# Novel Transplatinum(II) Complexes with $[N_2O_2]$ Donor Sets. Cellular Pharmacology and Apoptosis Induction in Pam 212-*ras* Cells

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Cellular pharmacological properties of eight *trans*-picoline platinum(II) complexes of formula *trans*-$[PtX_2(L)(L')]$, where X = Cl or $CH_3COO$ (OAc) and L = L′ = 3-picoline (3-pic), 4-picoline (4-pic) or L = $NH_3$ and L′ = 3-pic or 4-pic, were investigated in murine keratinocyte Pam 212 cells and Pam 212-*ras* cells, murine tumor keratinocytes derived from transformation with a viral vector containing the H-*ras* oncogene. The derivatives *trans*-$[Pt(OAc)_2(L)(L')]$ (L = L′ = 3-pic, **9**, and L = L′ = 4-pic, **10**) were able to circumvent resistance in Pam 212-*ras* cells. Although all the *trans*-picoline platinum(II) acetate derivatives showed a similar level of DNA binding, there were remarkable differences in cellular accumulation: the complexes having two picoline ligands (**9**, **10**) had a much higher intracellular accumulation than those having mixed picoline and ammine ligands (**11**, **12**). No significant differences in cellular pharmacological properties have been observed between isomers having 3- or 4-picoline.

## Introduction

Platinum-based drugs cisplatin and its structural analogues carboplatin and oxaliplatin have received worldwide acceptance in the treatment of various neoplastic diseases. Despite the synthesis of many hundreds of cisplatin derivatives over the past 30 years, there have been relatively few leads in the discovery of new, structurally distinct novel classes of platinum drugs.[1] The principal structural class of clinical interest is that of poly(di/tri)nuclear systems represented by the phase II drug BBR3464.[2] The development of this series is based on the hypothesis that platinum compounds structurally dissimilar to cisplatin may have, by virtue of formation of different types of Pt−DNA adducts, a spectrum of antitumor activity complementary to that of cisplatin.[3]

The trans isomer of cisplatin, *trans*-$[PtCl_2(NH_3)_2]$ (transplatin), is therapeutically inactive.[4] Substitution of $NH_3$ in *trans*-$[PtCl_2(L)(L')]$ gives complexes with cytotoxicity in the micromolar range. In general, these complexes exhibit enhanced cytotoxicity with respect to the parent transplatin and are usually non-cross-resistant with cisplatin. Since the first publication of this phenomenon using planar amines[4−6] (pyridine, thiazole, quinoline, isoquinoline, etc.) a range of amine ligands has been employed, including iminoethers, alicyclic amines, and heterocyclic aliphatic amines.[7−11] Complexes containing transplanaramines (TPA compounds) exhibit a unique cytotoxicity profile in the NCI tumor panel and induction of topoisomeraseI-DNA complexes in human tumor cells.[12,13] Thus, pharmacological and biological differences might be systematically exploited to design new complexes with activity in cisplatin-resistant tumors.

The majority of *trans*-$[PtCl_2(L)(L')]$ complexes are only sparingly water-soluble and still relatively reactive, under the trans influence of the Cl−Pt−Cl axis. Recently, we have reported on the first examples of cytotoxic transplatinum complexes containing $N_2O_2$ donor sets.[14] The complexes are very water-soluble and surprisingly stable toward hydrolysis. Modification of the pharmacological properties can in principle be affected by steric and electronic effects of the donor groups as well as in the leaving carboxylate ligands. The overall profile of the new carboxylate compounds in the NCI 60-cell line panel is similar to those of the parent chlorides.[12,27] In this paper, we report further on the synthesis and characterization of members of this series of novel trans platinum complexes of formula *trans*-$[PtX_2(L)(L')]$ (X = Cl, $CH_3COO$) and compare their pharmacological properties in murine keratinocytes transformed by H-*ras* oncogene with their chloride analogues. With this set of compounds, comparison can be made between "symmetric" (*trans*-$[PtX_2(pyridine)_2]$) and "nonsymmetric" *trans*-$[PtX_2(NH_3)(pyridine)]$ compounds, as well as assessing the steric and electronic effects upon variation of the planar ligand. In addition, the cytotoxicity is compared with the mixed amine complex *trans*-$[Pt(ipa)(dma)Cl_2]$ (ipa = isopropylamine and dma = dimethylamine) which has also shown a very different cytotoxicity profile to cisplatin.[8]

## Results

**Synthesis and Characterization.** The symmetric *trans*-amine complexes *trans*-$[PtX_2L_2]$ were prepared using the method of Scheme 1. The reaction between $K_2PtCl_4$ and the bases L = 3-picoline (3-pic) and 4-picoline (4-pic) at reflux temperature gave a clear solution of the tetraamine $[PtL_4]^{2+}$ complex. When X is iodide, the addition of KI afforded the precipitation of $[Pt(L)_4]I_2$, which with heating is converted to *trans*-$[PtI_2L_2]$.[15] Similarly, formation of *trans*-$[PtCl_2L_2]$ also occurs simply by heating the tetraamine complex with an excess of HCl. The addition of a small excess of silver acetate in acetone suspension to a DMF (X = Cl) or acetone (X = I) solution of *trans*-$[Pt(L)_2X_2]$, results in the metathesis reaction and formation of the corresponding acetates with satisfactory yield, especially for the iodide (Scheme 1).

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**Scheme 1.** Preparation of the "Symmetric" (*trans*-[$PtX_2$(pyridine)$_2$]) and "Nonsymmetric" *trans*-[$PtX_2(NH_3)$(pyridine)] Compounds

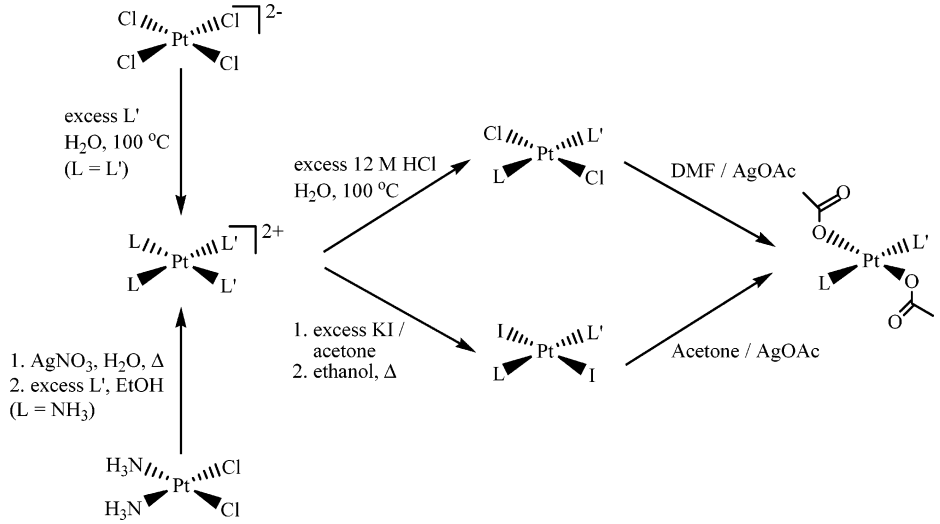


**Chart 1.** Compound Numbering System of the "Symmetric" (*trans*-[$PtX_2$(pyridine)$_2$]) and "Nonsymmetric" *trans*-[$PtX_2(NH_3)$(pyridine)] Compounds

X = Cl
**1**: L=3-pic **3**: L=3-pic
**2**: L=4-pic **4**: L=4-pic

X = I
**5**: L=3-pic **7**: L=3-pic
**6**: L=4-pic **8**: L=4-pic

X = OAc
**9**: L=3-pic **11**: L=3-pic
**10**: L=4-pic **12**: L=4-pic

The mixed amine complexes *trans*-[$PtX_2(NH_3)$(L′)] were similarly prepared with L′ = 3-pic and 4-pic bases. The optimal route for synthesis of mixed-amine complexes is also depicted in Scheme 1. The *cis*-[$Pt(NH_3)_2(L)_2](NO_3)_2$ complex is prepared via metathesis of cisplatin with $AgNO_3$, and the trans isomer is prepared by treatment of the tetraamine cation with an excess of acid or KI and heating, as in the general method described before. The yield of every step is around 80%, with the yield of the tetraammine intermediate being slightly lower (~60%). Compound numbering is given in Chart 1.

