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Synthesis, Characterization, and In Vitro Antitumor Activity of New Amidineplatinum(II) Complexes Obtained by Addition of Ammonia to Coordinated Acetonitrile

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Dedicated to professor Jan Reedijk on the occasion of his 65th birthday and retirement

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New *cis*- and *trans*-dichloridoplatinum(II) complexes, which contain two amidine ligands or one amidine and one ammine ligand, *cis*- and *trans*-[$PtCl_2${(*Z*)-HN=C(NH_2)CH_3}$_2$] (**1**) and *cis*- and *trans*-[$PtCl_2$(NH_3){(*Z*)-HN=C(NH_2)CH_3}] (**2**), have been prepared from the corresponding nitrile complexes by amminolysis in thf solution. All synthesized compounds were characterized by elemental analysis, ESI-MS, and IR and NMR spectroscopy. Amidines are isosters of iminoethers and ketimines. The *trans* isomers of the platinum complexes of the latter are endowed with an unexpectedly high antitumor activity. An important feature of complexes **1** and **2**, as compared to iminoethers, is the exclusive preference for the *Z* configuration of the amidine ligand(s). The tumor cell growth inhibitory potency of the amidine compounds was tested towards a pair of human ovarian tumor cell lines A2780 (cancer cells sensitive to cisplatin) and A2780cisR (cancer cells with acquired resistance to cisplatin), and compared to that of cisplatin. From the obtained results it appears that the resistance factor is lower for *cis*-amidine complexes as compared to cisplatin, and for *trans* compounds it is lower as compared to *cis* compounds.

Introduction

Since Rosenberg's discovery of the anticancer activity of cisplatin and the inactivity of its *trans* isomer,[1,2] the *cis* geometry of the leaving ligands was considered a necessary prerequisite for antitumor activity. Moreover, the discovery that some ligands (planar aromatic N-donor heterocycles, imino ligands, and sterically hindered aliphatic amines) are able to activate the *trans* geometry of platinum(II) compounds towards antitumor activity represented an unexpected, but desirable, breakthrough and led to intense research in this new field.[3–4]

Among the active *trans*-platinum compounds, the iminoether complex *trans*-[$PtCl_2${(*E*)-HN=C(OCH_3)CH_3}$_2$] (*trans*-*EE*) was the first one that has proven significant in vivo antitumor activity towards leukaemic and solid murine tumors.[5,6] Moreover, these species was shown to form stable monofunctional adducts with purine residues of DNA, which were able to stop DNA and RNA polymerases.[7]

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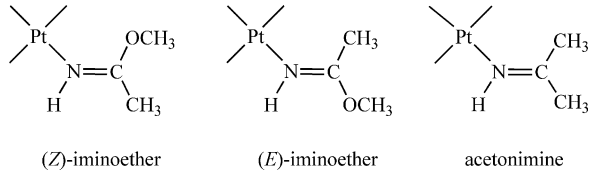
Subsequently, it was demonstrated that also the complexes *trans*-[$PtCl_2$(NH_3){(*E*,*Z*)-HN=C(OCH_3)CH_3}] (*trans*-*E*,*Z*), bearing only one molecule of iminoether, were endowed with relevant antitumor activity.[8]

Platinum(II) iminoether compounds could be prepared by alcoholysis of the precursor nitrile complexes. The reaction led initially to the formation of the kinetically favored *Z* configuration of the iminoether ligand(s). Subsequently, if the reaction medium was kept slightly basic, isomerization to the thermodynamically favored *E* configuration of the iminoether(s) was observed (Scheme 1).[9]



(*Z*)-iminoether (*E*)-iminoether acetonimine

Scheme 1. Molecular sketches of iminoether and acetonimine complexes.

The complication arising from possible *E*/*Z* isomerization of the ligands can be avoided by the use of other types of imino ligands, such as acetonimine (Scheme 1).[4]

Preliminary data have shown that acetonimine ligands, like iminoethers, are able to activate the *trans* geometry of platinum(II) complexes towards antitumor activity.[10]

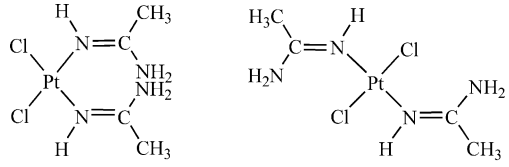
The very promising results obtained with iminoethers and acetonimines prompted us to extend the investigation to other isosteric ligands, such as amidines, which, like the structurally analogous iminoether and ketimine ligands, coordinate to platinum through the imino nitrogen atom.

