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Synthesis, characterisation and *in-vitro* cytotoxicity of mixed ligand Pt(II) oxadiazoline complexes with hexamethylenetetramine and 7-nitro-1,3,5-triazaadamantane.

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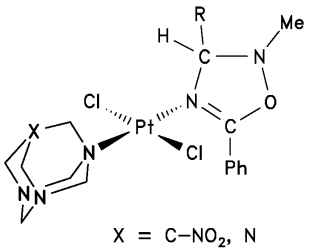
TOC Entry



The synthesis, spectroscopic and DFT-computational characterisation of *trans*-platinum(II) oxadiazoline complexes with one hexamethylenetetramine or 7-nitro-1,3,5-triazaadamantane ligand is described. Some of these complexes are more cytotoxic than cisplatin in *in vitro* tests with the human cancer cell lines HeLa and A549.

Abstract

Trans-platinum(II) oxadiazoline complexes with 7-nitro-1,3,5-triazaadamantane (NO_2-TAA) or hexamethylenetetramine (hmta) ligands have been synthesised from *trans*-[$PtCl_2(PhCN)_2$] *via* cycloaddition of nitrones to one of the coordinated nitriles, followed by exchange of the other nitrile by NO_2-TAA or hmta. Stoichiometric control allows for the selective synthesis of mono- and dinuclear complexes where 7-NO_2TAA and hmta act as mono- and bidentate ligands, respectively. Precursors and the target complexes *trans*-[$PtCl_2$(hmta)(oxadiazoline)], *trans*-[$PtCl_2$(NO_2-TAA)(oxadiazoline)] and *trans*-[{$PtCl_2$(oxadiazoline)}$_2$(hmta)] were characterised by elemental analysis, IR and multinuclear (^{1}H, ^{13}C, ^{195}Pt) NMR spectroscopy. DFT (B3LYP/6-31G*/LANL08) and AIM calculations suggest a stronger bonding of hmta with the [$PtCl_2$(oxadiazoline)] fragment, in agreement with the experimentally observed reactivity in the ligand exchange (hmta > 7-NO_2TAA). Replacement of the nitrile by hmta is predicted more exothermic than that with 7-NO_2-TAA, although the activation barriers are similar. Protonation of the non-coordinated N atoms is anticipated to weaken the Pt-N bond and lower the activation barrier for ligand exchange. This effect might help activate these compounds in a slightly acidic environment such as some tumour tissues.

Ten of the new compounds were tested for their *in vitro* cytotoxicity in the human cancer cell lines HeLa and A549. Some of the mononuclear complexes are more potent than cisplatin, and their activity is still high in A549 where cisplatin shows little effect. The dinuclear complexes are inactive, presumably due to their lipophilicity and reduced solubility in water.

Keywords

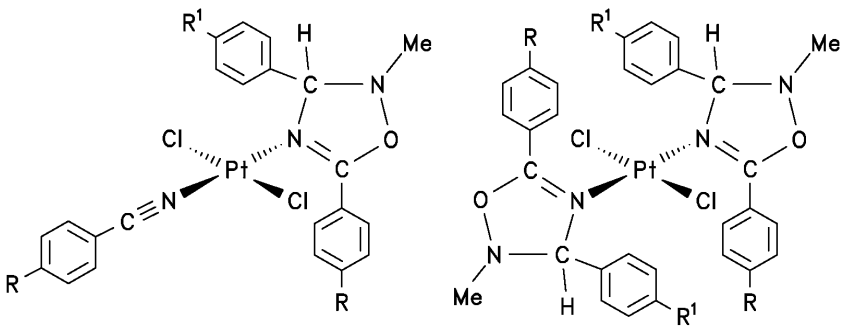
Platinum complexes, oxadiazoline, hexamethylenetetramine, azaadamantane, cancer therapy

Introduction

Cancer chemotherapy of solid tumours relies heavily on the use of platinum-based drugs, namely the globally approved cytotoxic Pt(II) compounds cisplatin, carboplatin and oxaliplatin, together with locally approved derivatives such as lobaplatin, nedaplatin and heptaplatin.[1] Despite their significant therapeutic success there are strong limitations due to the severe side effects experienced by the patient, and the occurrence of intrinsic or acquired

resistance. The latter, in the form of cross resistance, drastically restricts the therapeutic options because cancer cells that acquired resistance to one drug will respond poorly to secondary treatment with other platinum drugs also.

In the search for improved therapeutic methods, much work has gone into the development of new delivery systems for established drugs, but also into the design of new compounds.[1,2] *Trans*-configured Pt(II) compounds received increasing interest when their *in vitro* ability to overcome resistance was recognised. Among the compounds studied there are Pt(II) and Pt(IV) complexes bearing aliphatic or aromatic amines,[3] or higher order nitrogen containing ligands. Pt(II) iminoether complexes[4] have been investigated in much detail with respect to their mechanism of action. A marked cellular uptake and higher degree of DNA platination, together with the formation of mainly monofunctional adducts, seems to evoke DNA damage and intracellular repair mechanisms which are quite different to those caused by cisplatin. Pt(II) bisamidine complexes[5] also show a higher uptake and cellular accumulation than cisplatin, and this has been attributed to the presence of a phenyl group which increases the lipophilicity of the complex. Also the *trans*-configured Pt(II) oxadiazoline complexes, shown in Scheme 1 and studied in our group,[6] are active against a panel of human cancer cells including cisplatin and carboplatin resistant ones, and the IC_{50} values are typically in an acceptable micromolar range, between those of cisplatin and carboplatin. In these compounds, the substitution pattern can be easily varied, allowing for fine tuning of pharmacologically relevant parameters such as solubility and transport properties, or even for introduction of targeting agents that aid the selective uptake in the cancer cells.



Scheme 1. Platinum(II) oxadiazoline complexes with *in-vitro* cytostatic properties.[6]

The synthesis of platinum oxadiazoline complexes follows a straightforward modular scheme where the ligand is built up in the coordination sphere of the metal by cycloaddition of a nitrone to a metal coordinated nitrile, as long as the latter is kinetically sufficiently stable.[7] Both Pt(IV)[8] and Pt(II)[9] complexes are easily accessible and can be interconverted into each other.[10,11] The reaction typically occurs with a high degree of chemo- and stereoselectivity, so that functionalised[12] and chiral complexes[13] are accessible as well. Mixed ligand complexes can be made from suitable precursors bearing one nitrile and one other ligand (e.g. sulfoxide),[13] or by mono-cycloaddition to only one of two initially equivalent nitriles[14] and subsequent ligand exchange.[11]

The latter method lead to complexes bearing a reactive and labile NO_2-TAA ligand,[15] designed to achieve some selectivity in cellular uptake and enhanced reactivity in tumour cells with fairly simple means. The non-coordinated nitrogen atoms are expected to partially protonate in an aqueous medium, to give cationic complexes that are more prone to penetrate the cell membrane. Moreover, protonation has been shown to weaken the coordination to the platinum to make the complex more labile, and also the release of formaldehyde from the ligand is stimulated by protonation. Since tumour tissue is often more acidic than normal tissue, all these effects should be at work, resulting in a higher activity.

In this work, hexamethylenetetamine (hmta, also known as 1,3,5,7-tetraazaadamantane) is explored for a similar purpose, and compared with 7-nitro-1,3,5-triazaadamantane. Hmta is known to coordinate to transition metals in various ways,[16] although only very few reports exist on platinum complexes.[17,18] Hmta is used, as hippurate and other salts, against urinary infections, and the mode of action is assumed to be based on the slow release of formaldehyde.[19] Moreover, hmta is approved in the EU for usage as food preservative (under the name E239),[20] again using the antibiotic activity of formaldehyde released from the compound under acidic conditions. HMTA has also been reported to enhance the sensitivity to cisplatin when co-administered.[21]

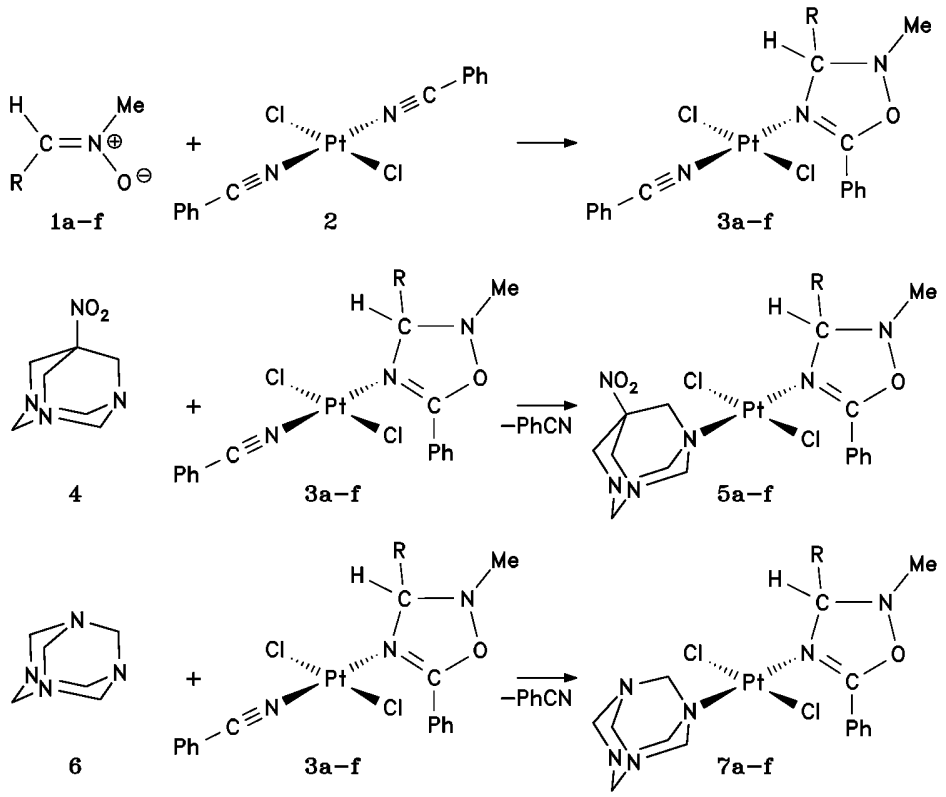
Results and Discussion

In this work, we present the synthesis of new Pt(II) oxadiazoline complexes bearing a 7-nitro-1,3,5-triaza-adamantane ligand (**5b**, **5e, 5f**), the mononuclear hexamethylenetetramine complexes **7a** – **7f** and the corresponding dinuclear species **8a** – **8f**, shown in Scheme 2 and 3. DFT calculations are used to rationalise the reactivity pattern, and the *in vitro* cytotoxicity of selected compounds is assessed.

