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**NEUARTIGE OXIMPLATIN(II) KOMPLEXE: SYNTHESE,
CHARAKTERISIERUNG UND ZYTOTOXIZITÄT**

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NOVEL OXIMEPLATINUM(II) COMPLEXES: SYNTHESIS, CHARACTERIZATION AND CYTOTOXICITY

Written by

Mag. Yulia Yu. Scaffidi-Domianello

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Dedicated to my son Antonio

This PhD thesis is based on the following papers and manuscript, which are presented in the original format or as the submitted manuscript:

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Novel Oximato-Bridged Platinum(II) Di- and Trimer(s): Synthetic, Structural, and in vitro Anticancer Activity Studies

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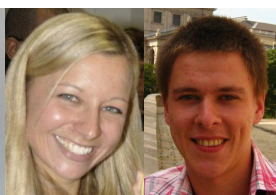
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ABSTRACT

In this work, a broad spectrum of novel platinum(II) complexes bearing various ketoxime and aldoxime ligands was prepared and characterized by elemental analysis, IR and electrospray ionization mass spectrometry (ESI-MS), multinuclear (^1H , ^{13}C , and ^{195}Pt) NMR spectroscopy and, in some cases, X-ray crystallography. Further, evaluation of their cytotoxic potency was carried out.

Initially, in order to study the effect of the leaving group as well as of the carbon chain length of the coordinated ketoxime ligand (both in neutral dichlorido and cationic diammino complexes) on the cytotoxicity, a number of structural modifications of the “parent” acetone oxime platinum(II) complex $[\text{PtCl}_2(\text{Me}_2\text{C}=\text{NOH})_2]$ was performed. For this purpose, *cis/trans*-couples of $[\text{PtX}_2(\text{R}_2\text{C}=\text{NOH})_2]$ [$\text{X} = \text{Cl}, \text{Br}, \text{I}$; $\text{R} = \text{Me}, \text{Et}, n\text{-Pr}, i\text{-Pr}$] and $[\text{Pt}(\text{NH}_3)_2(\text{R}_2\text{C}=\text{NOH})_2](\text{NO}_3)_2$ [$\text{R} = \text{Me}, \text{Et}, n\text{-Pr}$] were synthesized and characterized. The cytotoxicities of the novel species were studied in cisplatin-sensitive ovarian carcinoma (CH1) and inherently cisplatin-resistant colon carcinoma (SW480) cancer cells. Astonishingly, the dihalogenido complexes showed similar IC_{50} values in both tumor cell lines, therefore possessing a promising potential to circumvent cisplatin resistance. Changing the chlorido ligands for bromides in *cis/trans*- $[\text{PtX}_2(\text{Me}_2\text{C}=\text{NOH})_2]$ resulted in the expected decrease of the cytotoxic potency, while the diiodido complexes exhibited an enhanced activity as compared to dibromido species, which could be explained by their increased lipophilicity facilitating cellular accumulation. As the dichlorido complexes *cis/trans*- $[\text{PtCl}_2(\text{R}_2\text{C}=\text{NOH})_2]$ are concerned, the cytotoxicity depends on the substituent R as follows: $\text{Me} \approx i\text{-Pr} \approx n\text{-Pr} > \text{Et}$. The cationic species *cis/trans*- $[\text{Pt}(\text{NH}_3)_2(\text{R}_2\text{C}=\text{NOH})_2](\text{NO}_3)_2$ proved to be less or insignificantly cytotoxic, which corresponds to the classical structure-activity rules postulating inactivity of charged complexes lacking leaving groups. Interestingly, a more pronounced cytotoxicity of the *trans*-configuration was confirmed for two more platinum(II)(oxime) *cis/trans*-couples.

The second project is devoted to the generation of relatively inert platinum species (potential prodrugs) being capable of activation in the slightly acidic microenvironment of solid tumors. For this purpose, a series of zwitterionic diam(m)ine(3-hydroxy-2-(oxidoimino)propan-1-olato- $\kappa^2\text{N},\text{O}$)platinum(II) complexes [diam(m)ine = (ammonia) $_2$, (ethylamine) $_2$, ethane-1,2-diamine, and (*R,R*)-cyclohexane-1,2-diamine] featuring an *N,O*-chelate was generated, followed by the protonation of the oximato group by HCl and consequent opening of the chelate ring. The antiproliferative effect of the prepared compounds under physiological conditions (pH 7.4) was evaluated *in vitro* in three human cancer cell lines (CH1, SW480, and A549), demonstrating IC_{50} values in the medium or even low micromolar range. The solution behavior of a series of complexes featuring the ethane-1,2-diamine ligand was studied by ^1H NMR spectroscopy at pH 7.4, 6.0, and exemplarily 5.0, correlating well with the pH-dependent cytotoxicity; activation of the chelated complex under slightly acidic conditions was observed.