Amidines can be formed by addition of amines to coordinated nitriles, and complexes resulting from the addition of primary or secondary amines to coordinated nitriles have already been reported (i.e. *cis*- and *trans*-$[PtCl_2\{HN{=}C(NRR')R''\}_2]$ with R = H, CH_3, Et, *i*Pr, or *t*Bu; R′ = CH_3, Et, or *t*Bu; R″ = CH_3 or Ph).[11–13]

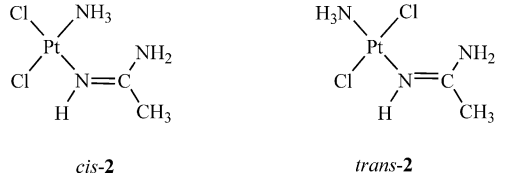
Amidines, like iminoethers, have the possibility of *E*/*Z* configuration; however, when the size and number of substituents are small (R = H and R′ = R″ = CH_3), the *Z* configuration is not only kinetically favored, but also thermodynamically more stable.[14] On this basis, the *Z* configuration was expected to be the only form present in the case of unsubstituted amidines (R = R′ = H).

However, the reaction of gaseous ammonia (required for the preparation of unsubstituted amidines) with a nitrile precursor complex such as $[PtCl_2(N{\equiv}CR)_2]$ (R = CH_3, Ph, CH_2Ph), even performed at low temperature (–10 °C), leads to the formation of $[PtCl(NH_3)\{(Z)\text{-}HN{=}C(NH_2)R\}_2]Cl$ species, in which an ammine molecule has also displaced a chlorido ligand.[15]

In this paper, we report on the synthesis and characterization of new *cis*- and *trans*-bis(amidine)dichloridoplatinum(II) and (amidine)(amine)dichloridoplatinum(II) complexes as sketched in Scheme 2.



cis-**1** *trans*-**1**



cis-**2** *trans*-**2**

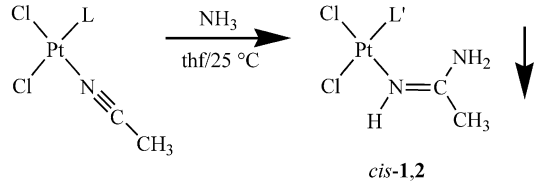
Scheme 2. Molecular structures of the newly synthesized bis(amidine) and (ammine)(amidine) platinum(II) complexes having *cis* and *trans* geometry.

Moreover, the tumor cell growth inhibitory potency of the newly synthesized compounds has been tested towards a pair of human ovarian tumor cell lines (A2780/A2780cisR), one sensitive and the other resistant to cisplatin, and compared to that of the reference compound cisplatin.

Results and Discussion

Synthesis

The bis(amidine) and the (ammine)(amidine) complexes of platinum(II) with *cis* geometry were prepared by addition of aqueous NH_3 to the corresponding bis(nitrile) or (ammine)(nitrile) species in thf solution (Scheme 3).



cis-**1**,**2**

L = N≡CCH$_3$, NH$_3$

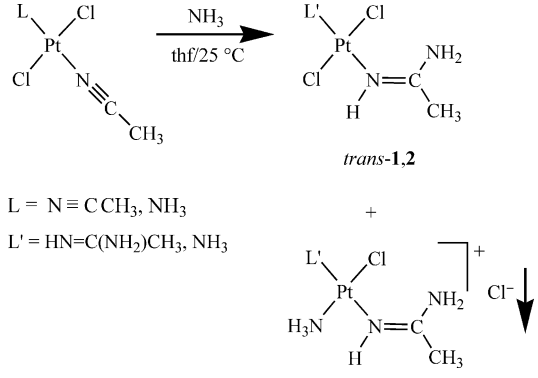
L′ = HN=C(NH$_2$)CH$_3$ (*cis*-**1**), NH$_3$ (*cis*-**2**)

Scheme 3. Reaction scheme for the preparation of *cis* complexes (*cis*-**1** and *cis*-**2**).

The reaction products (*cis*-**1** and *cis*-**2**) are insoluble in thf and form a precipitate that can be recovered by filtration of the mother liquor. The immediate precipitation of the formed amidine complex from solution prevents ammonia, present in excess, from displacing a chlorido ligand.

In the case of *trans* isomers (*trans*-**1** and *trans*-**2**), the reaction products are soluble in thf, therefore they can react further with excess ammonia by chlorido substitution to form the complex salts *trans*-$[PtCl(NH_3)\{(Z)\text{-}HN{=}C(NH_2)CH_3\}_2]Cl$ and *cis*-$[PtCl(NH_3)_2\{(Z)\text{-}HN{=}C(NH_2)CH_3\}]Cl$, respectively, which precipitate from the solution.

The amount of complex salt formed can be decreased, when the excess of ammonia is reduced (for this purpose ammonia was dissolved in water and aliquots of the water solution added to the solution of the nitrileplatinum complex in thf up to the complete conversion of the nitrile into the amidine), but it cannot be avoided. However, since the ammine-substituted salt is sparingly soluble in thf and can be removed by filtration, the pure amidine compound can be recovered from the solution by evaporation of the solvent under reduced pressure (Scheme 4).