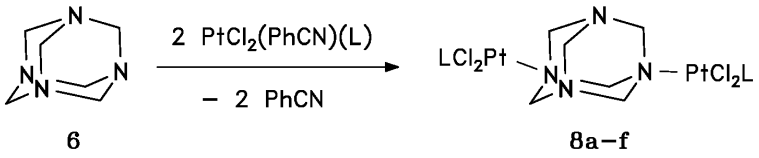
Synthesis of the Pt(II) complexes

The complexes **5a** – **5f** and **7a** – **7f** were synthesised in two steps via the cycloadducts **3a** – **f**, by reaction of *trans*-[$PtCl_2(PhCN)_2$] (**2**) with one equivalent of a nitrone **1a** – **1f**. The mono-cycloadducts *trans*-[$PtCl_2$(PhCN)(oxadiazoline)] **3a** – **3f** were obtained with high selectivity and in good yields. Their spectroscopic properties correspond closely to those described previously for related compounds.[14,15] When **3a** – **3f** are reacted with one equivalent of a tertiary amine such as 7-nitro-1,3,5-triaza-adamantane **4** and hexamethylenetetramine **6**, the benzonitrile ligand is replaced and the mixed ligand oxadiazoline complexes **5a** – **5f** and **7a** – **7f** are formed (see Scheme 2). The reaction with **6** is accompanied by the formation of the dinuclear side products **8a** – **8f**, in which two $PtCl_2$(oxadiazoline) moieties are coordinated to one molecule of **6**, as shown in Scheme 3. This side reaction, however, can be suppressed when an excess (1.5 equivalents) of **6** is used. It is worth mentioning that **6**, even in a three-fold excess, does not replace the oxadiazoline ligand from the platinum complex under the conditions applied, even if the reaction is left for 3 weeks.

The selective synthesis of the dinuclear compounds **8a** – **8f** was achieved when **3a** – **3f** and **6** was used in a 2:1 stoichiometry and the reaction time was extended to 2 weeks. The analogous NO_2-TAA complexes can be prepared from **3a** – **3f** and **4**, but the reaction at room temperature takes 5 weeks to complete. At a higher temperature, formation of the dinuclear complexes is accompanied by a number of unidentified side products, most likely due to the decomposition of the azaadamantane framework. This can be concluded from the appearance of additional signals in the aliphatic range during ^{1}H-NMR monitoring of the reaction.



Scheme 2. Synthesis of platinum(II) oxadiazoline complexes bearing 7-nitro-1,3,5-triaza-adamantane[15] or hexamethylenetetramine ligands (R = 2-methoxyphenyl (**a**), 4-methoxy-phenyl (**b**), 2,6-dimethoxyphenyl (**c**), 2,4,6-trimethoxyphenyl (**d**), 2,3,4-trimethoxyphenyl (**e**) and 3,4,5-trimethoxyphenyl (**f**).

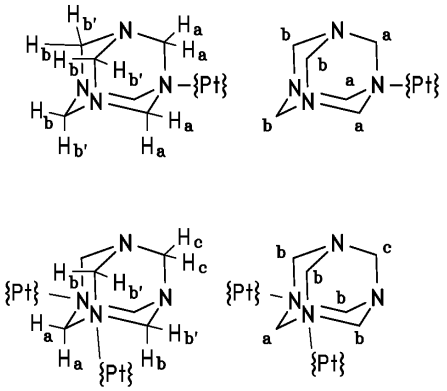


Scheme 3. Synthesis of dinuclear platinum(II) oxadiazoline complexes **8** with the hexa-methylenetetramine ligand **6** and oxadiazoline ligands (abbreviated as L).

The IR spectra of the mononuclear complexes **7a – f** are dominated by the fundamentals of the C-N vibrations of the hexamethylenetetramine ligand. These bands appear at 1234, 992, 808 and 669 cm^{-1} in the free hexamethylenetetramine. In the Pt(II) coordinated species they are split and also experience some shift. Literature data suggest a minor splitting resulting in closely spaced doublets or triplets when hmta acts as a monodentate ligand,[22] whereas

complexes with bidentate bridging hmta ligands show well defined and well separated bands.[23] This, however, seems not to apply as a general rule since compounds **7a – 7f** show clearly separated signals in spite of the monodentate coordination mode. Thus, the resonance at 1234 cm^{-1} splits into two bands and experiences a symmetric shift around the band of the free hmta, whereas those at 992 and 808 cm^{-1} split and shift, the former to higher, the latter to lower wavenumbers. A detailed interpretation, however, is complicated by the presence of signals of the oxadiazoline ligand, among which the C=N and C=C stretch at 1629-1643 and 1593-1610 cm^{-1} can be clearly assigned, together with the characteristic C-H stretching vibration of the OMe groups at 2841-2834 cm^{-1}. The dinuclear complexes **8a – 8f** show relatively similar IR spectra, but the signals attributed to the oxadiazoline ligand are somewhat more intense.

Free hmta is T_d symmetric, resulting in the equivalence of all CH_2 groups in the NMR. Therefore, only one singlet is seen in the ^1H NMR and also only one signal appears in the ^{13}C NMR spectrum. When coordination to one nitrogen atom occurs, the local symmetry of the hmta ligand is C_{3v}, assuming that the rotation around the Pt-N bond is not hindered (and ignoring the C1 symmetry of the $PtCl_2$(oxadiazoline) moiety). In this case, one would expect one singlet for the protons H_a and two doublets for the axial and equatorial protons H_b and $H_{b'}$ in the ^1H NMR and two signals in the ^{13}C NMR, as indicated in Scheme 4. This was indeed observed in the spectra of compounds **7a** – **7f**: The proton signals appear in a range of 4.96 to 5.02 ppm (H_a) and at 4.44 and 4.50 ppm (H_b and $H_{b'}$), at higher and lower field, respectively, as compared to the free ligand (4.72 ppm). The signal at 4.50 ppm was attributed to $H_{b'}$ because it displays an NOE with H_a in the NOESY spectrum. Consequently, the signal at 4.44 ppm that does not show an NOE with H_a is assigned to H_b. The ^{13}C signals are also more and less deshielded (79.4 and 73.0 ppm in the complex, as compared to 74.8 ppm in the free ligand). As a rule, the atoms closer to the Pt coordinated nitrogen appear at lower field, whereas those further away from the coordination site experience a high field shift. The *trans*-configuration of the complexes can be inferred from the absence of NOE signals between the two organic ligands. Also the ^{195}Pt NMR signal would be expected further downfield in the corresponding *cis*-complexes,[24] although this effect can be pretty small.[14]



Scheme 4. ^{1}H and ^{13}C NMR numbering of the coordinated hexamethylenetetramine ligand in compounds **7** (top) and **8** (bottom).

When two platinum moieties coordinate to the hmta, the local symmetry is further reduced to C2v (still assuming free rotation around the Pt-N bonds). This should result in four signals in the ^{1}H NMR and three signals in the ^{13}C NMR. In the spectra of **8a** – **8f**, however, further signal splitting was observed, suggesting the presence of two diastereoisomers with different configurations at the chiral carbon of the oxadiazoline. Additionally, some of the methoxy signals in the ^{1}H and ^{13}C spectra of **8c** and **8d** are split or broadened, which is attributed to the additional existence of conformers, due to a hindered rotation of the heterocycles around the Pt-N bonds, when two methoxy substituents are present in ortho-position of the aromatic ring. These signals should collapse at higher temperature. T-dependent NMR experiments, however, are complicated by the limited thermal stability of the complexes, and noticeable decomposition takes place at 60 ^{o}C already.

Computational analysis of the ligands and the platinum complexes.

A DFT study and a topological analysis of the charge densities was undertaken for ligands **4** and **6**, the representative Pt(II) compounds **3a**, **5a**, **7a** and **8a** and their protonated congeners, with the aim to elucidate the reactivity and the properties of the platinum complexes. The structures obtained by full geometry optimisation using the B3LYP functional, LANL08 for Pt and Cl, and 6-31G* for all other atoms compare well with X-ray crystallographic data of closely related complexes bearing oxadiazoline or azaadamantane ligands,[9,11,13,25] and with the results from other DFT calculations using the same or very similar methods.[15,26]

Table 1 shows the bond distances, bond orders and charge densities at the bond critical point of the Pt-N and Pt-Cl bonds in compounds **5a**, **7a**, **8a**, monoprotonated **5a-H+, 7a-H+,** diprotonated **5a-2H+, 7a-2H+,** and triprotonated **7a-3H+**. The Pt-Cl bonds show little response to the nature of the N-ligands, as expected from molecular orbital considerations for a *cis*-arrangement where the electronic communication is weak. The *trans*-positioned N-ligands, however, clearly communicate with each other, and the Pt-N bond to the oxadiazoline ligand is weaker when the Pt-N bond to the *trans*-positioned azaadamantane is stronger (and *vice versa*). Judging from the shorter bond length, the higher bond order and the higher charge density in the bond critical point, the Pt-N bond to the hmta ligand in **7a** is stronger than that to the 7-NO_2-TAA ligand in **5a**. The azaadamantane cage of free **6** is more electron rich than that of free **4**, as deduced from the higher average AIM charge[27] at the amine nitrogens (**4**: -0.968; **6**: -0.976) and the slightly higher charge density in the cage critical point (**4:** 0.0984 e/Å; **6:** 0.0993 e/Å). The higher negative charge at the N-atoms in **6** suggest stronger σ-donor properties resulting in the higher tendency to bind to Pt(II), in agreement with the experiment.

The bond between the Pt(II) and the hmta nitrogen is stronger in the mononuclear complex **7a** than in the dinuclear species **8a**, suggesting that the coordination of a second Lewis acidic metal moiety weakens the bond to the first one. A similar effect is observed when the non-coordinating nitrogen atoms in **7a** are protonated to give **7a-H+**, **7a-2H+** and **7a-3H+**. With increasing degree of protonation the Pt-N bond weakens and concomitantly the Pt-N(oxa) bond strengthens. A ligand exchange is thus expected to occur more easily when the hmta ligand is protonated or a second coordination takes place.

The monoprotonated species **5a-H+** and **7a-H+** also show an interesting charge distribution at the C-atoms of the azaadamantane ligand. The carbons remote from the coordination site are electron rich whereas the one that is flanked by the Pt-N and H-N+ moieties is particularly electron deficient. A nucleophilic attack at this carbon should thus be facilitated, and this has indeed been observed with **5a** where hydrolysis lead to the release of formaldehyde.[15] The same effect can be seen in the dinuclear complex **8a**, where the same C-atom is flanked by two $PtCl_2$(oxadiazoline) moieties. Here, however, a nucleophilic attack appears difficult for steric reasons and this might explain the relative stability of complexes of this type.

Table 1. Bond lenghts and electronic properties of the Pt-N and Pt-Cl bonds in compounds **5a**, **7a**, **8a**, monoprotonated **5a-H+, 7a-H+,** diprotonated **5a-2H+, 7a-2H+,** and triprotonated **7a-3H+**.

