# New *trans*-Platinum Drugs with Phosphines and Amines as Carrier Ligands Induce Apoptosis in Tumor Cells Resistant to Cisplatin

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Cisplatin resistance observed in some human tumors has prompted research in platinum derivatives that can circumvent this effect. Despite initial works reporting lack of activity of *trans*-platinum derivatives, complexes with the general formula $PtCl_2(L)(L')$ exhibit cytotoxic activity in cisplatin-sensitive and -resistant cell lines. Here we reported the chemical and biological properties of seven platinum complexes with $PPh_3$ or $PMe_2Ph$ in trans to several amines. They show important antitumoral properties in tumor cell lines. Among the compounds, those with a replacement of an ammine ligand in the inactive *trans*-DDP by a phosphine ligand have an important enhancement of their cytotoxic activity. In SKOV3, no G1 nor G2/M accumulation was observed after treatments, and apoptosis was launched probably by a mechanism independent of classical checkpoints activation. Our data indicate that our compounds are not cross-resistant with cisplatin and might be promising agents in the treatment of tumors unresponsive to cisplatin.

## Introduction

In 1964, Rosenberg et al.[1] discovered the anticancer properties of *cis*-$[PtCl_2(NH_3)_2]$, often referred to as cisplatin or *cis*-DDP. Although cisplatin is the most important platinum complex with cis geometry that exhibits antitumor activity, carboplatin, nedaplatin, and oxaliplatin are currently used as antitumor drugs in clinical treatments in an attempt to minimize the secondary effects of cisplatin.[2,3]

It has been generally accepted that antitumoral platinum compounds interact with DNA to form DNA adducts, which activate several signal transduction pathways.[4]

Early structure−activity relationship studies of platinum-based coordination complexes showed that transplatin, as well as other *trans*-platinum isomers studied, are inactive as antitumor agents.[5] Regardless of this finding, in the last 15 years *trans*-platinum complexes have been demonstrated to be endowed with antitumor activity.[6−10] Some of them have entered clinical trials and currently are in phase II.[11,12]

Complexes with general formula *trans*-$[PtCl_2(L)(L')]$ are symmetric, where $L = L' =$ pyridine, *N*-methylimidazole, thiazole, acetonimine, or iminoether, or asymmetric, where $L \neq L'$ with L = quinoline, piperidine, piperazine, or thiazole and $L' = NH_3$ or L and L′ are aliphatic amines.[6,13−23] These compounds exhibit a cytotoxic activity in cisplatin-sensitive cell lines and sometimes greater activity than cisplatin in several cisplatin-resistant tumor cells,[7,8] indicating that synthetic variations in coordinating ligands to the platinum atom have led to new active platinum complexes.

In the synthesis of new drugs with metallic ions, the hydrophobic character of the ligands could help to cross cellular membranes and can modify the electrophilic properties of the metal. The nature of the ligands could also change the stereochemistry of the complexes.[24] Generally, *trans*-diaminechloroplatinum(II) analogues with bulky ligands have lower water solubility than their cis counterparts, although this can be increased by using cyclic amines ligands.[20,25] Therefore, bulky ligands could increase the crossing of cellular membrane and the intracellular accumulation of the drugs. The intracellular accumulation is necessary to produce damage in tumoral cells by formation of DNA adducts.[26] From a mechanistic point of view, the bulky ligands can retard substitution reactions of the living groups, thus reducting the kinetic instability of transplatin, and form DNA adducts qualitatively and quantitatively different.[27]

In the literature, platinum complexes containing aminophospine, triphos ligands, and phosphorus-derived ligands have been described but there are few data about their cytotoxicity.[28−31] Previously to this paper, we published that the complex *trans*-$[PtCl_2$(2-butylamine)$(PPh_3)]$ was more active than cisplatin against the Pam212 and Pam212-ras cell lines.[32]

Therefore, since some *trans*-platinum compounds with aliphatics amines show antitumoral activity in some cell lines,[18,20,33] we decided to study the influence on the cytotoxic activity of bulky and hydrophobic ligands like the phosphines $PPh_3$ and $PMe_2Ph$ in trans configuration to $NH_3$ and the following aliphatic amines: isopropylamine, dimethylamine, and 1-aminoindane.

