Synthesis and Characterization of Platinum(II) Oxadiazoline Complexes and their In Vitro Antitumor Activity in Platinum-Sensitive and -Resistant Cancer Cell Lines

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Received October 29, 2006

A series of platinum(II) complexes bearing Δ^4-1,2,4-oxadiazoline ligands have been synthesized and characterized. Their in vitro antitumor activity has been assessed in platinum-sensitive and -resistant human ovarian cancer cell lines (PEO1, PEOCisR, PEOCarboR, and SK-OV3), as well as in colon cancer (SW948) and testicular cancer cell lines (N-TERA). All compounds tested showed potent cytotoxicity in the platinum-sensitive cell lines and retained activity in the cisplatin- and carboplatin-resistant lines, with IC_{50} values similar to the parental drug sensitive counterpart. We propose, therefore, that platinum(II) oxadiazoline complexes may possess a novel mechanism of action, which render them active in tumor cells, with resistance to currently used platinum anticancer agents.

Introduction

For a number of years, platinum-based chemotherapeutic regimens have been the mainstay for the management of epithelial ovarian cancer,[1] testicular cancers, head and neck tumors, and a number of other solid tumor types.[2] Cisplatin and carboplatin (Scheme 1) are frequently used in combination chemotherapy, and their antitumor activity contributed significantly to the improvement in survival rates for patients with ovarian and testicular cancers when they were first introduced into the clinic. More recently, the platinum analogue oxaliplatin has been developed and is now licensed for use in metastatic colon cancer in the U.S.[3]

However, in spite of their well-deserved place as main players in currently used chemotherapeutic regimens, resistance to platinum agents is a well-documented and unfortunate drawback in their use. In particular, the poor 5 year survival rates seen in epithelial ovarian cancer are at least partly attributed to the development of platinum resistance.[4] Moreover, a large body of laboratory- and clinically-based evidence has sought to clarify and understand the molecular basis for platinum resistance in tumors.[4–6] Undoubtedly, a major aim of current anticancer drug development programs is to isolate and identify new compounds with the ability to overcome drug resistance, while still maintaining the potent antitumor activities of agents like cisplatin.

In this context, *trans*-configured platinum compounds have found increasing interest, and a number of promising drug candidates could be identified[7] as, for example, Kelland's Pt(IV) complexes bearing NH_3 and aliphatic amines in the *trans*-position to each other.[8] Farrell introduced mixed ligand $PtCl_2(L^1)(L^2)$ complexes, where L^1 and L^2 can both be aromatic nitrogen heterocycles or one of the ligands can be an ammine or a sulfoxide.[9] Similarly, Navarro-Ranninger's group successfully developed compounds bearing branched aliphatic amines as ligands,[10] and Natile introduced imino-type ligands, which were synthesized in the coordination sphere of the ligand by addition of an alcohol to a coordinated nitrile[11a] and others by condensation of acetone with a platinum-ammine precursor.[11b]

In the framework of our research, we found a new class of *trans*-configured platinum(II) compounds whose in vitro properties suggest good potential for the development of new therapeutics that are able to overcome the resistance against conventionally used platinum-based antitumor agents: The modular synthetic strategy applied leads to structures with 4−7 sites of diversity and potentially lends itself for a combinatorial chemistry approach (Scheme 2). The coordination sphere around the Pt (and, thus, the principal DNA interaction capacity) can be kept constant, while pharmacologically relevant parameters such as solubility, polarity, charge distribution, p*K*, logP, or the transport properties can be addressed by the substitution pattern in the remote periphery of the complex. The heterocyclic ligands are designed to allow for additional interactions; for example, aromatic substituents attached to the five-membered ring could intercalate into the DNA and thus strengthen the interaction and circumvent repair mechanisms the Pt-resistant cells developed. Additionally, the ligand reactivity (e.g., N−O bond cleavage in a reducing environment) could cause secondary DNA damage. In oxygen-deficient cancer tissues this could offer an additional reaction pathway that takes place in cancer cells but less so in normal cells. It has also been shown that the synthetic strategy can be extended to analogous Pt(IV) compounds[12] and Pt(II) compounds bearing other ligands such as sulfoxides,[13] so there is future scope for further optimization of the new lead structure.

Results and Discussion

Synthesis and Characterization of the Platinum Compounds. The synthesis of the platinum(II) mono-oxadiazoline and platinum(II) bis-oxadiazoline complexes was achieved by cycloaddition of nitrones to $PtCl_2(nitrile)_2$ compounds, as shown in Scheme 2. The structures of the compounds prepared are shown in Scheme 3, together with the compound numbering.

