Synthesis and in Vitro Antitumor Activity of Platinum Acetonimine Complexes

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Received October 4, 2005

The *cis*- and *trans*-dichloro- and diiodo-platinum(II) complexes containing two acetonimines (*cis*- and *trans*-$[PtX_2\{HN{=}C(CH_3)_2\}_2]$, **1** and **2** for X = Cl and **1′** and **2′** for X = I, respectively) or one acetonimine and one ammine (*cis*- and *trans*-$[PtX_2(NH_3)\{HN{=}C(CH_3)_2\}]$, **3** and **4** for X = Cl and **3′** and **4′** for X = I, respectively) have been prepared from platinum-ammine precursors by condensation with acetone. Except for the *cis*-diiodo species, in all other cases the presence of a base was required. A crucial role of the ligand *trans* to the ammine undergoing condensation with acetone has been disclosed: the greater the *trans* effect the greater the reactivity. In a panel of human tumor cell lines representative of ovarian, colon, lung, and breast cancers, *cis* complexes **1** and **3** are less active than *cis*-DDP (mean IC_{50} = 20, 12.5, and 2.8 μM, respectively), whereas *trans* complexes **2** and **4** are more active than *trans*-DDP (mean IC_{50} = 10.6, 26, and 164 μM, respectively), thus indicating that substitution of acetonimine for one or two ammine ligands determines strikingly different effects depending upon the complex geometry.

Introduction

It is generally accepted that modification of the carrier ligands in antitumor platinum(II) complexes with *cis* geometry can alter both their efficacy and their spectrum of activity.[1] It has also been shown that carrier ligands such as iminoethers, aromatic N-donor etherocycles, cyclohexylamine, and ramified aliphatic amines can affect the activity of platinum complexes with *trans* geometry.[2] In particular the platinum-iminoether complex *trans*-$[PtCl_2\{E\text{-}HN{=}C(OMe)Me\}_2]$ was the first reported example of a *trans*-platinum complex exhibiting antitumor activity in vivo. Also corresponding complexes with one iminoether and one ammine ligand had comparable antitumor activities.[3−6]

Platinum-coordinated iminoether ligands (Pt−N(H)=C(OR)-R′) are characterized by having *E* or *Z* configuration, depending upon the relative positions of the alkoxide and platinum groups with respect to the C=N double bond, and the relationship between configuration of the iminoethers and pharmacological action of the platinum complexes have been investigated.[7,8] Since iminoether complexes can undergo in solution slow isomerization at the azomethine double bond, we extended the investigation to platinum complexes with cyclic ligands mimicking iminoethers but which cannot undergo *E*/*Z* isomerization (e.g. 2-methyl-4,5-dihydro-1,3-oxazole and 5-methoxy-3,4-dihydropyrrole). These latter ligands however, differently from iminoethers, no longer have a proton on the iminic nitrogen, and this could represent a major change.[9] A further generation of platinum complexes mimicking iminoether derivatives is represented by the platinum complexes with acetonimine ($HN{=}CMe_2$) reported in this paper. The main advantage of symmetrical ketimines (like acetonimine), with respect to iminoethers, is the lack of geometric isomerism about the C=N double bond. As to the other features, ketimines and iminoethers are very similar (sp^2-hybridization of the nitrogen atom like in pyridine, a proton still bound to nitrogen like in secondary aliphatic amines, the ligand extending in a plane with the steric bulk localized only on one side of the donor atom).

Acetonimine can be prepared from acetone and ammonia at high temperature and pressure and with the use of a catalyst.[10,11] However, unlike diphenylketimine (which is commercially available), acetonimine is unstable, even at room temperature, affording polymeric species such as 2,2,4,4,6-pentamethyl-2,3,4,5-tetrahydropyrimidine (acetonine).[12] Acetonimine can be stabilized by coordination to a metallic center; therefore, acetonimine complexes of a wide variety of transition metals (such as Mo, W, Cr,[13] Ni,[14] Ru,[15a,b] Os,[16] and more recently also Au,[17] Pd,[18] Ag, and Rh[19]) have been reported in the literature. Two synthetic procedures have been employed: (i) condensation with acetone of a coordinated ammine and (ii) in situ preparation of acetonimine starting from acetone and ammonia, followed by immediate coordination to a metallic center. To the best of our knowledge, no acetonimine complexes of platinum have been reported so far, the only published ketimine complexes being those with diphenylketimine,[20] N-(2-aminoethyl)-acetonimine (obtained by reaction of monocoordinated ethylenediamine with acetone)[21] and acetimineacetoniminate (obtained by reaction of acetylacetonate with platinum(IV)-coordinated *cis*-ammines).[22]

The newly synthesized *cis* and *trans* complexes of platinum are reported in Scheme 1 (it can be noted that *cis* complexes are indicated by odd numbers while *trans* complexes are indicated by even numbers). A crucial role of the ligand *trans* to the ammine undergoing condensation with acetone has been disclosed. As to the in vitro antitumor activity, this study has demonstrated that substitution of symmetrical ketimines for ammines dramatically modifies the antitumor properties of platinum compounds as already observed for platinum complexes with iminoethers and other ligands.[2,23]

Results and Discussion

Synthesis. The bisacetonimine complexes of platinum (**1** and **2** in Scheme 1) can be obtained in good yield by reaction of the corresponding ammine complexes (*cis*- or *trans*-diamminedichloroplatinum(II), *cis*- or *trans*-DDP) suspended in acetone/methanol (2:1, v/v) and treated with powdered KOH (5× the stoichiometric amount, Scheme 2). The reaction is faster in the case of the *cis* isomer (complete in 1 h) than in the case

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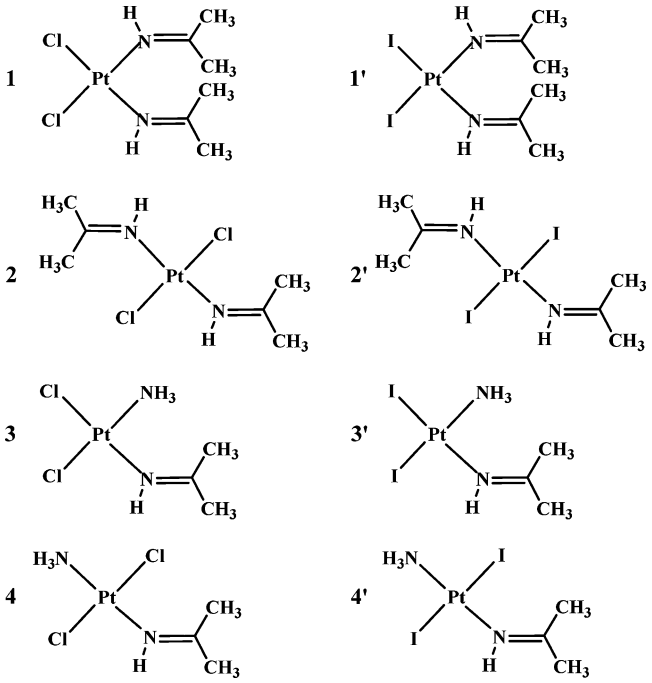
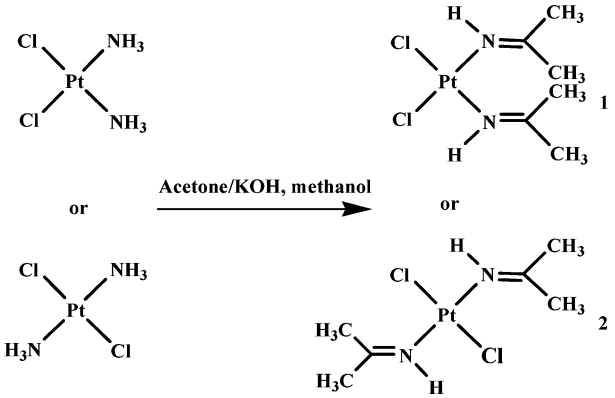
10.1021/jm050986t CCC: $33.50
Published on Web 01/04/2006

Scheme 1. Schematic Drawing of Newly Synthesized Acetonimine Compounds



Scheme 2. Reaction Scheme for the Synthesis of the Diacetonimine Complexes Starting from the Corresponding Diammine Derivatives



of the *trans* isomer (complete in 24 h). The complexes were isolated as pale yellow solids and were characterized by elemental analysis, IR, and NMR.