In this work, we report the results of the antitumoral properties of seven complexes in several cell lines in vitro. The $IC_{50}$ values found for these complexes were better than the values reported for other *trans*-platinum complexes. Furthermore, the toxicity in normal cell line IMR90 is low and the intrinsic resistance to cisplatin in the ovarian carcinoma cell lines SKOV3 and CH1cisR is overcome. At difference of the treatment with cisplatin and other platinum compounds like BBR3464 or JM149 that accumulate cells at G2/M phase of the cell cycle, our *trans*-platinum derivative complexes induce apoptosis without G2/M nor G1 accumulation, suggesting a novel mechanism of action.[12,34]

## Results and Discussion

**Synthesis and Structural Characterization**. The complexes tested are the combination of $PPh_3$ or $PMe_2Ph$ with four amine

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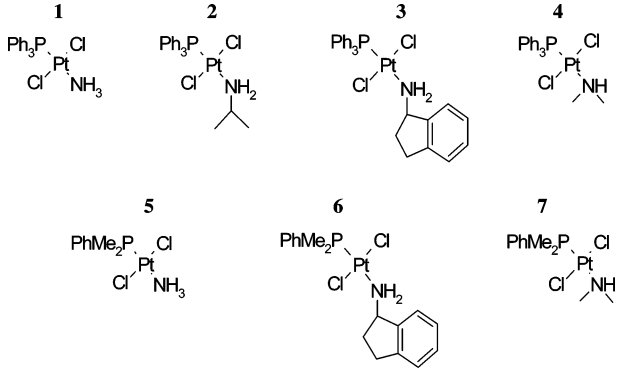


**Figure 1.** Schematic representation of the *trans*-platinum(II) complexes studied in this work.

**Table 1.** Selected Bond Lengths and Angles for Complex **2**[a]

| | | | |
|---|---|---|---|
| Pt−Cl(1) | 2.2949(9) | Pt−Cl(2) | 2.2990(9) |
| Pt−P | 2.2396(9) | Pt−N | 2.127(3) |
| Cl(1)−Pt−Cl(2) | 173.28(3) | Cl(1)−Pt−P | 89.33(3) |
| Cl(1)−Pt−N | 88.13(11) | Cl(2)−Pt−P | 96.30(3) |
| Cl(2)−Pt−N | 86.61(11) | P−Pt−N | 173.94(10) |

[a] Bond lengths are given in angstroms; bond angles are given in degrees.

ligands bonded to the platinum atom in trans configuration (Figure 1). We used both phosphines because the cone angles are 145° and 122°, respectively.[35]

The complexes were prepared in three different steps.[32] The first step was a reaction between $K_2PtCl_4$ and the phosphine, affording *cis*-$[PtCl_2(PR_3)_2]$. In a second step, direct treatment of the cis isomer with $PtCl_2$ produced the complexes $[(\mu\text{-Cl})\text{-}PtCl(PR_3)]_2$. Further addition of the desired amine splits the chloro-bridged complex to afford the final trans complex *trans*-$[PtCl_2(L)(PR_3)]$.

The characterization of these complexes was carried out by IR and by $^{195}$Pt, $^{31}$P, $^{1}$H, and $^{13}$C NMR. All the complexes show only one $\nu$(Pt−Cl) band around 345 cm$^{-1}$, as would be expected for a *trans*-Pt geometry.[31,36] All the microanalytical data were consistent with the empirical formulas.

The values of the coupling constant $^1J(^{31}P-^{195}Pt)$ measured from the $^{195}$Pt satellites were different for *cis*- and *trans*-platinum geometry.[37] The phosphine groups signals were doublets in $^{195}$Pt NMR with constants $^1J(^{31}P-^{195}Pt)$ of 3695 Hz in complex **1**, 3597 Hz in complex **2**, 3625 Hz in complex **3**, 3521 Hz in complex **4**, 3524 Hz in complex **5**, 3476 Hz in complex **6**, and 3368 Hz in complex **7**. The values are in agreement with the literature data.[31,38] *trans*-platinum complexes with the $PPh_3$ group showed a signal in $^{31}$P NMR at 12 ppm, whereas $PMe_2Ph$ are at −16 ppm. The coupling constants $^1J(^{31}P-^{195}Pt)$ with $PPh_3$ were higher than in the case of $PMe_2Ph$. This effect was probably due to the influence of two more aromatic groups in $PPh_3$, in such a way that they can delocalize more electronic density from P atom.

The structure of complex **2** was determined by X-ray analysis. Select bond lengths (angstroms) and angles (degrees) are summarized in Table 1.

