Interaction of antitumor platinum complexes with human liver microsomal cytochromes P450

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Interaction of nine human hepatic cytochromes P450 (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4) with six platinum complexes was studied using pooled human microsomes. The compounds used were cisplatin, oxaliplatin, carboplatin, transplatin, and *trans*-[$PtCl_2(NH_3)$ (Am)], where Am=2-methylbutylamine or sec-butylamine. No significant inhibition of all CYP activities by carboplatin was observed. With cisplatin and oxaliplatin, a minor inhibition of CYP2C9 enzyme (75% of control at 400 μmol/l of these complexes) was seen; cisplatin also inhibited slightly the CYP2B6 activity (85% of control). With respect to plasma levels of cisplatin obtained in clinical applications, these effects are probably not important. In contrast, clinically ineffective transplatin, inhibited the CYP2B6 as well as CYP2C9 activities significantly (to 50–35% of control at 100 μmol/l); also, an inhibition of CYP2E1 activity was found here (to 70% at 100 μmol/l). Two other derivatives of transplatin (new antitumor agents with trans geometry), inhibited CYP activities more strongly reaching nearly a complete inhibition of the respective CYP activities at concentration of 200 μmol/l. Half maximal inhibitory concentration values were found in the range of tens of μmol/l indicating that there is a possibility of potential interactions of these compounds with drugs metabolized by CYP3A4, CYP2E1, CYP2D6, CYP2C19, CYP2B6, CYP2A6, and CYP1A2. Interestingly, clinically non-significant inhibition was found with the CYP2C9 and CYP2C8 indicating low probability of interactions with, for example, warfarin. The results document that the new antitumor agents based on the transplatin should be more thoroughly tested for interactions with liver microsomal drug-metabolizing cytochromes P450. *Anti-Cancer Drugs* 20:305–311 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Anticancer activity of cisplatin [*cis*-diamminedichloridoplatinum(II), Fig. 1a] has been known since 1969 [1]. Since that time, a variety of analogous compounds has been synthesized with an aim to reduce the toxicity of cisplatin, still the most prominent, antineoplastic drug [2]. The therapeutic activity of these compounds is mainly based on the covalent modification of the DNA molecule. There is a large body of evidence documenting the formation of various types of covalent DNA adducts and their influence, for example, on the DNA replication [2]. Carboplatin [*cis*-diamminecyclobutanedicarboxylatoplatinum(II), Fig. 1d] and oxaliplatin [1,2-diaminocyclohexane-(oxalato)platinum(II), Fig. 1c] are more recent analogs exhibiting generally lower or less severe toxicity; their use is increasing as in some indications they represent an alternative to cisplatin or exhibit effects unattainable for cisplatin (e.g. oxaliplatin in treatment of colorectal cancer) [3].

Transplatin [*trans*-diamminedichloridoplatinum(II), Fig. 1b] is a clinically nonefficient isomer of cisplatin having identical ligands as the cis isomer but with two chloride atoms (and hence the two amino groups as well) at the opposite side of the square-like planar complex. However, recently, a new class of derivatives of transplatin exhibiting cytotoxic activity was introduced with one hydrogen atom of amino group substituted by short chain aliphatic structures as 2-methylbutyl – or sec-butyl – residues [4] or, by substituents of more complex structure as, for example, hydroxymethylpyridine [5]. In selecting the trans platinum complexes for this study, two of the complexes mentioned here were chosen (*trans*-[$PtCl_2(NH_3)$(Am)], where Am=2-methylbutylamine or sec-butylamine), as well as the transplatin, which, although inactive, represents the simplest complex with trans geometry whose properties to interact with CYP enzymes should be compared with the well-known cisplatin isomer. In the following text, these newly designed compounds are labeled as trans-metbut (the former) (Fig. 1e) and trans-secbut, the later one, respectively (Fig. 1f).