All complexes were characterized by elemental analysis, IR, and NMR spectroscopies and assessed for purity by HPLC. The trans geometry is supported by the IR spectral data: a single $\nu$(Pt−Cl) band in the range of 339−350 $cm^{-1}$ due to the $D_{2h}$ symmetry. The IR spectra of the acetate compounds show a characteristic single band from 1665 to 1638 $cm^{-1}$ assigned to the $\nu$(COO) of the acetate group acting as monodentate ligand. NMR spectra of the complexes **1**−**12** are included in the Experimental Section. The acetate complexes **9**−**12** are characterized by good aqueous solubility (Table 1). The greater water solubility of the nonsymmetric complexes in comparison to the symmetric bis(picoline) complexes probably reflects the greater lipophilicity of the pyridine ligand with respect to that of ammonia. The p$K_a$ values given in Table 1 are similar to those previously reported; there is no great variation between the various complexes.[14,16]

**Cytotoxicity of New Transplatinum Complexes.** In vitro activation of the *trans*-platinum geometry by substituting heterocyclic ligands such as pyridine ligands for $NH_3$ in the general structure *trans*-[$PtX_2$L(L′)] is already well-known.[4] Moreover, *trans*-platinum complexes such as *trans*-[Pt(ipa)-(dma)$Cl_2$] also exhibit biological activity against Pam 212-*ras* cells,[8] which are murine keratinocytes that overexpress the H-*ras* oncogene and are generally insensitive to cisplatin. While a range of amines has been employed to obtain enhanced cytotoxicity of the transplatinum structure, few comparisons between the various structural classes (planar ligands, aliphatic amines, iminoethers) have been made. For these reasons, we have tested the cytotoxicity of compounds **1**−**4** and **9**−**12** in Pam 212 and Pam 212-*ras* cell lines.

**Table 1.** Comparison of p$K_a$[a] and Solubility[b] of TPA Acetates

| compound | solubility, mg/mL | p$K_{a1}$ | p$K_{a2}$ |
|---|---|---|---|
| *trans*-[$Pt(OAc)_2(NH_3)$(4-pic)] | 13.2 | 3.94[c] | 6.88[c] |
| *trans*-[$Pt(OAc)_2(NH_3)$(3-pic)] | 11.4 | 3.97[c] | 6.78[c] |
| *trans*-[$Pt(OAc)_2$(4-pic)$_2$] | 5.7 | 3.84 | 6.43 |
| *trans*-[$Pt(OAc)_2$(3-pic)$_2$] | 4.6 | 3.78 | 6.49 |
| *trans*-[$Pt(OAc)_2$(py)$_2$] | 15.0 | 3.87 | 6.70 |
| *trans*-[$Pt(OAc)_2(NH_3)$(py)] | 11.6 | 3.73 | 6.80 |

[a] p*K*a obtained from potentiometric titrations of the *trans*-[$Pt(OH_2)_2$(L)(L′)] complex. [b] Samples were sonicated after the addition of $H_2O$ and stored at 37 °C for 15 min. [c] From ref 16.

**Table 2.** $IC_{50}$ Values and in Vitro Resistance Indices (RI) for *trans*-Platinum Complexes

| compd | $IC_{50}$ ($\mu$M) Pam 212 | Pam 212-*ras* | RI[a] |
|---|---|---|---|
| **1** | 41.0 ± 2.0 | 72.0 ± 5.0 | 1.75 |
| **2** | 32.0 ± 1.0 | 20 ± 1.0 | 0.62 |
| **3** | 52.0 ± 4.0 | 99.0 ± 10.0 | 1.90 |
| **4** | 28.0 ± 2.0 | 26.0 ± 2.0 | 0.93 |
| **9** | 19.4 ± 1.0 | 21.0 ± 3.0 | 1.08 |
| **10** | 16.9 ± 2.0 | 23.3 ± 1.0 | 1.39 |
| **11** | 34.1 ± 3.0 | 101.3 ± 9.0 | 2.97 |
| **12** | 36.5 ± 2.0 | 167.5 ± 12.0 | 4.59 |
| *cis*-DDP | 103 ± 8.0 | 106 ± 7.0 | 1.02 |

[a] RI: resistance inde, defined as $IC_{50}$ Pam 212-*ras*/$IC_{50}$ Pam 212.

The cytotoxicity of the complexes was evaluated using the MTT cell-survival assay. The $IC_{50}$ values were defined as the concentration of compound that produces 50% of cell death. Table 2 shows the $IC_{50}$ values obtained after 24 h of treatment with compounds **1**−**4** and **9**−**12** in the pair of Pam 212 cells.

For chloride complexes, the presence of the 4-picoline ligand appears to enhance cytotoxicity: complexes **2** and **4** show $IC_{50}$ values against Pam 212-*ras* cells of 20 and 26 $\mu$M, while the 4-picoline analogues **1** and **3** show $IC_{50}$ values of 72 and 99 $\mu$M, respectively. The 4-picoline derivatives are also the most

**Table 3.** $IC_{50}$ Values ($\mu$M ± SD) Obtained after 24 h of Incubation of Compounds **9**, **10**, and *cis*-DDP (cisplatin) with the Pair of Human Ovarian Tumor Cell Lines 41M (sensitive) and 41McisR (resistant) to *cis*-DDP

| | 41M | 41McisR | RF[a] |
|---|---|---|---|
| **9** | 15 ± 1 | 35 ± 2 | 2.3 |
| **10** | 21 ± 3 | 42 ± 3 | 2.0 |
| *cis*-DDP | 25 ± 2 | 102 ± 9 | 4.0 |

[a] RF = resistance factor, defined as the ratio $IC_{50}$ 41McisR cells/$IC_{50}$ 41M cells. 41McisR cells are resistant to *cis*-DDP through decreased platinum accumulation.[29]

active ones against Pam 212-*ras* cells, with resistance indices being less than 1. The values in general are similar to those found previously for the mixed-amine complexes using alicyclic amines.[8] For the acetate series, on the other hand, it may be seen that the most potent complexes are those containing two planar ligands; there is little difference between the 3-picoline and 4-picoline derivatives. Complexes **9** and **10** show the highest cytotoxicity of all compounds studied in Pam 212 and retain very similar activity in the transformed Pam 212-*ras* cells. It is noteworthy that, despite the difference in reactivity between chloride and acetate complexes, both Pam 212 and Pam 212-*ras* appear to be significantly more sensitive to **9** and **10**. The cytotoxicity in the "parent" Pam 212 is also greater than that found for the alicyclic amine series. Cisplatin generally shows cytotoxicity in the micromolar range; the high values of cytotoxicity in Pam 212 and Pam 212-*ras* indicate a lack of sensitivity of both cell lines to this drug, confirming previous reports.[8]

Some cytotoxicity data has been reported previously for **1**.[9,17] Using the most comparable data with a similar time point (24 h), the cytotoxicity of **1** in C-26 and OV-163 cells is 11.0 and 16.0 $\mu$M, respectively. Allowing for the inherent insensitivity of the Pam 212 pair of cell lines, the results again indicate that there is little difference in cytotoxic efficacy between related acetate and chloride compounds.