In the case of the chloro species, the addition of a base is absolutely necessary for the condensation reaction between acetone and coordinated ammines to take place; however, the iodospecies *cis*-$[PtI_2(NH_3)_2]$ reacts with acetone also in the absence of a base affording the corresponding acetonimine complex *cis*-$[PtI_2\{HN{=}C(CH_3)_2\}_2]$ (**1′**). In the latter case, the formation of the acetonimine is complete in 24 h at 55 °C. The reaction course, monitored by NMR spectroscopy using a ^{15}N-enriched sample, is reported in Figure 1. The starting complex exhibits a doublet at 3.85 ppm (each signal flanked by ^{195}Pt satellites; $J_{H,N} = 72$ Hz and $J_{H,Pt} = 57$ Hz).[24] After 1 h a new set of signals, comprising a doublet at 3.81 ppm (each signal flanked by ^{195}Pt satellites; $J_{H,N} = 72$ Hz and $J_{H,Pt} = 60$ Hz) and a doublet at 9.64 ppm ($J_{H,N} = 77$ Hz), is clearly visible. These two doublets are assigned to the iminic (low field signal) and to the amminic (high field signal) protons of the ammine/acetonimine species *cis*-$[PtI_2(NH_3)\{HN{=}C(CH_3)_2\}]$ (**3′**). This intermediate set of signals is replaced, with time, by a new set of signals characterized by a doublet at 9.67 ppm ($J_{H,N} = 76$ Hz) which is assigned to the iminic proton of the bisacetonimine species *cis*-$[PtI_2\{HN{=}C(CH_3)_2\}_2]$ (**1′**).

It is to be noted that, differently from the *cis* isomer, the *trans*-$[PtI_2(NH_3)_2]$ complex, like the chloro species *cis*- and *trans*-$[PtCl_2(NH_3)_2]$, does not react with neat acetone. This result confirms the greater reactivity of the *cis* isomer with respect to the *trans* species as already observed for the reaction with acetone of the chloro species in basic medium.

The iodo complex *cis*-$[PtI_2(NH_3)_2]$ was used as starting substrate for the preparation of the mixed ammine/acetonimine complexes *cis*- and *trans*-$[PtCl_2(NH_3)\{HN{=}C(CH_3)_2\}]$ (**3** and **4**, respectively, Scheme 3). Addition of a small amount of KOH dissolved in water to a suspension of the platinum substrate in acetone (KOH:Pt ratio of 1:2), followed by a rapid quenching of the reaction by addition of water, leads to formation of a mixture of *cis*-$[PtI_2(NH_3)\{HN{=}C(CH_3)_2\}]$ (**3′**) and *cis*-$[PtI_2\{HN{=}C(CH_3)_2\}_2]$ (**1′**). The separation of the two compounds can be accomplished owing to their different solubilities in $CHCl_3$. Unlike *cis*-$[PtI_2\{HN{=}C(CH_3)_2\}_2]$, *cis*-$[PtI_2(NH_3)\{HN{=}C(CH_3)_2\}]$ is insoluble in this solvent. The conversion of the iodospecies (**3′**) into the chlorospecies (**3**) was readily accomplished by treatment with $AgNO_3$ (to remove the iodide) followed by addition of KCl.

The complex *trans*-$[PtI_2(NH_3)\{HN{=}C(CH_3)_2\}]$ (**4′**) could not be prepared from *trans*-$[PtI_2(NH_3)_2]$ using a procedure analogous to that used for the *cis* isomer since in this case the mono- and bisacetonimine complexes (**4′** and **2′**, respectively) are difficult to separate one from the other. Therefore we adopted a different reaction procedure starting from *cis*-$[PtI_2\{HN{=}C(CH_3)_2\}_2]$ (**1′**). Complex **1′** was first converted into *cis*-$[Pt(NH_3)_2\{HN{=}C(CH_3)_2\}_2](NO_3)_2$ by treatment with 2 equiv of $AgNO_3$ (which remove the two iodide ligands) followed by addition of an excess of aqueous ammonia. The water soluble diamine/diacetonimine cationic species was then converted into the insoluble *trans*-$[PtI_2(NH_3)\{HN{=}C(CH_3)_2\}]$ by reaction with KI. This reaction takes advantage of the strong *trans* labilizing effect of the iodide ligand; therefore, after substitution of the first ammine or acetonimine ligand by iodide, the second displaced ligand is that *trans* to the first entered iodide. Finally the diiodo complex was converted into the dichloro species by treatment with $AgNO_3$ (which removes the iodides) followed by addition of KCl.

The Condensation Reaction. Important differences are found in the reactivities of strictly related complexes. Only *cis*-$[PtI_2(NH_3)_2]$ reacts with neat acetone to afford the corresponding acetonimine complex; in contrast all other compounds (*trans*-$[PtI_2(NH_3)_2]$ and *cis*- and *trans*-$[PtCl_2(NH_3)_2]$) require the presence of a base. The general trend is as follows: iodospecies more reactive than chlorospecies and *cis* complexes more reactive than *trans* complexes. Thus, the role of the *trans* ligand appears to be important: the greater the *trans* effect of this ligand ($I > Cl > NH_3$), the greater the reactivity of the substrate. The condensation reaction is believed to take place through deprotonation of the coordinated ammine (with formation of an amido species) followed by electrophilic attack of acetone (and formation of the corresponding acetonimine).[25] The pK_a of the ammine is lowered by the effect of coordination to a metal center.[22,25] For instance, pK_a values of 7 and 10 have been estimated for the first and second deprotonation of cationic $[Pt(NH_3)_6]^{4+}$;[25−28] however, only in the case of the *cis* iodospecies was the spontaneous deprotonation of the coordinated ammines sufficient to ensure their conversion to acetonimines