The platinum derivatives are well known to be toxic, but the mechanisms underlying their toxicity are still poorly

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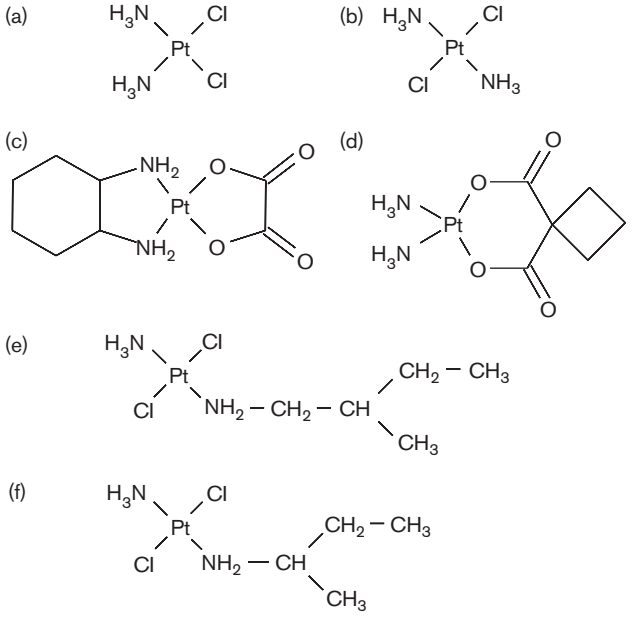
Fig. 1



Cisplatin [*cis*-diamminedichloridoplatinum (II)] (a), transplatin [*trans*-diamminedichloridoplatinum (II)] (b), oxaliplatin (c), carboplatin (d), trans-metbut (*trans*-[$PtCl_2(NH_3)$(2-methylbutylamine)]) (e), and trans-secbut (*trans*-[$PtCl_2(NH_3)$(sec-butylamine)]) (f).

understood. One of the causes of nephrotoxic and neurotoxic effects include generation of reactive oxygen species, which may lead to oxidative damage, lipid peroxidation, and apoptosis [6]. Cytochromes P450 are known to be enzymes primarily responsible for most of the drug biotransformations in a variety of tissues such as liver, kidney, brain, lung, and heart [7]. Interestingly, no systematic study on the possible interaction of platinum complexes with cytochromes P450 has been carried out although there were earlier indications that platinum anticancer drugs may modulate mRNA levels of CYP enzymes in the rat [8]; also, an inhibition of one form of CYP enzyme, the CYP2E1, has been documented to protect against nephrotoxicity and hepatotoxicity of cisplatin [9,10]. The effect of dehydrotarplatin, a relatively novel anticancer drug, on rat male-specific CYP enzymes indicated a decrease of CYP2C11 [11]. Baumhäkel *et al.* [12], in their study of inhibitory effects of antineoplastic agents on CYP3A4 in human liver microsomes, reported that cisplatin and carboplatin do not influence the enzymatic activity typical of this enzyme, that is the nifedipine oxidation. Preliminary results from this laboratory indicated an influence of cisplatin on the CYP2C9 and CYP2E1 activities [13], here, a detailed study is presented for a representative panel of nine human liver microsomal cytochrome P450 forms important for metabolism of drugs and other xenobiotics [14].

Materials and methods

Materials

Cisplatin and transplatin were supplied by Sigma Aldrich CZ (Prague, Czech Republic). Carboplatin and oxaliplatin were kind gifts from Pliva-Lachema A.S. (Brno, Czech Republic). Transplatin derivatives, trans-metbut and trans-secbut, were prepared by the methods described in detail previously and were kindly supplied by AM Pizarro and C Navarro-Raninger [15]. For determination of CYP activities, chlorzoxazone, 6-hydroxychlorzoxazone, diclofenac, 4-hydroxydiclofenac, bufuralol, 6-hydroxybufuralol, and 6β-hydroxytestosterone were supplied by Cerilliant Corporation (Round Rock, Texas, USA). P450-Glo substrates (luciferin BE, luciferin ME, and luciferin H) for evaluation of CYP3A4, CYP2C8, and CYP2C9 activities by luminescence spectrometry were products of Promega (Madison, Wisconsin, USA) obtained through East Port (Prague). 7-Ethoxy-4-(trifluoromethyl)coumarin was supplied by Fluka (Buchs, Switzerland). Cryopreserved human liver microsomes (pooled) were purchased from Advancell (Barcelona, Spain). Microsomes were obtained under approval of the local ethics committee and in accordance with the ethic regulations of the country of origin (Spain). They were from five males and five females with protein content 38.4 mg/ml; the CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2E1, and CYP3A4 enzyme activities are accessible at the Advancell web site (*www.advancell.net*, batch reference 102091201). All other chemicals were supplied by Sigma Aldrich (Prague).