The bis-nitrile complexes $[PtCl_2(PhCN)_2]$, **1**, and **2** were synthesized by ligand exchange starting from *cis*/*trans*-$[PtCl_2(MeCN)_2]$. At the elevated temperatures applied in the synthesis, the *trans*-isomers formed almost exclusively. In the case of $[PtCl_2(PhCN)_2]$ and **2**, the pure *trans* compounds were

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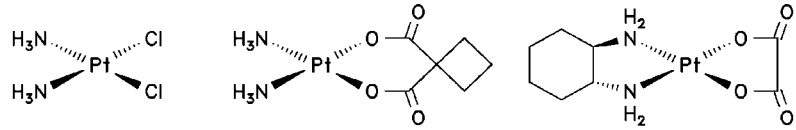
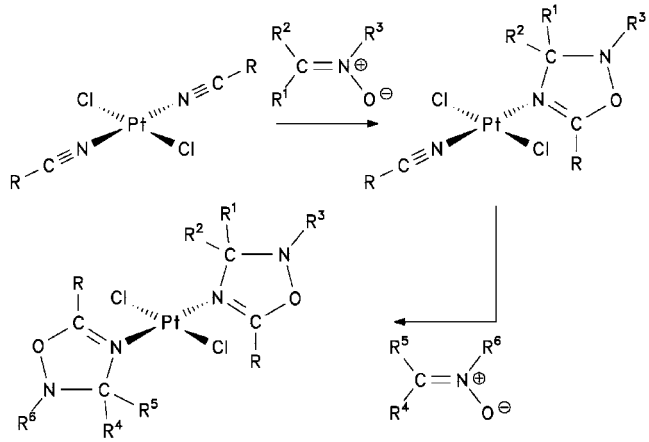
10.1021/jm061263s CCC: $40.75
Published on Web 12/12/2007

Scheme 1. Platinum Compounds Currently Used in Cancer Therapy

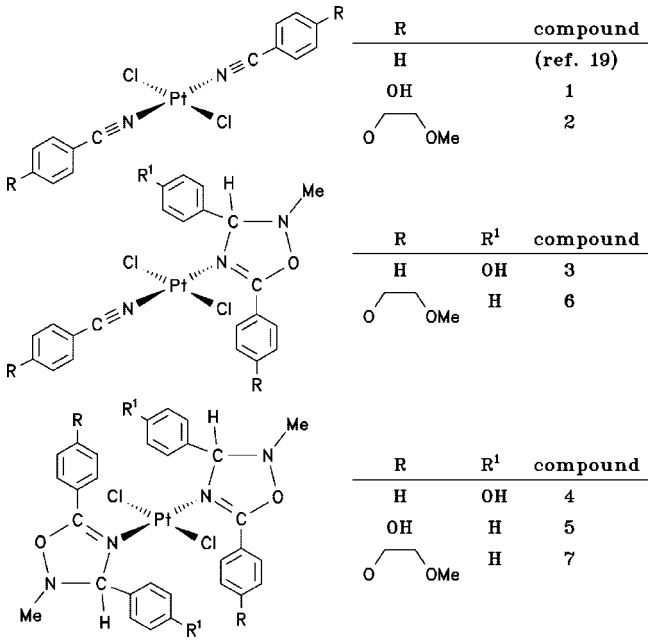


cisplatin carboplatin oxaliplatin

Scheme 2. General Strategy for the Synthesis of Platinum(II) Oxadiazoline Complexes



Scheme 3. Structures of the Compounds Prepared, Together with the Compound Numbering



R	compound
H	(ref. 19)
OH	1
O OMe	2

R	R^1	compound
H	OH	3
O OMe	H	6

R	R^1	compound
H	OH	4
OH	H	5
O OMe	H	7

obtained after chromatography. Compound **1** formed in a *trans*/*cis* ratio of 95:5 and was used for subsequent reactions without further purification. Evidence for the structure was obtained from the IR spectra that display a C≡N stretching vibration in the typical range of N-coordinated aromatic nitriles at 2287 to 2291 cm^{-1}. Both elemental analysis and the mass spectrum confirm the elemental composition, and the ^1H and ^{13}C NMR spectra show the expected signal pattern of the N-coordinated nitriles. The ^1H NMR of **1** displayed a second signal set of about 5% of the intensity of the major ones, revealing the presence of the *cis*-isomer. The ^{195}Pt NMR spectra consist of one signal at −2350 ([$PtCl_2(PhCN)_2$]), −2339 (**1**), or −2313 ppm (**2**). This range is consistent with literature values for the *trans*-isomers of this type of complexes.[14,15] The ^{195}Pt NMR spectrum of **1** shows an additional small peak at −2244 ppm for the *cis*-isomer. This compares well with the signal of the known *cis*-[$PtCl_2$(*p*-MeO-$C_6H_4CN)_2$] at −2239 ppm.[14]