**Induction of Apoptosis by the Acetate *trans*-Pyridine Derivatives in Pam 212-*ras* Cells.** Certain antitumor drugs achieve a significant therapeutic index, differentiating transformed cells from wild-type cells,[18] and this ability may be related to their capacity to induce tumor cell death through apoptosis at drug concentrations lower than those needed to kill wild-type cells.[19] In fact, using Pam 212-*ras* murine keratinocytes, it has been shown that complexes such as *trans*-[$PtCl_2$(ipa)(dma)] produce cell death through apoptosis induction.[8] On the basis of this, we have analyzed whether the cytotoxic effect against Pam 212-*ras* of the *trans*-pyridine platinum complexes **1**−**4** and **9**−**12** is related to the capacity of these complexes to induce apoptosis. Thus, genomic DNA from Pam 212-*ras* cells exposed during 24 h at the $IC_{50}$ of the *trans*-pyridine complexes was extracted and analyzed by agarose gel electrophoresis. Figure 1 shows that treatment of Pam 212-*ras* cells at the $IC_{50}$ value of complexes **9**, **11**, and **12** produced a ladder of genomic DNA indicative of apoptosis (Figure 1, lanes 3, 6, and 5, respectively). Treatment of Pam 212-*ras* with 2 times the $IC_{50}$ value of complex **9** or **10** also produced a DNA ladder (Figure 1, lane 4). In contrast, treatment of Pam 212-*ras* cells with the $IC_{50}$ of *cis*-DDP did not produce a DNA ladder (Figure 1, lane 7).

Apoptosis induction was confirmed by fluorescence microscopy of cells stained with propidium iodide after drug treatment. Figure 2C shows the formation of apoptotic bodies in cells treated with the $IC_{50}$ of compound **9**, while in cells treated with the $IC_{50}$ of *cis*-DDP (Figure 2B) the PI staining is diffusely

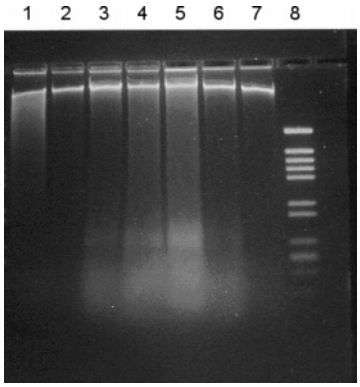


**Figure 1.** Agarose gel electrophoresis of genomic DNA extracted from Pam 212-*ras* cells treated with the $IC_{50}$ of **10** (lane 1), **9** (lane 3), **12** (lane 5), **11** (lane 6), *cis*-DDP (lane 7) and with twice the $IC_{50}$ of **10** (lane 2), **9** (lane 4). Lane 8: Φ29-*Hin*d III digested DNA.

distributed within the cell and some of the cells show an irregular shape as an indication of cell death by necrosis. In contrast, Figure 2A shows that control Pam 212-*ras* cells have a normal rounded shape with PI homogeneously distributed within them.

**Platinum Uptake in Pam 212-*ras* Cells.** The original reports on the novel cytotoxicity of *trans*-[$PtCl_2$(pyridine)$_2$] noted an enhanced cellular uptake in comparison to *cis*- or *trans*-DDP.[5] The cellular pharmacology of the new acetate compounds is also notable for enhanced cellular uptake.[14] We therefore determined platinum accumulation for the acetate complexes of this report in Pam 212-*ras* cells. For comparative purposes, we used a concentration of the *trans*-platinum drugs (100 $\mu$M) that is close to the $IC_{50}$ of *cis*-DDP in this cell line (106 $\mu$M). Figure 3 shows that the uptake of compounds **9**, **10**, **11**, **12**, and *cis*-DDP was, respectively, 0.80, 0.80, 0.50, 0.43, and 0.40 $\mu$mol/2 × $10^6$ cells after 24 h of drug treatment. These results indicate that under our experimental conditions 80% of the input molecules of compounds **9** and **10** were accumulated within Pam 212-*ras* cells. This accumulation is significantly higher in contrast to that of 45%, 43%, and 40% of the input molecules of compounds **11**, **12**, and *cis*-DDP, respectively.

**Platinum Binding to Genomic DNA in Pam 212-*ras* Cells.** Studies of DNA platination of the *cis*- and *trans*-[$PtCl_2$(pyridine)$_2$] pair in comparison to *cis*- and *trans*-[$PtCl_2(NH_3)_2$] analogues indicated reduced DNA affinity due to the steric effects of the pyridine groups.[20,21] To further correlate the differences in cytotoxic activity observed for the acetate derivatives, we incubated the Pam 212-*ras* cells for 24 h with equitoxic concentrations ($IC_{50}$ value) of compounds **9**−**12** and *cis*-DDP. The results of Figure 4 show that the level of DNA platination that induces an equitoxic effect ($IC_{50}$) is in the range between 800 and 1477 nmol/g DNA. The highest levels of DNA platination were observed for compounds **9** (1280 nmol/g DNA, which represents 1 bound Pt every 2307 nucleotides) and **12** (1477 nmol/g DNA, which represents 1 bound Pt every 2000 nucleotides). In addition, at the $IC_{50}$ of *cis*-DDP, the level of DNA platination was 923 nmol/g DNA (1 bound Pt every 3200 nucleotides); that is a value which is within the range found for the acetate derivatives. Moreover, it is interesting to note that at the $IC_{50}$ value of compounds **9**, **10**, **11**, **12**, and *cis*-DDP, the amount of platinum bound to DNA was 1.50%, 0.95%, 0.21%, 0.22%, and 0.22%, respectively, of the platinum input. These results also match those previously found with *trans*-[$PtCl_2$(pyridine)$_2$].

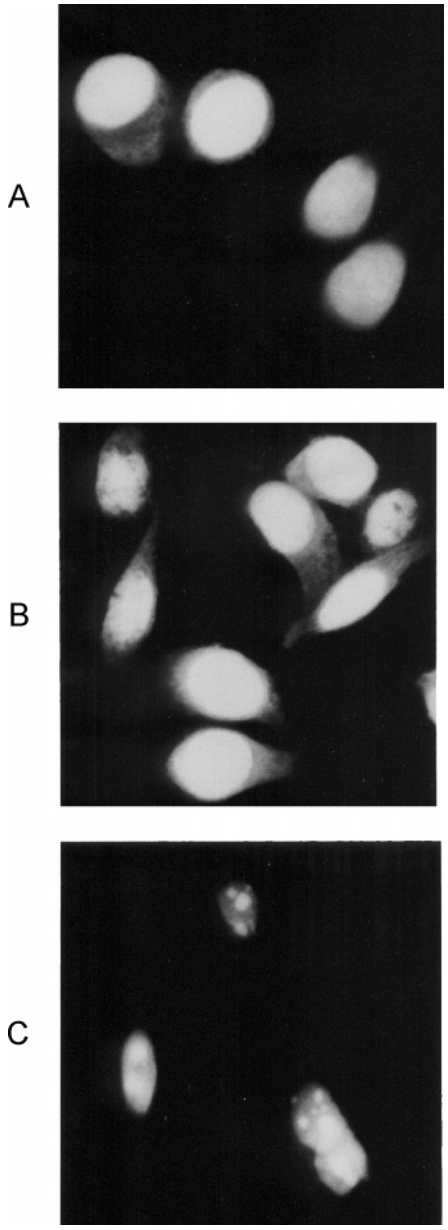


**Figure 2.** (A) Fluorescence microscopy of control untreated Pam 212-*ras* cells. (B) Fluorescence microscopy of Pam 212-*ras* cells treated with the $IC_{50}$ of *cis*-DDP for 24 h. At higher concentrations of *cis*-DDP, the number of necrotic cells increases. (C) Fluorescence microscopy of Pam 212-*ras* cells treated for 24 h with the $IC_{50}$ of the *trans*-platinum acetate complex **9**.