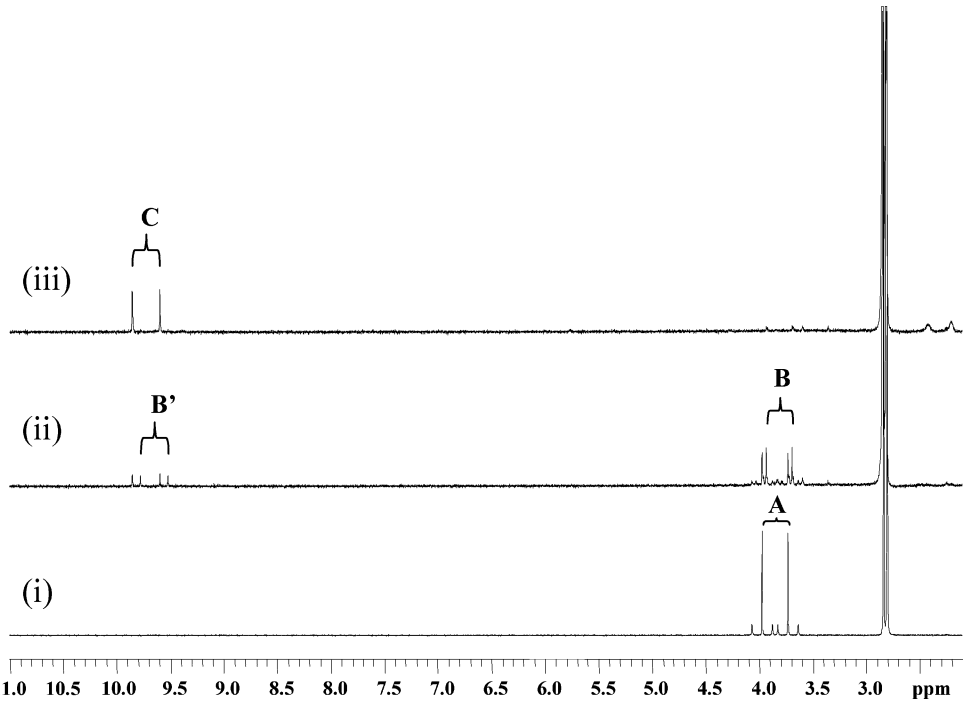
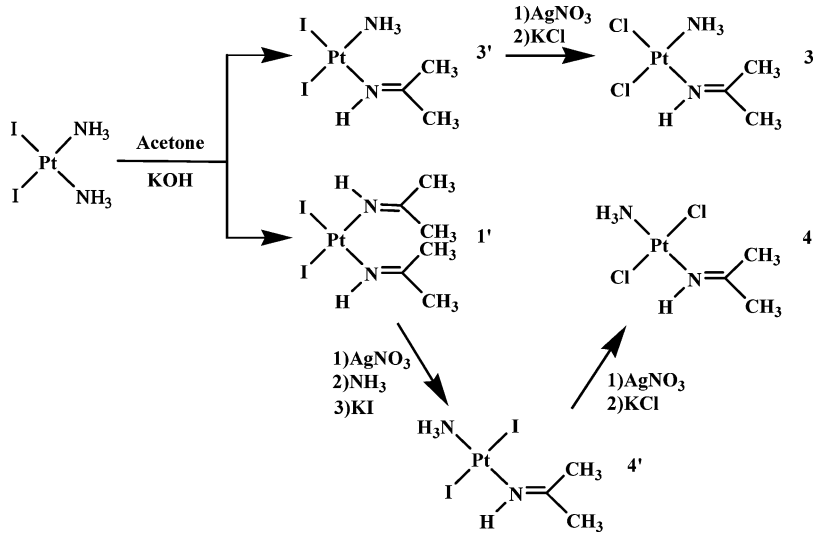
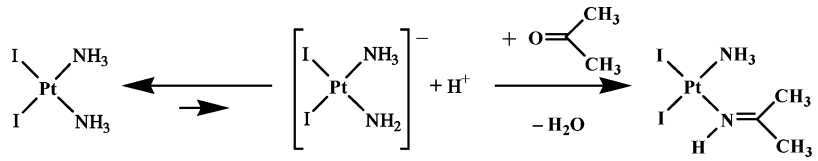


Figure 1. ^{1}H NMR spectra of a solution of *cis*-[$PtI_2(^{15}NH_3)_2$] in acetone-d_6 at 55 °C. Parts i, ii, and iii are the spectra soon after dissolution, after 5 h, and after 24 h, respectively. **A** indicates the $^{15}NH_3$ proton signals of the starting complex; **B** and **B′** mark the $^{15}NH_3$ and ^{15}NH proton signals (in the given order) for the complex *cis*-[$PtI_2(^{15}NH_3)\{H^{15}N{=}C(CH_3)_2\}$]; and **C** indicates the ^{15}NH proton signal for *cis*-[$PtI_2\{H^{15}N{=}C(CH_3)_2\}_2$]. Two very weak signals at high field (2.42 and 2.20 ppm) belong to the methyl residues of the acetonimine ligands, their weakness ascribed to the use of deuterated acetone (only a small portion of solvent containing a residual proton).

Scheme 3. Reaction Scheme for the Preparation of *cis* and *trans* Ammine/Acetonimine Compounds Starting from the *cis*-Diammine Complex



Scheme 4. Proposed Reaction Mechanism for the Condensation of Acetone with a Coordinated Ammine



in neat acetone (Scheme 4), while in all other cases the action of a base was required. We have measured the δH (ppm), the $J_{H,Pt}$ (Hz), and the $J_{H,N}$ (Hz) values (the values will be reported in parentheses in the given order) for the amminic protons in ^{15}N enriched samples of *cis*-DDP (3.78, 61, and 72), *trans*-DDP (3.40, 56, and 72), *cis*-[$PtI_2(NH_3)_2$] (3.85, 57, and 72), and *trans*-[$PtI_2(NH_3)_2$] (3.47, 56, and 72) in the same solvent (acetone-d_6). There were no significant differences in the values of coupling constants (except for a slightly greater value of $J_{H,Pt}$ for *cis*-DDP); however, the chemical shift values suggest a linear dependence of the proton deshielding upon the labilizing effect of the *trans*-ligand (I > Cl > N). Furthermore, *trans* labilizing ligands are able to remove electron charge from the metal center; therefore, ligands with higher *trans* effect can better stabilize

Table 1. Proton Chemical Shifts[a] of Complexes **1**−**4** and **1′**−**4′**

compound	solvent	NH	NH_3	CH_3
cis-[$PtCl_2${HN═C$(CH_3)_2$}$_2$] (**1**)	$CDCl_3$	10.31		2.59, 2.24
cis-[$PtCl_2${HN═C$(CH_3)_2$}$_2$] (**1**)	CD_3COCD_3	9.89		2.58, 2.35
cis-[PtI_2{HN═C$(CH_3)_2$}$_2$] (**1′**)	$CDCl_3$	10.18		2.53, 2.34
cis-[PtI_2{HN═C$(CH_3)_2$}$_2$] (**1′**)	CD_3COCD_3	9.70		2.65, 2.38
cis-[$PtCl_2(NH_3)${HN═C$(CH_3)_2$}] (**3**)	CD_3COCD_3	9.70	3.75	2.55, 2.27
cis-[$PtI_2(NH_3)${HN═C$(CH_3)_2$}] (**3′**)	$CDCl_3$	10.61	3.62	2.58, 2.34
cis-[$PtI_2(NH_3)${HN═C$(CH_3)_2$}] (**3′**)	CD_3COCD_3	9.60	3.80	2.52, 2.28
trans-[$PtCl_2${HN═C$(CH_3)_2$}$_2$] (**2**)	$CDCl_3$	8.72		2.63, 2.25
trans-[$PtCl_2${HN═C$(CH_3)_2$}$_2$] (**2**)	CD_3COCD_3	9.61		2.65, 2.40
trans-[PtI_2{HN═C$(CH_3)_2$}$_2$] (**2′**)	CD_3COCD_3	9.51		2.44, 2.32
trans-[$PtCl_2(NH_3)${HN═C$(CH_3)_2$}] (**4**)	CD_3COCD_3	9.45	3.41	2.46, 2.24
trans-[$PtI_2(NH_3)${HN═C$(CH_3)_2$}] (**4′**)	CD_3COCD_3	9.47	3.45	2.38, 2.28