Bond	**Property**	**5a**	**5a-H+**	**5a-2H+**	**7a**	**8a**		**7a-H+**	**7a-2H+**	**7a-3H+**
Pt-N(oxa)	bond lenght (Å)	2.035	2.008	1.989	2.042	2.036	2.035	2.014	1.992	1.981
	bond order	0.354	0.405	0.458	0.348	0.357	0.357	0.399	0.454	0.513
	ρ_{BCP} (e/Å^3)	0.7792	0.8489	0.9086	0.7645	0.7854	0.7794	0.8357	0.9009	0.9461
Pt-N(TAA)	bond lenght (Å)	2.135	2.182	2.234	2.124	2.136	2.136	2.165	2.215	2.276
or	bond order	0.420	0.334	0.253	0.431	0.419	0.419	0.345	0.260	0.184
Pt-N(hmta)	ρ_{BCP} (e/Å^3)	0.6450	0.5551	0.4768	0.6658	0.6428	0.6419	0.5822	0.4989	0.4239
Pt-Cl(1)	bond length (Å)	2.447	2.433	2.443	2.448	2.443	2.443	2.451	2.446	2.466
	bond order	0.818	0.843	0.812	0.816	0.827	0.829	0.808	0.811	0.768
	ρ_{BCP} (e/Å^3)	0.4513	0.4670	0.4590	0.4498	0.4556	0.4558	0.4491	0.4564	0.4415
Pt-Cl(2)	bond lenght (Å)	2.435	2.429	2.413	2.432	2.432	2.432	2.413	2.413	2.403
	bond order	0.876	0.860	0.879	0.873	0.877	0.878	0.902	0.881	0.891
	ρ_{BCP} (e/Å^3)	0.4668	0.4683	0.4854	0.4653	0.4659	0.4656	0.4856	0.4852	0.4974

The energy difference ΔE = E(protonated species) – E(unprotonated species) was used as a measure for the protonation energies[28] given in Table 2. Comparing the free ligands, it becomes evident that hmta **6** is more easily protonated than 7-NO_2TAA **4**, as the reaction **6** + H^+ → **6**-H^+ is more exothermic than **4** + H^+ → **4**-H^+. This suggests that **4** is less basic than **6**, in agreement with the pKa values of the protonated species **4**-H^+ and **6**-H^+ of 3.42 (±0.05) (see Experimental Part) and 4.89.[29] The same trend is seen in the protonation of the platinum complexes **5a** and **7a**, where also the NO_2TAA complex **5a** is less prone to accept H^+. Compared to the free ligands, the platinum complexes are more difficult to protonate, in line with the electron withdrawing effect the Lewis-acidic Pt(II) center exhibits. A second and third protonation (where possible) is energetically less favourable than the first protonation, for free ligands and Pt(II) complexes alike, which also meets our expectations. The protonation of the complexes **5a** and **7a** is easier than H^+-transfer to the mono-protonated free ligands, since the binding of the stronger Lewis acid H^+ reduces the basicity of the remaining nitrogens more than the coordination to the weakly Lewis-acidic Pt(II) center.

The ligand exchange reactions of $PtCl_2$(oxa)(PhCN), namely the derivative **3a**, were assessed from the reaction energies ΔE = Σ(E(products) – Σ(E(reactants). Overall, the exchange of PhCN by **4** or **6** is exothermic, to a higher degree for hmta **6** than for the 7-NO_2TAA ligand **4**. The thermodynamic motivation of the analogous reactions with the mono-protonated ligands is lower and comes close to thermoneutrality in the case of **4**-H^+. Thus, protonation of **5a** and possibly also **7a** should lead to an equilibrium situation in which **3a** co-exists with **5a** and **7a**.

In the formation of the 2:1 complex **8a**, the second coordination is less thermodynamically motivated than the first one, in agreement with the experimental observations.

The replacement of the oxadiazoline ligand from **3a** by reaction with **4** is thermodynamically disfavoured, and the analogous reaction with **6** is close to thermoneutral. In both cases, the replacement of the nitrile ligand in **3a** is thermodynamically far more favourable, and this agrees well with the observed selectivity in favour of formation of **5a** and **7a**.

Table 2. Reaction energies ΔE and selected activation energies Ea of the protonation and ligand exchange reactions involving the free ligands **4** and **6** and the platinum complexes **5a, 7a** and **8a** (kcal/mol).

Protonation reaction	ΔE	Protonation reaction	ΔE
4 + H^+ → **4**-H^+	-220.6	**6** + H^+ → **6**-H^+	-229.0
4-H^+ + H^+ → **4**-$2H^+$	-113.3	**6**-H^+ + H^+ → **6**-$2H^+$	-120.1
4-$2H^+$ + H^+ → **4**-$3H^+$	-7.1	**6**-$2H^+$ + H^+ → **6**-$3H^+$	-11.0
5a + H^+ → **5a**-H^+	-217.0	**7a** + H^+ → **7a**-H^+	-224.5
5a-H^+ + H^+ → **5a**-$2H^+$	-121.3	**7a**-H^+ + H^+ → **7a**-$2H^+$	-127.1
		7a-$2H^+$ + H^+ → **7a**-$3H^+$	-32.6
Ligand exchange	ΔE Ea	Ligand exchange	ΔE Ea
3a + **4** → **5a** + PhCN	-5.13 +15.2	**3a** + **6** → **7a** + PhCN	-8.02 +15.3
3a + **4**-H^+ → **5a**-H^+ + PhCN	-1.48	**3a** + **6**-H^+ → **7a**-H^+ + PhCN	-3.53
3a + **7a** → **8a** + PhCN	-4.71	**3a** + **6**-$3H^+$ → **7a**-$3H^+$ + PhCN	-30.2
3a + **4** → **9a** + oxadiazoline	2.74	**3a** + **6** → **10a** + oxadiazoline	-0.58

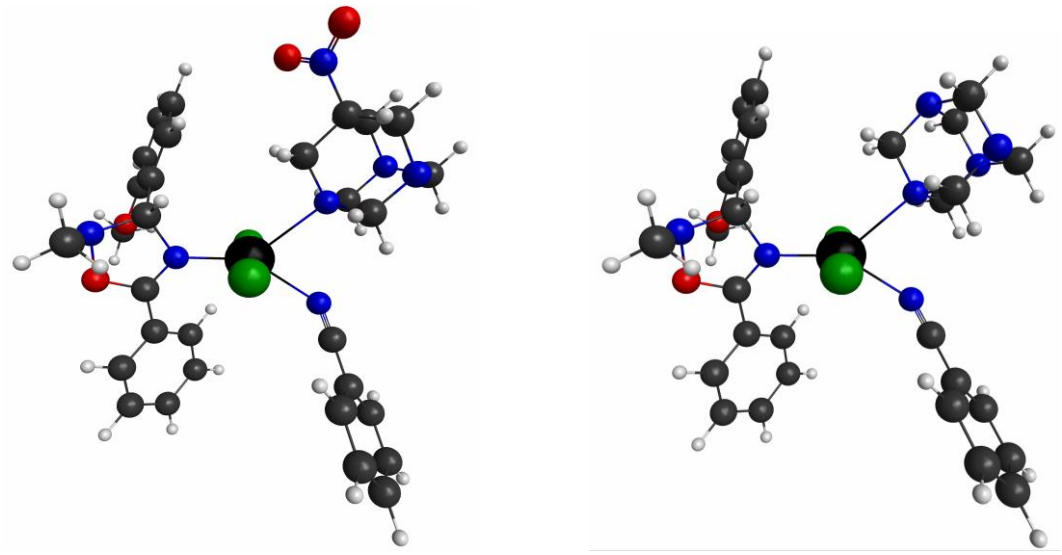


Figure 1. Transition states for the ligand exchange reactions **3a** + **4** → **5a** + PhCN (left) and **3a** + **6** → **7a** + PhCN (right).

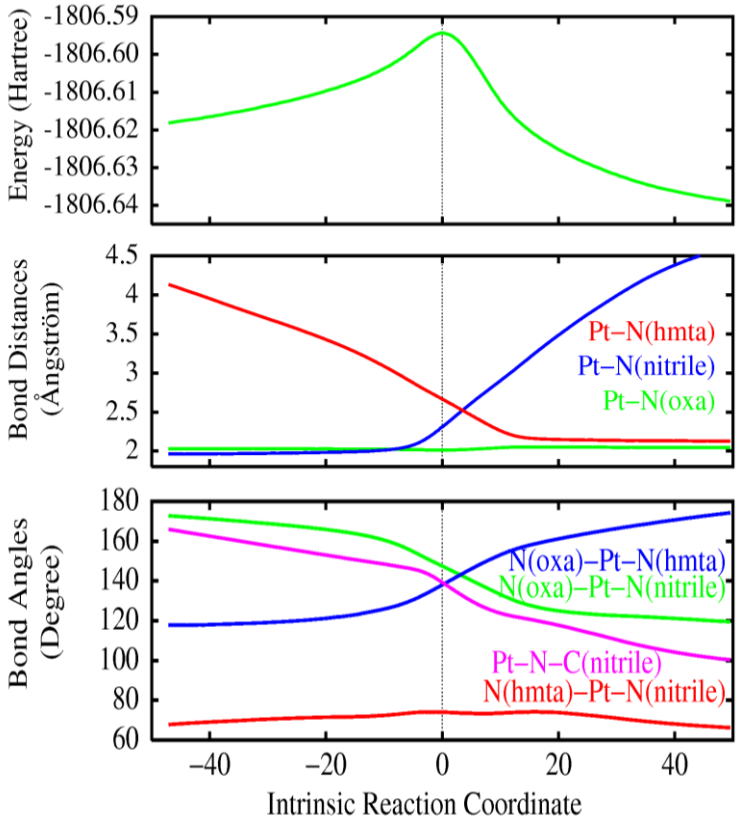


Figure 2. Energy profile and characteristic bond distances and angles along the intrinsic reaction coordinate of the reaction **3a** + **6** → **7a** + PhCN.

The reaction kinetics for the ligand exchange (**3a** + **4** or **6** to give **5a** or **7a** + PhCN) were assessed from the activation barriers Ea, which are 15.2 kcal/mol and 15.3 kcal/mol, respectively. Both reactions are thus expected to occur with approximately the same reaction rate. The observed slower reaction with 7-NO_2TAA **4** is probably due to the poor solubility of this ligand, thus the reaction is hampered by the low availability of the free ligand in solution.

The transition state for ligand exchange (Figure 1) can be best described as a slightly distorted trigonal bipyramidal structure with the chloro ligands in apical positions at the central Pt atom and the nitrogen ligands in the trigonal plane. From a mechanistic point of view, addition and elimination are relatively simultaneous processes, which can be seen from the transition state geometry and also from the single energy barrier in the energy profile and the changes in bond distances and angles along the intrinsic reaction coordinate (IRC), shown in Figure 2 for the reaction **3a** + 6 → **7a** + PhCN. The Pt-N-C(nitrile) angle starts to bend upon approach of the azaadamantane ligand, and the Pt-N bond to the nitrile elongates as the Pt-N bond to the

azaadamantane shortens. The overall process resembles an S_Ni reaction at a tetrahedral center where both nucleophile and nucleofuge share the same orbital lobe of the electrophilic center. The oxadiazoline as a spectator ligand practically does not change any of its parameters along the IRC, except of a small conformational modification of the phenyl ring. Also the chloro ligands remain unaffected. Overall, the ligand exchange occurs under retention of the *trans*-configuration in the product, in agreement with the experimental observation.