[$PtCl_2(PhCN)_2$] and **2** reacted with 1 equiv of nitrone at room temperature to form the monocycloadducts **3** and **6**, selectively. Elemental analysis and mass spectra are consistent with the proposed structure. The IR spectra show signals for the stretching vibration of the nitrile C≡N bond (2281 to 2288 cm^{-1}) and the C═N bond of the oxadiazoline (1596 to 1603 cm^{-1}), indicating that both ligands are present in the complex. Also, the ^1H and ^{13}C NMR spectra consist of the signal patterns of both ligands, the nitrile and the oxadiazoline. The most characteristic ^1H NMR signals for the oxadiazoline are the NMe peak at 3.02 to 3.05 ppm, and the N−CH−N appears as a broad singlet at 5.89 to 5.94 ppm. The *ortho*-protons of the aromatic ring attached to the imino carbon of the oxadiazoline are strongly deshielded and shifted to high ppm values (8.98 to 9.04 ppm) due to their close proximity to the platinum center. In the ^{13}C NMR spectrum the oxadiazoline can be recognized by the signal of the NMe group at 46.0 to 46.8 ppm, the N−CH−N group at 94.7 to 94.8 ppm, and the C═N functionality at 163.1 to 163.2 ppm. The ^{195}Pt NMR signal is seen at −2216 ppm (**6**) and −2220 ppm (**3**). These values again compare well with those observed previously for similar complexes of this type.[15,16] However, the ^{195}Pt NMR data in this case are of little use for an assignment of the stereochemistry of the complex, because the signals of the *cis*- and *trans*-complexes are known to differ by 10 ppm only.[15,16] Our previous work has shown that *ortho*-protons of the coordinated nitrile report most sensitively about the coordination geometry. In the *cis*-complex, these protons are shielded from the magnetic field and the signals are shifted to low ppm values (7.17 for PhCN compared to 7.68 ppm in the *trans*-complex or 7.60 ppm in the uncoordinated nitrile).[15] In both **3** and **6**, no such shielding is observed and the respective protons appear at similar ppm values as the free nitriles, in agreement with a proposed *trans*-geometry of these complexes. It was also shown previously that *cis*-$PtCl_2$(nitrile)(oxadiazoline) complexes can be prepared, but this requires the use of *cis*-$PtCl_2(nitrile)_2$ as a precursor, and the resulting complexes are not stable and decompose readily in solution.[15] Finally, the synthesis of the mono-oxadiazoline complex derived from **1** was attempted but not successful. Due to the poor solubility of **1**, the cycloaddition is significantly less-selective, and only mixtures of mono- and bis-oxadiazoline complexes were obtained.

The bis-oxadiazoline complexes **4**, **5**, and **7** are formed upon reaction of the bis-nitrile complexes [$PtCl_2(PhCN)_2$], **1**, and **2** with 2 equiv of the corresponding nitrone at elevated temperature and prolonged reaction times. Again, elemental analyses and mass spectra confirm the molecular formula. In the IR spectra, the signal of the C≡N stretch has disappeared and a C═N stretching vibration is present at 1607 to 1604 cm^{-1}. The ^1H and ^{13}C NMR spectra show signals of the oxadiazoline ligands

Table 1. Antiproliferative Activity of Platinum Agents in Platinum-Sensitive and -Resistant Tumor Cell Line Models[a]

cell line	cisplatin	carboplatin	4	5	6	7
PEO1 ovarian cancer	1.34 (0.82)	32.6 (11.6)	16.6 (3.4)	8.7 (3.0)	16.3 (3.3)	39.6 (19.3)
PEO1CisR (cisplatin resistant)	5.1 (1.4)	138	15.6 (1.9)	6.9 (0.92)	11.2 (5.3)	nd[b]
PEO1carboR (carboplatin resistant)	6.3 (2.6)	130.7 (43.5)	25.5 (13.4)	8.9 (4.9)	5.2 (1.8)	43.6 (15.4)
SK-OV3 ovarian cancer	13.1 (2.1)	92.8 (6.1)	32.3 (3.7)	10.4 (3.2)	35.8 (5.0)	>100
SW948 colon cancer	44.8 (13.3)	nd[b]	29.4 (4.30)	9.6 (1.9)	22.3 (8.9)	nd[b]
N-TERA testicular cancer	0.78 (0.27)	nd[b]	7.3 (0.7)	1.95 (0.9)	6.1 (0.6)	>100

[a] Data shown are IC_{50} values (μM); all data are mean values of >3 repeat assays, and the standard deviation is given in parenthesis. [b] nd = not determined.

only and compare well with data of similar *trans*-[$PtCl_2$-(oxadiazoline)$_2$] complexes reported previously.[15–17] Also, the ^{195}Pt resonance signals at −2028 to −2114 ppm agree well with expected values.[15–17] There are two signals because of the existence of the bis-oxadiazoline complexes as diastereomers (*meso-R,S* form and racemic *R,R*/*S,S* form), due to the presence of a chiral carbon atom in position 3 of each of the oxadiazoline rings. These could not be separated, and the isomeric mixtures were used for the assays.

Solubility and Stability Studies. The solubility of **4**, **5**, and **7** was investigated gravimetrically in distilled water and compared with the solubility in phosphate-buffered saline and isotonic saline (0.9 g/mL) by UV spectroscopy. The solubility of all compounds was found to be similar and in an order of 0.5 to 4 mg/mL. The solubilities are thus comparable to the one of cisplatin of 1 mg/mL.[18]