[a] δ, downfield from $SiMe_4$, ppm; room temperature.

the negative charge of the amido species so favoring the formation of this key intermediate in the condensation reaction.[29]

Spectroscopy. The IR spectra of complexes **1**−**4** and **1′**−**4′** are characterized by ν(NH) stretchings in the region 3350−3050 cm^{-1}, ν(C═N) stretchings in the region 1680−1640 cm^{-1}, and ν(Pt−Cl) stretchings (only for complexes **1**−**4**) in the region 340−320 cm^{-1}. Compounds **1**, **2**, **1′**, and **2′** having only acetonimine carrier ligands have sharp NH stretchings in the range 3215−3240 cm^{-1}. On the other hand, compounds **3**, **4**, **3′**, and **4′** containing one ammine and one acetonimine carrier ligands have, in addition to a rather sharp resonance in the range 3215−3240 cm^{-1} assignable to the acetonimine N−H stretching, also overlapping very broad signals covering the range 3050−3350 cm^{-1} and assignable to the ammine N−H stretchings.

The ^{1}H NMR spectra exhibit NH proton resonances as broad singlets in the region between 9.5 and 10.0 ppm (Table 1). The corresponding ^{15}N enriched complexes give rise, in the same region, to sharp doublets owing to $J_{H,N}$ coupling. The protons of the two unequivalent methyl groups give rise to two signals in the region 2.20−2.50 ppm. The signal at higher field has always greater coupling with the iminic proton ($^4J_{H,H}$ of ca. 1.5 Hz) and smaller coupling with platinum ($^4J_{H,Pt}$ of ca. 5−7 Hz) than the signal at lower field ($^4J_{H,H} \leq 0.5$ Hz and $^4J_{H,Pt}$ of ca. 10−12 Hz).

The chemical shifts and the *J* values agree with those reported in the literature for analogous acetonimine and ketimine complexes;[13,17,30] however, previous criteria used for assignment of the resonances to individual methyls (in the case of acetonimine derivatives) do not appear to apply to our case. In most instances (Cr, Mo, W, Mn, Fe,[13] Au,[17a] Pd,[18] and Ag[19a] complexes) the methyl resonance having the largest coupling with the iminic proton was assigned to the methyl group *trans* to NH and *cis* to the metal. The values of chemical shifts were not considered diagnostic since they are solvent dependent and the signal of a given methyl can be downfield or upfield (with respect to the signal of the other methyl) depending upon the type of solvent used (a clear example is that of the complex [$W(CO)_5${HN═C$(CH_3)_2$}]).[13] We have recorded the ^{1}H NMR spectra of *trans*-[PtI_2{HN═C$(CH_3)_2$}$_2$] in three different solvents (acetone-d_6, $CDCl_3$, and benzene-d_6) and found that the $\Delta\delta$ between the two methyl resonances varies from 1.1 ppm in benzene-d_6 to 0.12 ppm in acetone-d_6. In our case, however, the signal at lower field had invariably the smallest coupling with the iminic proton and the largest coupling with platinum.

On the basis of the criterion of larger coupling between methyl group and iminic proton when they are *trans* to one another, the methyl signal at lower field, having smaller coupling with the iminic proton, should be assigned to the methyl *cis* to the iminic proton and *trans* to platinum. The greater coupling between this methyl and the platinum nucleus (*trans* to one another) would support such an assignment. However, we noticed that the above assignment contradicts the results obtained in the case of analogous compounds of platinum(II) with iminoether ligands for which the resonance of the methyl *cis* to platinum with respect to the azomethine double bond was invariably at lower field (effect of the magnetic anisotropy of the metal center). We were also aware of the fact that in some occasions the coupling constant between a methyl group and a platinum atom can be greater when the two groups are *cis* to one another with respect to an azomethine double bond than when they are *trans*.[31] Therefore, to place on a more solid foundation the assignment of the methyl resonances, a NOESY experiment was performed on the *cis*- and *trans*-[PtI_2{HN═C$(CH_3)_2$}$_2$] compounds. In both cases, a much stronger cross-peak was observed between the more shielded methyl and the iminic proton (Figure 2), clearly indicating that the more shielded methyl is *cis* to the iminic proton with respect to the azomethine double bond.[18] Such a behavior was completely analogous to that observed in the case of iminoether complexes of platinum for which stronger NOE cross-peaks were observed between more shielded methyl or methoxy group and iminic proton.[32] Therefore we can conclude that the criterion of bigger coupling between methyl group and iminic proton *trans* to one another can be misleading and, at least in the case of acetonimine complexes of platinum(II), it is invariably found that a smaller coupling with the iminic proton, a larger coupling with platinum,

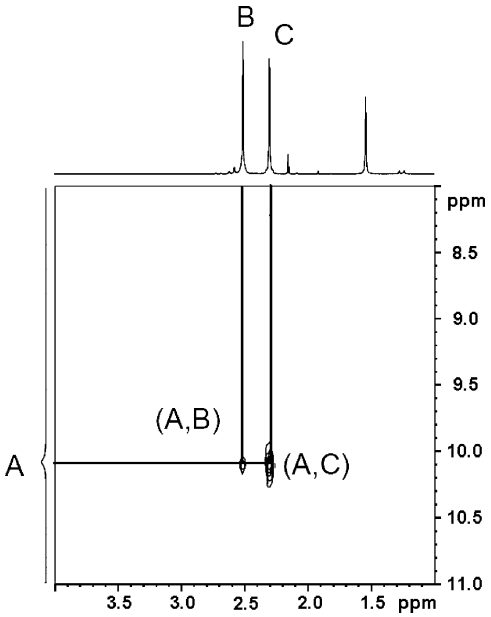


Figure 2. Portion of the 2D NOESY spectrum of *cis*-[PtI_2{HN═C$(CH_3)_2$}$_2$] in $CDCl_3$ showing the strong cross-peak between the iminic proton (**A**) and the upfield methyl protons (**C**).