In-vitro cytotoxicity of the Pt(II) complexes

The *in vitro* cytotoxicity of cisplatin and the new compounds **5a**, **7a**, **8a**, **5b**, **7b**, **5c**, **7c, 7d**, **5e** and **7e** in the epithelial human cancer cell lines HeLa[30] and A549[31] was determined by means of the CellTiter-Glo® luminescent cell viability assay,[32] as described in the experimental part. HeLa cervical cancer cells are known to respond to cisplatin with an IC_{50} of 1.1 to 1.3 μM,[33] whereas the lung cancer cell line A549, with an IC_{50} of 64 μM, is fairly inert to cisplatin treatment.[34]

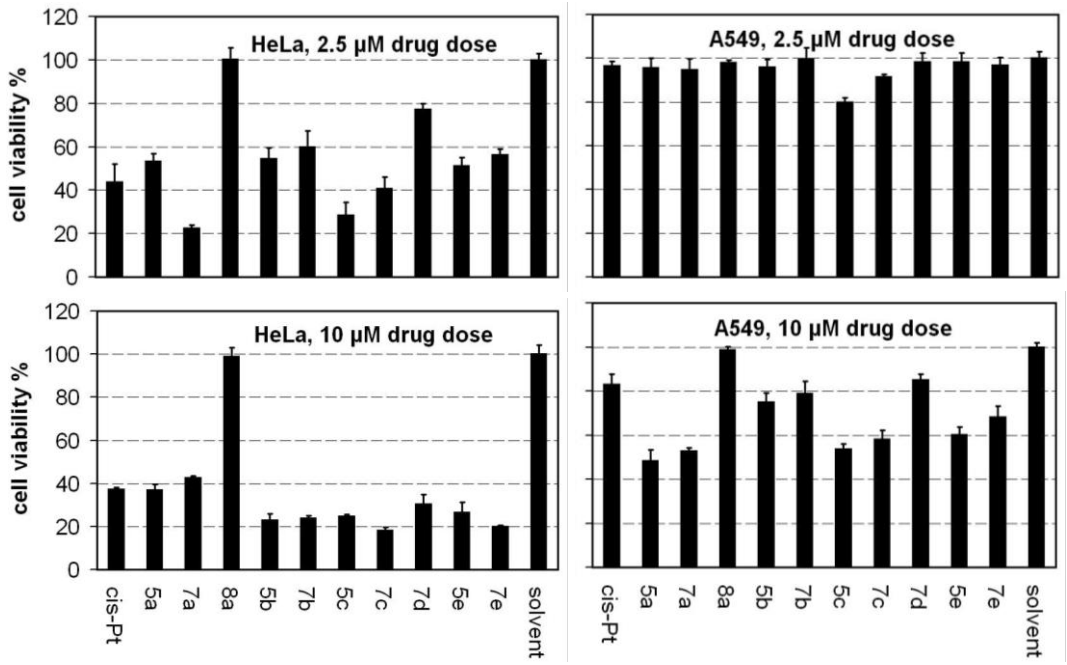


Figure 3. Cell viability after 24 h of incubation in the presence of 2.5 μM and 10 μM doses of the platinum compounds cisplatin, **5a**, **7a**, **8a**, **5b**, **7b**, **5c**, **7c**, **7d**, **5e** and **7e**. Data are mean values over three experiments and given relative to untreated cells = 100 %. Cell viabilities are given relative to a solvent blank = 100 %. Error bars indicate standard deviations.

Due to the poor solubility of the Pt(II) compounds in aqueous media DMSO had to be used as a co-solvent. The stability of compound **5a** in DMSO over a period of weeks has been established before.[15] Nevertheless, care has been taken to avoid ligand exchange by reducing the contact to DMSO to a minimum. Thus, the platinum compounds were dissolved in DMSO and immediately diluted into water to give stock solutions which are 100 μM in platinum and contain 5 % (v/v) DMSO. These were used immediately without further storage for the cytotoxicity experiments. Aliquots used to achieve 10 μM or 2.5 μM platinum doses in the cell suspension introduce 0.5 % (v/v) and 0.125 % (v/v) of DMSO, which should have little effect on the growth and survival of the cancer cells.[35] Any effects on the intracellular ATP levels in the presence of more than 0.1 % (v/v) DMSO[36] are compensated by solvent blank measurements.

The mononuclear hmta complex **7a**, as the most potent compound tested, is significantly more active than cisplatin and able to reduce the cell viability to 24.3%, using a 2.5 μM dose, where with cisplatin the cell viability is 47.3%. In contrast to that, the analogous dinuclear complex **8a**, bearing the same oxadiazoline ligand as **7a**, is totally inactive in the concentration range tested (2.5 to 10 μM). This might be due to the low polarity of **8a**, seen in the high R_f values in thin layer chromatography. Additionally, since the di-coordinated hmta in **8a** will not be protonated, no cationic species are present in an aqueous medium, resulting in an overall low solubility and poor transport properties and cellular uptake. The substitution pattern of compounds **7** has a strong influence on the activity**,** which decreases in an order **7a** > **7c** > **7e** > **7b** > **7d**. It seems that the presence of an OMe group in 4-position of the aromatic ring attached to the oxadiazoline ligand reduces the *in vitro* activity of the compound, whereas an OMe group in 2-position seems to strongly enhance it. The analogous hmta and NO_2-TAA complexes reduce the cell viability to a similar extent (e.g **7b** and **5b**), suggesting that the hmta and NO_2-TAA ligands do not affect the activity of the compound greatly. NO_2-TAA and hmta may dissociate off at a fairly early stage of delivery and binding, and activity is determined by the nature of the $PtCl_2$(oxadiazoline) fragment or the hydrolysed form thereof. An attempted NMR study of the stability of our compounds in the cell culture medium did not

give conclusive results due to the low concentration (and solubility) of the Pt(II) compounds in the presence of a large amount of culture medium.

Compound **7a**, as the only compound, is more active at low concentration (2.5 μM, as compared to 10 μM), and this effect is reproducible. At the current stage of investigation, we are not sure whether there is a biochemical reason for this, or whether it is caused by the low solubility of the compound in an aqueous medium. The 100 μM stock solutions, when left overnight, show clear signs of precipitation, and the solid, isolated by centrifugation and analysed by SEM, TEM and EDX, has the correct Pt:Cl:S elemental composition expected for un-decomposed **7a** (that is, Pt:Cl 1:2, no S detetable). Presumably, the 2.5 M solution is supersaturated and all the platinum compound is bioavailable to the cells, whereas the 10 M solution precipitates **7a** during the cytotoxicity experiment and the cells can only take up the dissolved compound and not the particulate matter. Clearly, far more detailed studies are necessary to clarify this effect.

A549 cells show the expected weak response to 2.5 μM cisplatin, and also our new compounds do not perform any better, except of the TAA complex **5c**, which reduces the cell viability to about 80 %. With the four-fold dose (10 μM), the dinuclear complex **8a**, is still inactive, as in the case of the HeLa cells, but all mononuclear complexes show appreciable activity, and the structure-activity relation pattern is similar to the one observed for the HeLa cells at lower concentration. Most of our mononuclear complexes (**5a**, **5c**, **5e**, **7a**, **7c**, **7e**) are in fact more active in A549 than cisplatin, and this could make this class of compounds interesting for therapeutic applications. Only a few other Pt(II) complexes show a similar behaviour, as for example a group of cis-Pt(II) complexes with pyrazole derived ligands with an up to 3-fold potency as compared to cisplatin.[37] More often, the activity against A549 is best of all similar to that of cisplatin, as in the case of cis-Pt(II) amidine complexes.[5a]

Conclusion

In this work, we described a highly efficient and selective synthesis route to a series of mono- and dinuclear mixed ligand Pt(II) complexes bearing oxadiazoline and azaadamantane ligands, and their in-vitro cytotoxicity in two human cancer cell lines (HeLa and A549).

These complexes were designed to bear one oxadiazoline as an easily modifiable ligand that allows for fine-adjustment of the pharmacological properties, and one azaadamantane ligand whose lability can be triggered by protonation in the slightly acidic environment found in some tumour tissues. This hypothesis has been supported by DFT studies and is in line with preliminary experimental observations of the chemical reactivity of related NO_2TAA complexes.[15] DFT calculations were also used to corroborate the reactivity and selectivity in the ligand exchange reaction and mechanistic issues, by looking into transition state geometries, activation barriers and energy profiles of the reaction.

The in-vitro cytotoxicity of ten of the new compounds was tested using the human cancer cell lines HeLa and A549. Whereas the dinuclear complexes were inactive (most likely due to low solubility and poor cellular uptake), all mono-nuclear complexes showed a fairly high activity. This was often higher than that of cisplatin used for comparison, in particular with the lung cancer cell line A549 which is known to respond poorly to cisplatin. Analogous NO_2TAA and hmta complexes show fairly similar activity, suggesting a dissociation of the labile ligand at a relatively early stage. For practical reasons (commercial availability, low cost, faster reactions and easier product purification), hmta as a labile ligand appears slightly superior, as compared to the NO_2TAA. Overall, our in-vitro cytotoxicity results are promising, but further studies will be necessary to fully assess the potential of these new compounds with respect to a potential therapeutic use.

Experimental Part

Materials and Instrumentation. Solvents and reagents were obtained from commercial sources and used as received. *Trans*-[$PtCl_2(PhCN)_2$] **2**,[11,38] nitrones **1a-1f**[39] and 7-NO_2TAA **4**[40] were synthesised according to published methods. C, H, N elemental analyses were run on a Vario Micro Cube automatic analyser. Infrared spectra (4000-400 cm^{-1}) were recorded on a Bruker Tensor 27 FT-IR using the ATR technique. ^{1}H, ^{13}C and ^{195}Pt NMR spectra were acquired on Bruker Avance 500 and Bruker Avance 400 spectrometers at ambient temperature. ^{195}Pt chemical shifts are given relative to aqueous $K_2[PtCl_4]$ = –1630

ppm. All ^{195}Pt signals show half height line widths of 600 – 750 Hz, as a result of unresolved spin-spin interactions with the quadrupolar ^{14}N nuclei.

Determination of the pKa values of the free ligands 4 and 6.

The pKa values were determined from titrations of **4** and **6** with 0.1 M HCl, according to a procedure described in the literature.[29] Since the pKa values are relatively low, back titration of the protonated forms **4**-H^+ and **6**-H^+ with 0.1 M NaOH was also applied. For this, 0.8 mmol of compounds **4** or **6** were dissolved in 30 ml of demineralised water and 8 ml of 0.1 M HCl (1 equivalent) were added. The solution was then titrated with 0.1 M NaOH and the pKa value was obtained from the titration curve at the half-equivalence point.

4-H^+ + H_2O → **4** + H_3O^+ (pKa = 3.42).

6-H^+ + H_2O → **6** + H_3O^+ (pKa = 4.87; lit: 4.89,[29] 4.86[41]).

Synthesis of the mixed benzonitrile / oxadiazoline complexes.