Methods

Activities of individual CYP forms were measured according to published protocols. The following microsomal CYP activities were tested: CYP3A4, both testosterone 6β-hydroxylation [16] and luciferin-6′ benzyl ether debenzylation (P450-Glo substrate) (Technical Bulletin no. 325, *www.promega.com*); CYP2C8 activity, luciferin-6′ methyl ether demethylation (P450-Glo substrate) (Technical Bulletin no. 325, *www.promega.com*); CYP2C9 activity, both diclofenac 4′-hydroxylation [17]; and 6′-deoxyluciferin hydroxylation (another P450-Glo substrate) (Technical bulletin no 325, *www.promega.com*); CYP2C19, (*S*)-mephenytoin 4′-hydroxylation (*http://www.cypex.co.uk/intro.htm*, Cypex 2C19 QC assays); CYP2E1, chlorzoxazone 6-hydroxylation [18]; CYP1A2, 7-ethoxyresorufin *O*-deethylation [19]; CYP2D6, bufuralol 1′-hydroxylation [20]; CYP2A6, coumarin 7-hydroxylation [21]; CYP2B6, 7-ethoxy-4-(trifluoromethyl)coumarin *O*-deethylation [22]. To reaction mixtures (final incubation mixture volumes: CYP3A4 activities, 500 and 100 µl for testosterone or luciferin derivatives as substrates; CYP2C8, CYP2C9, and CYP2B6, 100 µl and CYP2E1, 1000 µl for chlorzoxazone hydroxylation; CYP1A2, CYP2C19, CYP2D6, and CYP2A6, 200 µl), a 5-mmol/l stock solution (in 100 mmol/l K-phosphate buffer, pH 7.4, except for determinations of CYP1A2 activity, where 100 mmol/l Tris/KCl buffer, pH 7.6 was used and of

CYP3A4-mediated testosterone hydroxylation, where 50 mmol/l Tris/KCl buffer, pH 7.4 was taken according to respective protocols) of each compound tested was added to obtain the concentration desired.

A TECAN Infinite M200 absorbance/fluorescence/luminescence reader (Tecan Austria, Vienna, Austria) was used in the detection of the respective spectral data. High-performance liquid chromatography analyses (of 6β-hydroxytestosterone, 6-hydroxychlorzoxazone, 4′-hydroxydiclofenac, 4′-hydroxymephenytoin, and bufuralol 1′-hydroxylation) were performed using a Shimadzu Class VP system (Kyoto, Japan).

For each enzyme assay, a preliminary experiment was done to determine the K_M and V_{max} for a given enzyme reaction and to obtain the values of substrate concentrations suitable for the inhibition experiments (as a rule, substrate concentration was chosen in the range corresponding to the value of the K_M). Inhibition experiments were routinely carried out with up to seven concentrations of the tested drug. A control experiment with known reference inhibitors was implemented in cases where a significant degree of inhibition was presumed, namely, with sulfaphenazole (CYP2C9), methoxsalen (CYP2A6), diethyldithiocarbamate (CYP2E1) [7,23], and 3-isopropenyl-3-methyldiamantane (CYP2B6) [24].

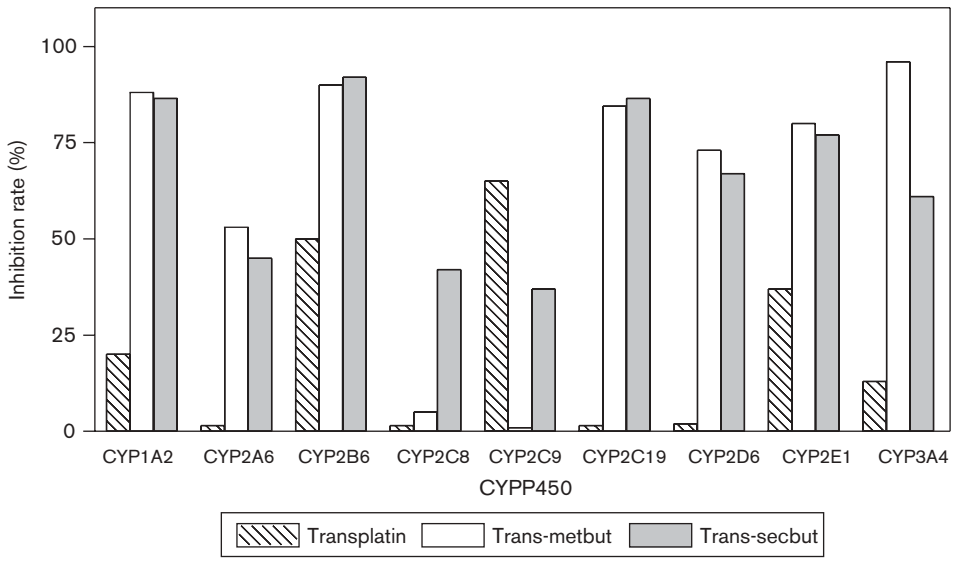
Inhibition of individual CYP activities by platinum complexes in all cases was evaluated by plotting the respective remaining activity against the inhibitor concentration; as a rule, the results were expressed as means of two to five independent determinations with the difference between duplicates being lower than 15%. When an inhibition was pronounced, the K_i values were determined as averages from Dixon plots with three substrate concentrations used (corresponding to $0.5K_M$, K_M, and $2K_M$). For information on the course of the enzyme kinetics, parameters of enzyme kinetics (K_M, V_{max}) as well as the intercepts of Dixon plots were obtained using the Sigma Plot 8.0.2 scientific graphing software (SPSS, Chicago, Illinois, USA).