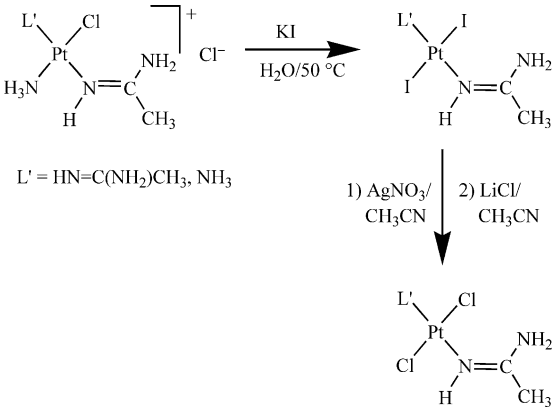
trans-**1**,**2**

L = N≡CCH$_3$, NH$_3$

L′ = HN=C(NH$_2$)CH$_3$, NH$_3$

Scheme 4. Reaction scheme for the preparation of *trans* complexes (*trans*-**1** and *trans*-**2**).

We sought also the conditions for a clean and complete transformation of the salt into the neutral amidine species. For this purpose, the salt was dissolved in water and treated with a slight excess of KI to afford the neutral diiodido species, which separates from solution. The diiodido compound was then converted into the dichlorido species by treatment with $AgNO_3$ (to remove the iodido ligand), followed by addition of LiCl (Scheme 5).



Scheme 5. Reaction scheme for the conversion of cationic complexes *trans*-$[PtCl(NH_3)L'\{HN=C(NH_2)CH_3\}]^+$ into neutral trans species (*trans*-**1** and *trans*-**2**).

Spectroscopy

All amidine complexes were characterized by elemental analysis, electrospray ionization mass spectrometry (ESI-MS), and IR and NMR spectroscopy.

The IR spectra of the amidine complexes are characterized by N–H stretchings in the region 3500–3200 cm^{-1}, C=N stretchings in the region 1670–1640 cm^{-1}, and Pt–Cl stretchings in the region 340–310 cm^{-1}.

The ESI-MS spectra of all complexes in CH_3OH display the parent peak $[M + Na]^+$ whose isotopic distribution is in good agreement with the calculated one.

For the bis(amidine) complexes *cis*-**1** and *trans*-**1**, the parent peak at *m*/*z* = 405 generates a peak at *m*/*z* = 368 by loss of a neutral moiety of 37 Da, corresponding to HCl. In turn, the latter peak, by loss of a fragment of mass 37 Da (corresponding to a second molecule of HCl) or 58 Da [corresponding to $HN=C(NH_2)CH_3$], generates the two peaks at *m*/*z* = 331 and 310, respectively.

For the (ammine)(amidine) complexes *cis*-**2** and *trans*-**2** (parent peak at *m*/*z* = 364), the consecutive loss of a neutral moiety of 17 Da (corresponding to NH_3) and 37 Da (corresponding to HCl) gives rise to the peaks at *m*/*z* = 347 and 310, respectively.

In the ^1H NMR spectra, the amidine ligand exhibits three broad signals of equal intensity in the region 7.4–5.3 ppm (NH protons) and a sharp signal in the region 2.2–1.9 ppm (methyl protons).

The highfield signal of the latter protons is in agreement with the *Z* configuration (CH_3 *trans* to Pt with respect to the C=N double bond). For a CH_3 *cis* to Pt, a strong downfield shift is generally observed.[11,14,15] The assignment of the amino and imino protons of the amidine ligands was carried out with the help of 2D NOESY experiments performed in $[D_6]$acetone for *cis*-**1** and *trans*-**1** (Figure 1). In both cases, the two less shielded NH signals have a strong cross peak (**A**) in phase with the diagonal (exchange cross peak)[16,17] and are assigned to the two nonequivalent ammine protons, consequently the more shielded NH signal is assigned to the imine proton. This latter exhibits a strong cross peak (**B**) with the signal of the methyl protons, clearly indicating that the methyl group and the imine proton are in a *cis* position with respect to the azomethine double bond, thus confirming the *Z* configuration of the amidine ligand.

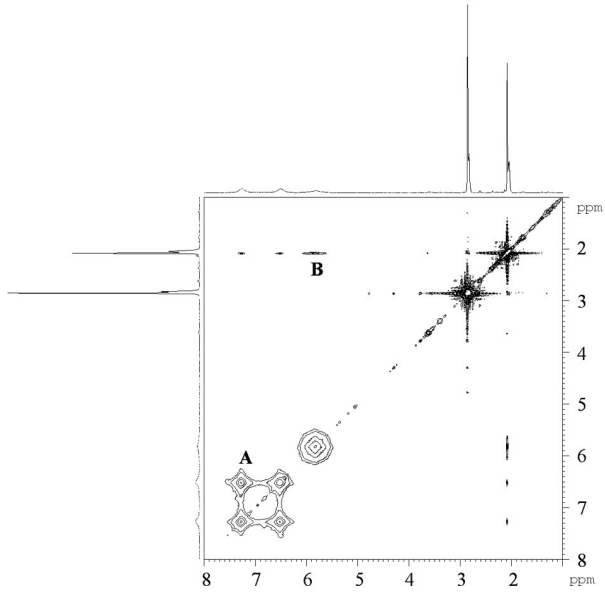


Figure 1. 2D NOESY spectrum in $[D_6]$acetone of complex *trans*-**1** (1.3×10^{-2} M) at 22 °C; the resonance close to 2.90 ppm belongs to trace water, and the small signal close to the CH_3 resonance at δ = 2.04 ppm to protic acetone.