Trans-$PtCl_2$(PhCN)(oxadiazoline) complexes **3a** - **3f** were prepared according to the literature,[15] where also the characterisation of compounds **3a**, **3c** and **3d** can be found.[15]

***trans*-(Benzonitrile)-dichloro[2,3-dihydro-3-(4-methoxyphenyl)-2-methyl-5-phenyl-1,2,4-oxadiazole-κN^4] platinum (3b).** Yield 89 %. Elemental analysis calculated for $C_{23}H_{21}Cl_2N_3O_2Pt$: C 43.34; H 3.32; N 6.59; found: C 43.12; H 3.22; N 6.68. IR (selected bands), cm^{-1}: 3046, 3003, 2964 and 2931 ν(C–H), 2837 ν(C–H of OMe), 2289 ν(C≡N), 1627 m ν(C=N). ^{1}H NMR in $CDCl_3$, δ (ppm): 3.06 (s, br., 3H, NMe), 3.86 (s, 3H, OMe), 5.95 (s, br., 1H, N-CH-N), 7.01 (d, 8.9 Hz, 2H) and 7.67 (d, 8.5 Hz, 2H)(aryl-H of N-CH(Ar)-N), 7.52 (t, 7.7 Hz, 2H) and 7.72 (m, 3H)(PhC≡N-Pt), 7.62 (t, 7.7 Hz, 2H), 7.71 (m, 1H) and 9.01 (d, 8.0 Hz, 2H)(aryl-H of PhC=N). ^{13}C NMR in $CDCl_3$, δ (ppm): 45.8 (NMe), 55.3 (OMe), 94.4 (N-CH-N), 114.1 and 130.7 (CH of N-CH(*Ar*)-N), 130.2 and 160.7 (C_q of N-CH(*Ar*)-N), 129.3, 133.5 and 134.7 (CH of *Ph*C≡N-Pt), 109.8 (C_q of *Ph*C≡N-Pt), 116.5 (C≡N), 128.6, 130.3 and 134.1 (CH of *Ph*C=N), 122.3 (C_q of *Ph*C=N), 164.3 (C=N). ^{195}Pt NMR in $CDCl_3$, δ (ppm): –2236.

***trans*-(Benzonitrile)-dichloro[2,3-dihydro-3-(2,3,4-trimethoxyphenyl)-2-methyl-5-phenyl-1,2,4-oxadiazole-κN^4] platinum (3e).** Yield 90 %. Elemental analysis calculated for

$C_{25}H_{25}Cl_2N_3O_4Pt$: C 43.05; H 3.61; N 6.02; found: C 43.13; H 3.48; N 6.17. IR (selected bands), cm^{-1}: 3060, 2999, 2968 and 2938 ν(C–H), 2836 ν(C–H of OMe), 2289 ν(C≡N), 1630 m ν(C=N). ^{1}H NMR in $CDCl_3$, δ (ppm): 3.08 (s, br., 3H, NMe), 3.89, 3.92 and 4.13 (s, 3H each, OMe), 6.36 (s, br., 1H, N-CH-N), 6.75 (d, 8.8 Hz, 1H) and 7.34 (d, br., 8 Hz, 2H)(aryl-H of N-CH(Ar)-N), 7.52 (t, 7.9 Hz, 2H) and 7.73 (m, 3H)(PhC≡N-Pt), 7.63 (t, 7.8 Hz, 2H), 7.69 (m, 1H) and 9.02 (d, 8.0 Hz, 2H)(aryl-H of PhC=N). ^{13}C NMR in $CDCl_3$, δ (ppm): 46.8 (NMe), 55.9, 60.8 and 61.4 (3 × OMe), 90.4 (N-CH-N), 106.6 and 124.1 (CH of N-CH(*Ar*)-N), 121.7, 141.9, 152.3 and 154.9 (C_q of N-CH(*Ar*)-N), 129.3, 133.5 and 134.8 (CH of *Ph*C≡N-Pt), 109.8 (C_q of *Ph*C≡N-Pt), 116.5 (C≡N), 128.6, 130.6 and 134.0 (CH of *Ph*C=N), 122.4 (C_q of *Ph*C=N), 161.4 (C=N). ^{195}Pt NMR in $CDCl_3$, δ (ppm): –2233.

***trans*-(Benzonitrile)-dichloro[2,3-dihydro-3-(3,4,5-methoxyphenyl)-2-methyl-5-phenyl-1,2,4-oxadiazole-κN^4] platinum (3f).** Yield 79 %. Elemental analysis calculated for $C_{25}H_{25}Cl_2N_3O_4Pt$: C 43.05; H 3.61; N 6.02; found: C 42.88; H 3.58; N 6.14. IR (selected bands), cm^{-1}: 3061, 3000, 2965 and 2940 ν(C–H), 2839 ν(C–H of OMe), 2289 ν(C≡N), 1625 m ν(C=N). ^{1}H NMR in $CDCl_3$, δ (ppm): 3.06 (s, br., 3H, NMe), 3.90 (s, 3H, OMe), 3.94 (s, 6H, OMe), 5.92 (s, br., 1H, N-CH-N), 6.97 (s, 2H, aryl-H of N-CH(Ar)-N), 7.50 (t, 7.8 Hz, 2H) and 7.69 (m, 3H)(PhC≡N-Pt), 7.62 (t, 7.9 Hz, 2H), 7.68 (m, 1H) and 9.02 (d, 7.9 Hz, 2H)(aryl-H of PhC=N). ^{13}C NMR in $CDCl_3$, δ (ppm): 45.8 (NMe), 56.4 and 60.8 (OMe), 94.7 (N-CH-N), 106.1 (CH of N-CH(*Ar*)-N), 138.9 and 153.4 (C_q of N-CH(*Ar*)-N, third C_q not detected), 129.3, 133.5 and 134.8 (CH of *Ph*C≡N-Pt), 109.7 (C_q of *Ph*C≡N-Pt), 116.6 (C≡N), 128.6, 130.8 and 134.2 (CH of *Ph*C=N), 122.2 (C_q of *Ph*C=N), 163.8 (C=N). ^{195}Pt NMR in $CDCl_3$, δ (ppm): –2226.

Synthesis of the mononuclear mixed triazaadamantane / oxadiazoline complexes.

Trans-$PtCl_2$(7-NO_2-TAA)(oxadiazoline) complexes **5a** – **5f** were prepared by reaction of **3a** – **3f** with 7-nitro-1,3,5-triazaadamantane **4**, according to the literature.[15] The compounds **5a**, **5c** and **5d** have already been described and characterised.[15]

***trans*-Dichloro[2,3-dihydro-3-(4-methoxyphenyl)-2-methyl-5-phenyl-1,2,4-oxadiazole-κN^4][7-nitro-1,3,5-triazaadamantane-κN^1]platinum (5b).** Yield 88 %. Elemental analysis calculated for $C_{23}H_{28}Cl_2N_6O_4Pt$: C 38.45; H 3.93; N 11.70; found: C 38.33; H 4.06; N 11.57.

IR (selected bands), cm^{-1}: 3055, 2966, 2926 and 2853 ν(C–H), 2839 ν(C–H of OMe), 1636 m ν(C=N), 1608 w ν(C=C), 1540 s $\nu(NO_2)$. ^{1}H NMR in $CDCl_3$, δ (ppm): 3.02 (s, br., 3H, NMe), 3.87 (s, 3H, OMe), 3.66 (d, 14.2 Hz, 2H) and 3.69 (d, 14.2 Hz, 2H)(TAA H_d and $H_{d'}$), 4.17 ("s", 2H, TAA H_c), 3.90 (m, 1H) and 4.26 (d, 13.4 Hz, 1H)(TAA H_b and $H_{b'}$), 4.52 (dm, 12.9 Hz, 2H) and 4.76 (dm, 12.9 Hz, 2H)(TAA H_a and $H_{a'}$), 5.81 (s, br., 1H, N-CH-N), 7.01 (d, 8.4 Hz, 2H) and 7.65 (d, 8.4 Hz, 2H)(aryl-H of N-CH(Ar)-N), 7.59 (t, 7.8 Hz, 2H), 7.70 (t, 7.4 Hz, 1H) and 8.92 (d, 7.9 Hz, 2H)(aryl-H of PhC=N). ^{13}C NMR in $CDCl_3$, δ (ppm): 45.7 (NMe), 55.3 (OMe), 58.09 and 58.11 (TAA C_d), 62.5 (TAA C_c), 71.4 (TAA C_b), 78.06 and 78.08 (TAA C_a), 72.8 (TAA C-NO_2), 94.3 (N-CH-N), 113.8 and 130.4 (CH of N-CH(*Ar*)-N), 130.5 and 160.7 (C_q of N-CH(*Ar*)-N), 128.4, 130.6 and 133.8 (CH of *Ph*C=N), 122.7 (C_q of *Ph*C=N), 163.5 (C=N). ^{195}Pt NMR in $CDCl_3$, δ (ppm): –2170.

***trans*-Dichloro[2,3-dihydro-3-(2,3,4-trimethoxyphenyl)-2-methyl-5-phenyl-1,2,4-oxadiazole-κN^4][7-nitro-1,3,5-triazaadamantane-κN^1]platinum (5e).** Yield 82%. Elemental analysis calculated for $C_{25}H_{32}Cl_2N_6O_6Pt$: C 38.57; H 4.14; N 10.79; found: C 38.20; H 4.07; N 10.51. IR (selected bands), cm^{-1}: 3053, 2967 and 2878 ν(C–H), 2837 ν(C–H of OMe), 1635 m ν(C=N), 1601 w ν(C=C), 1538 s $\nu(NO_2)$. ^{1}H NMR in $CDCl_3$, δ (ppm): 3.00 (s, br., 3H, NMe), 3.89 (s, 3H), 3.92 (s, 3H) and 4.12 (s, 3H)(3 × OMe), 3.68 (d, 14.3 Hz, 2H) and 3.71 (d, 14.3 Hz, 2H)(TAA H_d and $H_{d'}$), 4.24 ("s", 2H, TAA H_c), 3.89 (m, 1H) and 4.27 (dm, 13.5 Hz, 1H)(TAA H_b and $H_{b'}$), 4.58 (m, 2H) and 4.81 (d, 13.2 Hz, 2H)(TAA H_a and $H_{a'}$), 6.22 (s, br., 1H, N-CH-N), 6.72 (d, 8.5 Hz, 1H) and 7.21 (d, br., 7.0 Hz, 1H)(aryl-H of N-CH(Ar)-N), 7.61 (t, 7.9 Hz, 2H), 7.71 (t, 7.6 Hz, 1H) and 8.93 (d, 7.3 Hz, 2H)(aryl-H of PhC=N). ^{13}C NMR in $CDCl_3$, δ (ppm): 46.8 (NMe), 55.9, 60.8 and 61.4 (3 × OMe), 58.1 (TAA C_d), 62.6 (TAA C_c), 71.4 (TAA C_b), 78.15 and 78.22 (TAA C_a), 72.8 (TAA C-NO_2), 90.0 (N-CH-N), 106.8 and 124.1 (CH of N-CH(*Ar*)-N), 121.9, 141.9, 152.3 and 154.8 (C_q of N-CH(*Ar*)-N), 128.4, 130.4 and 133.7 (CH of *Ph*C=N), 122.9 (C_q of *Ph*C=N), 164.7 (C=N). ^{195}Pt NMR in $CDCl_3$, δ (ppm): –2172.