Because DMSO has been used for the preparation of stock solutions, the stability of **1**−**7** in d_6-DMSO was studied using ^{1}H and ^{195}Pt NMR spectroscopy. The bis-oxadiazoline complexes **4**, **5**, and **7** were stable during incubation at 25 °C over 10 weeks and even for 10 days at 60 °C before the first signs of degradation became apparent in the ^{1}H NMR spectrum. No new signal indicative of the DMSO coordinated species could be detected in the ^{195}Pt NMR. Therefore, handling and storage of stock solutions should not pose any problems. The mono-oxadiazoline complexes **3** and **6**, however, exhibit a significantly higher reactivity toward DMSO. Within 3 h at 25 °C, there was 70% conversion and exchange of the remaining nitrile ligand by DMSO had taken place. The newly formed platinum compounds were isolated and identified as the complexes *cis*- and *trans*-[$PtCl_2$(d_6-DMSO)(oxadiazoline)] (dichloro[2,3-dihydro-2-methyl-3-*R*-5-*R*′-1,2,4-oxadiazole-κN^4][(sulfinyl-κS)bis[methane]]platinum) by ^{1}H, ^{13}C, and ^{195}Pt NMR spectroscopy and comparison of the data with literature values of the parent compound lacking the OH or glycol group.[13] The bis-nitrile complexes **1** and **2** are very prone to ligand exchange in DMSO. Complete conversion into the known complex [$PtCl_2$(DMSO)$_2$] (dichlorobis[(sulfinyl-κS)bis[methane]]platinum) takes place at 25 °C within 2 h. Consequently, the bis-nitrile complexes have not been used for cytotoxicity testing.

^{1}H NMR was also used to study the stability of **5** in an aqueous medium. Because the solubility of **5** in water or saline is not sufficiently high, NMR experiments were performed in d_6-acetone/H_2O 9:1 and in d_6-acetone/H_2O/Na_2CO_3. Although these experiments did not reveal whether hydrolysis of one or more of the chloro ligands plays any role, we were able to establish that the oxadiazoline ligands in **5** are stable over 10 d at 40 °C, in both neutral and alkaline medium. The alkaline medium was used with the idea in mind that the phenolic OH group can be deprotonated quite easily and this could alter the solubility and the chemical and pharmacological properties of the platinum complex.

In Vitro Activities. The in vitro antiproliferative activity of the compounds was determined by means of a colorimetric microculture assay technique (MTT assay), as described in the Experimental Section. Typically, the data shown indicate the somewhat higher potency that cisplatin has over carboplatin (Table 1), which is a repeatable finding throughout the literature. Apart from **7**, the compounds in this study showed comparable and, against SW948, even superior activity in comparison with the reference compound cisplatin.

Importantly, against platinum-resistant cell lines, the activity of **5** is essentially retained. The same holds true for **4** and **5** when tested in PEO1cisR, but **4** is less-active against the PEO1carboR, whereas **6** becomes even more active. Compound **5** is comparable to cisplatin in potency, being slightly less potent in PEO1 cells, but more potent against the colon cancer cell line SW948. Compound **7** is similar in potency to carboplatin, and **6** and **4** occupy an intermediate position. Importantly, **6** appears to be more active in the PEO1CarboR cell line that in the parental cell line.

Overall, for different cancer cell lines and also for the Pt-resistant ones, the following trends can be seen: **5** has highest potency, followed by **6** and **4**, and finally **7**, the least-active compound. We consistently saw lower IC_{50} values for the test compounds in SW948 colon cancer cells than the value for the reference compound cisplatin. There is a large differential between the activities of **7** versus **6**. Hence, we may conclude at this stage that a mono-oxadiazoline complex is more active than the bis-oxadiazoline substituted one. Comparing **4** and **5**, there is a consistent finding that **5** is significantly more potent than **4**, although these two compounds are isomers of each other, differing in the position of an OH group only. Cisplatin and carboplatin were made up as stock solutions in sterile aqueous 0.9% w/v NaCl (saline solution) and diluted in tissue culture medium to give the required final concentrations for the MTT assay. Compounds **4**–**7** were generally used as stock solutions made up in DMSO and then subjected to further dilution as for cisplatin and carboplatin. We also used **4** as an aqueous stock solution and found that there was no significant variation in the cytotoxicity testing when compared with a DMSO stock solution.

Cell Cycle Analysis. For the PEO1 parental cells, the cell cycle perturbation we saw following a 48 h incubation with 2.5 μM cisplatin, is a characteristic G_2M blockade of the cell cycle, relative to control untreated cells (see Figure 1). The effect of cisplatin in the carboplatin-resistant PEO1CarboR line gave rise to a more modest G_2M block, consistent with its drug-resistant phenotype. These data are in contrast with those obtained with our most potent compound **5** where we failed to see any marked cell cycle blockade. However, there was evidence of a small G1 accumulation together with a build up of cells in the sub-G1 phase, which is indicative of early apoptosis. The results were similar to the effects seen with 24 h incubation and were similar for both cell lines, with a larger sub-G1 phase being seen for PEO1CarboR.

Concluding Remarks

The development of platinum analogues remains an active area of medicinal chemistry. To date, over 30 platinum

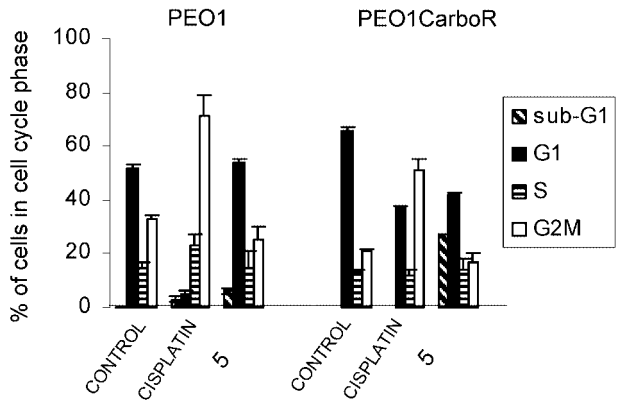


Figure 1. Bar chart showing effects of platinum agents on cell cycle in PEO1 human ovarian cancer cells. Data shown are the mean of >3 replicate analyses, with the standard deviation shown as error bars.