Results

Six platinum complexes (cisplatin, oxaliplatin, carboplatin, transplatin, trans-metbut, trans-secbut) and activities of nine forms of CYP enzymes present in human liver microsomes (CYPs 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4) were studied. Among the platinum compounds, carboplatin was found not to interact with any of CYP enzymes even at concentrations approaching 0.5 mmol/l. Similarly, oxaliplatin exhibited only limited effects on activities of one CYP form, namely, on those exhibited by CYP2C9 (diclofenac 4′-hydroxylation and luciferin H biotransformation). In this case, both activities determined by two independent methods were inhibited by 20%, however, at an oxaliplatin concentration of 400 μmol/l, which is not a clinically relevant plasma concentration in patients and usually do not exceed 10 μmol/l [25].

Cisplatin inhibited to a slight extent the activities of three CYP enzyme forms. The effects exhibited, as in the

Fig. 2

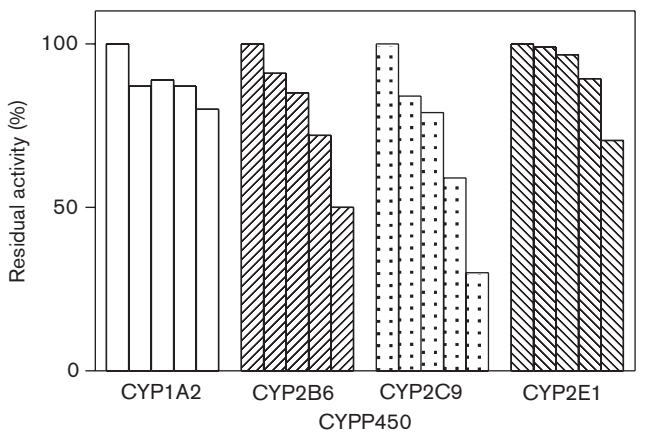


Overview of inhibition rates of CYP activities by 'trans' complexes at 100 μmol/l concentration. Results expressed as extent of inhibition, that is 100%, full inhibition. Individual activities determined as described in Materials and methods.

case of oxaliplatin, did not exceed a decrease of activity greater than 25% (for both activities of CYP2C9, at concentration of 400 μmol/l). In addition, the enzyme activity of CYP2B6 form was decreased to 85% (i.e. by 15% at 400 μmol/l); finally, a minor but reproducible decrease of CYP1A2 activity (by 10%) was found with this platinum complex at the same concentrations as above.

The most prominent inhibition of activities characteristic for human liver microsomal CYPs was observed with three platinum complexes with structures based on the trans geometry (Fig. 2). With transplatin, at 100 μmol/l concentration, the CYP2C9 activity was inhibited down to 30% of the control as determined by both methods used (see above). Activity of the CYP2B6 form was inhibited to 50%; also the CYP2E1 activity was reduced to 65% and this of the CYP1A2, which was found to be inhibited to 80% of the value obtained in the absence of the inhibitor only (Fig. 3). Enzymatic activity of the CYP3A4 form was inhibited by 10% only; activities of other forms (CYP2A6, CYP2C19, CYP2D6) were unchanged. Interestingly, the inhibition data given here were obtained with a transplatin concentration in the reaction mixture reaching 100 μmol/l; higher levels of this compound were not attainable because of low solubility of transplatin. Inhibition of the respective CYP activities by better soluble trans-metbut and trans-secbut complexes is more pronounced reaching nearly complete inhibition of the respective CYP activities at concentrations of 200 μmol/l. However, in the Fig. 2, the values of inhibition are given for 100 μmol/l concentration of platinum complexes for better comparison.