## Discussion

We report here the synthesis, characterization, and cytotoxic properties of novel *trans*-picoline platinum(II) complexes containing an $N_2O_2$ donor set. The results confirm and expand the previous report on this new series of cytotoxic transplatinum agents.[14] The acetate derivatives (compounds **9**−**12**) are water-soluble complexes that show remarkable cytotoxic properties against Pam 212 and Pam 212-*ras* cells. In contrast to *cis*-DDP, complexes **9**−**12** produce cell death in Pam 212-*ras* cells through apoptosis induction. It should be pointed out that *trans*-platinum complexes with mixed aliphatic amines also induce apoptosis in Pam 212-*ras* but in one case at lower drug concentrations than the *trans*-picoline platinum(II) acetate derivatives.[8] In fact, *trans*-[Pt(ipa)(dma)$Cl_2$] produces cell death through apoptosis in Pam 212-*ras* cells at an $IC_{50}$ of 6 $\mu$M.[8]

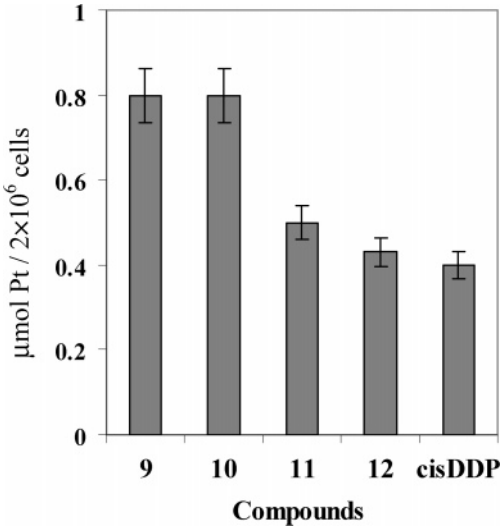


**Figure 3.** Platinum accumulation in Pam 212-*ras* cells after exposure to 100 $\mu$M of compounds **9**−**12** and *cis*-DDP for 24 h at 37 °C. At this concentration, cells usually survive for 36 h.

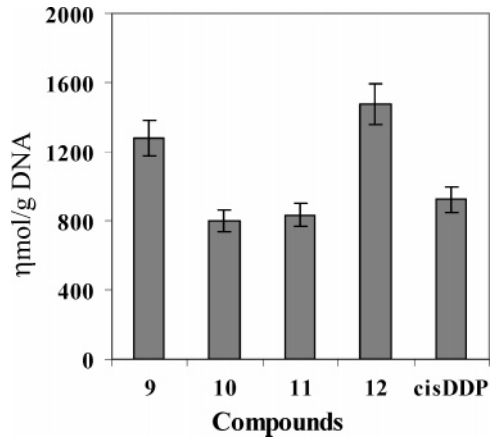


**Figure 4.** Platinum binding to genomic DNA of Pam 212-*ras* cells after exposure to equitoxic doses ($IC_{50}$) of compounds **9**−**12** and *cis*-DDP for 24 h at 37 °C.

The differences in cytotoxicity as well as in apoptosis induction between platinum drugs may be related to their ability to bind to genomic DNA.[22] Surprisingly, the planar picoline ligands do not seem to decrease the binding of the *trans*-Pt(II) center to DNA, although the methyl group on the pyridine ring is known to exert steric hindrance on the platinum center.[23] From the results presented in Figure 4, it is clear that compounds **9** and **10** achieve levels of DNA platination in Pam 212-*ras* cells similar to that of *cis*-DDP but at concentrations 5 times lower than this drug. On the other hand, it is interesting to note that although other types of *trans*-platinum complexes such as *trans*-[$PtCl_2$(ipa)(dma)] show steric hindrance and a level of binding to DNA lower than those of *cis*-DDP, they are also able to induce apoptosis at drug concentrations lower than *cis*-DDP.[8,22] In this latter case, the lack of correlation between the amount of platinum bound to DNA and apoptosis induction may be explained by the types of DNA adducts formed by the *trans*-platinum complex. Notably the *trans*-planar amine compounds produce interstrand cross-links between adjacent G of $(GC)_n$ base pairs[23] whereas *trans*-[$PtCl_2$(ipa)(dma)] produces a interstrand cross-links between the G and C bases of the same (GC) base pair.[24]

Our results also show that in Pam 212-*ras* cells the amount of platinum bound to DNA relative to the platinum input is

significantly higher (≥5 times higher) for compounds **9** and **10** than for compounds **11**, **12**, and cis-DDP. These data suggest that there may exist differences in cellular pharmacokinetics between the acetate derivatives having two picoline ligands (**9** and **10**) and those having mixed picoline and ammine ligands (**11** and **12**). In fact, at a dose of 100 $\mu$M, platinum accumulation for compounds **9** and **10** is about 2 times higher than that for compounds **11** and **12**. In compounds **9** and **10**, the presence of two lipophilic picoline ligands may favor the transport through the cell membrane and even the nuclear membrane. A major pharmacological factor contributing to platinum complex cytotoxicity is cellular accumulation, which is also of significant clinical relevance in cisplatin resistance.[26] An unusual feature of the carboxylate compounds in general is enhanced cellular accumulation.[14,27] The results presented here confirm the generality of this phenomenon for this series of compounds. In this respect, it is of interest to note that $IC_{50}$ values of *trans*-$[Pt(OAc)_2(py)_2]$ in human ovarian cell lines sensitive and resistant to *cis*-DDP generally show low resistance factors; for example, in A2780 the $IC_{50}$ is 12.8 $\mu$M with a resistance factor of 0.90. Corresponding values for CH1 and 41M are 19.0 $\mu$M (RF 0.22) and 14.0 $\mu$M (RF 0.32), respectively.[14] Corresponding values for *trans*-$[Pt(OAc)_2(NH_3)(py)]$ are 15.5 (1.45), 24.0 (0.34), and 21.5 (0.33) in A2780, CH1, and 41M, respectively.[28] To strictly compare with the time points of Table 2, the cytotoxicity of compounds **9** and **10** in 41M were measured after 24 h. Resistance factors were lower than that of *cis*-DDP. The low resistance factors are provocative, especially in the 41M cell pair, where accumulation/efflux defects contribute to resistance.[29] It is tempting therefore to suggest that the effects of cellular uptake in sensitive and resistant cells should be considered an important structural feature for systematic exploitation to overcome cisplatin resistance. In this respect, the biochemical mechanisms whereby *ras* oncogenes impart *cis*-DDP resistance are not well understood. Mutated *ras* proteins may modulate membrane association of cellular signaling proteins.[30] Whether cellular transformation by H-*ras* oncogene affects platinum complex uptake through membrane perturbation is an area worthy of exploration.

In summary, this contribution expands on the cellular pharmacology of novel transplatinum complexes containing $N_2O_2$ donor sets. The modifications with two acetate leaving groups appear to give cytotoxicity insensitive to cellular transformation by the H-*ras* oncogene. The enhanced intracellular accumulation of the most active complexes discussed here suggest that this feature is an important one dictating the overall pharmacological profile of this novel series.