In complexes *cis*-**2** and *trans*-**2**, the additional ammine ligand has resonances in the expected region (4.2–3.3 ppm).

Tumor Cell Lines and In Vitro Growth Inhibition Assay

The in vitro growth inhibitory potency of the newly synthesized *cis* and *trans* bis(amidine) and (ammine)(amidine)-platinum(II) complexes was evaluated against a pair of human ovarian tumor cell lines: A2780 (cancer cells sensitive to cisplatin) and A2780cisR (cancer cells characterized by acquired resistance to cisplatin) and compared to that of cisplatin. The results are shown in Table 1, along with values of the resistance factors (RF = IC_{50} resistant cells/IC_{50} sensitive cells). The *cis*-platinum complexes are less active than cisplatin towards A2780 cells (IC_{50} = 2.3, 1.2, and 0.2 μM, for *cis*-**1**, *cis*-**2**, and cisplatin, respectively) and retain some activity towards A2780cisR cells (IC_{50} = 18.5, 7.1, and 3.2 μM, for *cis*-**1**, *cis*-**2**, and cisplatin, respectively) with a resistance factor that is only slightly lower than that of

cisplatin (RF = 8, 5.9, and 16, respectively). The growth inhibitory potency of the *trans*-bis(amidine) complex (*trans*-**1**, IC_{50} = 3.4 μM) is comparable to that of the *cis* isomer (*cis*-**1**) towards A2780 cells, whereas that of the *trans*-(ammine)(amidine) complex (*trans*-**2**, IC_{50} = 20 μM) is smaller than that of the corresponding *cis* isomer (*cis*-**2**).

Table 1. In vitro tumor cell growth inhibition by *cis*-[$PtCl_2${(*Z*)-HN=C(NH_2)CH_3}$_2$] (*cis*-**1**), *cis*-[$PtCl_2$(NH_3){(*Z*)-HN=C(NH_2)-CH_3}] (*cis*-**2**), *trans*-[$PtCl_2${(*Z*)-HN=C(NH_2)CH_3}$_2$] (*trans*-**1**), *trans*-[$PtCl_2$(NH_3){(*Z*)-HN=C(NH_2)CH_3}] (*trans*-**2**) in comparison to cisplatin.

	IC_{50}[a]		
Complex	A2780	A2780cisR	RF[b]
cisplatin	0.2 ± 0.05	3.2 ± 0.2	16
cis-**1**	2.3 ± 0.6	18.5 ± 2	8
cis-**2**	1.2 ± 0.4	7.1 ± 1.8	5.9
trans-**1**	3.4 ± 0.8	8.6 ± 2	2.5
trans-**2**	20 ± 5	36 ± 7	1.8

[a] Values (means of at least three experiments ± SD) are in μM (96 h drug exposure). [b] Resistance factor (IC_{50} resistant cells/IC_{50} sensitive cells).

Importantly, both *trans*-**1** and *trans*-**2** maintain some activity towards the A2780cisR cells (IC_{50} = 8.6 and 36 μM, respectively). Accordingly, the resistance factors for *trans*-**1** and *trans*-**2** are quite low (RF = 2.5 and 1.8, respectively), and complex *trans*-**2** shows no cross-resistance (RF < 2).

The results of the inhibition assay show that the number of amidine ligands (one or two) has an effect, which depends upon the complex geometry. In the case of *cis* compounds, the inhibitory potency decreases as the number of amidine ligands increases, and the bis(amidine) complex is less active than the (ammine)(amidine) complex. In contrast, in the case of *trans* compounds, the inhibitory potency increases with the number of amidine ligands, and the bis(amidine) complex is more active than the (ammine)-(amidine) complex. Moreover, the effect of the amidine ligand(s) upon the ability to partially circumvent the cisplatin resistance in A2780cisR cells appears to be smaller in the case of *cis* compounds than in the case of *trans* compounds.

The results obtained with analogous compounds containing iminoethers and acetonimines are summarized in Table 2. Overall there is a strict similarity between the three different types of complexes, suggesting that the key factor influencing the activity of these compounds is the imine group. The activity of the compounds presently investigated appears to be in contrast with the reported inactivity of analogous platinum complexes with unsubstituted amidine ligands derived from benzonitrile.[18] Although different cell lines were employed, and this could be the reason for the difference in behavior, we also want to note that the medium used to dissolve the compounds was different. In the work with benzamidine complexes, dimethylsulfoxide was used to dissolve the compounds, and it is well known that this solvent can cause fast solvation with the formation of cationic species that could be biologically inactive. This did not happen in our case, where the compounds were soluble in saline water, which could ensure the integrity of the platinum drug until it approached the cancer cell. The configurational stability and the water solubility are two major advantages of the (unsubstituted) amidine complexes used in this investigation. Hopefully, these advantages will foster further pharmacological investigations.