Table 2. In Vitro Tumor Cell Growth Inhibition by *cis*-[$PtCl_2\{HN{=}C(CH_3)_2\}_2$] (**1**), *cis*-[$PtCl_2(NH_3)\{HN{=}C(CH_3)_2\}$] (**3**), *trans*-[$PtCl_2\{HN{=}C(CH_3)_2\}_2$] (**2**), and *trans*-[$PtCl_2(NH_3)\{HN{=}C(CH_3)_2\}$] (**4**) Complexes, in Comparison to *cis*- and *trans*-DDP[a]

cell line	**1**	**3**	**2**	**4**	*cis*-DDP	*trans*-DDP
A2780	1.5 ± 0.3	0.3 ± 0.07	4.8 ± 0.2	13 ± 1.5	0.2 ± 0.07	14 ± 4
41M	4.3 ± 1	1.4 ± 0.2	4.2 ± 0.4	9.3 ± 1.6	0.6 ± 0.02	36 ± 5
OVCAR-8	8.3 ± 1.5	3.2 ± 0.4	15.4 ± 1.6	18 ± 2.1	3 ± 0.4	186 ± 24
SK-OV-3	30.6 ± 4	9 ± 1.2	22 ± 2	77 ± 8	4 ± 1	170 ± 25
KM12	50 ± 8	43 ± 5	11 ± 2	27 ± 1.9	8 ± 1.5	355 ± 36
COLO-205	37 ± 3.1	41 ± 4	10 ± 1	25 ± 2.4	7.6 ± 1.4	190 ± 33
HCT-116	28 ± 3.5	8 ± 1.3	6 ± 1.1	16 ± 1.3	1.9 ± 0.3	35 ± 5
A549	9.2 ± 2	5.5 ± 0.7	3.2 ± 0.3	8.2 ± 1	1.3 ± 0.1	260 ± 24
H460	5.6 ± 1.5	2.4 ± 0.2	12 ± 2	31 ± 2	0.8 ± 0.3	376 ± 35
MCF7	10.1 ± 2.5	4.3 ± 0.3	6.6 ± 0.9	13 ± 1.1	1.2 ± 0. 3	45 ± 7
MDA	36 ± 5	19 ± 1.5	22 ± 2.6	48 ± 6.2	2.5 ± 0.3	140 ± 21
A2780cisR	4.9 ± 1.1	0.72 ± 0.02	19.2 ± 1.8	18.2 ± 2.1	3.2 ± 0.2	118.3 ± 17
41McisR	12.9 ± 1.3	2.38 ± 0.2	15.9 ± 1.6	10.2 ± 1.4	2.7 ± 0.15	50.4 ± 6

[a] Values (means of at least three experiments ± SD) are IC_{50} in μM (96 h drug exposure).

and a shift to lower field are typical of the methyl group *cis* to platinum and *trans* to the iminic proton with respect to the azomethine double bond.

In Vitro Growth Inhibition Assay. The in vitro growth inhibitory effect of *cis*- and *trans*-dichloro complexes with two acetonimine ligands (complexes **1** and **2**) or with one ammine and one acetonimine (complexes **3** and **4**) was evaluated in comparison to that of *cis*- or *trans*-DDP in a panel of human tumor cell lines containing examples of ovarian (A2780, 41M, OVCAR-8, and SK-OV-3), colon (KM12, COLO-205, and HCT-116), lung (A549/ATCC and NCI-H460), and breast (MCF7 and MDA) cancers. The panel also contains ovarian cancer cells characterized by acquired resistance to *cis*-DDP (A2780cisR and 41McisR).[33,34] The results are shown in Table 2. Compounds **1′**−**4′** were not tested because of their much lower water solubility in comparison to the chloro derivatives. *cis*-[$PtCl_2\{HN{=}C(CH_3)_2\}_2$] (**1**) and *cis*-[$PtCl_2(NH_3)\{HN{=}C(CH_3)_2\}$] (**3**) (note that *cis* complexes are indicated by odd numbers) showed a growth inhibitory activity lower than that of *cis*-DDP, the mean IC_{50} (μM) values being 20 (1.5−50), 12.5 (0.3−43), and 2.8 (0.2−8) for **1**, **3**, and *cis*-DDP, respectively. As far as the growth inhibitory potency toward the different tumor cell lines is concerned, complex **1** showed an efficacy similar to that of **3** against KM12 and COLO-205 colon cancer cells, but it was about 3-fold less effective than **3** toward the remaining tumor cells. Despite their different inhibitory potency, both **1** and **3** are characterized by a similar growth inhibitory profile with respect to the different types of tumor cells. The two complexes showed indeed major activity toward A2780, 41M, H460, and OVCAR-8 cells and minor activity toward KM12, COLO-205, and MDA cells. The similarities between **1** and **3** were further confirmed by Spearman rank analysis.[35] For such an analysis, a high, statistically significant, correlation coefficient (r_s) within a given pair of compounds is indicative of a similar pattern of response across the cell lines, whereas a low, statistically nonsignificant coefficient indicates that the two compounds are acting in different ways. Spearman rank analysis for the pair **1**/**3** gave an r_s value of 0.98 ($p = 0.0001$), thus indicating a good correlation. Interestingly, Spearman rank analysis for the pairs **1**/*cis*-DDP and **3**/*cis*-DDP gave r_s values of 0.90 ($p = 0.0003$) and 0.84 ($p = 0.001$), respectively, the good rank correlations indicating that substitution of acetonimine for ammine does not affect the growth inhibitory profile of platinum complexes with *cis* geometry.

The *trans* platinum complexes *trans*-[$PtCl_2\{HN{=}C(CH_3)_2\}_2$] (**2**) and *trans*-[$PtCl_2(NH_3)\{HN{=}C(CH_3)_2\}$] (**4**) (note that *trans* complexes are indicated by even numbers) exhibited a remarkable growth inhibitory activity with respect to *trans*-DDP, the mean IC_{50} (μM) values being 10.6 (3.2−22), 26 (8.2−77), and 164.3 (14−376) for **2**, **4**, and *trans*-DDP, respectively. Complexes **2** and **4** showed a comparable efficacy toward OVCAR-8 cells, but **2** was over 2-fold more active than **4** toward the remaining tumor cells. As to the growth inhibitory profile, both **2** and **4** showed a similar pattern of response across the cell lines, the Spearman rank analysis giving an r_s value of 0.9 ($p = 0.0003$). Interestingly, Spearman rank analysis for the pairs **2**/*trans*-DDP and **4**/*trans*-DDP gave r_s values of 0.31 ($p = 0.34$) and 0.36 ($p = 0.27$), the poor rank correlations indicating that substitution of acetonimine for ammine deeply modifies the activity profile of complexes with *trans* geometry.

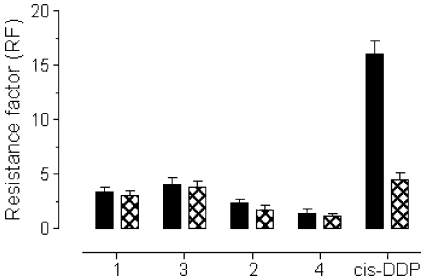


Figure 3. Cross-resistance profiles for A2780cisR versus A2780 (solid boxes) and 41McisR versus 41M (crosshatched boxes) of complexes **1**−**4** and *cis*-DDP. Resistance factor (RF) = IC_{50} resistant cells/IC_{50} sensitive cells. Columns = mean values from at least three experiments; bars = SD.