The complex **2** *trans*-$\{PtCl_2[NH_2CH(CH_3)_2](PPh_3)_2\}$ presents a square-planar geometry (Figure 2), which is slightly distorted around the platinum atom (see Table 1). The distances and angles are similar to the values found in complexes with triphenylphosphine ligands and aliphatic amines in trans configuration around the platinum atom.[32] However, the Pt−P distance is shorter than that seen for the complexes with two phosphines in trans (2.32 Å).[38] The difference is probably produced by the different trans effect of $PPh_3$ in trans to amine,

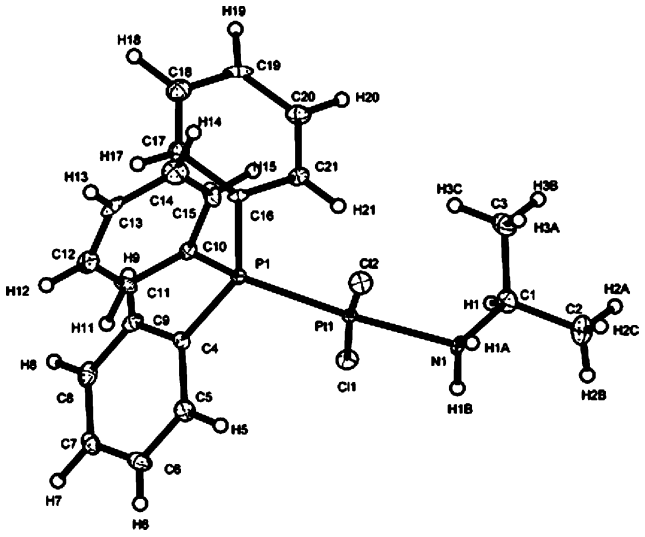


**Figure 2.** ORTEP view of complex **2**.

**Table 2.** Cytotoxic Potency of Pt(II) Complexes **1**−**7** on the Different Cell Lines Tested

| | IC$_{50}$, $\mu$M | | | | |
|---|---|---|---|---|---|
| complex | IMR90 | SKOV3 | CH1 | CH1cisR (RF) | SR2910 |
| **1** | 15 | 4 | 8.3 | 5.2 (0.6) | 3.8 |
| **2** | 5 | 2.2 | 2.8 | 2.3 (0.8) | 7 |
| **3** | 5 | 2.3 | 4.2 | 2.4 (0.6) | >50 |
| **4** | 5 | 2.3 | 4.3 | 2.6 (0.6) | >50 |
| **5** | 30 | 9 | 6.7 | 7.3 (1.1) | 13.4 |
| **6** | >100 | 4 | 3.7 | 3.1 (0.8) | 2.8 |
| **7** | >100 | 5 | 3.4 | 2.5 (0.7) | 4.5 |
| *cis*-DDP | >50 | 26 | 7 | 20 (2.8) | a |

[a] Not determined.

whereas in the case of two phosphines in trans, both trans effects are counteracted. The Pt−N distance is longer in comparison with *trans*-$[PtCl_2(dma)(ipa)]$ and this value is consistent with the trans influence of phosphines relative to other ligands.[39]

The complex **2** shows one intermolecular hydrogen bond in the crystal structure N(1)−H(1B)···Cl(2), where the distances N−H, H···Cl(2), and N···Cl(2) are 0.87(5), 2.67(5), and 3.465(4) Å, respectively, and the angle N−H···Cl(2) is 152(4)°. The symmetry transformations used to generate equivalent atoms were $-x + 1$, $-y + 2$, $-z + 2$.

**Cytotoxic Activity**. We have tested the cytotoxicity of all these complexes and *cis*-DDP against five cell lines selected because of the known major mechanism underlying resistance to cisplatin. To that end we used the normal cells IMR90 (human primary fibroblasts, nonimmortal, nontumoral, and without genetic alterations) and the tumoral cell lines SKOV3 (ovarian carcinoma cell line that shows intrinsic resistance to cisplatin), CH1 and CH1cisR (ovarian carcinoma cell line sensitive to cisplatin and the respective acquired cisplatin-resistance cell line due to enhanced DNA repair/tolerance), and SR2910 (Ewing's sarcoma cells). The cell survival in the culture treated with platinum compounds was evaluated by using the crystal violet assay.[40] A complete listing of the 50% inhibitory concentration values for the distinct complexes investigated (concentration of drug yielding a 50% decrease in either cell density or cell viability) is given in Table 2.