complexes have entered clinical trials worldwide in an effort to circumvent side-effects and resistance shown to cisplatin. In the present study, we show that platinum(II) oxadiazoline-substituted complexes are capable of overcoming platinum resistance. The clinical problem of platinum-refractory disease is highlighted by the poor responses of advanced/late stage ovarian cancer to carboplatin- or cisplatin-containing regimes. Like us, other groups have focused their efforts on the identification of novel platinum structures that may offer improved properties over those agents currently available. For example, Yu et al.[19] have described the anticancer activity of a series of platinum complexes that possess a demethylcantharidin moiety, shown to overcome platinum resistance in tumor cell line models. Kalinowska et al.[20] have studied analogues of *trans*-platinum(II) containing two diethyl (pyridin-4-ylmethyl) phosphates, which show IC_{50} values in a similar range to cisplatin in tumor cell lines, also seen for our study.

Two new potential lead structures have been established, both of which exhibit a potency similar to carboplatin or better, for most cell lines studied. The activity is retained in cisplatin and carboplatin resistant cell lines. The bis-oxadiazoline complexes appear to perform better with respect to stability. The high activity of compounds **5** and **6** relative to cisplatin may relate to many features of the compounds interactions on a cellular level. The finding of activity of *trans*-platinum compounds in cancer cells with resistance to *cis*-platinum compounds (such as cisplatin) has been reported by others.[21] We plan to evaluate in vivo activity of compounds **5** and **6** in platinum-resistant ovarian cancer xenograft models. The effects of platinum compounds on cell cycle have been looked at in some detail by others[22] and can provide valuable information on the mechanism of action of platinum-based cytotoxic compounds. Of great interest are our preliminary experiments that have addressed the issue of cell cycle blockade. While cisplatin predictably gave rise to a G_2M blockade, as seen by others,[23] we were unable to see significant changes in cell cycle apart from a slight accumulation of sub-G_1 and G_1 when cells were treated with compound **5** under the same experimental conditions. This is in contrast to the findings of others who have looked at the effects of *trans*-platinum compounds[22] and see prolonged S-phase and G_2M phase arrest. They have studied these effects over a more prolonged time period than in the current study, that is, 24–96 h time points as opposed to the 24 and 48 h time points we used. It may well be that cell cycle perturbations due to treatment with compound **5** will be manifest if we look at longer treatment times. However, our data provide prima facie evidence that the oxadiazoline containing *trans*-platinum compounds differ from cisplatin and carboplatin in their cell cycle effects. Our studies are currently ongoing to elucidate the cell cycle blockade effects of the oxadiazoline *trans*-platinum compounds used in the present study.

Experimental Section

Materials and Instrumentation. Solvents and reagents for synthesis were obtained from commercial sources and used as received. *cis/trans*-$[PtCl_2(MeCN)_2]$,[24,25] *trans*-$[PtCl_2(PhCN)_2]$,[26,27] 4-(2-methoxyethoxy)benzonitrile,[28] and nitrones[29,30] were prepared according to published methods. The synthesis and spectroscopic characterization of the new compounds **1** and **2** is described in the Supporting Information. C, H, and N elemental analyses were carried out on a Leeman CE 440 automatic analyzer. Infrared spectra (4000–400 cm^{-1}) were recorded on Perkin-Elmer 2000 FTIR and Nicolet Avatar 320 FT-IR spectrometers in KBr pellets. Positive FAB-MS spectra of the samples in 3-nitrobenzyl alcohol (NBA) matrices were obtained on a Thermoquest MAT 95XL instrument. ^1H, ^{13}C, and ^{195}Pt NMR experiments were acquired on a Bruker DRX 500 spectrometer at ambient temperature. Signals in ^1H and ^{13}C were assigned with the help of COSY, NOESY, and HMQC spectra. ^{195}Pt chemical shifts are given relative to aqueous $K_2[PtCl_4] = -1630$ ppm, with half-height line widths in parenthesis.