Conclusions

Amidine chlorido complexes of platinum can be obtained by addition of N-donor nucleophiles (such as ammonia and primary and secondary amines) to coordinated platinum nitriles. However, particularly in the case of platinum(II) substrates having *trans* geometry, the reaction with gaseous ammonia, even at low temperature and in dichloromethane, affords cationic species in which, besides the ammination of the nitrile, also substitution of an ammine for a chlorido ligand has taken place.[15] We have found that, by performing the reaction in tetrahydrofuran, it is possible to obtain the amidine dichlorido complexes in good yields and in a pure form. The *cis* complexes precipitate from thf before they can undergo chlorido substitution, on the other hand the *trans* complexes are soluble in thf while the product of chlorido substitution is not soluble and can be easily removed. These complexes with unsubstituted amidines have invariably *Z* configuration of the organic ligand, and this configuration is not only kinetically but also thermodynamically favored. In addition to their configurational stability, these complexes have also good solubility in water.

In a recent paper reporting the reactions of four iminoether platinum(II) compounds with horse heart cytochrome c (cyt c),[19] the occurrence of extensive hydrolysis/

Table 2. In vitro tumor cell growth inhibition by platinum(II) complexes with iminoether and acetonimine ligands.

	IC_{50}[a]		
Complex	A2780	A2780cisR	RF[b]
cis-[$PtCl_2${HN=C(CH_3)CH_3}$_2$]	1.5 ± 0.3	4.9 ± 1.1	3.2
cis-[$PtCl_2$(NH_3){HN=C(CH_3)CH_3}]	0.3 ± 0.07	0.72 ± 0.02	2.4
trans-[$PtCl_2${(*E*)-HN=C(OCH_3)CH_3}$_2$]	1.5 ± 0.2	10.8 ± 1.8	7.2
trans-[$PtCl_2$(NH_3){(*Z*)-HN=C(OCH_3)CH_3}]	1.3 ± 0.3	7.02 ± 1.3	5.4
trans-[$PtCl_2$(NH_3){(*E*)-HN=C(OCH_3)CH_3}]	2.8 ± 0.7	10.6 ± 2	3.8
trans-[$PtCl_2${HN=C(CH_3)CH_3}$_2$]	4.8 ± 0.2	19.2 ± 1.8	2.4
trans-[$PtCl_2$(NH_3){HN=C(CH_3)CH_3}]	13 ± 1.5	18.2 ± 2.1	1.4

[a] Values (means of at least three experiments ± SD) are in μM (96 h drug exposure). [b] Resistance factor (IC_{50} resistant cells/IC_{50} sensitive cells).

amminolysis (the latter fostered by the presence of ammonium carbonate buffer) of the iminoether ligand(s) and formation of the corresponding amides/amidines was noted. The hydrolysis/amminolysis reaction appeared to be fostered by the presence of the protein; therefore, the occurrence of similar reactions in the biological milieu cannot be excluded. However, as discussed below, the occurrence of such a reaction does not appear to have an effect on the mechanism of action.

The in vitro growth inhibitory potency of the amidine complexes has been evaluated towards a pair of human ovarian tumor cell lines (A2780 and A2780cisR, one sensitive and the other with acquired resistance to cisplatin). The overall behavior resembles the one already observed for isosteric platinum complexes with iminoether and acetonimine ligands.[4–10] The strict analogy between the behavior of the three classes of isosteric platinum complexes indicates that the key feature, as far as the biological activity is concerned, is the common N-bonded imine functionality (Pt–NH=CXR; X = OR′, NR′R′′, and R′ for iminoethers, amidines, and ketimines, respectively; R, R′, and R′′ = organic groups). The nature of X and R can deeply affect the water solubility and the lipophilicity of the drug and plays a role in the pharmacokinetics of the compounds; however, it does not appear to affect the efficacy much. From this point of view, the substitutional lability of the X group, as observed in the case of iminoethers under particular conditions, does not appear to play a major role. Extensive mechanistic investigations performed on iminoether compounds indicate that these drugs form stable monofunctional adducts with DNA, which readily cross-link proteins.[20,21] The formation of DNA–protein crosslinks markedly inhibits not only the DNA synthesis by DNA polymerases, but also the removal of adducts from DNA by the nucleotide excision repair system.[22] Thus, the major role of the imine ligands would be to foster the formation of DNA–protein crosslinks mediated by platinum.

The configurational stability and water solubility of amidine complexes render them amenable for further investigation.