These new *trans*-platinum complexes with phosphines and amines were more potent than cisplatin in all the cell lines tested. All compounds showed similar cytotoxic activity in the intrinsic resistant SKOV3 cells and the acquired resistant cells CH1cisR. This fact perhaps reflects the inability of cisplatin-resistant cells

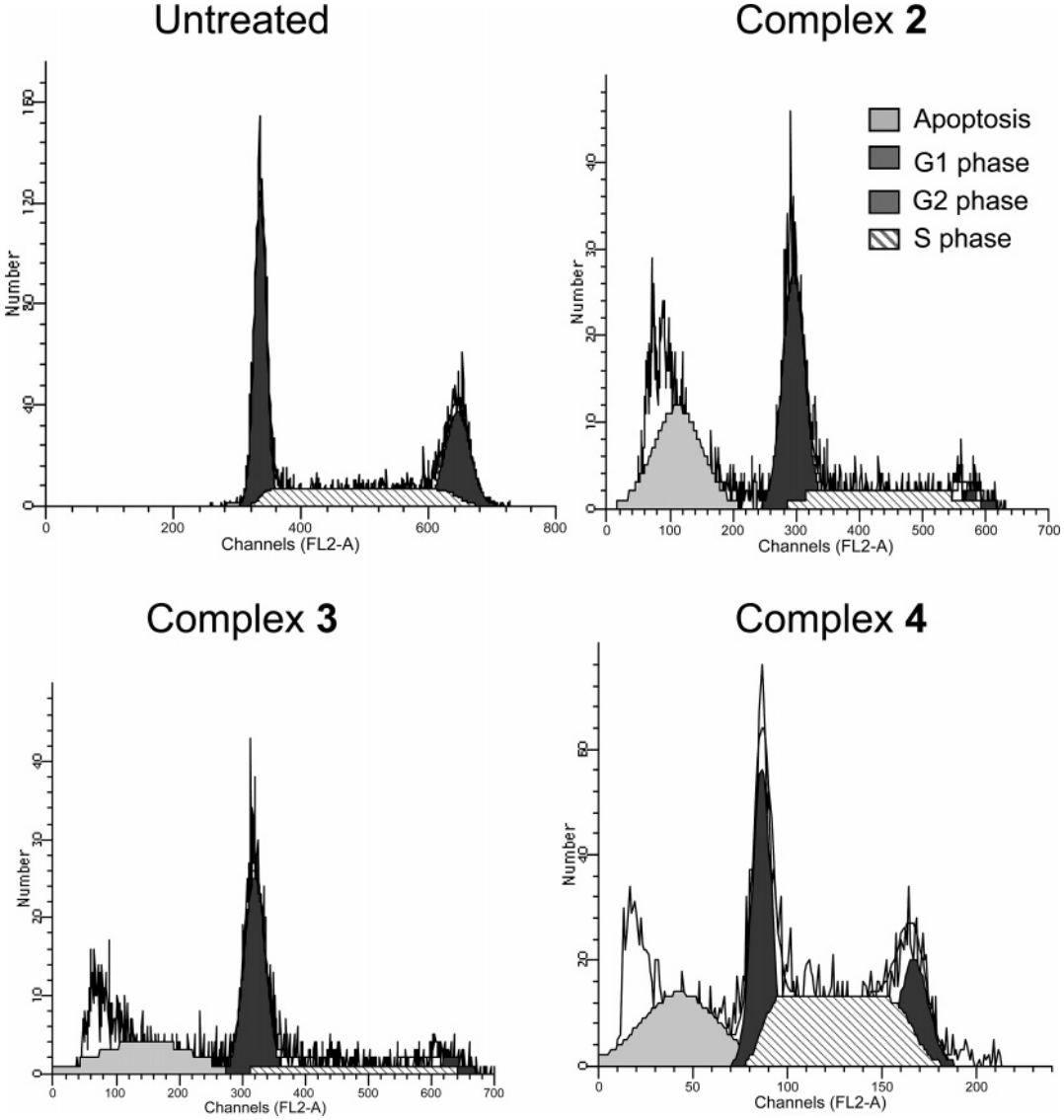


**Figure 3.** DNA content of cells treated with the *trans*-platinum(II) complexes. SKOV3 cells were seeded at 25% density; after 24 h, the medium was changed and compounds were added to 10 $\mu$M final concentration. After 48 h, cells were collected and cell cycle status was analyzed by FACS for DNA content.