***trans*-Dichloro[2,3-dihydro-3-(3,4,5-trimethoxyphenyl)-2-methyl-5-phenyl-1,2,4-oxadiazole-κN^4][7-nitro-1,3,5-triazaadamantane-κN^1]platinum (5f).** Yield 85 %. Elemental analysis calculated for $C_{25}H_{32}Cl_2N_6O_6Pt$: C 38.57; H 4.14; N 10.79; found: C 38.33; H 4.06;

N 10.55. IR (selected bands), cm^{-1}: 3064, 3001, 2964 and 2942 ν(C–H), 2840 ν(C–H of OMe), 1629 m ν(C=N), 1595 w ν(C=C), 1538 s ν(NO_2). ^{1}H NMR in $CDCl_3$, δ (ppm): 3.03 (s, br., 3H, NMe), 3.91 (s, 3H, OMe), 3.95 (s, 6H, OMe), 3.67 (s, br., 4H, TAA H_d and $H_{d'}$), 4.14 (d, 14.3 Hz, 1H) and 4.19 (d, 14.3 Hz, 1H)(TAA H_c and $H_{c'}$), 3.89 (m, 1H) and 4.24 (d, 13.3 Hz, 1H)(TAA H_b and $H_{b'}$), 4.50 (m, 2H) and 4.72 (dm, 13.3 Hz, 2H)(TAA H_a and $H_{a'}$), 5.76 (s, br., 1H, N-CH-N), 6.98 (s, 2H, aryl-H of N-CH(Ar)-N), 7.60 (t, 7.9 Hz, 2H), 7.70 (t, 7.4 Hz, 1H) and 8.94 (d, 7.8 Hz, 2H)(aryl-H of PhC=N). ^{13}C NMR in $CDCl_3$, δ (ppm): 45.5 (NMe), 56.4 and 60.9 (OMe), 58.1 (TAA C_d), 62.5 (TAA C_c), 71.4 (TAA C_b), 78.10 and 78.12 (TAA C_a), 72.8 (TAA C-NO_2), 94.5 (N-CH-N), 106.6 (CH of N-CH(*Ar*)-N), 138.9 and 153.2 (C_q of N-CH(*Ar*)-N, third C_q not detected), 128.4, 130.4 and 133.9 (CH of *Ph*C=N), 122.6 (C_q of *Ph*C=N), 164.3 (C=N). ^{195}Pt NMR in $CDCl_3$, δ (ppm): –2158.

Synthesis of the mononuclear mixed hexamethylenetetramine / oxadiazoline complexes.

Trans-$PtCl_2$(PhCN)(oxadiazoline) **3a-3f** (0.1 mmol) and hexamethylenetetramine **6** (0.15 mmol) were dissolved in chloroform (1 ml) and stirred at room temperature for 4 days. The solvent was evaporated and the residual crude products were purified by chromatography on silica using a CH_2Cl_2/diethylether gradient of 100:0 to 50:50 as eluent.

***trans*-Dichloro[2,3-dihydro-3-(2-methoxyphenyl)-2-methyl-5-phenyl-1,2,4-oxadiazole-κN^4][hexamethylenetetramine-κN^1]platinum (7a).** Yield 83 %. Elemental analysis calculated for $C_{22}H_{28}Cl_2N_6O_2Pt$: C 39.18; H 4.18; N 12.46; found: C 38.95; H 4.27; N 12.75. IR (selected bands), cm^{-1}: 3063, 2964 and 2888 ν(C–H), 2840 ν(C–H of OMe), 1629 m ν(C=N), 1603 w ν(C=C), 1247, 1225, 1024, 998, 831, 774, 754, 688, 653. ^{1}H NMR in $CDCl_3$, δ (ppm): 3.05 (s, br., 3H, NMe), 3.94 (s, 3H, OMe), 4.45 (d, 12.6 Hz, 3 H) and 4.52 (d, 12.2 Hz, 3 H)(hmta H_b and $H_{b'}$), 5.02 (s, 6H, hmta H_a), 6.32 (s, br., 1H, N-CH-N), 6.97 (d, 8.5 Hz, 1H), 7.00 (t, 7.5 Hz, 1H), 7.39 (td, 7.8 Hz, 1.5 Hz, 1H) and 7.50 (d, br., 7.1 Hz, 1H)(aryl-H of N-CH(Ar)-N), 7.60 (t, 7.6 Hz, 2H), 7.70 (t, 7.5 Hz, 1H) and 8.95 (d, 7.4 Hz, 2H)(aryl-H of PhC=N). ^{13}C NMR in $CDCl_3$, δ (ppm): 47.2 (NMe), 55.7 (OMe), 73.1 (hmta C_b), 79.5 (hmta C_a), 89.9 (N-CH-N), 110.9, 120.3, 129.3 and 130.7 (CH of N-CH(*Ar*)-N), 124.3 and 157.5 (C_q of N-CH(*Ar*)-N), 128.4, 130.4 and 133.6 (CH of *Ph*C=N), 123.0 (C_q of *Ph*C=N), 164.9 (C=N). ^{195}Pt NMR in $CDCl_3$, δ (ppm): –2208.

***trans*-Dichloro[2,3-dihydro-3-(4-methoxyphenyl)-2-methyl-5-phenyl-1,2,4-oxadiazole-κN^4][hexamethylenetetramine-κN^1]platinum (7b).** Yield 78 %. Elemental analysis calculated for $C_{22}H_{28}Cl_2N_6O_2Pt$: C 39.18; H 4.18; N 12.46; found: C 39.35; H 4.11; N 12.17. IR (selected bands), cm^{-1}: 3060, 2963, 2931 and 2885 ν(C–H), 2834 ν(C–H of OMe), 1629 m ν(C=N), 1610 w ν(C=C), 1253, 1228, 1022, 995, 830, 775, 757, 689, 655. ^{1}H NMR in $CDCl_3$, δ (ppm): 3.00 (s, br., 3H, NMe), 3.85 (s, 3H, OMe), 4.44 (d, 12.5 Hz, 3 H) and 4.50 (d, 12.3 Hz, 3 H)(hmta H_b and $H_{b'}$), 4.96 (s, 6H, hmta H_a), 5.82 (s, br., 1H, N-CH-N), 6.98 (d, 8.5 Hz, 2H) and 7.64 (d, 8.5 Hz, 2H)(aryl-H of N-CH(Ar)-N), 7.58 (t, 7.8 Hz, 2H), 7.68 (t, 7.3 Hz, 1H) and 8.95 (d, 7.9 Hz, 2H)(aryl-H of PhC=N). ^{13}C NMR in $CDCl_3$, δ (ppm): 45.7 (NMe), 55.3 (OMe), 73.0 (hmta C_b), 79.4 (hmta C_a), 94.1 (N-CH-N), 113.7 and 130.4 (CH of N-CH(*Ar*)-N), 130.42 and 160.6 (C_q of N-CH(*Ar*)-N), 128.3, 130.4 and 133.7 (CH of *Ph*C=N), 122.8 (C_q of *Ph*C=N), 163.5 (C=N). ^{195}Pt NMR in $CDCl_3$, δ (ppm): –2213.

***trans*-Dichloro[2,3-dihydro-3-(2,6-dimethoxyphenyl)-2-methyl-5-phenyl-1,2,4-oxadiazole-κN^4][hexamethylenetetramine-κN^1]platinum (7c).** Yield 70 %. Elemental analysis calculated for $C_{23}H_{30}Cl_2N_6O_3Pt$: C 39.21; H 4.29; N 11.93; found: C 38.91; H 4.33; N 11.71. IR (selected bands), cm^{-1}: 3003, 2970, 2931 and 2886 ν(C–H), 2838 ν(C–H of OMe), 1634 m ν(C=N), 1596 w ν(C=C), 1253, 1226, 1108, 1022, 995, 830, 776, 761, 689, 656. ^{1}H NMR in $CDCl_3$, δ (ppm): 3.02 (s, 3H, NMe), 3.64 (s, 3H) and 4.05 (s, 3H)(2 × OMe), 4.43 (d, 12.7 Hz, 3 H) and 4.49 (d, 12.7 Hz, 3 H)(hmta H_b and $H_{b'}$), 4.96 (s, 6H, hmta H_a), 6.63 (s, 1H, N-CH-N), 6.55 (d, 8.3 Hz, 1H), 6.66 (d, 8.3 Hz, 1H) and 7.34 (t, 8.3 Hz, 1H)(aryl-H of N-CH(Ar)-N), 7.58 (t, 7.5 Hz, 2H), 7.66 (m, 1H) and 8.87 (d, 7.7 Hz, 2H)(aryl-H of PhC=N). ^{13}C NMR in $CDCl_3$, δ (ppm): 48.3 (NMe), 55.9 and 56.7 (2 × OMe), 73.0 (hmta C_b), 79.3 (hmta C_a), 86.7 (N-CH-N), 103.9, 105.1 and 131.0 (CH of N-CH(*Ar*)-N), 113.3, 158.5 and 160.6 (C_q of N-CH(*Ar*)-N), 128.3, 130.1 and 133.0 (CH of *Ph*C=N), 123.3 (C_q of *Ph*C=N), 163.2 (C=N). ^{195}Pt NMR in $CDCl_3$, δ (ppm): –2214.

***trans*-Dichloro[2,3-dihydro-3-(2,4,6-trimethoxyphenyl)-2-methyl-5-phenyl-1,2,4-oxadiazole-κN^4][hexamethylenetetramine-κN^1]platinum (7d).** Yield 71 %. Elemental analysis calculated for $C_{24}H_{32}Cl_2N_6O_4Pt$: C 39.24; H 4.39; N 11.44; found: C 39.37; H 4.43; N 11.60. IR (selected bands), cm^{-1}: 3008, 2963, 2931 and 2881 ν(C–H), 2836 ν(C–H of OMe), 1606 m

ν(C=N), 1593 w ν(C=C), 1452, 1256, 1227, 1121, 1022, 996, 828, 774, 758, 690, 653. ^{1}H NMR in $CDCl_3$, δ (ppm): 2.99 (s, br., 3H, NMe), 3.62 (s, 3H), 3.82 (s, 3H) and 4.01 (s, 3H)(3 × OMe), 4.43 (d, 12.7 Hz, 3 H) and 4.50 (d, 12.0 Hz, 3 H)(hmta H_b and $H_{b'}$), 4.97 (s, 6H, hmta H_a), 6.51 (s, br., 1H, N-CH-N), 6.09 (d, 2.2 Hz, 1H) and 6.20 (d, 2.2 Hz, 1H)(aryl-H of N-CH(Ar)-N), 7.56 (t, 7.8 Hz, 2H), 7.65 (t, 7.5 Hz, 1H) and 8.86 (d, 7.9 Hz, 2H)(aryl-H of PhC=N). ^{13}C NMR in $CDCl_3$, δ (ppm): 48.5 (NMe), 55.2, 55.9 and 56.6 (3 × OMe), 73.0 (hmta C_b), 79.3 (hmta C_a), 86.9 (N-CH-N), 90.7 and 91.6 (CH of N-CH(*Ar*)-N), 106.3, 159.2, 161.5 and 162.2 (C_q of N-CH(*Ar*)-N), 128.3, 130.0 and 133.0 (CH of *Ph*C=N), 123.5 (C_q of *Ph*C=N), 163.2 (C=N). ^{195}Pt NMR in $CDCl_3$, δ (ppm): –2214.