Furthermore, with the aim of increasing the aqueous solubility of the platinum(II)(oxime) species, aldoxime ligands *E/Z*-R(H)C=NOH were applied instead of ketoximes (R₂C=NOH), resulting in *trans*-[PtCl₂{(Z)-R(H)C=NOH}₂] and *trans*-[PtCl₂{(E)-R(H)C=NOH}{(Z)-R(H)C=NOH}] [R = Me, Et]. As a next step, dimerization of the novel platinum(II)(aldoxime) species as well as of previously reported *trans*-[PtCl₂(R₂C=NOH)₂] [R = Me, Et] complexes was performed leading to the corresponding dimers [PtCl(μ-RR'C=NO)(RR'C=NOH)]₂ [R = Me, Et; R' = H, Me, Et]. All compounds were completely characterized, including X-ray diffraction for two monomeric and two dimeric species. Interestingly, the crystal structure of an oximato-bridged trimer featuring three platinum coordination units connected in a chain manner was determined. Additionally, solution behavior of *trans*-[PtCl₂{(Z)-Me(H)C=NOH}₂] and its respective dimer was studied at pH 7.4 and physiological NaCl concentration by ¹H NMR spectroscopy, expectedly resulting in nearly identical equilibrium mixtures. Further, hydrolysis of the platinum(II)(aldoxime) complexes was studied exemplarily with *trans*-[PtCl₂{(Z)-Me(H)C=NOH}₂] under the same conditions in absence of NaCl. The complex was ultimately converted into a new species, supposed to possess a ring-shaped oximato-bridged trimeric structure. Finally, the cytotoxic potency of the monomers and the corresponding dimers was studied in cisplatin-sensitive CH1 and intrinsically cisplatin-resistant SW480 and A549 cells. It was revealed that dimerization leads to a significant (1.3–6.6 times) enhancement of the cytotoxicity. Intriguingly, the novel complexes were found to be not cross-resistant to cisplatin in the SW480 cell line.

ZUSAMMENFASSUNG

Im Zuge dieser Dissertation wurde ein breites Spektrum an neuartigen Oximiplatin(II) Komplexverbindungen synthetisiert und mittels Elementaranalyse, IR- und Elektrospray-Ionisation Massenspektrometrie (ESI-MS), ^1H , ^{13}C , und ^{195}Pt NMR-Spektroskopie und, in einigen Fällen, Röntgenstrukturanalyse charakterisiert. Weiterhin wurde die Zytotoxizität aller hergestellten Komplexe mittels MTT-Assay bestimmt.

Zur Beurteilung der Auswirkung der Abgangsgruppe sowie der Kohlenstoffkettenlänge des Oximliganden auf die Zytotoxizität, wurden *cis/trans*-Paare $[\text{PtX}_2(\text{R}_2\text{C}=\text{NOH})_2]$ $[\text{X} = \text{Cl}, \text{Br}, \text{I}; \text{R} = \text{Me}, \text{Et}, n\text{-Pr}, i\text{-Pr}]$ und $[\text{Pt}(\text{NH}_3)_2(\text{R}_2\text{C}=\text{NOH})_2](\text{NO}_3)_2$ $[\text{R} = \text{Me}, \text{Et}, n\text{-Pr}]$ synthetisiert und vollständig charakterisiert. Die Zytotoxizität wurde in Cisplatin-sensitiven Ovarialkarzinom (CH1) und intrinsisch Cisplatin-resistenten Kolonkarzinom (SW480) Krebszellen untersucht. Da die Dihalogenidokomplexe ähnliche IC_{50} -Werte in den beiden Tumorzelllinien zeigten, besitzen sie ein vielversprechendes Potential zur Überwindung der Cisplatin Resistenz. Austausch der Chlorido- gegen Bromidoliganden in *cis/trans*- $[\text{PtX}_2(\text{Me}_2\text{C}=\text{NOH})_2]$ führte zu der erwarteten Verringerung der zytotoxischen Wirksamkeit, während die Diiodidokomplexe eine verstärkte Aktivität im Vergleich zu den Dibromidospezies zeigten. Dies ist erklärbar durch die erhöhte Lipophilie, die zu einer erhöhten zellulären Akkumulation führt. Bei den Dichloridokomplexen *cis/trans*- $[\text{PtCl}_2(\text{R}_2\text{C}=\text{NOH})_2]$ hängt die Zytotoxizität von dem Substituent R wie folgt ab: $\text{Me} \approx i\text{-Pr} \approx n\text{-Pr} > \text{Et}$. Die kationischen Spezies *cis/trans*- $[\text{Pt}(\text{NH}_3)_2(\text{R}_2\text{C}=\text{NOH})_2](\text{NO}_3)_2$ erwiesen sich als wenig zytotoxisch; dies entspricht den klassischen Struktur-Aktivitäts-Regeln. Zusätzlich wurde eine höhere Zytotoxizität der *trans*-Konfiguration für zwei weitere Oximiplatin(II) *cis/trans*-Paare bestätigt.