to tolerate the DNA damage produced by the new trans complexes.[34,41] Our interpretation of the lower $IC_{50}$ in CH1cisR than CH1 is that these complexes could circumvent the enhanced DNA repair/tolerance possibly due to NER, MMR, or recombination repair systems.[26,42] The new complexes with phosphines have an RF (resistance factor, defined as $IC_{50}$ resistant line/$IC_{50}$ parental line) between 0.6 and 1.1. Their values are much lower than cisplatin (RF = 3.8) and others like *trans*-[$PtCl_2(NH_3)$-(2-methylbutylamine)] (RF = 3.6) or complexes with piperazine (RF = 1.1−3.9), which are also cytotoxic in CH1 and can circumvent the resistance in CH1cisR.[19,33] Our data also shows that all tumor cell lines were more sensitive to the complexes than IMR90 cells, suggesting the existence of a small therapeutic window for these compounds.

The replacement of one ammine ligand by phosphines in the molecule of transplatin (such **1** and **5**) results in a radical enhancement of its activity in tumor cell lines both sensitive and resistant to cisplatin, but the substitution of both ammines has shown to be even better than the previous one. Interestingly, the $IC_{50}$ values produced in SKOV3 and CH1cisR cell lines by complexes with $PPh_3$ is lower than in those with $PMe_2Ph$ ligand. A possible explanation is that this change in the cytotoxicity could be due to the steric effect. The conic angle of $PPh_3$ complexes is 23° bigger than for $PMe_2Ph$. It seems that the greater steric impediments of these *trans*-platinum complexes with $PPh_3$ could produce greater DNA damage or distortion than those complexes with the $PMe_2Ph$ ligand in trans. It is not reasonable to attribute only the lower $IC_{50}$ to hydrophobic effects because there are not significant differences in the cytotoxicity among complexes with the same phosphine ligand and several amines. The presence of the phosphine would have a lipophilic effect in the complex and help to cross the cytoplasmic membrane; therefore a higher platinum concentration might bind to DNA. This hypothesis could be explored in the future by techniques described previously.[43,44]

We observed that the steric effect of the phosphine has an influence, but changing the amines is also important for the cytotoxicity.

Complexes **1** and **5** with an ammine ligand in trans to $PPh_3$ and $PMe_2Ph$, respectively, show higher $IC_{50}$ than the other new *trans*-platinum compounds studied. Once more the assay with both complexes showed better activity in the case of **1**, the *trans*-platinum complex with $PPh_3$. Again the explanation could be due to a greater steric effect in the case of the $PPh_3$ ligand.

**Table 3.** Percentage of Cells at Each Cell Cycle Phase after the *trans*-Platinum(II) Complexes Treatment

| phase | untreated | complex **2** | complex **3** | complex **4** |
|---|---|---|---|---|
| G0/G1 | 48.8 | 41.1 | 42.2 | 24.9 |
| S | 16.7 | 16.7 | 19.6 | 37.5 |
| G2/M | 26 | 3.7 | 4.4 | 13.3 |
| apoptosis | 0.9 | 38.6 | 31.7 | 24.12 |

The $IC_{50}$ values in IMR90 cell line are in the range 5−30 $\mu$M. For complexes **6** and **7** we did not reach an $IC_{50}$ in IMR90 at the concentrations that we used in the assay (0−100 $\mu$M). It is interesting that $PMe_2Ph$-derived compounds show a higher $IC_{50}$ in almost all lines tested than $PPh_3$ compounds, presenting also an increased therapeutic window. This property should be explored further.

Complexes **3** and **4** against the SR2910 cell line have a very high $IC_{50}$, unlike **2**. It is possible to think that **3** and **4** could show more preference for carcinoma tumor cells (like SKOV3, CH1, and CH1cisR) than to nonepithelial origin cells (such as SR2910). Alternatively, specific mechanisms of detoxification might exist, being expressed in different cell lines.