## Experimental Section

**Abbreviations.** *cis*-DDP, *cis*-diamminedichloroplatinum(II); DMEM, Dulbecco modified Eagle's medium; DMS, dimethyl sulfate; pic, picoline; EDTA, ethylenediaminetetraacetic acid; FCS, fetal calf serum; $IC_{50}$, drug concentration that induces 50% of cell death; ICL, interstrand cross-link; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PAGE, polyacrilamide gel electrophoresis; PBS, phosphate-buffered saline; PI, propidium iodide; *trans*-DDP, *trans*-diamminedichloroplatinum(II); TXRF, total X-ray fluorescence.

**Starting Materials.** Cisplatin was synthesized by published procedures. $K_2PtCl_4$ was from Engelhard Corp. All the chemicals and solvents were purchased from common vendors and used as supplied. All syntheses used distilled water as solvent. IR spectra were recorded of Nujol mulls on CsI windows and KBr pellets in the 4000−200 $cm^{-1}$ range with a NEXUS 670 FTIR spectrophotometer. $^1H$ NMR spectra were recorded at 300 MHz on a Varian instrument at 294 K. Chemical shifts ($\delta$, ppm) are referenced to TMS. Quantitative Technologies, Inc. performed elemental analyses.

**p$K_a$ Measurements.** pH titration curves were obtained from the potentiometric titrations of $10^{-3}$ M solutions of *trans*-$[Pt(OH_2)_2(L)(L')]$ by a standardized NaOH solution ($9.5 \times 10^{-4}$ M). Solutions of *trans*-$[Pt(OH_2)_2(L)(L')]$ were obtained by stirring suspensions of *trans*-$[Pt(NO_3)_2(L)(L')]$ in $H_2O$ for 24−48 h at 60−70 °C; any insoluble species were filtered off through a pad of Celite. The purities of *trans*-$[Pt(OH_2)_2(L)(L')]$ solutions were verified prior to titration by HPLC analysis.[14,15]

**Synthesis. *trans*-$[PtCl_2L_2]$.** $K_2PtCl_4$ (4.8 mol) was dissolved in 500 mL of water, the base (2.4 mmol) was added to the solution, and the mixture was heated at reflux temperature until the yellow precipitate that formed turned to a clear solution. HCl in excess amount was added and the mixture heated at reflux temperature until the solution turned to yellow. Then the solution was concentrated to a small volume and the bright yellow solid that precipitated was filtered off and washed with water and acetone. The obtained crude products were recrystallized in chloroform/ether.

*trans*-$[PtCl_2(3\text{-pic})_2]$ (**1**). Yield: 0.9 g, 63%. $^1H$ NMR ($CDCl_3$): $\delta$ 8.69 (s, 1H), 8.67 (d, 2H), 7.53 (d, 1H), 7.17 (t, 1H), 2.33 (s, 3H). $\nu$(Pt−Cl) = 339.5 $cm^{-1}$. Anal. Calcd for $C_{12}H_{14}N_2Cl_2Pt$: C, 31.87; H, 3.12; N, 6.19, Cl, 15.68. Found: C, 31.92; H,3.10; N, 6.19; Cl, 15.44.

*trans*-$[PtCl_2(4\text{-pic})_2]$ (**2**). Yield: (0.8 g.) 53%. $^1H$ NMR ($CDCl_3$): $\delta$ 8.70 (d, 2H), 7.10 (d, 2H), 2.41 (s, 3H). $\nu$(Pt−Cl) = 348.5 $cm^{-1}$. Anal. Calcd for $C_{12}H_{14}N_2Cl_2Pt$: C, 31.87; H, 3.12; N, 6.19; Cl, 15.68. Found: C, 32.07; H,3.06; N, 6.07; Cl, 16.25.

***trans*-$[PtI_2L_2]$.** $K_2PtCl_4$ (2.4 mmol) was dissolved in water, the base (12 mmol) was added to the solution, and the mixture was heated at reflux temperature until the yellow precipitate that had formed turned to a clear solution. A stoichiometric amount of KI (2 mmol) was added and the mixture allowed to stand overnight until precipitation was complete. Then a white solid, $[PtL_4]I_2$, was isolated, washed with ethanol and diethyl ether, and dried under vacuum. This salt, suspended in ethanol, was heated at reflux temperature until it turned to an orange color. Then the orange solid was filtered off, washed with ethanol, and recrystallized in chloroform/ether.

*trans*-$[PtI_2(3\text{-pic})_2]$ (**5**). Yield: 1.31 g, 94%. $^1H$ NMR ($CDCl_3$): $\delta$ 8.79 (s, 1H), 8.77 (d, 2H), 7.47 (d, 1H), 7.13 (t, 1H), 2.34 (s, 3H). Anal. Calcd for $C_{12}H_{14}N_2I_2Pt$: C, 22.69; H, 2.22; N, 4.41. Found: C, 22.79; H, 1.98; N, 4.42.

*trans*-$[PtI_2(4\text{-pic})2]$ (**6**). Yield: 1.31 g, 94%. $^1H$ NMR ($CDCl_3$): $\delta$ 8.76 (d, 2H), 7.04 (d, 2H), 2.40 (s, 3H). Anal. Calcd for $C_{12}H_{14}N_2I_2Pt$: C, 22.69; H, 2.22; N, 4.41. Found: C, 23.09; H, 2.03; N, 4.46.

***trans*-$[Pt(OAc)_2L_2]$.** To a *trans*-$[PtX_2L_2]$ solution in DMF was added a suspension of AgOAc in acetone and water. Then the mixture was stirred overnight, turning to a dusky white color. The silver iodide or chloride was filtered through Celite and the filtrate was evaporated to dryness (vacuum pump). The crude product was dissolved in methanol, stirred with charcoal during 5−10 min, and filtered. The resultant solution was concentrated and diethyl ether was added to induce precipitation. After cooling to 0 °C overnight, a white solid was isolated, filtered, and dried under vacuum. The yields varied between 60% using the chloride complexes as precursor material and 77% using the iodide complexes.

*trans*-$[Pt(OAc)_2(3\text{-pic})_2]$ (**9**). Yield: 84% from the iodide, 77% from the chloride. $^1H$ NMR ($D_2O$): $\delta$ 8.53 (s, 1H), 8.48 (d, 2H), 7.80 (d, 1H), 7.37 (t, 1H), 2.34 (s, 3H), 1.91 (s, 3H). $\nu$(C=O): 1638 $cm^{-1}$. Anal. Calcd for $C_{16}H_{20}N_2O_4Pt$: C, 38.48; H, 4.04; N, 5.61. Found: C, 38.35; H, 3.99; N, 5.63.

*trans*-$[Pt(OAc)_2(4\text{-pic})_2]$ (**10**). Yield: 80%. $^1H$ NMR ($D_2O$): $\delta$ 8.47 (d, 2H), 8.33 (d, 2H), 2.42 (s, 3H), 1.87 (s, 3H). $\nu$(C=O): 1650 $cm^{-1}$. Anal. Calcd for $C_{16}H_{20}N_2O_4Pt$: C, 38.48; H, 4.04; N, 5.61. Found:C, 38.46, H, 4.02, N, 5.53.

***trans*-$[PtCl_2(NH_3)L]$.** *cis*-$PtCl_2(NH_3)_2$ (3.3 mmol) was dissolved in water at 55 °C. Silver nitrate (6.6 mmol) was dissolved in the minimum amount of water and added dropwise to the platinum solution. The mixture was covered, diluted with a solution of the

base (6.6 mmol) in ethanol, and stirred overnight. To dissolve the *cis*-[$Pt(NH_3)_2(L)_2$]($NO_3$)$_2$ that formed, the mixture was heated to reflux temperature during 4 h. The hot solution was filtered through Celite to remove the silver chloride. HCl (8 mL) was added to the filtrate and the solution was heated until it turned yellow. Then the solution was concentrated and a yellow precipitate was isolated and recrystallized in chloroform/diethyl ether.