***trans*-Dichloro[2,3-dihydro-3-(2,3,4-trimethoxyphenyl)-2-methyl-5-phenyl-1,2,4-oxadiazole-κN^4][hexamethylenetetramine-κN^1]platinum (7e).** Yield 81%. Elemental analysis calculated for $C_{24}H_{32}Cl_2N_6O_4Pt$: C 39.24; H 4.39; N 11.44; found: C 39.36; H 4.32; N 11.74. IR (selected bands), cm^{-1}: 3059, 2939 and 2879 ν(C–H), 2836 ν(C–H of OMe), 1630 m ν(C=N), 1600 w ν(C=C), 1495, 1256, 1227, 1097, 1023, 996, 829, 774, 758, 690, 652. ^{1}H NMR in $CDCl_3$, δ (ppm): 3.02 (s, br., 3H, NMe), 3.88 (s, 3H), 3.91 (s, 3H) and 4.12 (s, 3H)(3 × OMe), 4.45 (d, 12.8 Hz, 3 H) and 4.52 (d, 12.5 Hz, 3 H)(hmta H_b and $H_{b'}$), 5.01 (s, 6H, hmta H_a), 6.24 (s, br., 1H, N-CH-N), 6.70 (d, 8.6 Hz, 1H) and 7.20 (d, br., 8.0 Hz, 1H)(aryl-H of N-CH(Ar)-N), 7.56 (t, 7.8 Hz, 2H), 7.70 (t, 7.3 Hz, 1H) and 8.96 (d, 7.8 Hz, 2H)(aryl-H of PhC=N). ^{13}C NMR in $CDCl_3$, δ (ppm): 46.8 (NMe), 55.9, 60.8 and 61.4 (3 × OMe), 73.0 (hmta C_b), 79.5 (hmta C_a), 89.9 (N-CH-N), 106.7 and 124.0 (CH of N-CH(*Ar*)-N), 122.0, 141.8, 152.2 and 154.7 (C_q of N-CH(*Ar*)-N), 128.4, 130.4 and 133.7 (CH of *Ph*C=N), 123.0 (C_q of *Ph*C=N), 164.4 (C=N). ^{195}Pt NMR in $CDCl_3$, δ (ppm): –2217.

***trans*-Dichloro[2,3-dihydro-3-(3,4,5-trimethoxyphenyl)-2-methyl-5-phenyl-1,2,4-oxadiazole-κN^4][hexamethylenetetramine-κN^1]platinum (7f).** Yield 79%. Elemental analysis calculated for $C_{24}H_{32}Cl_2N_6O_4Pt$: C 39.24; H 4.39; N 11.44; found: C 38.99; H 4.23; N 11.56. IR (selected bands), cm^{-1}: 3009, 2995, 2943 and 2887 ν(C–H), 2841 ν(C–H of OMe), 1643 m ν(C=N), 1596 w ν(C=C), 1451, 1331, 1259, 1238, 1226, 1025, 997, 826, 778, 690, 654. ^{1}H NMR in $CDCl_3$, δ (ppm): 3.02 (s, br., 3H, NMe), 3.90 (s, 3H, 4-OMe), 3.94 (s, 6H, 3-OMe and 5-OMe), 4.44 (d, 12.9 Hz, 3 H) and 4.49 (d, 12.3 Hz, 3 H)(hmta H_b and $H_{b'}$),

4.95 (s, 6H, hmta H_a), 5.78 (s, br., 1H, N-CH-N), 6.98 (s, 2H, aryl-H of N-CH(Ar)-N), 7.58 (t, 7.8 Hz, 2H), 7.69 (t, 7.5 Hz, 1H) and 8.96 (d, 7.8 Hz, 2H)(aryl-H of PhC=N). ^{13}C NMR in $CDCl_3$, δ (ppm): 45.6 (NMe), 56.4 (4-OMe), 60.8 (3-OMe and 5-OMe), 73.0 (hmta C_b), 79.4 (hmta C_a), 94.4 (N-CH-N), 106.5 (CH of N-CH(*Ar*)-N), 138.8 and 153.3 (C_q of N-CH(*Ar*)-N, third C_q not detected), 128.3, 130.4 and 133.8 (CH of *Ph*C=N), 122.7 (C_q of *Ph*C=N), 164.4 (C=N). ^{195}Pt NMR in $CDCl_3$, δ (ppm): −2202.

Synthesis of the dinuclear mixed hexamethylenetetramine / oxadiazoline complexes.

Trans-$PtCl_2$(PhCN)(oxadiazoline) **3a-3f** (0.22 mmol) and hexamethylenetetramine **6** (0.1 mmol) were dissolved in chloroform (1 ml) and stirred at room temperature for 2 weeks. The solvent was evaporated and the residual crude products were purified by chromatography on silica using a CH_2Cl_2/diethylether gradient of 100:0 to 90:10 as eluent.

[μ-(hexamethylenetetramine-κN^1:κN^3]tetrachlorobis[2,3-dihydro-3-(2-methoxyphenyl)-2-methyl-5-phenyl-1,2,4-oxadiazole-κN^4]diplatinum (8a, isomeric mixture). Yield 61 %. Elemental analysis calculated for $C_{38}H_{44}Cl_4N_8O_4Pt_2$: C 37.76; H 3.67; N 9.27; found: C 38.05; H 3.80; N 9.38. IR (selected bands), cm^{-1}: 3058, 3008, 2968, 2938 and 2914 ν(C–H), 2840 ν(C–H of OMe), 1620 m ν(C=N), 1605 and 1589 s ν(C=C), 1247, 1062, 1030, 983, 792, 772, 748, 733, 686. ^{1}H NMR in $CDCl_3$, δ (ppm): 3.05 (s, br., 6H, NMe), 3.93 and 3.94 (s, 3H each, OMe), 4.24 (s, 2H, hmta H_c), 4.75 (d, 12.8 Hz, 4H), 4.92 (d, 11.6 Hz, 2H) and 4.93 (d, 11.6 Hz, 2H)(hmta Hb and Hb'), 5.38 (s, 2H, hmta H_a), 6.30 (s, br., 2H, N-CH-N), 6.96 (m, 2H), 7.00 (m, 2H), 7.39 (tm, 7.8 Hz, 2H) and 7.49 (d, br., 6.7 Hz, 2H)(aryl-H of N-CH(Ar)-N), 7.60 (m, 4H), 7.69 (m, 2H) and 8.94 (dm, 7.4 Hz, 4H)(aryl-H of PhC=N). ^{13}C NMR in $CDCl_3$, δ (ppm): 47.3 (NMe), 55.8 (OMe), 71.3 (hmta C_c), 77.72, 77.78, 77.83 and 77.87 (hmta C_b), 80.8 (hmta C_a), 92.7 (N-CH-N), 110.9, 120.4, 129.1 and 130.7 (CH of N-CH(*Ar*)-N), 123.8 and 156.0 (C_q of N-CH(*Ar*)-N), 128.5, 130.4 and 133.8 (CH of *Ph*C=N), 122.8 (C_q of *Ph*C=N), 164.5 (C=N). ^{195}Pt NMR in $CDCl_3$, δ (ppm): -2207.

[μ-(hexamethylenetetramine-κN^1:κN^3]tetrachlorobis[2,3-dihydro-3-(4-methoxyphenyl)-2-methyl-5-phenyl-1,2,4-oxadiazole-κN^4]diplatinum (8b, isomeric mixture). Yield 68 %. Elemental analysis calculated for $C_{38}H_{44}Cl_4N_8O_4Pt_2$: C 37.76; H 3.67; N 9.27; found: C 38.01; H 3.33; N 8.94. IR (selected bands), cm^{-1}: 3063, 2963, 2934 and 2915 ν(C–H), 2837

ν(C–H of OMe), 1631 m ν(C=N), 1612 s ν(C=C), 1514, 1348, 1305, 1251, 1176, 1032, 990, 909, 850, 774, 731, 689. ^{1}H NMR in $CDCl_3$, δ (ppm): 3.02 (s, br., 6H, NMe), 3.84 (s, 6H, OMe), 4.22 (s, 2H, hmta H_c), 4.69 (d, 12.7 Hz, 4H), 4.83 (m, 4H, hmta Hb and Hb'), 5.30 ("m", 2H, hmta H_a), 5.81 (s, br., 2H, N-CH-N), 6.99 (d, 8.8 Hz, 2H), 7.00 (d, 8.8 Hz, 2H) and 7.66 (d, 8.2 Hz, 4H)(aryl-H of N-CH(Ar)-N), 7.59 (t, 7.9 Hz, 4H), 7.66 (t, 7.4 Hz, 2H) and 8.95 (d, 7.7 Hz, 4H)(aryl-H of PhC=N). ^{13}C NMR in $CDCl_3$, δ (ppm): 45.7 (NMe), 55.3 (OMe), 71.3 (hmta C_c), 77.45, 77.55, 77.67 and 77.75 (hmta C_b), 80.8 (hmta C_a), 94.1 (N-CH-N), 113.9 and 130.40 (CH of N-CH(*Ar*)-N), 130.44 and 160.6 (C_q of N-CH(*Ar*)-N), 128.5, 130.4 and 133.8 (CH of *Ph*C=N), 122.6 (C_q of *Ph*C=N), 163.5 (C=N). ^{195}Pt NMR in $CDCl_3$, δ (ppm): –2211.

[μ-(hexamethylenetetramine-κN^1:κN^3]tetrachlorobis[2,3-dihydro-3-(2,6-dimethoxy-phenyl)-2-methyl-5-phenyl-1,2,4-oxadiazole-κN^4]diplatinum (8c, isomeric mixture). Yield 60 %. Elemental analysis calculated for $C_{40}H_{48}Cl_4N_8O_6Pt_2$: C 37.86; H 3.81; N 8.83; found: C 38.14; H 3.61; N 8.65. IR (selected bands), cm^{-1}: 3002, 2966, 2937 and 2911 ν(C–H), 2838 ν(C–H of OMe), 1642 m ν(C=N), 1596 w ν(C=C), 1477, 1253, 1108, 1031, 990, 909, 793, 726, 689. ^{1}H NMR in $CDCl_3$, δ (ppm): 3.03 (s, 6H, NMe), 3.654 (s, 3H), 3.658 (s, 3H), 4.00 (s, 3H) and 4.05 (s, 3H)(2 × OMe), 4.21 (s, 2H, hmta H_c), 4.70 (m, 4H), 4.83 (m, 4H)(hmta Hb and Hb'), 5.27 ("m", 2H, hmta H_a), 6.61 (s, 2H, N-CH-N), 6.55 (d, 8.4 Hz, 2H), 6.65 (d, 8.4 Hz, 2H) and 7.34 (t, 8.5 Hz, 2H)(aryl-H of N-CH(Ar)-N), 7.59 (t, 7.7 Hz, 4H), 7.67 (t, 7.4 Hz, 2H) and 8.86 (d, 7.5 Hz, 4H)(aryl-H of PhC=N). ^{13}C NMR in $CDCl_3$, δ (ppm): 48.4 (NMe), 56.0 and 56.9 (2 × OMe), 71.3 (hmta C_c), 77.57, 77.64, 77.67 and 77.68 (hmta C_b), 80.5 and 80.6 (hmta C_a), 86.8 (N-CH-N), 104.1, 105.0 and 131.0 (CH of N-CH(*Ar*)-N), 113.2, 158.5 and 160.5 (C_q of N-CH(*Ar*)-N), 128.5, 130.1 and 133.2 (CH of *Ph*C=N), 123.1 (C_q of *Ph*C=N), 163.2 (C=N). ^{195}Pt NMR in $CDCl_3$, δ (ppm): –2214.