All our new *trans*-platinum complexes show lower $IC_{50}$ values in the SKOV3 cell line, in comparison with other compounds previously reported in the literature.[8,19,45] Compounds **1** and **5** have higher $IC_{50}$ values than the rest of the assayed platinum complexes with phosphines. However, these values are lower than those displayed with cyclic ligands mimicking iminoethers with an ammine ligand in trans configuration.[19]

**Cell Cycle Analysis.** One of the early effects of the platination of DNA is a reduction in the rate of DNA synthesis and a consequent slowdown in the traverse of the cell through the S phase of their cycle;[46] subsequently, there is an activation of DNA-damage checkpoint, eliciting a dose-dependent arrest in G2.[46−49]

Our results clearly demonstrate that the cells treated with compounds **2**, **3**, and **4** die by apoptosis (Figure 3). All compounds induce G2/M-phase cell depletion, suggesting that apoptosis is launched from G2/M. However, some differences in behavior can be observed between cells treated with compounds **2** and **3** and cells treated with compound **4**. Cells treated with compound **4** accumulate preferentially at S phase, while cells treated with compounds **2** and **3** have a stable percentage of cells at G1 and S phases (see Table 3).

One of the pathways activated by cisplatin-induced DNA damage culminates in the activation of the cell cycle checkpoints. A generally accepted consequence is that the cell cycle is arrested because it is necessary to enable the nucleotide excision repair (NER) complex to remove the adducts and promote cell survival. When repair is incomplete, the cells undergo apoptosis. In the case of cisplatin, the activation of cell cycle DNA-damage checkpoint induces G1 and G2/M arrest.[47]

Among the *trans*-platinum compounds, the complex BBR3464 induced a dose-dependent cell cycle arrest in G2/M phase with no arrest in the S phase. The complexes JM335 and JM149, which are cis and trans analogues, respectively, of ammine-(cyclohexylamine)dihydroxodichloroplatinum (IV), showed a frozen cell cycle at G2/M phase with little movement from G1 into S, accompanied by a buildup of cells in G2, representative of a G2/M block.[51] *trans*-platinum complexes like *trans*-$[PtCl_2\{E\text{-}HN{=}C(OMe)Me\}_2]$ also cause an early accumulation in S phase of treated cells, followed by G2/M accumulation, which suggests that the cell death occurs in the same phases as that of *cis*-DDP.[50]

It is possible that interstrand adducts induced by *trans*-platinum compounds provide G1- and S-phase arrest, allowing low transition toward G2/M phase. DNA damage will launch the apoptosis and cell death from G2/M. An apoptotic ratio higher than transition through S phase will lead to a cell cycle profile such as the one shown in our experiments (Figure 3). This hypothesis may agree with recent findings in SKOV3 with the complex *trans*-$[PtCl_2\{Z\text{-}HN{=}C(OMe)Me\}(NH_3)]$, which shows a persistent increase in genes related to DNA stability and repair and G2/M checkpoint in SKOV3 treated with *trans*-platinum compounds.[27] However, our data indicates that the compound **4** induces a higher accumulation of cells in S phase than compounds **2** and **3**, although this is not translated into any differential cytotoxic effect, since all three compounds shows very similar $IC_{50}$ values in all cell lines analyzed. The different accumulation of cells in S phase can be due to multiple causes, for instance, different DNA adducts formed by different ligands, binding to different cellular proteins or cellular membranes altering the cell response. Further exploration is needed to determine a possible differential mechanism of action.

In any case, our compounds are not cross-resistant with cisplatin and seem to possess a different mechanism of action.

## Conclusions

The replacement of an ammine ligand in the inactive *trans*-DDP by a amine ligand has a radical enhancement of their activity, but the replacement of the second ammine ligand by a phosphine ligand should produce a much better cytotoxic effect.[7,19,22]

The use of bulky ligands with hydrophobic properties can produce steric impediments and maybe these are the causes of the increased cytotoxicity.

These compounds are not cross-resistant with cisplatin and seem to possess a different mechanism of action

## Experimental Section

**General**. Infrared spectra were recorded in Nujol mulls on CsI windows and in KBr pellets in the 4000−200 $cm^{-1}$ range with a Perkin-Elmer model 283 spectrophotometer. NMR spectra were recorded on a Bruker AMX-300 (300 MHz) spectrometer at room temperature (25 °C) in acetone-$d_6$ solution. $^{31}P$ NMR and $^1H$ NMR experiments were performed in DMSO-$d_6$ for 24, 48, and 72 h to confirm the stability of these complexes (spectra not shown). Elemental analysis was performed on a Perkin-Elmer 2400 series II microanalyzer. The structural determination was performed on a Bruker SMART 6K CCD (Supporting Information).

