basu A, Teicher BA, Hacker MP, Hamer DH, Lazo JS. Overexpression of metallothionein confers resistance to anticancer drugs. Science 1988; 241: 1813-5.

[96] Saga Y, Hashimoto H, Yachiku S, Iwata T, Tokumitsu M. Reversal of acquired cisplatin resistance by modulation of metallothionein in transplanted murine tumors. Int J Urol 2004; 11: 407-15.

[97] Zhang L, Hanigan MH. Role of cystein S-conjugate β-lyase in the metabolism of cisplatin. J Pharmacol Exp Ther 1996; 306: 988-94.

[98] Vrana O, Brabec V. L-methionine inhibits reaction of DNA with anticancer cis-diamminedichloroplatinum (II). Biochemistry 2002; 41: 10994-9.

[99] El-Khateeb M, Appleton TG, Gahan LR, Charles BG, Berners-Price SJ, Bolton A-M. Reactions of hydrolytes with methionine, cysteine, and plasma ultrafiltrate studied by a combination of HPLC and NMR techniques. J Inorg Biochem 1999; 77: 13-21.

[100] Pendyala L, Kidani Y, Perez R, Wilkes J, Bernacki RJ, Creaven PJ. Cytotoxicity, cellular accumulation and DNA binding of oxaliplatin isomers. Cancer Lett 1995; 97: 177-84.

[101] Lindauer E, Holler E. Cellular distribution and cellular reactivity of platinum (II) complexes. Biochem Pharmacol 1996; 52: 7-14.

[102] Screnci D, Er HM, Hambley TW, Galettis P, Brouwer W, McKeage MJ. Stereoselective peripheral sensory neurotoxicity of

diaminocyclohexane platinum enantiomers related to ormaplatin and oxaliplatin. Br J Cancer 1997; 76: 502-10.

[103] Guo Y, Smith K, Petris MJ. Cisplatin stabilizes a multimeric complex of the human Ctr1 copper transporter. J Biol Chem 2004; 279: 46393-9.

[104] Rudin CM, Yang Z, Schumaker LM, VanderWeele DJ, Newkirk K, Egorin MJ, *et al.* Inhibition of glutathione synthesis reverses Bcl-2-mediated cisplatin resistance. Cancer Res 2003; 63: 312-8.

[105] Tew KD. Glutathione-associated enzymes in anticancer drug resistance. Cancer Res 1994; 54: 4313-20.

[106] Boubakari, Bracht K, Neumann C, Grünert R, Bednarski PJ. No correlation between GSH level in human cancer cell lines and the cell growth inhibitory activities of platinum diamine complexes. Arch Pharm Pharm Med Chem 2004; 337: 668-71.

[107] Faivre S, Chan D, Salinas R, Woynarowska B, Woynarowski JM. DNA strand breaks and apoptosis induced by oxaliplatin in cancer cells. Biochem Pharmacol 2003; 66: 225-37.

[108] Rosenhagen MC, Soti C, Schmidt U, Wochnik GM, Hartl FU, Holsboer F, *et al.* The heat shock protein 90-targeting drug cisplatin selectively inhibits steroid receptor activation. Mol Endocrinol 2003; 17: 1991-2001.

[109] Colangelo D, Ghiglia AL, Viano I, Mahboobi H, Ghezzi A, Cassino C, *et al.* Might telomerase enzyme be a possible target for trans-Pt(II) complexes? J Inorg Biochem 2004; 98: 61-7.

[110] Colangelo D, Osella D. Telomerase inhibition and cancer: might platinum based drugs have a future as anti-telomerase pharmacological approach? Curr Med Chem 2005; 12: 3091-102.

[111] Colangelo D, Ghiglia AL, Viano I, Cavigiolio G, Osella D. Cis-[Pt(Cl_2(pyridine)(5-SO_3H-isoquinoline)] complex, a selective inhibitor of telomerase enzyme. Biometals 2003; 16: 553-60.

[112] Fujie Y, Yamamoto H, Ngan CY, Akimitsu T, Hayashi T, Suzuki R *et al*. Oxaliplatin, a potent inhibitor of survivin, enhances paclitaxel-induced apoptosis and mitotic catastrophe in colon cancer cells. Jpn J Clin Oncol 2005; 35: 453-63

[113] Reile H, Müller R, Gust R, Laske R, Krischke W, Bernhardt W *et al*. Tumor inhibiting [1,2-bis(fluorophenyl)ethylenediamine]platinum (II) complexes. Part II: biological evaluation - *in vitro* studies on the P388 D1 leukemia cell line. Arch Pharm 1990; 323: 133-40.