Preparation of the *trans*-[$PtCl_2$(Nitrile)(oxadiazoline)] Complexes. *trans*-[$PtCl_2$(PhCN){N═C(Ph)-O-N(Me)-CH(*p*-HO-C_6H_4)}], *trans*-(Benzonitrile)-dichloro[2,3-dihydro-3-(4-hydroxyphenyl)-2-methyl-5-phenyl-1,2,4-oxadiazole-κN^4]platinum (3). A mixture of *trans*-$[PtCl_2(PhCN)_2]$ (47.2 mg, 0.1 mmol) and *C*-(*p*-hydroxyphenyl)-*N*-methylnitrone (15.1 mg, 0.1 mmol) in $CHCl_3$ (1 mL) was stirred for 3 days at 25 °C under a nitrogen atmosphere. The reaction was monitored periodically by ^1H NMR and stopped at the first appearance of signals of the bis(oxadiazoline) complex. The product was purified by chromatography on SiO_2 using first CH_2Cl_2 to remove unreacted *trans*-$[PtCl_2(PhCN)_2]$, then CH_2Cl_2/ethyl acetate 10/1 to elute the platinum oxadiazoline complex. Yellow crystalline solid, yield 71%. Anal. ($C_{22}H_{19}Cl_2N_3O_2Pt$) C, H, N. FAB$^+$-MS *m/z*: 624 $[M + H]^+$, 646 $[M + Na]^+$, 587.0 [M − HCl], 551 [M − 2HCl], 521 [M − PhCN]. IR spectrum (KBr), selected bands, cm^{-1}: 2288 ν(C≡N), 1596 ν(C═N). ^1H NMR (500 MHz, d_6-acetone) δ (ppm): 3.02 (s, 3H, NMe), 5.89 (s, 1H, NCHN), 8.57 (s, H, OH), 8.98 (d, 8.0 Hz, 2H), 7.69 (m, 2H) and 7.85 (t, 7.0 Hz, 1H)(PhC═N), 7.53 (d, 8.5 Hz, 2H) and 6.90 (d, 8.5 Hz, 2H)(*p*-HO-C_6H_4), 7.69 (m, 2H), 7.81 (m, 1H) and 7.92 (d, 7.2 Hz, 2H)(PhC≡N). ^{13}C NMR (125.7 MHz, d_6-acetone) δ(ppm): 46.0 (NMe), 94.8 (NCHN), 116.0 (C≡N), 130.6, 130.7 and 128.6 (PhC═N, CH), 115.7 and 129.3 (*p*-HO-C_6H_4, CH), 134.7, 134.1 and 133.6 (PhC≡N, CH), 109.7 (ArC(1)CN), 157.5 (ArC(4)-O), 163.1 (C═N), 122.3 (PhC≡N, C_q), 132.4 (PhC═N, C_q). ^{195}Pt NMR (107.3 MHz, d_6-acetone) δ (ppm): −2220 (1075 Hz).

***trans*-[$PtCl_2$(*p*-$MeOC_2H_4O$-C_6H_4CN){N═C(*p*-$MeOC_2H_4O$-C_6H_4)-O-N(Me)-CH(Ph)}], *trans*-Dichloro[4-(2-methoxyethoxy)benzonitrile][2,3-dihydro-5-(4-(2-methoxy ethoxy)-phenyl)-2-methyl-3-phenyl-1,2,4-oxadiazole-κN^4]platinum (6).** This compound was prepared by reaction of $[PtCl_2(p\text{-}MeOC_2H_4O\text{-}C_6H_4CN)_2]$ with phenyl-*N*-methyl-nitrone, using the reaction protocol above. The reaction time was 7 days, and CH_2Cl_2/ethyl acetate 4/1 was used as eluent for the chromatographic purification of the complex. Pale yellow solid, yield 67%. Anal. ($C_{28}H_{31}Cl_2N_3O_5Pt$) C, H, N. FAB$^+$-MS, *m/z*: 756 $[M + H]^+$, 778 [M + Na], 719 [M − HCl], 683 [M − 2HCl].

IR spectrum (KBr), selected bands, cm^{-1}: 2281 ν(C≡N), 1603 ν(C=N). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 3.05 (s, 3H, NMe), 3.77 (m, 4H, CH_2-O), 4.20 (m, 4H, O-CH_2), 3.46 (s, 6H, MeO), 5.94 (s, 1H, NCHN), 6.98 (d, 8.5 Hz, 2H) and 7.63 (d, 5.8 Hz, 2H)(R-C_6H_4C≡N), 7.09 (d, 8.5 Hz, 2H) and 9.04 (d, 8.5 Hz, 2H)(N=C-C_6H_4-R), 7.45 (m, 3H) and 7.70 (d, 6.8 Hz, 2H)(Ph). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ (ppm): 46.8 (NMe), 59.5 (OMe), 67.9 (CH_2-O), 70.8 (O-CH_2), 94.7 (NCHN), 117.2 (C≡N), 115.5 and 135.5 (R-C_6H_4C≡N, CH), 101.3 and 163.7 (R-C_6H_4C≡N, C_q), 114.6 and 133.1 (N=C-C_6H_4-R, CH), 128.9 and 163.9 (N=C-C_6H_4-R, C_q), 128.7, 128.6 and 129.9 (Ph, CH), 134.2 (Ph, C_q), 163.2 (C=N). ^{195}Pt NMR (107.3 MHz, $CDCl_3$) δ (ppm): −2216 (790 Hz).