**Synthesis.** All the new *trans*-platinum complexes were prepared by the methods described previously.[32] Addition of a stoichiometric quantity of amine to a partial solution of $[(\mu\text{-}Cl)PtCl(L)]_2$, where L is $PPh_3$ or $PMe_2Ph$ (0.213 mmol, 225 mg) in acetone at room temperature, gives rise after 30 min to a yellow solution and a lightening in the color. The acetone is removed under reduced pressure. Diethyl ether is added and the solution is filtered to remove the insoluble impurities. The diethyl ether is also removed under reduced pressure with the formation of *trans*-$[PtCl_2(L')(L)]$ crystals or yellow precipitates.

**Complex 1, *trans*-$[PtCl_2(PPh_3)(NH_3)]$.** Yield 65%; $\nu$(Pt−Cl) 344 $cm^{-1}$. Anal. ($C_{18}H_{18}NPtCl_2P$) C, H, N. $^1H$ NMR (ppm) 7.70 (m, 6H, $H_{ortho}$), 7.44 (m, 9H, $H_{meta}$ and $H_{para}$), 3.65 (br s, 3H, $NH_3$). $^{31}P$ NMR (300 MHz, acetone-$d_6$, 25 °C) (ppm) 11.95 ($J_{P-Pt}$ = 3694 Hz). $^{13}C$ NMR (ppm) 128.6 ($C_{ortho}$), 130.4 ($C_{ipso}$), 131.4 ($C_{para}$), 135.5 ($C_{meta}$). $^{195}Pt$ NMR (ppm) −3799 ($J_{P-Pt}$ = 3650 Hz).

**Complex 2, *trans*-$[PtCl_2(PPh_3)$(isopropylamine)].** The characterization of this complex has been published previously.[32]

**Complex 3, *trans*-$[PtCl_2(PPh_3)$(1-aminoindane)].** Yield 56%; $\nu$(Pt−Cl) 342 $cm^{-1}$, $\nu$(Pt−P)$\mu$ 453 $cm^{-1}$, $\nu$ (Pt−N) 431 $cm^{-1}$. Anal. ($C_{27}H_{26}NPtCl_2P$) C, H, N. $^1H$ NMR and $^{13}C$ NMR show a complex

signal system similar to the amine used for blank. $^{31}$P NMR (ppm) 11.95 ($J_{P-Pt}$ = 3625 Hz). $^{195}$Pt NMR (ppm) −3801 ($J_{P-Pt}$ = 3618 Hz).

**Complex 4, *trans*-[PtCl$_2$(PPh$_3$)(dimethylamine)].** Yield 68%; $\nu$(Pt−Cl) 345 cm$^{-1}$, $\nu$(Pt−P): 452 cm$^{-1}$, $\nu$ (Pt−N) 426 cm$^{-1}$. Anal. ($C_{20}H_{22}NPtCl_2P$) C, H, N. $^1$H NMR (ppm) 7.70 (m,, 6H, $H_{ortho}$) 7.44 (m, 9H, $H_{meta}$ and $H_{para}$), 4.47 (br s, NH), 2.67 (dd, 6H, $CH_3$). $^{31}$P NMR (ppm) 11.71 ($J_{P-Pt}$ = 3525 Hz). $^{13}$C NMR (ppm) 40.5 ($CH_3$), 128.6 ($C_{ortho}$), 130.4 ($C_{ipso}$), 131.4 ($C_{para}$), 135.5 ($C_{meta}$). $^{195}$Pt NMR (ppm) −3837.7 ($J_{P-Pt}$ = 3516 Hz).

**Complex 5, *trans*-[PtCl$_2$(PMe$_2$Ph)(NH$_3$)].** Yield 67%; $\nu$(Pt−Cl) 344 cm$^{-1}$, $\nu$(Pt−P): 446 and 428 cm$^{-1}$. Anal. ($C_8H_{14}NPtCl_2P$) C, H, N. $^1$H NMR (ppm) 7.95 (m, 2H, $H_{ortho}$) 7.48 (m, 3H, $H_{meta}$ and $H_{para}$), 1.77 (d, 6H, $CH_3$) 3.39 (br s, $NH_3$). $^{31}$P NMR (ppm) −16.28 ($J_{P-Pt}$ = 3524 Hz). $^{13}$C NMR (ppm) 132.40 (d, $C_{meta}$), 131.22 (d, $C_{para}$), 134.00 (d, $C_{ipso}$), 129.06 (d, $C_{ortho}$), 12.32 (d, $CH_3$). $^{195}$Pt NMR (ppm) −3752 ($J_{P-Pt}$ = 3538 Hz).