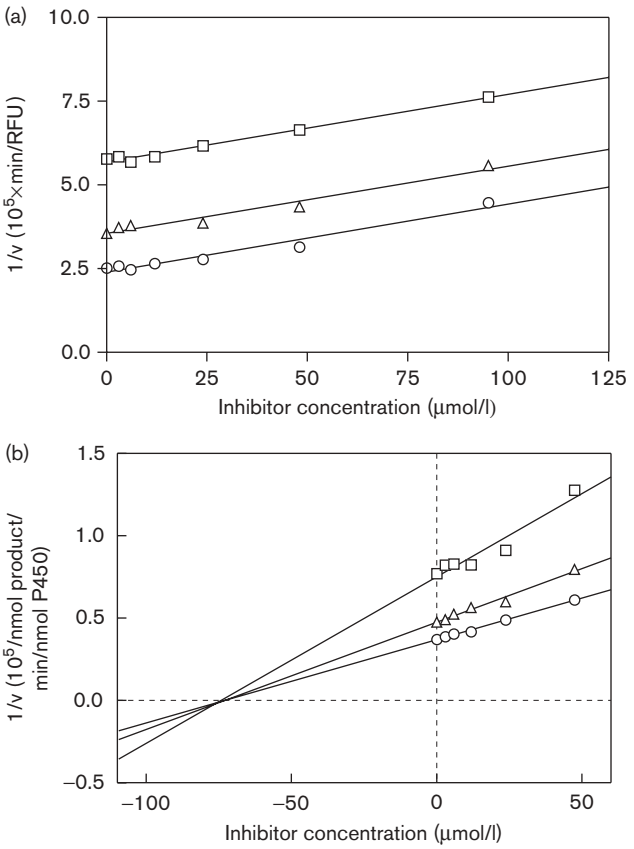
Fig. 3



Effect of transplatin on four specific activities of cytochromes P450. Activities tested: CYP1A2, 7-ethoxyresorufin *O*-deethylation; CYP2B6, 7-ethoxy-4-(trifluoromethyl)coumarin *O*-deethylation; CYP2C9 activity, 6′-deoxyluciferin hydroxylation; CYP2E1, chlorzoxazone 6-hydroxylation. Concentrations of transplatin in reaction mixture were 0, 12.5; 25, 50, and 100 μmol/l.

Experimental data obtained with trans complexes were analyzed by Dixon plots to evaluate the possible mechanisms of enzyme inhibition [26]. Figure 4a documents the inhibition of the microsomal CYP2B6 activity by transplatin; the course of the Dixon plot indicates a fully uncompetitive inhibition, which means that a conformational change caused by binding of the substrate facilitates the binding of an inhibitor to another place in the active site. Another example of the Dixon plot showing an inhibition of the CYP2C9 activity by the same compound, transplatin, is shown in Fig. 4b. Here, the course of the plot indicates the presence of a noncompetitive mechanism of inhibition; that is, an inhibition of product formation by the presence of an inhibitor. The inhibitor is here bound again in a site close to the substrate; however, the inhibition is not conditioned by a conformational change of the active site because of the previous binding of a substrate. The K_i value is at about 70 μmol/l; a similar case of noncompetitive inhibition was also observed for inhibition of the CYP2E1 activity where the value of the K_i is, however, greater (at about 250 μmol/l).