The growth inhibitory effect of platinum complexes **1**−**4** was evaluated also toward ovarian cancer cells characterized by acquired resistance to cisplatin (Table 2), and the resistance factors (RF = IC_{50} resistant cells/IC_{50} sensitive cells) for A2780cisR/A2780 and 41McisR/41M pairs are shown in Figure 3. In the A2780cisR/A2780 pair, the resistance factors of complexes **1** and **3** were considerably lower than that of cisplatin, (RF = 3.3 ± 0.5, 4 ± 0.7, and 16 ± 1.2 for **1**, **3**, and cisplatin, respectively); even lower were the resistance factors of **2** and **4** (RF = 2.4 ± 0.3 and 1.4 ± 0.4, respectively), complex **4** showing no cross-resistance. In the 41McisR/41M pair (RF of cisplatin = 4.5 ± 0.6), relatively high resistance factors were observed for compounds **1** and **3**, whereas no cross-resistance was observed for compounds **2** and **4** (RF = 3 ± 0.5, 3.8 ± 0.6, 1.7 ± 0.4, and 1.1 ± 0.3 for **1**, **3**, **2**, and **4**, respectively).

Conclusions

Although platinum(II)-bound NH_3 is considered to be an inert ligand, we have shown that in particular circumstances it can undergo reaction. The role of the *trans* ligand appears to be important: the greater the *trans* effect of this ligand (I > Cl >

Table 3. In Vitro Growth Inhibitory Activity of *trans*-Platinum Complexes in a Panel of Human Ovary, Colon, Lung, and Breast Cancers[a]

complex	IC_{50}	RF A2780cisR/A2780	RF 41McisR/41M
trans-$[PtCl_2\{HN{=}C(CH_3)_2\}_2]$[b]	10.6 (3.2-22)	2.4 ± 0.3	1.7 ± 0.4
trans-$[PtCl_2(NH_3)\{HN{=}C(CH_3)_2\}]$[b]	26 (8.2-77)	1.4 ± 0.4	1.1 ± 0.3
trans-$[PtCl_2(E\text{-}HN{=}C(OCH_3)CH_3)_2]$[c]	6.3 (1.5−19)	7.2 ± 0.6	1.9 ± 0.4
trans-$[PtCl_2(NH_3)(E\text{-}HN{=}C(OCH_3)CH_3)]$[d]	16.5 (2.8−43)	3.8 ± 0.6	1.4 ± 0.5
trans-$[PtCl_2(NH_3)(Z\text{-}HN{=}C(OCH_3)CH_3)]$[d]	34.7 (1.3−120)	5.4 ± 0.4	2.5 ± 0.4
trans-$[PtCl_2(NH_3)(N{=}C(CH_3)OCH_2CH_2)]$[d]	4.1 (1.5−8.1)	2.5 ± 0.5	2 ± 0.3
trans-$[PtCl_2(NH_3)(N{=}C(OCH_3)CH_2CH_2CH_2)]$[d]	9.9 (5−16.4)	3 ± 0.5	1.5 ± 0.4
trans-$[PtCl_2(NH_3)(NH_3)]$[d]	164.3 (14−376)	9.6 ± 1.1	1.8 ± 0.2

[a] Mean value and minimum and maximum values (in parentheses) of IC_{50} are given in μM. RF (resistance factor) is the ratio IC_{50} resistant cells/IC_{50} sensitive cells. [b] This paper. [c] Unpublished data. [d] Data from ref 9.

NH_3), the greater the reactivity of the substrate. Thus spontaneous conversion of the ammine into acetonimine in neat acetone occurs only for the *cis* iodo species. ^{1}H NMR chemical shift values indicate a linear dependence of the deshielding of the ammine protons upon the labilizing effect of the *trans* ligand. Therefore a good *trans* labilizing ligand such as iodide can lower the pK_a of the *trans*-coordinated ammine to such an extent to promote its spontaneous condensation with acetone solvent.

As far as the tumor cell growth inhibitory activity of platinum-acetonimine and platinum-ammine/acetonimine complexes with *cis* or *trans* geometry is concerned, several interesting conclusions can be drawn from the obtained results. First, substitution of one or two ammine ligands by acetonimine produces strikingly different effects depending upon the complex geometry. In the case of *cis* geometry, the inhibitory potency is decreased with respect to *cis*-DDP, and the bisacetonimine complex is less active than the ammine/acetonimine complex. In contrast, in the case of *trans* geometry the inhibitory potency is increased with respect to *trans*-DDP, and the bisacetonimine complex is more active than the ammine/acetonimine complex. Second, bisacetonimine and ammine/acetonimine *cis*-compounds are characterized by an activity profile similar to that of *cis*-DDP; however, they are able to circumvent, at least partially, the cisplatin resistance of A2780cisR cells but not that of 41McisR cells. Since cisplatin resistance is mediated by a multifocal mechanism (reduced accumulation, increased levels of glutathione, and increased repair of platinum-DNA adducts) in A2780cisR cells[33] and by reduced drug accumulation in 41McisR cells,[34] this result suggests that acetonimine ligands modify more of the DNA and/or detoxifying interactions of *cis* complexes than their cellular uptake. Third, bisacetonimine and ammine/acetonimine *trans* compounds have an activity profile different from that of *trans*-DDP and are able to circumvent (either partially or completely) the cisplatin resistance depending upon both multifocal mechanism and reduced accumulation. These findings indicate that substitution of acetonimine for ammine dramatically modifies the antitumor properties of *trans* complexes and, as already observed for platinum-iminoether complexes, determines the activation of the *trans* geometry.

The tumor cell growth inhibitory potency of platinum-acetonimine complexes appears to be very similar to that of corresponding platinum complexes with iminoethers or iminoether-like ligands, as summarized in Table 3. Furthermore, platinum-ketimine and platinum-iminoether complexes have the same activity profile ($r_s = 0.9$, $p = 0.0005$). It can be concluded that ketimines and iminoethers are bioisosteric structures.[36] The exchange of the alkoxy group of the iminoether for the alkyl group of the ketimine could induce significant modifications in terms of electronic distribution, chemical reactivity, lipophilicity, and hydrogen bonding capacity. However, most of the investigations carried on with iminoethers compounds concerned ligands with *E* configurations in which the alkoxy group is *trans* to platinum, with respect to the C═N double bond, and protruding outside the platinum-coordination shell, therefore not directly interfering with other platinum-bound moieties. This might explain the strong analogy in biological activity of iminoethers and acetonimine derivatives notwithstanding the chemical differences pointed out above.

From a chemical point of view, platinum complexes with ketimines are more amenable than iminoether derivatives to an extensive investigation not only for the simplicity of their synthesis and the greater number of variants that can be obtained starting from ammine complexes of platinum and commercially available ketones, but also for the absence of isomerism about the azomethine double bond (in the case of symmetrical ketones) and the greater resistance to hydrolyses.

Experimental Section

Instrumental Measurements. NMR spectra were run on a Bruker AVANCE DPX-WB 300 MHz instrument at room temperature (22 °C), and data are presented in Table 1. Standard Bruker automation programs were used for two-dimensional NMR experiments. ^{1}H chemical shifts were referenced to TMS by using the residual protic peak of the solvent as internal reference (2.04 ppm for acetone-d_6). IR spectra were obtained with a Perkin-Elmer Spectrum One infrared spectrophotometer using KBr as a solid support for pellets. Elemental analyses were performed with a Carlo Erba elemental analyzer model 1106 instrument.

Preparation of the Complexes. The complexes *cis*- and *trans*-$[PtCl_2(NH_3)_2]$ and *cis*-$[PtI_2(NH_3)_2]$ were prepared as already reported.[37,38] The same procedure was adopted for preparing the ^{15}N enriched complex *cis*-$[PtI_2(^{15}NH_3)_2]$ ($^{15}NH_3$ was obtained from $^{15}NH_4Cl$ by treatment with a stoichiometric amount of KOH).