Das zweite Projekt ist der Synthese von relativ inerten Platinspezies (potentiellen Prodrugs), die in der leicht sauren Mikroumgebung solider Tumoren aktivierbar sind, gewidmet. Zu diesem Zweck wurde eine Reihe von zwitterionischen Diam(m)in(3-hydroxy-2-(oxidoimino)propan-1-olato- $\kappa^2\text{N},\text{O}$)platin(II)-Komplexen $[\text{Diam(m)in} = (\text{Ammoniak})_2, (\text{Ethylamin})_2, \text{Ethan-1,2-diamin} \text{ und } (R,R)\text{-Cyclohexan-1,2-diamin}]$, gefolgt von der Protonierung der Oximato Gruppe mittels HCl und der anschließenden Öffnung des Chelatringes, synthetisiert. Die antiproliferative Wirkung der hergestellten Verbindungen unter physiologischen Bedingungen (pH 7.4) wurde *in vitro* in drei humanen Krebszelllinien (CH1, SW480 und A549) untersucht. Die IC_{50} -Werte waren im mittleren oder sogar niedrigen mikromolaren Bereich. Das Verhalten der Ethan-1,2-diamin-Komplexserie in wässriger Lösung wurde mittels ^1H -NMR-Spektroskopie bei pH 7.4, 6.0 und exemplarisch bei pH 5.0 untersucht. Die Ergebnisse korrelieren gut mit der pH-abhängigen Zytotoxizität; eine Aktivierung des Chelatkomplexes unter leicht sauren Bedingungen wurde beobachtet.

Zur Erhöhung der Wasserlöslichkeit der Oximiplatin(II) Spezies, wurden Aldoxim Liganden *E/Z*- $\text{R}(\text{H})\text{C}=\text{NOH}$ anstelle der Ketoxime $\text{R}_2\text{C}=\text{NOH}$ für die Synthese von *trans*- $[\text{PtCl}_2\{(\text{Z})$ -

$R(H)C=NOH\}_2]$ und *trans*- $[PtCl_2\{(E)-R(H)C=NOH\}\{(Z)-R(H)C=NOH\}]$ [R = Me, Et] verwendet. Als nächster Schritt wurde die Dimerisierung der neuen Aldoximplatin(II) Spezies sowie der *trans*- $[PtCl_2(R_2C=NOH)_2]$ [R = Me, Et] Komplexe durchgeführt und die entsprechenden Dimere $[PtCl(\mu-RR'C=NO)(RR'C=NOH)]_2$ [R = Me, Et, R' = H, Me, Et] erhalten. Alle Verbindungen wurden vollständig, einschließlich Einkristall-Röntgenstrukturanalyse für zwei monomere und zwei dimere Spezies, charakterisiert. Zusätzlich wurde die Kristallstruktur des Oximato-verbrückten Trimers mit drei kettenförmig verbundenen Platinkoordinationseinheiten bestimmt. Weiters wurde das Verhalten des *trans*- $[PtCl_2\{(Z)-Me(H)C=NOH\}_2]$ Komplexes sowie des entsprechenden Dimers in wässriger Lösung bei pH 7.4 und physiologischer NaCl-Konzentration mittels 1H -NMR-Spektroskopie untersucht. Erwartungsgemäß waren die Gleichgewichtsmischungen nahezu identisch. Zusätzlich wurde die Hydrolyse des Aldoximplatin(II)-Komplexes, *trans*- $[PtCl_2\{(Z)-Me(H)C=NOH\}_2]$, unter den gleichen Bedingungen ohne Zusatz von NaCl untersucht. Schließlich wurde die zytotoxische Wirkung der Monomere und der entsprechenden Dimere in humanen CH1, SW480 und A549-Tumorzellen bestimmt. Es zeigte sich, dass die Dimerisierung zu einer signifikanten (1.3- bis 6.6-fachen) Steigerung der Zytotoxizität führte. Interessanterweise konnte für die neuen Komplexe keine Cisplatin-Kreuzresistenz in SW480-Zellen festgestellt werden.