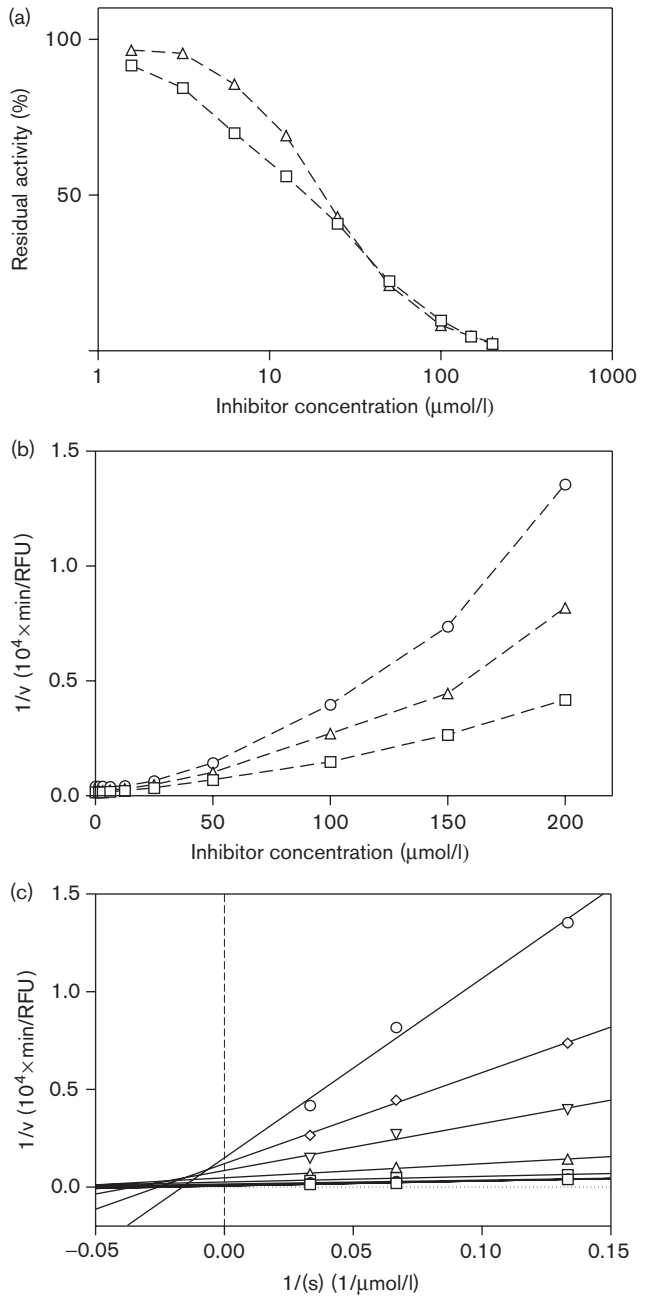
Fig. 4



Dixon plots for inhibition of CYP2B6 (a) and CYP2C9 (b) by transplatin at three substrate concentrations (8 μmol/l, squares; 15 μmol/l, triangles; 30 μmol/l, circles). Activities of CYP enzymes determined as in Fig. 3. 1/v, reciprocal velocity; RFU, relative fluorescence unit.

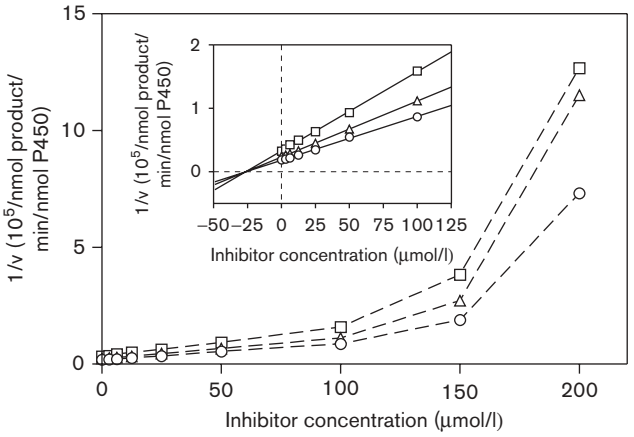
The two compounds with trans geometry (trans-metbut and trans-secbut) were, however, much stronger and less selective inhibitors of the liver microsomal CYP activities as they effectively inhibited seven out of nine CYP activities except CYP2C8 and CYP2C9 where the extent of inhibition was lower than 20% for trans-metbut and at about 50–60% for trans-secbut at 200 μmol/l. The Dixon plot as well the Lineweaver–Burk plot indicate in all cases a strongly bound inhibitor [the inhibition of CYP2B6 activity is shown as a typical example (Fig. 5, Dixon plot showing an upright curvature typical for a strongly bound inhibitor, and a Fig. 5b, Lineweaver–Burk plot, Fig. 5c which for this case of strongly bound inhibitor exhibits the same course as for the noncompetitive inhibition with an intercept at the *x* axis)].