*trans*-[$PtCl_2(NH_3)$(3-pic)] (**3**). Yield: 1.0 g, 83%. $^1$H NMR ($CDCl_3$): $\delta$ 8.76 (s, 1H), 8.71 (d, 2H), 7.55 (d, 1H), 7.17 (t, 1H), 3.22 (br s, 3H), 2.35 (s, 3H). $\nu$(NH): 3292, 3310 cm$^{-1}$. $\nu$(Pt−Cl): 337 cm$^{-1}$. Anal. Calcd for $C_6H_{10}N_2Cl_2Pt$: C, 19.16; H, 2.68; N, 7.45; Cl, 18.85. Found: C, 19.36; H, 2.34; N, 7.33; Cl, 18.63.

*trans*-[$PtCl_2(NH_3)$(4-pic)] (**4**). Yield: 1.1 g, 83%. $^1$H NMR ($CDCl_3$): $\delta$ 8.70 (d, 2H), 7.10 (d, 2H), 3.14 (br s, 3H), 2.40 (s, 3H). $\nu$(NH): 3185, 3222 cm$^{-1}$. $\nu$(Pt−Cl): 345 cm$^{-1}$. Anal. Calcd for $C_6H_{10}N_2Cl_2Pt$: C, 19.16; H, 2.68; N, 7.45. Found: $C_6H_{10}N_2Cl_2Pt$: C, 19.26; H, 2.30; N, 7.40.

***trans*-[$PtI_2(NH_3)$L].** *cis*-$PtCl_2(NH_3)_2$ (3.3 mmol) was dissolved in water at 55 °C. Silver nitrate (6.6 mmol) was dissolved in the minimum amount of water and added dropwise to the platinum solution. The mixture was covered, diluted with a solution of the base (6.6 mmol) in ethanol, and stirred overnight. To dissolve the *cis*-[$Pt(NH_3)_2(L)_2$]($NO_3$)$_2$ that formed, the mixture was heated to reflux temperature during 4 h. The hot solution was filtered through Celite to remove the silver chloride. KI (10 mmol) dissolved in the minimum amount of water was added and the mixture was evaporated to dryness. The white crude was suspended in ethanol and heated at reflux temperature during 4 h. An orange solid was isolated and recrystallized in dry acetone/diethyl ether to remove any excess KI. The characterization of these intermediates was made only by NMR:

*trans*-[$PtI_2(NH_3)$(3-pic)] (**7**). $^1$H NMR ($CDCl_3$): $\delta$ 8.74 (s, 1H), 8.70 (d, 2H), 7.56 (d, 1H), 7.18 (t, 1H), 3.18 (br s, 3H), 2.36 (s, 3H). $\nu$(NH): 3226, 3283 cm$^{-1}$.

*trans*-[$PtI_2(NH_3)$(4-pic)] (**8**). Yield: 1.08 g, 56%. $^1$H NMR ($CDCl_3$): $\delta$ 8.70 (d, 2H), 7.10 (d, 2H), 3.14 (br s, 3H), 2.40 (s, 3H). $\nu$(NH): 3230, 3287 cm$^{-1}$. Calcd for $C_6H_{10}N_2l_2Pt$: C, 12.89; H, 1.80; N, 5.01. Found: 13.73; H, 1.69; N, 5.92.

***trans*-[$Pt(OAc)_2(NH_3)$L].** A suspension of AgOAc (2 mmol) in acetone and water was added to a solution of *trans*-[$PtI_2(NH_3)$L] (1 mmol) in acetone. The mixture was stirred for 24 h, turning gray. Silver iodide or chloride was filtered off with Celite and the filtrate was evaporated to dryness. The crude product was dissolved in methanol, treated with charcoal during 5−10 min, and filtered. The resultant solution was concentrated and diethyl ether was added to induce precipitation. After cooling to 0 °C overnight, a white solid was isolated, filtered, and dried under vacuum. The yields varied between 40% using the chloride complexes as precursor material and 75% using the iodide complexes.

*trans*-[$Pt(OAc)_2(NH_3)$(3-pic)] (**11**). Yield: 64%. $^1$H NMR (MeOD): $\delta$ 8.50 (s, 1H), 8.46 (d, 2H), 7.88 (d, 1H), 7.44 (t, 1H), 4.45 (b.s, 2.7 H), 2.46 (s, 3H) 2.02 (s, 6H). $\nu$(NH): 3118, 3246 cm$^{-1}$. $\nu$(C═O): 1644 cm$^{-1}$. Anal. Calcd for $C_{10}H_{16}N_2O_4Pt$: C, 28.37; H, 3.81; N, 6.62. Found: C, 27.90; H, 3.78; N, 6.36.

*trans*-[$Pt(OAc)_2(NH_3)$(4-pic)] (**12**). Yield: 68%. $^1$H NMR ($D_2O$): $\delta$ 8.43 (d, 2H), 7.39 (d, 2H), 4.45 (b.s, 3H), 2.50 (s, 3H), 1.87 (s, 6H). $\nu$(NH): 3134, 3263 cm$^{-1}$. $\nu$(C═O): 1648 cm$^{-1}$. Anal. Calcd for $C_{10}H_{16}N_2O_4Pt$: C, 28.37; H, 3.81; N, 6.62. Found: C, 28.10; H, 3.25; N, 6.38.

**Biologicals and Drugs.** The 100-mm culture and microwell plates were obtained from NUNCLON (Roskilde, Denmark). MTT was purchased from Sigma Co., FCS was supplied by GIBCO−BRL, and ethanol was obtained from Merck. *cis*-DDP was synthesized from $K_2PtCl_4$ supplied by Johnson Mathey Co. *cis*-DDP was dissolved in 10 mM $NaClO_4$. The *trans*-platinum acetate complexes were dissolved in water as 1 mg/mL solutions. The *trans*-platinum chloride complexes were dissolved in DMSO (50% v/v). These solutions were freshly prepared before use.

**Cell Lines and Culture Conditions.** Pam 212, a normal murine keratinocytes cell line, and Pam 212-*ras*, a transformed murine keratinocytes cell line overexpressing the H-*ras* oncogene,[31] were cultured in DMEM supplemented with 10% FCS (fetal calf serum), 2 mM glutamine, 100 units/mL penicillin, and 100 $\mu$g/mL streptomycin at 37 °C in an atmosphere of 95% of air and 5% $CO_2$. All cultures were passaged twice weekly, showing a doubling time between 16 and 24 h.

**Cytotoxicity Assays.** Cell survival was evaluated by using a system based on the tetrazolium compound MTT, which is reduced by living cells to yield a soluble formazan product that can be detected colorimetrically.[31] Cells were plated in 96-well sterile plates at a density of $10^4$ cells/well in 100 $\mu$L of medium and were incubated for 3−4 h. The compounds were added to final concentrations from 0 to 200 $\mu$M in a volume of 100 $\mu$L/well. Twenty-four hours later, 50 $\mu$L of a MTT solution freshly diluted (1/5 in culture medium) to a concentration of 1 mg/mL was pipetted into each well and the plate was incubated for 5 h at 37 °C in a humidified 5% $CO_2$ atmosphere. Cell viability was measured by standard methods.[8] In control experiments, it was observed that 10% of DMSO did not have any effect on cell growth. This was the percentage of DMSO present in the cell cultures after adding 200 $\mu$M of the compounds. All data were obtained from four replicate wells on the same plate in a single experiment.