Preparation of the *trans*-[$PtCl_2$(Oxadiazoline)$_2$] Complexes. *trans*-[$PtCl_2${N=C(Ph)-O-N(Me)-CH(*p*-HO-C_6H_4)}$_2$], *trans*-Dichlorobis[2,3-dihydro-3-(4-hydroxyphenyl)-2-methyl-5-phenyl-1,2,4-oxadiazole-κN^4]platinum (4). *trans*-$PtCl_2$-(PhCN)$_2$ (47.2 mg, 0.1 mmol) and C-(*p*-hydroxyphenyl)-*N*-methyl-nitrone (33.2 mg, 0.22 mmol) were dissolved in a 1:1 mixture of acetone and $CHCl_3$ (1 mL). The reaction mixture was stirred at 50 °C for 7 days. The product was isolated by filtration and washed with ether. A second crop of product was recovered from the remaining solution by chromatography (SiO_2, CH_2Cl_2/ethyl acetate 4/1). Pale yellow solid, overall yield 48%, two diastereomers **4a/4b** = 70:30. Anal. ($C_{30}H_{28}Cl_2N_4O_4Pt$) C, H, N. FAB$^+$-MS, *m/z*: 797 [M + Na]$^+$, 775 [M]$^+$, 737 [M – HCl]$^+$, 702 [M – 2HCl]$^+$. IR spectrum (KBr), selected bands, cm^{-1}: 1604 ν(C=N). ^{1}H NMR (500 MHz, d_6-DMSO) δ (ppm): 2.88 (s, 3H, NMe), 5.86 (s, 1H, NCH), 6.84 (d, 8.6 Hz, 2H) and 7.41 (d, 8.6 Hz, 2H)(*p*-HO-C_6H_4), 7.57 (t, 8.0 Hz, 2H), 7.81 (t, 8.0 Hz, 1H) and 8.74 (d, 8.0 Hz, 2H)(Ph), 9.69 (s, 1H, OH). ^{13}C NMR (125.7 MHz, d_6-DMSO) δ (ppm): 45.5 (NMe), 93.6 (NCHN), 114.9 and 129.8 (*p*-HO-C_6H_4, CH), 158.3 (*p*-HO-C_6H_4, C_q), 133.8, 128.4, and 129.9 (Ph, CH), 121.9 (Ph, C_q), 162.5(C=N). ^{195}Pt NMR (107.3 MHz, d_6-acetone) δ (ppm): −2115 and −2122 (1075 Hz).

***trans*-[$PtCl_2${N=C(*p*-HO-C_6H_4)-O-N(Me)-CH(Ph)}$_2$], *trans*-Dichlorobis[2,3-dihydro-5-(4-hydroxyphenyl)-2-methyl-3-phenyl-1,2,4-oxadiazole-κN^4]platinum (5).** This compound was prepared by reaction of [$PtCl_2$(*p*-HO-C_6H_4CN)$_2$] with C-phenyl-*N*-methyl-nitrone using the reaction protocol above, with acetone as a solvent. The product was isolated by evaporation of most of the solvent and precipitation with diethyl ether. Yellow cream solid, yield 55%, two diastereoisomers **5a/5b** = 55/45. Anal. ($C_{30}H_{28}Cl_2N_4O_4Pt$) C, H, N. FAB$^+$-MS, *m/z*: 814 [M + K]$^+$, 798 [M + Na]$^+$, 775 [M]$^+$, 704 [M – 2HCl]$^+$. IR spectrum (KBr), selected bands, cm^{-1}: 1607 ν(C=N). ^{1}H NMR (500 MHz, d_6-acetone) δ (ppm): **5a**: 2.94 (s, br, 3H, NMe), 5.92 (s, br, 1H, NCH), 6.88 (d, 8.5 Hz, 2H) and 8.82 ("d", br, 2H)(*p*-HO-C_6H_4), 7.45 (m, br, 3H) and 7.60 (m, br, 2H)(Ph), 9.50 (s, 1H, OH). **5b**: 2.95 (s, br, 3H, NMe), 5.95 (s, br, 1H, NCH), 7.00 ("d", br, 2H) and 8.78 ("d", br, 2H)(*p*-HO-C_6H_4), 7.43 (m, br, 3H) and 7.66 (m, br, 2H)(Ph), 9.50 (s, 1H, OH). ^{13}C NMR (125.7 MHz, d_6-acetone, 10% D_2O, Na_2CO_3) δ (ppm): **5a** and **5b**: 45.6 (NMe), 94.2 (NCHN), 119.6 and 133.3/133.4 (*p*-HO-C_6H_4, CH), 104.3 and 163.3/164.3 (*p*-HO-C_6H_4, C_q), 128.5, 128.6 and 129.0 (Ph, CH). The quaternary carbons of the Ph and the C=N group have not been detected. ^{195}Pt NMR (107.3 MHz, d_6-acetone) δ (ppm): **5a** and **5b**: −2028 and −2049 (1075 Hz).