**Complex 6, *trans*-[PtCl$_2$(PMe$_2$Ph)(1-aminoindane)].** Yield 65%; $\nu$(Pt−Cl) 345 cm$^{-1}$, $\nu$(Pt−P): 446 and 428 cm$^{-1}$. Anal. ($C_{17}H_{22}NPtCl_2P$) C, H, N. $^1$H NMR and $^{13}$C NMR show a complex signal system similar to the amine used for blank. $^{31}$P NMR (ppm) −15.74 ($J_{P-Pt}$ = 3476 Hz). $^{195}$Pt NMR (ppm) −3804 ($J_{P-Pt}$ = 3461 Hz).

**Complex 7, *trans*-[PtCl$_2$(PMe$_2$Ph)(dimethylamine)].** Yield 68%; $\nu$(Pt−Cl) 343 cm$^{-1}$, $\nu$(Pt−P) 445 cm$^{-1}$, $\nu$ (Pt−N) 436 cm$^{-1}$. Anal. ($C_{10}H_{18}NPtCl_2P$) C, H, N. $^1$H NMR (ppm) 7.95 (m, 2H, $H_{ortho}$) 7.48 (m, 3H, $H_{meta}$ and $H_{para}$), 1.77 (d, 6H, PhC$H_3$), 4.1 (br s, $NH_3$), 2.6 (dd, 6H, $CH_3$). $^{31}$P NMR (ppm) −16.16 ($J_{P-Pt}$ = 3368 Hz). $^{13}$C NMR (ppm) 132.40 (d, $C_{meta}$), 131.22 (d, $C_{para}$), 129.06 (d, $C_{ortho}$), $C_{ipso}$ was not shown, 12.32 (d, $CH_3$), 40.53 ($CH_3$). $^{195}$Pt NMR (ppm) −3837 ($J_{P-Pt}$ = 3380 Hz).

**Assessment of Cytotoxicity**. The compounds were tested on 96-well plates. Cells growing in a flask are harvested just before they become confluent, counted by use of a hemocytometer, and diluted down with medium to adjust the concentration to the required number of cells per 0.2 mL (volume for each well). The cells are then seeded in the 96-well plates at a density, depending on the cell size, between 1000 and 4000 cells/well. The cells are then left to plate down and grow for 24 h before addition of the drugs.

The compounds are weighed out and diluted with dimethyl sulfoxide (DMSO) to get them into solution to a concentration of 10 $\mu$M. From here a "mother plate" with serial dilutions is prepared at 200× the final concentration in the culture. Each mother plate was stored at −20 °C until further use. The final concentration of DMSO in the tissue culture medium should not exceed 0.5%. The appropriate volume of the compound solution (usually 2 $\mu$L) was added automatically (Beckman FX 96 tip) to medium to make it up to the final concentration for each drug.

The medium is removed from the cells and replaced with 0.2 mL of medium dosed with drug. Each concentration was assayed in triplicate. A set of control wells are left on each plate, containing medium without drug. A second set of control wells are left on each plate, containing medium with the same concentration of DMSO. Another control set is obtained with the cells untreated just before addition of the drugs (seeding control, number of cells starting the culture).

The cells are exposed to the drugs for 72 h and then washed twice with phosphate-buffered saline before being fixed with 10% glutaraldehyde. Cells are washed twice and fixed with 0.5% crystal violet for 30 min. Then they are washed extensively and absorbance is measured at 595 nm.

**Cell Cycle Analysis**. Cells were seeded at low density (approximately 25%) in 10-cm dishes, grown for 24 h, and then treated with the indicated agent for 48 h at 10 $\mu$M (final concentration). Cells were then trypsinized, fixed with 70% methanol, and washed twice with phosphate-buffered saline (PBS) containing 0.1% fetal calf serum (FCS). After incubation in PBS supplemented with RNase (10 mg/mL) and propidium iodide (5 mg/mL) for 30 min, the DNA content was analyzed by fluorescence-activated cell sorting (FACS).

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**Supporting Information Available:** X-ray crystallographic determination and refinement of complex **2** and table listing elemental analysis results of *trans*-platinum complexes **1**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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