Experimental Section

Instrumental Measurements: NMR spectra were obtained with a Bruker AVANCE DPXWB 300 MHz instrument. Standard Bruker automation programs were used for two-dimensional NOESY experiments. ^{1}H chemical shifts were referenced to tetramethylsilane (tms) by using the residual protic peak of the solvent ($CDCl_3$, [D_6]-acetone, or [D_6]dmso) as internal reference (7.26, 2.04, and 2.49 ppm, respectively). ^{195}Pt chemical shifts were referenced to K_2PtCl_4 (1 M in water, δ = –1614 ppm). IR spectra were obtained with a Perkin Elmer Spectrum One infrared spectrometer using KBr as a solid support for pellets. Elemental analyses were performed with a Carlo Erba Elemental Analyzer model 1106 instrument. ESI-MS was performed with an electrospray interface and an ion trap mass spectrometer (1100 Series LC/MSD Trap system Agilent, Palo Alto, CA).

***cis*-[PtCl$_2${(*Z*)-HN=C(NH$_2$)CH$_3$}$_2$] (*cis*-1):** A suspension of *cis*-[PtCl$_2$(N≡CCH$_3$)$_2$] (0.5 g, 1.4×10^{-3} mol) in thf (100 mL) was treated with a fivefold excess of aqueous NH_3 (0.12 g, 7.0×10^{-3} mol). The reaction mixture was stirred at 25 °C for 24 h. The yellow precipitate of *cis*-[PtCl$_2${(*Z*)-HN=C(NH$_2$)CH$_3$}$_2$] was separated by filtration of the solution, washed with thf, and dried in a stream of dry air. Yield: 0.51 g (95%). ESI-MS (CH_3OH): parent peak at *m*/*z* = 405 corresponding to [M + Na]$^+$. ^{1}H NMR (300 MHz, [D_6]acetone, 22 °C): δ = 7.29 and 6.56 (s, 2H each, NH_2), 6.04 (s, 2 H, NH), 2.07 (s, 6 H, CH_3) ppm. ^{195}Pt NMR (64.3 MHz, [D_6]dmso, 22 °C): δ = –2108 ppm. $C_4H_{12}Cl_2N_4Pt$ (382): calcd. C 12.57, H 3.16, N 14.66; found C 12.55, H 3.33, N 14.50.

***cis*-[PtCl$_2$(NH$_3$){(*Z*)-HN=C(NH$_2$)CH$_3$}] (*cis*-2):** A suspension of *cis*-[PtCl$_2$(NH$_3$)(N≡CCH$_3$)] (0.35 g, 1.1×10^{-3} mol) in thf (70 mL) was treated with a threefold excess of aqueous NH_3 (0.055 g, 3.3×10^{-3} mol). The reaction mixture was stirred at 25 °C for 24 h. The yellow precipitate of *cis*-[PtCl$_2$(NH$_3$){(*Z*)-HN=C(NH$_2$)CH$_3$}] was separated by filtration of the solution, washed with thf, and dried in a stream of dry air. Yield: 0.33 g (88%). ESI-MS (CH_3OH): parent peak at *m*/*z* = 364 corresponding to [M + Na]$^+$. ^{1}H NMR (300 MHz, [D_6]acetone, 22 °C): δ = 7.35 and 6.75 (s, 1 H each, NH_2), 6.28 (s, 1 H, NH), 3.75 (s, 3 H, NH_3), 2.21 (s, 3 H, CH_3) ppm. ^{195}Pt NMR (64.3 MHz, [D_6]dmso, 22 °C): δ = –2089 ppm. $C_2H_9Cl_2N_3Pt$ (341): calcd. C 7.02, H 2.92, N 12.28; found C 7.04, H 2.47, N 11.95.

***trans*-[PtCl$_2${(*Z*)-HN=C(NH$_2$)CH$_3$}$_2$] (*trans*-1):** A suspension of *trans*-[PtCl$_2$(N≡CCH$_3$)$_2$] (1.4 g, 4.0×10^{-3} mol) in thf (170 mL) was treated with a fivefold excess of aqueous NH_3 (0.34 g, 0.02 mol). The reaction mixture was stirred at 25 °C for 2 h. A white precipitate of *trans*-[PtCl(NH$_3$){(*Z*)-HN=C(NH$_2$)CH$_3$}$_2$]Cl formed. This was separated by filtration of the solution, characterized, and then converted into *trans*-**1** as described in the following preparation. Yield: 0.77 g (48%). The yield of salt can be greatly reduced by using a smaller excess of aqueous NH_3. The mother solution was concentrated to dryness under reduced pressure to give yellow *trans*-[PtCl$_2${(*Z*)-HN=C(NH$_2$)CH$_3$}$_2$]. Yield: 0.46 g (30%). ESI-MS (CH_3OH): parent peak at *m*/*z* = 405 corresponding to [M + Na]$^+$. ^{1}H NMR (300 MHz, [D_6]acetone, 22 °C): δ = 7.27 and 6.51 (s, 2H each, NH_2), 5.82 (s, 2 H, NH), 2.09 (s, 6 H, CH_3) ppm. ^{195}Pt NMR (64.3 MHz, [D_6]acetone, 22 °C): δ = –2000 ppm. $C_4H_{12}Cl_2N_4Pt$ (382): calcd. C 12.57, H 3.16, N 14.66; found C 12.72, H 3.19, N 14.29.