Fig. 5



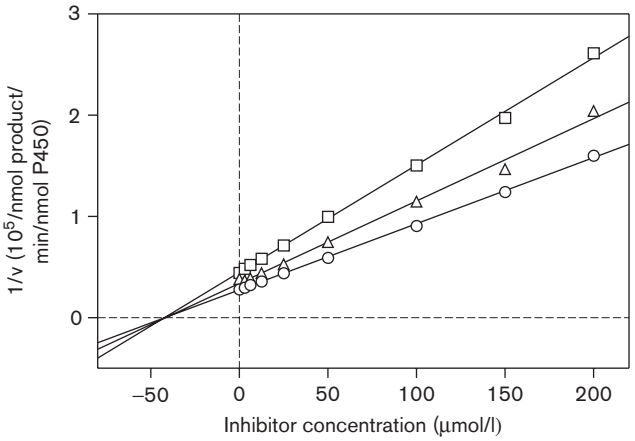
(a) Inhibition of CYP2B6 enzymatic activity (7-ethoxy-4-(trifluoromethyl)coumarin *O*-deethylation) by trans-metbut (squares) and trans-secbut (triangles) complexes. (b) Dixon plot for inhibition of CYP2B6 enzymatic activity (as in Fig. 5a) by trans-secbut at three substrate concentrations (25 μmol/l, squares; 50 μmol/l, triangles; 100 μmol/l circles). (c) Lineweaver–Burk plots for inhibition of CYP2B6 enzymatic activity (as in Fig. 5a) by trans-secbut complex at three substrate concentrations as in Fig. 5b for 10 concentrations of trans-secbut inhibitor (from up to down: 200, 150, 100, 50, 25, 13, 6, 3, 2 μmol/l and control with no inhibitor). 1/v, reciprocal velocity; 1/[S], reciprocal substrate concentration; RFU, relative fluorescence unit.

Fig. 6



Dixon plot for inhibition of CYP2E1 (chlorzoxazone 6-hydroxylation) by trans-metbut at three substrate concentrations (25 μmol/l, squares; 50 μmol/l, triangles; 100 μmol/l circles). Insert: Detail of Dixon plot for lower range of inhibitor concentrations. 1/v, reciprocal velocity.

Fig. 7



Dixon plot for inhibition of CYP2E1 (as in Fig. 6) by trans-secbut at three substrate concentrations (25 μmol/l, squares; 50 μmol/l, triangles; 100 μmol/l circles). 1/v, reciprocal velocity.

Table 1 **The IC_{50} values for trans-metbut and trans-secbut complex obtained from inhibition experiments**

	Transplatin		Trans-metbut complex		Trans-secbut complex	
CYP450	Inhibition type	IC_{50} (μmol/l)[b]	Inhibition type	IC_{50} (μmol/l)[b]	Inhibition type	IC_{50} (μmol/l)[b]
CYP1A2	ND (inhibition only at higher concentration)	ND	Strongly bound inhibitor	32 ± 2	Strongly bound inhibitor	33 ± 3
CYP2A6	No inhibition observed	ND	Strongly bound inhibitor (uncompetitive inhibition[a])	85 ± 16	Strongly bound inhibitor (uncompetitive inhibition[a])	106 ± 12
CYP2B6	Uncompetitive inhibition	42 ± 25	Strongly bound inhibitor	16 ± 2	Strongly bound inhibitor	21.1 ± 0.4
CYP2C8	No inhibition observed	ND	ND (inhibition only at higher concentration)	ND	ND (inhibition only at higher concentration)	ND
CYP2C9	Noncompetitive inhibition	61 ± 25	ND (inhibition only at higher concentration)	ND	ND (inhibition only at higher concentration)	ND
CYP2C19	No inhibition observed	ND	Strongly bound inhibitor	30 ± 8	Strongly bound inhibitor	27 ± 5
CYP2D6	No inhibition observed	ND	Strongly bound inhibitor (noncompetitive inhibition[a])	44 ± 9	Strongly bound inhibitor (noncompetitive inhibition[a])	51 ± 10
CYP2E1	ND (inhibition only at higher concentration)	ND	Strongly bound inhibitor (noncompetitive inhibition[a])	28 ± 4	Noncompetitive inhibition	32 ± 2
CYP3A4	ND (inhibition only at higher concentration)	ND	Strongly bound inhibitor	28 ± 2	Strongly bound inhibitor	68 ± 17

IC_{50}, half maximal inhibitory concentration; ND, not determined.
[a]Inhibition type involved in lower inhibitor concentrations (up to 100 μmol/l).
[b]Variations of IC_{50} values are expressed by standard deviations of the sampling distribution obtained by Sigma Plot statistical and graphing software.