***cis*-$[PtCl_2\{HN{=}C(CH_3)_2\}_2]$ (1).** *cis*-$[PtCl_2(NH_3)_2]$ (0.486 g, 1.62 mmol) suspended in 30 mL of acetone/methanol (2:1, v/v) was treated with KOH (0.100 g, 1.78 mmol) and the mixture left under stirring at 25 °C for 1 h. The pale yellow precipitate was isolated by filtration of the mother liquor, washed with methanol, and dried (yield 60%). The compound decomposes above 184−186 °C without melting. Anal. Calcd. for $C_6H_{14}Cl_2N_2Pt$: C, H, N. IR (KBr pellet, cm^{-1}) stretching wavenumbers = 3239 (s, N−H), 1670 and 1651 (m, C═N), and 323 (m, Pt−Cl).

***trans*-$[PtCl_2\{HN{=}C(CH_3)_2\}_2]$ (2).** *trans*-$[PtCl_2(NH_3)_2]$ (0.303 g, 1.01 mmol) suspended in 30 mL of acetone/methanol (2:1, v/v) was treated with KOH (0.200 g, 3.56 mmol) and the mixture left under stirring at 25 °C for 20 h. The solid was separated by filtration of the mother liquor, dried, and then extracted with $CHCl_3$ to remove insoluble KOH. The yellow solution, taken to dryness by evaporation of the solvent under reduced pressure, afforded a yellow solid which proved to be the desired compound (yield 57%). The compound decomposes above 212−215 °C without melting. Anal. Calcd. for $C_6H_{14}Cl_2N_2Pt$: C, H, N. IR (KBr pellet, cm^{-1}) stretching wavenumbers = 3224 (s, N−H), 1670 and 1651 (m, C═N), and 344 (m, Pt−Cl).

***cis*-[PtCl$_2$(NH$_3$){HN=C(CH$_3$)$_2$}] (3)** was prepared by a two-step process that contemplates first the preparation of the corresponding iodospecies *cis*-[PtI$_2$(NH$_3$){HN=C(CH$_3$)$_2$}] (**3′**) and then its conversion to the chlorospecies.

***cis*-[PtI$_2$(NH$_3$){HN=C(CH$_3$)$_2$}] (3′).** *cis*-[PtI$_2$(NH$_3$)$_2$] (1.004 g, 2.08 mmol) dissolved in acetone (180 mL) was treated with a solution of KOH (0.046 g, 1.4 mmol, in 4 mL of water) and the mixture was stirred at 25 °C for ca. 1 min. After addition of water (70 mL), the solution was concentrated to small volume (5 mL) and meanwhile a solid precipitated. This was collected by filtration of the mother liquor, washed with water, and dried in a stream of dry air. The solid proved to be a mixture of *cis*-[PtI$_2$(NH$_3$){HN=C(CH$_3$)$_2$}] (**3′**) and *cis*-[PtI$_2$(HN=C(CH$_3$)$_2$}$_2$] (**1′**). The separation of the two compounds was accomplished taking advantage of their different solubility in CHCl$_3$. The fraction insoluble in CHCl$_3$ was the ammine/acetonimine compound **3′** (yield 50%), while the fraction soluble in CHCl$_3$ was the diacetonimine species **1′**. The compound decomposes above 139−140 °C with melting. Anal. Calcd. for C$_3$H$_{10}$I$_2$N$_2$Pt (**3′**): C, H, N. IR (KBr pellet, cm^{-1}) stretching wavenumbers = 3215 and 3300−3050 (s, N−H of acetonimine and ammine ligands, respectively) and 1645 (m, C=N).

***cis*-[PtCl$_2$(NH$_3$){HN=C(CH$_3$)$_2$}] (3).** *cis*-[PtI$_2$(NH$_3$){HN=C(CH$_3$)$_2$}] (**3′**) (0.601 g, 1.15 mmol) suspended in acetone (70 mL) was treated with a solution of AgNO$_3$ (0.390 g, 2.30 mmol, in 2 mL of water) and kept under stirring at room temperature and in the dark for 2 h. The yellow solid residue (AgI) was removed by filtration of the mother liquor, and the solution was evaporated to dryness. The oily residue was dissolved in water (80 mL) and treated with KCl (1.00 g, 13.4 mmol). Concentration of the solution to ca. 5 mL under reduced pressure afforded a yellow solid which proved to be the desired compound. This was collected by filtration of the mother liquor and dried in a stream of dry air (yield 55%). The compound decomposes above 188−191 °C without melting. Anal. Calcd. for C$_3$H$_{10}$Cl$_2$N$_2$Pt (**3**): C, H, N. IR (KBr pellet, cm^{-1}) stretching wavenumbers = 3234 and 3350−3050 (s, N−H of acetonimine and ammine ligands, respectively), 1655 (m, C=N), and 328 and 310 (m, Pt−Cl).

***trans*-[PtCl$_2$(NH$_3$){HN=C(CH$_3$)$_2$}] (4)** was obtained by a three-step process which contemplates conversion of *cis*-[PtI$_2$(NH$_3$)$_2$] into *cis*-[PtI$_2$(HN=C(CH$_3$)$_2$}$_2$] (**1′**), conversion of **1′** into *trans*-[PtI$_2$(NH$_3$){HN=C(CH$_3$)$_2$}] (**4′**), and finally conversion of the iodospecies **4′** into the chlorospecies **4**.

***cis*-[PtI$_2$(HN=C(CH$_3$)$_2$}$_2$] (1′).** *cis*-[PtI$_2$(NH$_3$)$_2$] (0.190 g, 0.393 mmol) suspended in 30 mL of acetone was treated with KOH (0.080 g, 1.4 mmol), and the mixture was kept under stirring at 25 °C for 5 min. Solid KOH was removed by filtration, and the resulting yellow solution was treated with water (ca.50 mL). Addition of water caused the formation of a yellow precipitate which was collected by filtration of the mother liquor, washed with water, and dried in a stream of dry air (yield 70%). The compound decomposes above 142−144 °C without melting. Anal. Calcd. for C$_6$H$_{14}$I$_2$N$_2$Pt: C, H, N. IR (KBr pellet, cm^{-1}) stretching wavenumbers = 3213 (s, N−H) and 1650 (m, C=N).

***trans*-[PtI$_2$(NH$_3$){HN=C(CH$_3$)$_2$}] (4′).** *cis*-[PtI$_2$(HN=C(CH$_3$)$_2$}$_2$] (**1′**) (0.102 g, 0.181 mmol) dissolved in acetone (10 mL) was treated with a solution of AgNO$_3$ (0.095 g, 0.37 mmol) and kept under stirring at room temperature and in the dark for 2 h. The formed yellow solid (AgI) was removed by filtration of the mother liquor, and the solution evaporated to dryness. The residue was dissolved in water (10 mL) and treated with concentrated NH$_3$ (7.64 M, 2 mL). After the mixture was stirred for 12 h at room temperature, KI (0.306 g) was added. Concentration of the solution to ca. 1 mL under reduced pressure afforded a yellow solid. This resulted to be a mixture of two compounds which could be separated by chromatography on an open column of silica gel using acetone/dichloromethane (8:2 v/v) as eluant. The first eluted fraction contained the desired compound **4′** (yield 20%). The second eluted fraction contained the starting complex **1′** (yield 15%). The compound decomposes above 180−182 °C without melting. Anal. Calcd. for C$_3$H$_{10}$I$_2$N$_2$Pt (**4′**): C, H, N. IR (KBr pellet, cm^{-1}) stretching wavenumbers = 3259 and 3350−3050 (s, N−H of acetonimine and ammine ligands, respectively) and 1649 and 1651 (m, C=N).