**DNA Fragmentation Assay.** Pam 212-*ras* cells ($5 \times 10^5$ cells/mL) were plated in 100 mm sterile dishes. The cells were treated with the compounds for 24 h to a final concentration of the $IC_{50}$ in the above-mentioned conditions. The fraction of detached cells was collected by centrifugation of the culture media and washed twice with PBS. The cell pellet was disrupted with 700 $\mu$L of lysis buffer (150 mM Tris, tris(hydroxymethyl)aminomethane, pH 8.0; 100 mM NaCl; 100 mM EDTA, ethylenediaminetetracetate). The fraction of nondetached cells was also washed twice with PBS and lysed by addition to the plate of 700 $\mu$L of lysis buffer. Both cell fractions were joined, and the whole cell lysate was treated with proteinase K (500 $\mu$g/mL) for 2 h at 55 °C. Afterward, samples were exposed to RNase A (50 $\mu$g/mL) for 16 h at 37 °C. The DNA was first extracted with phenol, followed by phenol/chloroform/isoamyl alcohol (25:24:1) and a chloroform/isoamyl alcohol (24:1) phase. Subsequently, the DNA was precipitated overnight at −20 °C in 2.5 volumes of cold 100% ethanol/150 mM potassium acetate. After centrifugation at 12 000 rpm for 15 min to recover the precipitated DNA, the supernatant was discarded and the pellet was washed with 70% ethanol. Samples were dried using a SAVANT Speed Vac concentrator and resuspended in distilled water. The DNA concentration was calculated by determining the $OD_{260}$. Electrophoresis of DNA (10 $\mu$g/well) was performed for 16 h at 75 V in 1.8% agarose gel with TAE (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) as running buffer. The bands were visualized by ethidium bromide staining for 16 h and UV transillumination. DNA bands were analyzed by laser densitometry using a Molecular Dynamics densitometer.

**Propidium Iodide (PI) Staining and Fluorecence Microscopy.** Pam 212-*ras* cells ($10^5$ cells/plate) were plated at 37 °C in P60 sterile plates that contained cover slips attached to the plate surface by incubation with polylysine. After cell attachment and removal of medium, the cells were rinsed three times with PBS and then treated with equitoxic concentrations of the compounds ($IC_{50}$) dissolved in DMEM medium (treatment period of 24 h). Cells were fixed for staining by published methods.[8] Apoptotic nuclei were easily distinguished from normal nuclei; they were condensed, brightly fluorescent, and often fragmented.

**Measurements of Platinum Accumulation in Culture Cells.** Cultures plates containing exponentially growing Pam 212-*ras* cells in 10 mL of DMEM medium (cell density = $2 \times 10^6$ cells/mL) were exposed to 10 $\mu$M of the platinum drugs dissolved in DMEM medium for 1, 3, or 24 h. Cells were washed with ice-cold PBS; scraped; resuspended in 700 $\mu$L of lysis buffer containing 20 mM Tris, HCl, pH 7.5, 2 mM EDTA, and 0.4% Triton X-100; incubated at 4 °C for 15 min; and centrifuged at 12 000 rpm for 15 min. Afterward, supernatants were treated for 3 h at 37 °C with 20 $\mu$g/mL of proteinase K (Boehringer). The platinum content in the samples was determined by TXRF. Experiments were carried out in triplicate.

**Determination of Platinum Binding to DNA in Culture Cells.** Culture plates containing exponentially growing Pam 212-*ras* cells in 10 mL of DMEM medium (cell density = $2 \times 10^6$ cells/mL) were exposed to 10 $\mu$M of the platinum drugs dissolved in DMEM. The plates were incubated for 24 h under the conditions described above. Following drug incubation, culture medium was removed from the plates, and the cell plates were washed with PBS. Subsequently, the cells were lysed with 700 $\mu$L of a buffer solution containing 150 mM Tris, HCl pH 8.0, 100 mM EDTA, and 100 mM NaCl, incubated for 15 min at 4 °C, and centrifuged at 12 000 rpm for 15 min. Supernatants were treated with 20 $\mu$g/mL of proteinase K (Boehringer) at 37 °C for 3 h. Afterward, supernatants were incubated for 16 h at 37 °C with 100 $\mu$g/mL of RNase A (Boehringer). Finally, DNA was extracted with a volume of phenol−chloroform−isoamyl alcohol (50:49:1), precipitated with 2.5 volumes of cold ethanol and 0.1 volume of 3 M sodium acetate, washed with 75% of ethanol, dried, and resuspended in 1 mL of water. The DNA content in each sample was measured by UV spectrophotometry at 260 nm in a Shimadzu UV-240 spectrophotometer, and platinum bound to DNA was determined by TXRF. Experiments were carried out in triplicate.

**Total Reflection X-ray Fluorescence Measurements.** The analysis by TXRF was performed using a Seifert Extra-II spectrometer (Seifert, Ahrensburg, Germany). TXRF determinations were carried out according to a procedure previously reported.[32] Briefly, a 100 $\mu$L sample of either cell supernatants or cellular DNAs from the CH1cisR cell cultures was introduced in a 2-mL test tube. This solution was standardized with 100 ng/mL of vanadium [Merck (Darmstadt, Germany) ICP vanadium standard solution]. Afterward, the sample was introduced into a high-purity nitrogen flow concentrator at a temperature of 70 °C until the volume was reduced five times. An aliquot of 5 $\mu$L was then taken, deposited on a previously clean quartz reflector, and dried on a ceramic plate at a temperature of 50 °C. The entire process was done in a laminate flow chamber (Model A-100). The samples were analyzed following the X-ray molybdenum line under working conditions of 50 kV and 20 mA with a live-time of 1000 s and a dead time percentage of 35%. Spectra were recorded between 0 and 20 keV. The following 15 elements were simultaneously analyzed: P, S, K, Ca, V, Fe, Cu, Zn, As, Br, Rb, Sr, Ni, Mn, and Pt, to obtain a correct deconvolution of profiles associated with the general spectrum. The Pt line was used for Pt quantification. The analytical sensitivity of the TXRF measurements was 0.3−22.4 ng Pt in a solution volume of 100 $\mu$L, with repeatability between 2 and 8% ($n = 3$).

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**Supporting Information Available:** Results from elemental analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