A detailed analysis of the course of inhibition at lower concentrations of platinum complexes (to 100 μmol/l, for both trans-metbut and trans-secbut) revealed a noncompetitive (CYP2D6, CYP2E1; for illustration, see Fig. 6) or uncompetitive (CYP2A6) mechanisms of inhibition. Interestingly, for inhibition of CYP2E1 activity by the inhibitor possessing a shorter aliphatic chain (sec-butyl) (complex labeled as trans-secbut) in the whole range of concentrations the noncompetitive mechanism of inhibition was (Fig. 7). The half maximal inhibitory concentration values obtained are shown in Table 1.

Discussion

The results obtained in this study with platinum complexes with cis and trans geometry point to the differences in their interactions with the main liver microsomal systems of drug biotransformation, namely, with the CYP enzymes. Cisplatin and its derivatives exhibit only minor effect on activities of selected microsomal CYPs being thus safe drugs in the light of drug interactions based on this effect. In fact, cisplatin inhibited the CYP2C9, CYP2B6 and CYP1A2 activities, however, the decrease was not greater than 25% at the highest concentration of the drug. Oxaliplatin also exhibited a small effect on CYP2C9 activity and carboplatin did not inhibit CYP activities at all.

There are only limited data on the interaction of platinum cytostatics with CYP enzymes present in the literature, however, the results presented here are in line with information given by Baumhakel *et al.* [12] that the cisplatin as well as carboplatin do not influence the enzymatic activity of liver microsomal CYP3A4. The experiments carried out with transplatin gave a more complex picture of the interaction of platinum complexes with CYP enzymes and on their possible importance for clinical practice.

The significance of the results on inhibition of CYP activities obtained with transplatin for direct implications in clinical practice is low as this compound is clinically ineffective. In contrast, in the light of very promising results documenting the mode of binding to the DNA and a strong cytotoxic activity of transplatin derivatives [4,5], the results presented here with two complexes of trans geometry document the need of a detailed study of possible interactions of all structurally similar compounds with liver microsomal CYP enzymes.

The transplatin-based complexes exhibit different behavior to the cisplatin ones in many aspects (local unwinding of the DNA, higher DNA interstrand cross-linking efficiency, lower stability of intrastrand crosslinks, lower level of repair synthesis, higher cytotoxicity, [4]). Apparently, the results presented here also show that the interaction with CYP enzymes is different being relatively stronger than with the complexes based on the cis geometry. The fact that there is a difference between CYP forms in their ability to interact with platinum complexes most probably reflects the structural determinants of the respective CYP forms as well as the structure of the inhibitors. In fact, both the enzymes which were significantly inhibited by transplatin (and to certain extent also by cisplatin), namely, the CYP2B6 and CYP2C9 proteins, are known to possess a relatively open active site; in the case of the CYP2C9, the active site involves amino acid residues of basic character (tryptophan, histidine) [27] which are known to be targets of platinum complexes in proteins [28,29]. The two complexes with the trans geometry most probably bind to the CYP enzymes by the aliphatic chains, which may be accommodated relatively easily in the active sites of the respective enzymes.

In conclusion, the data presented in this study bring new support regarding the safety of the use of carboplatin and

oxaliplatin as their influence on the CYP enzyme activities in human liver microsomes seems to be none or minimal. The cisplatin, although it is able to inhibit partly the activities of CYP enzymes (to 75% of activity of CYP2C9, at 400 μmol/l), is not expected to elicit a clinically relevant interaction because of low plasma levels of this drug – as with the oxaliplatin, the maximum concentrations were reported to reach about 11 μmol/l [25].

As the complexes of platinum (II) with trans geometry are promising in circumventing the resistance to cisplatin [4], the derivatives of transplatin should be, according to the results presented here, thoroughly tested for possible interactions with liver microsomal drug metabolizing enzymes to get information needed for any new class of drug and to avoid the unwanted drug–drug interactions.

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