[114] Bednarski PJ. Reactions of a cisplatin analog bearing an estrogenic 1,2-diarylethylenediamine ligand with sulfur-containing amino acids and glutathione. J Inorg Biochem 80 (1995) 1-19.

[115] Chen Y, Guo Z, Sadler PJ. In: Lippert B Ed, Cisplatin chemistry and biochemistry of a leading anticancer drug. Zürich, Wiley-VCH. 1999; 293-318.

[116] Huang H, Zhu L, Reid BR, Drobny GP, Hopkins PB. Solution structure of a cisplatin-induced DNA interstrand cross-link. Science 1975; 270: 1842-5.

[117] Chen Y, Guo Z, Parsons S, Sadler PJ. Stereospecific and kinetic control over the hydrolysis of a sterically hindered platinum picoline anticancer complex. Chem Eur J 1998; 4: 672-6.

[118] Spellmeyer CF, Marzilli LG, Byrd RA, Summers MF, Zon G, Shinozuka K. HMQC and ^{1}H and ^{31}P NMR studies of platinum amine adducts of tetradeoxyribonucleotides. Relationship between ^{31}P shift and potential hydrogen-bonding interactions in pGpG moieties cross-linked by platinum. Inorg Chem 1988; 27: 366-76.

[119] Appleton TG, Connor JW, Hall JR, Prenzler PD. S,O- versus S,N-chelation in the reactions of the cis-diamminediaquaplatinum(II) cation with methionine and S-methylcysteine. Inorg Chem 1989; 28: 2030-7.

[120] Barefoot RR. Speciation of platinum compounds: a review of recent applications in studies of platinum anticancer drugs. J Chromatogr B 2001; 751: 205-11.

[121] Hagrman D, Goodisman J, Souid A-K. Kinetic study on the reactions of platinum drugs with glutathione. J Pharmacol Exp Ther 2004; 308: 658-66.

[122] Luo FR, Yen T-Y, Wyrick SD, Chaney SG. High-performance liquid chromatographic separation of the biotransformation products of oxaliplatin. J Chromatogr B 1999; 724: 345-56.

[123] Hagrman H, Goodisman J, Dabrowiak JC, Souid A-K. Kinetic study on the reaction of cisplatin with metallothionein. Drug Metab Dispos 2003; 31: 916-23.

[124] Townsend DM, Marto JA, Deng M, Macdonald TJ, Hanigan MH. High pressure liquid chromatography and mass spectrometry characterization of the nephrotoxic biotransformation products of cisplatin. Drug Metab Dispos 2003; 31: 705-13.

[125] Miao R, Yang G, Miao Y, Mei Y, Hong J, Zhao C, *et al.* Interaction of platinum(II) complexes with sulfur-containing peptides studied by electrospray ionization mass spectrometry and tandem mass spectrometry. Rapid Commun Mass Spectrogr 2005; 19: 1031-40.

[126] Liu Q, Wie H, Lin J, Zhu L, Guo Z. Novel polynuclear platinum adducts detected during the reactions of [Pt(Met-S,N)Cl_2] with γ-glutathione and L-cysteine. J Inorg Biochem 2004; 98: 702-12.

[127] Hartinger CG, Timerbaev AR, Keppler BK. Capillary electrophoresis in anti-cancer metallodrug research: advances and future challenges. Electrophoresis 2003; 24: 2023-37.

[128] Rudnev AV, Aleksenko SS, Semenova O, Hartinger CG, Timerbaev AR, Keppler BK. Determination of binding constants and stoechiometries for platinum anticancer drugs and serum proteins by capillary electrophoresis using the Hummel-Dreyer method. J Sep Sci 2005; 28: 121-7.

[129] Küng A, Strickmann DB, Galanski M, Keppler BK. Comparison of the binding behavior of oxaliplatin, cisplatin and analogues to 5'-GMP in the presence of sulfur-containing molecules by means of capillary electrophoresis and electrospray mass spectrometry. J Inorg Biochem 2001; 86: 691-8.

[130] Rappel C, Galanski M, Yasemi A, Habala L, Keppler BK. Analysis of anticancer platinum(II)-complexes by microemulsion electrokinetic chromatography: separation of diastereoisomers and estimation of octanol-water partition coefficients. Electrophoresis 2005; 26: 878-84.