Conversion of *trans*-[PtCl(NH$_3$){(*Z*)-HN=C(NH$_2$)CH$_3$}$_2$]Cl into *trans*-[PtCl$_2${(*Z*)-HN=C(NH$_2$)CH$_3$}$_2$] (*trans*-1)

The side product of the previous reaction, *trans*-[PtCl(NH$_3$){(*Z*)-HN=C(NH$_2$)CH$_3$}$_2$]Cl, was characterized by NMR spectroscopy, elemental analysis, and ESI-MS. ESI-MS (CH_3OH): parent peak at *m*/*z* = 363 corresponding to [M]$^+$. ^{1}H NMR (300 MHz, [D_6]-dmso, 22 °C): δ = 7.17 and 7.03 (s, 2H each, NH_2), 6.50 (s, 2 H, NH), 4.04 (s, 3 H, NH_3), 1.93 (s, 6 H, CH_3) ppm. $C_4H_{15}Cl_2N_5Pt$ (399): calcd. C 12.03, H 3.76, N 17.54; found C 11.08, H 3.80, N 17.30. A portion of this product (0.77 g, 1.9×10^{-3} mol) was dissolved in water (35 mL) and treated with an excess of KI (1.28 g, 7.7×10^{-3} mol). The reaction mixture was stirred at 50 °C for 4 h. The orange precipitate of *trans*-[PtI$_2${(*Z*)-HN=C(NH$_2$)CH$_3$}$_2$] was separated by filtration of the solution, washed with water, and dried in a stream of dry air. Yield: 0.54 g (50%). ESI-MS [CH_3OH/CH_3COOH (1:1)]: parent peak at *m*/*z* = 438 corresponding to [M – I]$^+$. ^{1}H NMR (300 MHz, [D_6]acetone, 22 °C): δ = 6.47 (s, 4 H, NH_2), 5.78 (s, 2 H, NH), 2.13 (s, 6 H, CH_3) ppm. $C_4H_{12}I_2N_4Pt$ (565): calcd. C 8.50, H 2.14, N 9.91; found C 8.67, H 2.09, N 9.65.

A suspension of *trans*-[PtI$_2${(*Z*)-HN=C(NH$_2$)CH$_3$}$_2$] (0.54 g, 9.56×10^{-4} mol) in CH_3CN (35 mL) was treated with the stoichiometric amount of $AgNO_3$ (0.32 g, 1.88×10^{-3} mol). The reaction

mixture was stirred at 25 °C for 10 min. The solution was filtered to remove precipitated AgI, treated with excess LiCl (0.81 g, 1.91×10^{-2} mol) and stirred for 30 min at 45 °C. The yellow solution was filtered to remove excess LiCl and concentrated to dryness under reduced pressure to yield the complex *trans*-[$PtCl_2${(*Z*)-HN=C(NH_2)CH_3}$_2$] (*trans*-**1**). Yield 0.24 g (65%).

***trans*-[$PtCl_2(NH_3)${(*Z*)-HN=C(NH_2)CH_3}] (*trans*-2)**

A suspension of *trans*-[$PtCl_2(NH_3)(N{\equiv}CCH_3)$] (0.5 g, 1.5×10^{-3} mol) in thf (150 mL) was treated with a fivefold excess of aqueous NH_3 (0.13 g, 7.5×10^{-3} mol). The reaction mixture was stirred at 25 °C for 24 h. A white precipitate of *cis*-[$PtCl(NH_3)_2$-{(*Z*)-HN=C(NH_2)CH_3}]Cl formed. This was isolated by filtration of the solution and treated as described in the following preparation. Yield: 0.30 g (55%). The yield of salt could be greatly reduced by using a far smaller excess of aqueous NH_3.

The yellow solution was concentrated to dryness under reduced pressure to afford a yellow solid of *trans*-[$PtCl_2(NH_3)${(*Z*)-HN=C(NH_2)CH_3}]. Yield: 0.18 g (35%). ESI-MS (CH_3OH): parent peak at *m*/*z* = 364 corresponding to $[M + Na]^+$. ^{1}H NMR (300 MHz, [D_6]acetone, 22 °C): δ = 7.37 and 6.54 (s, 1 H each, NH_2), 5.86 (s, 1 H, NH), 3.39 (s, 3 H, NH_3), 2.08 (s, 3 H, CH_3) ppm. ^{195}Pt NMR (64.3 MHz, [D_6]acetone, 22 °C): δ = –2042 ppm. $C_2H_9Cl_2N_3Pt$ (341): calcd. C 7.04, H 2.64, N 12.32; found C 6.89, H 2.67, N 11.93.