## References

(1) *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*; Lippert, B., Ed.; John Wiley & Son Ltd: Weinheim, 1999.
(2) Farrell, N. Polynuclear platinum drugs. *Met. Ions Biol. Syst.* **2004**, *42*, 251−296.
(3) Farrell, N. Non-classical platinum antitumor complexes. Perspectives for design and development of new drugs complementary to cisplatin. *Cancer Invest.* **1993**, *11*, 578−589.
(4) Beusichem, M. V.; Farrell, N. Activation of the trans geometry in platinum antitumor complexes. Synthesis, characterization, and biological activity of complexes with planar ligands of pyridine, *N*-methylimidazole, thiazole, and quinoline. *Inorg. Chem.* **1992**, *31*, 934−939.
(5) Farrell, N.; Kelland, L. R.; Roberts, J. D.; van Beusichem, M. Activation of the trans geometry in platinum antitumor complexes: A survey of the cytotoxicity of trans complexes containing planar ligands in murine L1210 and human tumor panels and studies on their mechanism of action. *Cancer Res.* **1992**, *52*, 5056−5072.
(6) Farrell, N. Current status of structure−activity relationships of platinum anticancer drugs: Activation of the trans geometry. *Met. Ions Biol. Syst.* **1996**, *32*, 251−296.
(7) Mellish, K. J.; Barnard, C. F. J.; Murrer, B. A.; Kelland, L. R. DNA-binding properties of novel cis- and trans-platinum-based anticancer agents in 2 human ovarian carcinoma cell lines. *Int. J. Cancer* **1995**, *62*, 717−723.
(8) Montero, E. I.; Diaz, S.; Gonzalez-Vadillo, A. M.; Perez, J. M.; Alonso, C.; Navarro-Ranninger, C. Preparation and characterization of novel trans-[$PtCl_2$(amine)(isopropylamine)] compounds: Cytotoxic activity and apoptosis induction in *ras*-transformed cells. *J. Med. Chem.* **1999**, *42*, 4264−4268.
(9) Khazanov, E.; Barenholz, Y.; Gibson, D.; Najajreh, Y. Novel apoptosis-inducing *trans*-platinum piperidine derivatives: Synthesis and biological characterization. *J. Med. Chem.* **2002**, *45*, 5196−5204.
(10) Najajreh, Y.; Perez, J. M.; Navarro-Ranninger, C.; Gibson, D. Novel soluble cationic trans-diaminedichloroplatinum(II) complexes that are active against cisplatin resistant ovarian cancer cell lines. *J. Med. Chem.* **2002**, *45*, 5189−5195.
(11) Natile, G.; Coluccia, M. Current status of trans-platinum compounds in cancer therapy. *Coord. Chem. Rev.* **2001**, *216−217*, 383−410.
(12) Fojo, T.; Farrell, N.; Orthuzar, W.; Tanimura, H.; Stein, J.; Myers, T. G. Identification of non-cross-resistant platinum compounds with novel cytotoxicity profiles using the NCI anticancer drug screen and clustered image map visualizations. *Crit. Rev. Hematol. Oncol.* **2005**, *53*, 25−34.
(13) Farrell, N.; Povirk, L. F.; Dange, Y.; Gupta, M. S.; Kohlhagen, G.; Pommier, Y.; Gewirtz, D. Cytotoxicity, DNA strand breakage and DNA-protein crosslinking by a novel transplatinum compound in human A2780 ovarian and MCF-7 breast carcinoma cells. *Biochem. Pharmacol.* **2004**, *68*, 857−866.
(14) Ma, E. S. F.; Bates, W. D.; Edmunds, A.; Kelland, L. R.; Fojo, T.; Farrell, N. Enhancement of aqueous solubility and stability employing a *trans* Acetate axis in trans-planar amine platinum compounds while maintaining the biological profile. *J. Med. Chem.* **2005**, *48*, 5651−5654.
(15) Souchard, J. P.; Wimmer, F. L.; Ha, T. T. B.; Johnson, N. P. A rapid method for the synthesis of water-soluble platinum(II) amine and pyridine complexes. *J. Chem. Soc., Dalton Trans.* **1990**, 307−310.
(16) McGowan, G.; Parsons, S.; Sadler, P. J. The contrasting chemistry of *cis* and trans $Pt^{II}$ diamine anticancer compounds: Hydrolysis studies of picoline complexes. *Inorg. Chem.* **2005**, *44*, 7459−7467.
(17) Kasparkova, J.; Marini, V.; Najajreh, Y.; Gibson, D.; Brabec, V. DNA binding mode of the cis and trans geometries of new antitumor nonclassical platinum complexes containing piperidine, piperazine, or 4-picoline ligand in cell-free media. Relations to their activity in cancer cell lines. *Biochemistry* **2003**, *42*, 6321−6332.
(18) Quiroga, A. G.; Pérez, J. M.; Montero, E. I.; West, D. W.; Alonso, C.; Navarro-Ranninger, C. Synthesis and characterization of Pd(II) and Pt(II) complexes of *p*-isopropylbenzaldehyde N-protected thiosemicarbazones. Cytotoxic activity against *ras*-transformed cells. *J. Bioinorg. Chem.* **1999**, *75*, 293−301.
(19) Lowe, S. W.; Ruley, H. E.; Jacks, T.; Housman, D. E. p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. *Cell* **1993**, *74*, 957−967.
(20) Zou, Y.; Van Houten, B.; Farrell, N. Ligand effects in platinum binding to DNA. A comparison of DNA binding properties for cis and trans-[$PtCl_2$(amine)$_2$] (amine = $NH_3$, pyridine). *Biochemistry* **1993**, *32*, 9632−9638.
(21) Zakovska, A.; Novakova, O.; Balcarova, Z.; Bierbach, U.; Farrell, N.; Brabec, V. DNA interactions of antitumor trans-[$PtCl_2(NH_3)$-quinoline]. *Eur. J. Biochem.* **1998**, *254*, 547−557.
(22) Gonzalez, V. M.; Fuertes, M. A.; Alonso, C.; Perez, J. M. Is cisplatin-induced cell death always produced by apoptosis? *Mol. Pharmacol.* **2001**, *59*, 657−663.
(23) Chen, Y.; Guo, Z.; Parsons, S.; Sadler, P. J. Stereospecific and kinetic control over the hydrolysis of a sterically hindered platinum picoline anticancer complex. *Chem. Eur. J.* **1998**, *4*, 672−676.
(24) Kasparkova, J.; Novakova, O.; Vrana, O.; Farrell, N.; Brabec, V. Effect of geometric isomerism in dinuclear platinum antitumor complexes on DNA interstrand cross-linking. *Biochemistry* **1999**, *38*, 10997−11005.
(25) Prokop, R.; Kasparkova, J.; Novakova, O.; Marini, V.; Pizarro, A. M.; Navarro-Ranninger, C.; Brabec, V. DNA interactions of new antitumor platinum complexes with trans geometry activated by a 2-methylbutylamine or *sec*-butylamine ligand. *Biochem. Pharmacol.* **2004**, *67*, 1097−1109.

(26) Farrell, N. Pre-clinical perspectives on the use of platinum compounds in cancer chemotherapy. *Semin. Oncol.* **2004**, *31*, 1−9.
(27) Murphy, R. F.; Farrell, N.; Aguila, A.; Okada, M.; Balis, F. M.; Fojo, T. Accumulation of novel transplatinum complexes in cisplatin and oxaliplatin resistant cell lines overcomes resistance. *Proc. Am. Assoc. Cancer Res.* **2005**, *46*, 4109.
(28) Farrell, N.; unpublished results.
(29) Ills, C.; Kelland, L. R.; Abel, G.; Siracky, J.; Wilson, A. P.; Harrap, K. R. Biological properties of ten human ovarian carcinoma cell lines: Calibration in vitro against four platinum complexes. *Br. J. Cancer* **1989**, *59*, 527−534.
(30) Caron, R. W.; Yacoub, A.; Zhu, X.; Mitchell, C.; Han, S. I.; Sasazuki, T.; Shirasawa, S.; Hagan, M. P.; Grant, S.; Dent, P. H-*RAS* V12-induced radioresistance in HCT116 colon carcinoma cells is heregulin dependent. *Mol. Cancer Therapeut.* **2005**, *4*, 243−255.
(31) Jacobson, M. D.; Burne, J. F.; King, M. P.; Miyashita, T.; Reed, J. C.; Raff, M. C. Bcl-2 blocks apoptosis in cells lacking mitochondrial DNA. *Nature* **1993**, *361*, 365−369.
(32) Fernández-Ruíz, R.; Tornero, J. D.; González, V. M.; Alonso, C. Quantification of Pt bound to DNA using total-reflection X-ray fluorescence (TXRF). *Analyst* **1999**, *124*, 583−585.

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