***trans*-[PtCl$_2$(NH$_3$){HN=C(CH$_3$)$_2$}] (4).** *trans*-[PtI$_2$(NH$_3$){HN=C(CH$_3$)$_2$}] (**4′**) (0.068 g, 0.13 mmol) suspended in acetone (10 mL) was treated with a solution of AgNO$_3$ (0.044 g, 0.26 mmol, in 1 mL of water). The mixture was kept under stirring at room temperature and in the dark for 2 h. After removal of insoluble AgI by filtration, the resulting solution was taken to dryness under reduced pressure, and the oily residue was dissolved in water (20 mL) and treated with KCl (0.050 g, 0.60 mmol). Concentration of the solution (kept at 55 °C) to ca. 1 mL under reduced pressure afforded a yellow solid of the desired compound. This was collected by filtration of the mother liquor, washed with cold water, and dried in a stream of dry air (yield 56%). The compound decomposes above 183−185 °C without melting. Anal. Calcd. for C$_3$H$_{10}$Cl$_2$N$_2$Pt (**4**): C, H, N. IR (KBr pellet, cm^{-1}) stretching wavenumbers = 3236 and 3350−3050 (s, N−H of acetonimine and ammine ligands, respectively), 1649 (m, C=N), and 336 (s, Pt−Cl).

***trans*-[PtI$_2$(HN=C(CH$_3$)$_2$}$_2$] (2′).** This compound was prepared for completing the series of iodo compounds. A suspension of *trans*-[PtCl$_2$(HN=C(CH$_3$)$_2$}$_2$] (0.044 gr, 0.12 mmol) in water (10 mL) was treated with KI (0.192 g, 1.16 mmol) and kept under stirring at room temperature for 20 h. The yellow solid residue was collected by filtration of the mother liquor, washed with water, and dried in a stream of dry air (yield 60%). The compound decomposes above 232−234 °C without melting. Anal. Calcd. for C$_6$H$_{14}$I$_2$N$_2$Pt (**2′**): C, H, N. IR (KBr pellet, cm^{-1}) stretching wavenumbers = 3217 (s, N−H) and 1667 and 1650 (w/m, C=N).

Tumor cell lines and in Vitro Growth Inhibition Assay. Tumor cell lines representative of ovary (OVCAR-8, SK-OV-3), colon (COLO-205, HCT-116, KM12), lung (A549/ATCC, NCI-H460), and breast (MCF7, MDA) human cancers were obtained from the National Cancer Institute, Biological Testing Branch (Frederick, MD), and maintained in the logarithmic phase at 37 °C in a 5% CO_2 humidified air in RPMI 1640 medium supplemented with 10% foetal calf serum, 2 mM glutamine, penicillin (100 U/mL), and streptomycin (0.1 mg/mL). In addition, two pairs of human ovarian cancer cell lines (parent line from untreated patients, and derived cisplatin-resistant subline), kindly supplied by Dr. L. Kelland (The Institute of Cancer Research, Surrey, U.K.), were used: A2780/A2780cisR and 41M/41McisR. Cisplatin resistance of A2780cisR cells is of multifocal origin, depending upon reduced drug uptake, increased levels of glutathione, and increased DNA repair.[33] Cisplatin resistance of 41McisR cells is mediated mainly by reduced drug uptake.[34] A2780/A2780cisR and 41M/41McisR were maintained at 37 °C in a 10% CO_2 humidified air in Dulbecco's Modified Eagle medium (DMEM) containing 10% heat-inactivated foetal bovine serum, 2 mM glutamine, 10 μg/mL insulin, 0.5 μg/mL hydrocortisone, 2.5 μg/mL amphoterycin B, and 50 μg/mL gentamicin. All culture media and reagents were from Euroclone (Paignton, U.K.) and Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany).

The growth inhibitory effect of compounds under investigation was evaluated by using the Sulforhodamine-B (SRB) assay.[39] Briefly, cells were seeded into 96-well microtiter plates in 100 μL of the appropriate culture medium at plating densities ranging from 1000 to 12000 cells/well depending upon the doubling time of individual cell lines. After seeding, microtiter plates were incubated at 37 °C for 24 h prior to addition of the compounds. After 24 h, several samples of each cell line were fixed in situ with cold trichloroacetic acid (TCA), to represent a measurement of the cell population at the time of compound addition. The testing compounds (weighted amount in the order of 1.00 mg) were freshly dissolved in culture medium and stepwise diluted to the desired final concentrations (complexes **1**−**4**, 0.09−100 μM; *cis*-DDP, 0.02−25 μM; and *trans*-DDP, 12.5−400 μM). After the addition of different compound concentrations to quadruplicate wells, the plates were further incubated at 37 °C for 96 h. Cells were fixed in situ by the gentle addition of 50 μL of cold 50% (w/v) TCA (final concentration, 10%) and incubated for 1 h at 4 °C. The supernatant

was discarded, and the plates were washed 4 times with tap water and air-dried. Sulforhodamine-B solution (100 μL) at 0.4% (w/v) in 1% acetic acid was added to each well, and plates were incubated for 30 min at room temperature. After staining, unbound dye was removed by washing 5 times with 1% acetic acid and the plates were air-dried. Bound stain was then solubilized with 10 mM trizma base, and the absorbance was read on an automatic plate reader at 515 nm. The compound concentration able to inhibit cell growth by 50% ($IC_{50} \pm SD$) was then calculated from semilogarithmic dose−response plots.

Acknowledgment. The authors thank the Ministero dell'Istruzione, Università e Ricerca (MIUR), Rome (Cofin N. 2004059078_006), the EC (COST Chemistry projects D20/0001/2000 and D20/0003/01), and the University of Bari for financial support. Dr. Francesco Cannito is gratefully acknowledged for his assistance in the preparation of the manuscript.

Supporting Information Available: Table S1 listing elemental analysis results of *cis*-$[PtCl_2\{HN{=}C(CH_3)_2\}_2]$ (**1**), *trans*-$[PtCl_2\{HN{=}C(CH_3)_2\}]$ (**2**), *cis*-$[PtI_2(NH_3)\{HN{=}C(CH_3)_2\}]$ (**3′**), *cis*-$[PtCl_2(NH_3)\{HN{=}C(CH_3)_2\}]$ (**3**), *cis*-$[PtI_2\{HN{=}C(CH_3)_2\}_2]$ (**1′**), *trans*-$[PtI_2(NH_3)\{HN{=}C(CH_3)_2\}]$ (**4′**), *trans*-$[PtCl_2(NH_3)\{HN{=}C(CH_3)_2\}]$ (**4**), and *trans*-$[PtI_2\{HN{=}C(CH_3)_2\}_2]$ (**2′**). This material is available free of charge via the Internet at http://pubs.acs.org.

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JM050986T