***trans*-[$PtCl_2${N=C(*p*-MeO-C_2H_4O-C_6H_4)-O-N(Me)-CH(Ph)}$_2$], *trans*-Dichlorobis-[2,3-dihydro-5-(4-(2-methoxyethoxy)phenyl)-2-methyl-3-phenyl-1,2,4-oxadiazole-κN^4]platinum (7).** This compound was prepared by reaction of [$PtCl_2$(*p*-MeO-C_2H_4O-C_6H_4CN)$_2$] with C-phenyl-*N*-methyl-nitrone using the reaction protocol above. $CHCl_3$ was used as solvent, and the reaction was complete after 7 d at 50 °C. The solvent was evaporated under vacuum and the residue purified by chromatography on silica using CH_2Cl_2/ethyl acetate 4:1 as eluent. Yellow-brown solid, yield 45%, two diastereoisomers **7a/7b** = 50/50. Anal. ($C_{36}H_{40}Cl_2N_4O_6Pt$) C, H, N. FAB$^+$-MS, *m/z*: 913 [M + Na]$^+$, 891 [M]$^+$, 854. [M – HCl]$^+$, 817 [M – 2HCl]$^+$. IR spectrum (KBr), selected bands, cm^{-1}: 1605 ν(C=N). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): **7a/7b** = 50/50: 2.91/2.94 (s, 3H, NMe), 3.79 (m, 2H, CH_2-O), 4.19 (m, 2H, O-CH_2), 3.47 (m, 3H, MeO), 5.88 (s, 1H, NCH), 7.48 (m, 3H) and 7.60 (m, 2H)(Ph), 6.82 (m, 2H) and 8.66 (d, 8.5 Hz, 2H of **7b**), 8.82 (m, br, 2H of **7a**)(*p*-R-C_6H_4). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ (ppm): 46.3 (NMe), 59.3 (OMe), 67.6 (CH_2-O), 70.8 (O-CH2), 94.7 (NCHN), 128.5, 128.6 and 129.5 (Ph, CH), 136.5 (Ph, C_q), 114.3 and 132.7/133.0 (*p*-R-C_6H_4, CH), 128.9 and 162.9 (*p*-R-C_6H_4, C_q), 163.0 (C=N). ^{195}Pt NMR (107.3 MHz, $CDCl_3$) δ (ppm): −2107 (775 Hz) and −2124, 2 (775 Hz).

Cell Culture. All tissue culture reagents were obtained from Sigma Aldrich (Poole, UK), unless stated otherwise. The PEO1 ovarian cancer cell line was originally developed by Langdon et al. and obtained from Prof. F. Balkwill (formerly of ICRF Laboratories, Lincoln Inn Fields, London, U.K.). The PEO1CisR (cisplatin-resistant) and PEO1CarboR (carboplatin-resistant) cell lines were derived in-house and possess approximately 5-fold resistance to their respective inducing agent. All PEO1 cell lines were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum (heat inactivated, obtained from Invitrogen, Paisley, U.K.) and 2 mM Glutamax (Invitrogen) as monolayers.

SK-OV-3 human ovarian adenocarcinoma cell line was obtained from the European Collection of Cell Cultures (Salisbury, U.K.) and cultured in Dulbecco's Modified Eagle's Medium (DMEM) with supplements, as above. The NTERA-2 cell line is described as an embryonal human testicular carcinoma cell line and was obtained from ATCC (LGC Promochem, Teddington, U.K.). This cell line was cultured in Dulbecco's Modified Eagle's Medium with 4 mM glutamine and 10% fetal calf serum. SW-948 is a colonic adenocarcinoma cell line established from a grade III adenocarcinoma of the colon and was obtained from ATCC. The culture medium was L-15 medium-supplemented with 2 mM glutamine and 10% fetal calf serum. All cell lines were grown as attached monolayers, which were removed by treatment with trypsin−EDTA solution to produce a single cell suspension for use in chemosensitivity testing.

Cytotoxicity Testing. Cytotoxicity was determined by means of the colorimetric assay MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide). Cultured cell monolayers were reduced to a single cell suspension (as described above) and then seeded into 96-well tissue culture plates at a density of 6×10^3 cells per well for all cell lines, apart from N-TERA-2, which were seeded at a density of 1×10^4 cells per well. This was calculated to allow for exponential growth of the cultures throughout the incubation period. Cells were allowed to settle for 24 h, under standard culture incubation conditions, and then drug-treated with a dose range spanning 2 logs of drug concentration, each drug aliquot being administered in a 50 μL volume. All drug dilutions were carried out in complete culture medium. After a 72 h incubation under standard culture conditions, MTT solution (5 mg/ml in PBS) was added in a 20 μL volume and incubated for a further 4 h. The MTT/medium mixture was then removed and the resulting formazan crystals

were dissolved in 200 μL of DMSO. The optical density of the purple color product was measured at 550 nm in a plate reading spectrophotometer. The quantity of live cells was expressed as *T*/*C* values by comparison with untreated control microcultures. The concentration of complexes that decreased absorption by 50% were calculated by interpolation and expressed as IC_{50} values.

Cell Cycle Analysis using Propidium Iodide. PEO1 human ovarian cancer cells at a density of 4×10^4 cells/mL were left to adhere in flasks for 4–6 h. Cells were treated with drug at approximately twice the IC_{50} dose (obtained from the MTT assay data with a continuous exposure over 72 h). Cells were harvested at 48 h and washed in PBS before resuspension in 70% ethanol in PBS, added while vortex mixing, and left at 4 °C for at least 24 h. After washing in PBS, cells were stained in 10 μg/mL of propidium iodide (Sigma) and 1 mg/mL ribonuclease A (Sigma) for at least 30 mins at 37 °C in the dark. Fluorescence >575 nm versus light scatter was measured with excitation of 488 nm on a Beckman-Coulter Epics-XL. Data shown on each histogram are representative of >3 separate experiments, with typical results obtained shown in the figure.

Acknowledgment. We are grateful to the EPSRC for the provision of a studentship and to the Proof of Concept Fund of the University of Surrey for supporting this work.

Supporting Information Available: Synthesis and spectroscopic characterization (^{1}H, ^{13}C, and ^{195}Pt NMR, IR, MS) of compounds **1** and **2** and a table of combustion analysis data for compounds **1**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM061263S