Conversion of *cis*-[$PtCl(NH_3)_2${(*Z*)-HN=C(NH_2)CH_3}]Cl into *trans*-[$PtCl_2(NH_3)${(*Z*)-HN=C(NH_2)CH_3}] (*trans*-2)

The side product of the previous reaction, *cis*-[$PtCl(NH_3)_2${(*Z*)-HN=C(NH_2)CH_3}]Cl, was fully characterized by ESI-MS (H_2O): parent peak at *m*/*z* = 323 corresponding to $[M]^+$. ^{1}H NMR (300 MHz, [D_6]dmso, 22 °C): δ = 7.32 and 6.73 (s, 1 H each, NH_2), 6.61 (s, 1 H, NH), 4.21 (s, 3 H, NH_3), 3.87 (s, 3 H, NH_3), 1.98 (s, 3 H, CH_3) ppm. $C_2H_{12}Cl_2N_4Pt$ (358): calcd. C 6.70, H 3.35, N 15.64; found C 6.96, H 3.41, N 15.25.

Compound *cis*-[$PtCl(NH_3)_2${(*Z*)-HN=C(NH_2)CH_3}]Cl (0.30 g, 8.4×10^{-4} mol) was dissolved in water (40 mL) and treated with an excess of KI (0.56 g, 3.4×10^{-3} mol). The reaction mixture was stirred at 50 °C for 4 h. The green precipitate of *trans*-[$PtI_2(NH_3)${(*Z*)-HN=C(NH_2)CH_3}] was separated by filtration of the solution, washed with water, and dried in a stream of dry air. Yield: 0.27 g (60%). ESI-MS (CH_3OH): parent peak at *m*/*z* = 506 corresponding to $[M - H - NH_3]^-$. ^{1}H NMR (300 MHz, [D_6]acetone, 22 °C): δ = 6.64 and 6.23 (s, 1 H each, NH_2), 5.87 (s, 1 H, NH), 3.36 (s, 3 H, NH_3), 2.14 (s, 3 H, CH_3) ppm. $C_2H_9I_2N_3Pt$ (524): calcd. C 4.58, H 1.72, N 8.02; found C 4.38, H 1.71, N 7.74.

A suspension of *trans*-[$PtI_2(NH_3)${(*Z*)-HN=C(NH_2)CH_3}] (0.27 g, 5.15×10^{-4} mol) in CH_3CN (20 mL) was treated with the stoichiometric amount of $AgNO_3$ (0.18 g, 1.03×10^{-3} mol). The reaction mixture was stirred at 25 °C for 10 min. The yellow solution was filtered through Celite to separate AgI and then treated with an excess of LiCl (0.44 g, 1.03×10^{-2} mol), and stirred for 30 min at 45 °C. The yellow solution was filtered to separate unreacted LiCl and then concentrated to dryness under reduced pressure to afford a yellow solid of *trans*-[$PtCl_2(NH_3)${(*Z*)-HN=C(NH_2)CH_3}]. Yield: 0.16 g (80%).

Tumor Cell Lines and In Vitro Growth Inhibition Assay: Two human ovarian cancer cell lines (the parent line from untreated patients A2780 and the derived cisplatin-resistant subline 2780cisR), were kindly supplied by Dr. L. Kelland (The Institute of Cancer Research, Surrey, U. K.). The cisplatin resistance of A2780cisR cells is of multifocal origin, depending on reduced drug uptake, increased levels of glutathione, and increased DNA repair.[23] A2780 and A2780cisR cells were maintained at 37 °C in humidified air containing 10% CO_2 in Dulbecco's Modified Eagle medium (DMEM) containing heat-inactivated foetal bovine serum (10%), glutamine (2 mM), insulin (10 μg/mL), hydrocortisone (0.5 μg/mL), amphoterycin B (2.5 μg/mL), and gentamicin (50 μg/mL). All culture media and reagents were from Euroclone (Paignton, U. K.) and Sigma–Aldrich Chemie GmbH (Schnelldorf, Germany). The growth-inhibitory effect of the compounds under investigation was evaluated by using the Sulforhodamine-B (SRB) assay.[24] Briefly, cells were seeded into 96-well microtiter plates in culture medium (100 μL) at a plating density of 1000 cells/well. After seeding, microtiter plates were incubated at 37 °C for 24 h prior to addition of the compounds. After 24 h, 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 compounds to be tested (weight in amounts in the order of 1.00 mg) were freshly dissolved in culture medium and stepwise diluted to the desired final concentrations (complexes 1–2, 0.09–200 μM; cisplatin, 0.09–15 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 slow addition of cold tca [50 μL, 50% (w/v), final concentration 10%] and incubated for 1 h at 4 °C. The supernatant was discarded, and the plates were washed four times with tap water and air-dried. Sulforhodamine-B solution [100 μL, 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 five times with acetic acid (1%), and the plates were air-dried. Bound stain was then solubilized with trizma base (10 mM), 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} ± SD) was then calculated from semilogarithmic dose–response plots.

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