[μ-(hexamethylenetetramine-κN^1:κN^3]tetrachlorobis[2,3-dihydro-3-(2,4,6-trimethoxy-phenyl)-2-methyl-5-phenyl-1,2,4-oxadiazole-κN^4]diplatinum (8d, isomeric mixture). Yield 70 %. Elemental analysis calculated for $C_{42}H_{52}Cl_4N_8O_8Pt_2$: C 37.96; H 3.94; N 8.43; found: C 38.11; H 3.81; N 8.09. IR (selected bands), cm^{-1}: 3001, 2965, 2940, 2915 and 2886 ν(C–H), 2839 ν(C–H of OMe), 1607 m ν(C=N), 1593 w ν(C=C), 1451, 1256, 1229, 1205,

1155, 1121, 1059, 1032, 991, 909, 813, 774, 690. ^{1}H NMR in $CDCl_3$, δ (ppm): 2.99 (s, br., 6H, NMe), 3.63 (s, 6H), 3.79 (s, 6H), 3.98 (s, 3H) and 4.00 (s, 3H)(3 × OMe), 4.23 ("m", 2H, hmta H_c), 4.72 (m, 4H), 4.85 (m, 4H)(hmta Hb and Hb'), 5.38 ("m", 2H, hmta H_a), 6.50 (s, br., 2H, N-CH-N), 6.09 (d, 2.1 Hz, 2H), 6.21 (d, 2.1 Hz, 1H) and 6.22 (d, 2.1 Hz, 1H)(aryl-H of N-CH(Ar)-N), 7.58 (t, 7.8 Hz, 4H), 7.65 (t, 7.4 Hz, 2H) and 8.85 (d, 7.7 Hz, 4H)(aryl-H of PhC=N). ^{13}C NMR in $CDCl_3$, δ (ppm): 48.3 (NMe), 55.2, 55.9 and 56.8 (3 × OMe), 71.3 (hmta C_c), 77.59, 77.66, 77.68 and 77.95 (hmta C_b), 80.5 and 80.6 (hmta C_a), 86.9 (N-CH-N), 90.74, 90.88, 91.57 and 91.72 (CH of N-CH(*Ar*)-N), 106.2, 159.3, 161.41, 161.43, 162.28 and 162.34 (C_q of N-CH(*Ar*)-N), 128.4, 130.0 and 133.1 (CH of *Ph*C=N), 123.3 (C_q of *Ph*C=N), 163.0 (C=N). ^{195}Pt NMR in $CDCl_3$, δ (ppm): –2214.

[μ-(hexamethylenetetramine-κN^1:κN^3]tetrachlorobis[2,3-dihydro-3-(2,3,4-trimethoxy-phenyl)-2-methyl-5-phenyl-1,2,4-oxadiazole-κN^4]diplatinum (8e, isomeric mixture). Yield 66 %. Elemental analysis calculated for $C_{42}H_{52}Cl_4N_8O_8Pt_2$: C 37.96; H 3.94; N 8.43; found: C 38.22; H 4.08; N 8.03. IR (selected bands), cm^{-1}: 3061, 2998, 2967 and 2943 ν(C–H), 2836 ν(C–H of OMe), 1633 m ν(C=N), 1601 s ν(C=C), 1495, 1466, 1384, 1290, 1097, 1033, 1012, 992, 909, 795, 730, 690. ^{1}H NMR in $CDCl_3$, δ (ppm): 3.03 (s, br., 6H, NMe), 3.87 (s, 6H), 3.90 (s, 6H) and 4.11 (s, 6H)(3 × OMe), 4.25 (s, br., 2H, hmta H_c), 4.78 (m, 4H), 4.88 (m, 4H)(hmta Hb and Hb'), 5.38 ("m", 2H, hmta H_a), 6.20 (s, br., 2H, N-CH-N), 6.70 (d, 8.8 Hz, 2H) and 7.19 (d, br., 8.2 Hz, 2H)(aryl-H of N-CH(Ar)-N), 7.60 (t, 7.5 Hz, 4H), 7.68 (t, 7.3 Hz, 2H) and 8.95 (d, 7.5 Hz, 4H)(aryl-H of PhC=N). ^{13}C NMR in $CDCl_3$, δ (ppm): 46.9 (NMe), 55.9, 60.8 and 61.5 (3 × OMe), 71.3 (hmta C_c), 77.56, 77.64, 77.83 and 77.87 (hmta C_b), 81.0 (hmta C_a), 89.9 (N-CH-N), 106.8 and 123.9 (CH of N-CH(*Ar*)-N), 121.9, 141.8, 152.2 and 154.8 (C_q of N-CH(*Ar*)-N), 128.5, 130.4 and 133.8 (CH of *Ph*C=N), 122.7 (C_q of *Ph*C=N), 164.5 (C=N). ^{195}Pt NMR in $CDCl_3$, δ (ppm): –2214.

[μ-(hexamethylenetetramine-κN^1:κN^3]tetrachlorobis[2,3-dihydro-3-(3,4,5-trimethoxy-phenyl)-2-methyl-5-phenyl-1,2,4-oxadiazole-κN^4]diplatinum (8f, isomeric mixture). Yield 67 %. Elemental analysis calculated for $C_{42}H_{52}Cl_4N_8O_8Pt_2$: C 37.96; H 3.94; N 8.43; found: C 37.33; H 3.60; N 8.61. IR (selected bands), cm^{-1}: 3063, 2999, 2965 and 2940 ν(C–H), 2838 ν(C–H of OMe), 1628 m ν(C=N), 1595 s ν(C=C), 1451, 1332, 1237, 1124, 1059, 1034, 990,

730, 689. ^{1}H NMR in $CDCl_3$, δ (ppm): 3.03 (s, br., 6H, NMe), 3.90 (s, 6H, 4-OMe), 3.93 (s, 12H, 2-OMe, 5-OMe), 4.22 (s, 2H, hmta H_c), 4.72 (m, 4H) and 4.80 (m, 4H)(hmta Hb and Hb'), 5.34 (s, br., 2H, hmta H_a), 5.77 (s, br., 2H, N-CH-N), 6.96 (s, 4H, aryl-H of N-CH(Ar)-N), 7.59 (t, 7.8 Hz, 4H), 7.68 (t, 7.3 Hz, 2H) and 8.96 (m, 4H)(aryl-H of PhC=N). ^{13}C NMR in $CDCl_3$, δ (ppm): 45.5 (NMe), 56.4 (4-OMe), 60.9 (3-OMe and 5-OMe), 71.2 (hmta C_c), 77.52, 77.57, 77.70 and 77.79 (hmta C_b), 81.0 (hmta C_a), 94.3 (N-CH-N), 106.3 (CH of N-CH(*Ar*)-N), 138.9 and 153.2 (C_q of N-CH(*Ar*)-N, third C_q not detected), 128.5, 130.5 and 134.0 (CH of *Ph*C=N), 122.5 (C_q of *Ph*C=N), 164.3 (C=N). ^{195}Pt NMR in $CDCl_3$, δ (ppm): –2205.

Computational Details

DFT calculations were carried out with the PC GAMESS/Firefly package,[42] which is partially based on the GAMESS(US) source code.[43] Results were visualised with MacMOLPlt.[44] Molecular geometries were fully optimised using the B3LYP hybrid functional,[45] in its implementation which is based on the VWN1 formula.[46] The LANL08 core potential basis set[47] was used for Pt and Cl and the 6-31G* basis set[48] for all other atoms. Relative energies are zero point energy (ZPE) corrected and refer to the energy of the starting materials = 0 kcal/mol. The harmonic vibrational frequencies of all stationary points were computed in order to characterise them as local minima or transition states. For all transition states, the vibration associated with the imaginary frequency was examined for being consistent with the product formation. Intrinsic reaction coordinates (IRC) were traced from the transition states towards both reactant and product direction along the imaginary mode of vibration using the algorithm developed by Gonzáles and Schlegel.[49] Mayer bond orders[50] were calculated as implemented in PC GAMESS/Firefly. The topological analysis of the charge densities[51] was performed with the software package MORPHY.[52]

Cytotoxicity Studies

Preparation of the Pt(II) stock solutions. The platinum compounds were dissolved in DMSO and immediately diluted into water to give solutions which are 100 μM in platinum and contain 5 % (v/v) DMSO. Aliquots of these solutions used to achieve 10 μM or 2.5 μM platinum doses in the cell suspension introduce 0.5 % (v/v) and 0.125 % (v/v) of DMSO,

which should have little effect on the growth and survival of the cancer cells.[35] Any effects on the intracellular ATP levels in the presence of more than 0.1 % (v/v) DMSO[36] are compensated by solvent blank measurements.

Cell culture. Cell culture reagents were obtained from PAA Laboratories (Cölbe, Germany). The HeLa and A549 cell lines were obtained from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen). Both cell lines were cultured as attached monolayers in Dulbecco's Modified Eagle's Medium (DMEM, high glucose 4.5 g/L) with 10% fetal bovine serum, 1% MEM non-essential amino acids and 1% Penicillin/Streptomycin supplements.

Cytotoxicity Testing. Cytotoxicity was determined by means of the luminescent cell viability assay CellTiter-Glo®,[53] obtained from Promega, which is based on the luciferase reaction and measures the ATP content of metabolically active cells (as a measure for the number of living cells). Cultured cell monolayers were converted into single cell suspension by treatment with trypsin-EDTA solution, and then seeded into 96-well tissue culture plates at a density of 1 x 10^5 cells per 100 µl. Cells were allowed to settle under standard culture incubation conditions for 24 h and then treated with freshly prepared solutions of the platinum compounds, at Pt concentrations in the cell medium of 2.5 µM and 10 µM, respectively. After 24 h incubation under standard culture conditions cells were lysed for 10 minutes with the CellTiter-Glo® reagent solution and the luminescence signal was read using a multiwell plate luminometer. The quantity of live cells was expressed relative to DMSO treated control cells ("solvent"). Cell viability data given are mean values over three experiments.

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