ABBREVIATIONS

Am	am(m)ine
BOC	<i>tert</i> -butoxycarbonyl
DACH	diaminocyclohexane or cyclohexanediamine
DMF	dimethylformamide
DNA	deoxyribonucleic acid
ESI-MS	electrospray ionization mass spectrometry
Et	ethyl group
5'-GMP	guanosine monophosphate
gDNA	genomic deoxyribonucleic acid
HMG	high mobility group
IC ₅₀	half maximal inhibitory concentration
IR	infrared (spectroscopy)
Me	methyl group
Me-pip	methylpiperidine
MMR	mismatch repair
mtDNA	mitochondrial deoxyribonucleic acid
NER	nucleotide excision repair
NMR	nuclear magnetic resonance
NSCLC	nonsmall-cell lung cancer
pdn	pyridazine
4-phe-1,2,3-trz	4-phenyl-1,2,3-triazolate
pmn	pyrimidine
Pr	propyl group
pz	pyrazolate
pzn	pyrazine
RNA	ribonucleic acid
SCLC	small-cell lung cancer
ta	tetrazolate
TPA	<i>trans</i> -platinum planar amine complex
1,2,3-trz	1,2,3-triazolate

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I. INTRODUCTION

Cancer, or malignant neoplasm, is a general term giving definition to a broad spectrum of diseases that can affect any part of the body (more than 100 different types of cancer are known). Cancer is notable for an uncontrolled growth of a group of abnormal cells and penetration into nearby tissues leading to their destruction. On the later stages, the tumor cells are spread to other parts in the body through the lymphatic or blood system resulting in the formation of metastases.

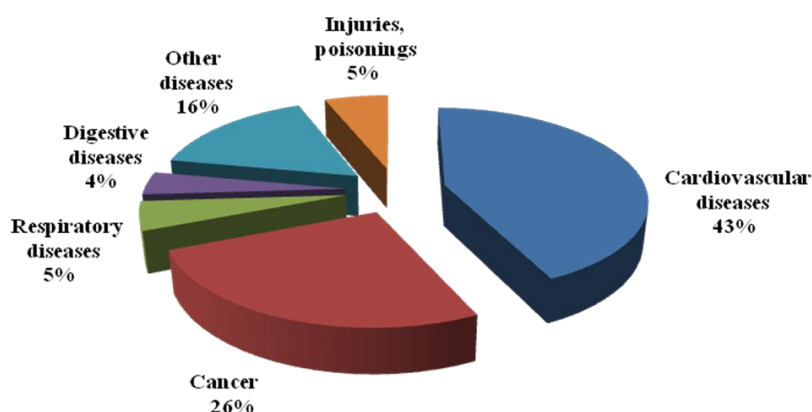


Figure 1. Distribution of causes of deaths in Austria (WHO, 2010).¹

Cancer represents the second most prevailing cause of death in Austria, being inferior solely to the cardiovascular diseases (**Figure 1**).¹ As provided by GLOBOCAN 2008,² it accounted for about 12.7 million cases and 7.6 million deaths (around 13% of all deaths) worldwide in 2008. The predominant types of cancer are lung (1.4 million deaths), stomach (740 000 deaths), liver (700 000 deaths), colorectal (610 000 deaths) and breast (460 000 deaths) cancer, however, it should be noted that the statistical data differ depending on the sex of patients. WHO evaluations for the near future show that the number of deaths caused by cancer worldwide is projected to continue rising, with estimated over 11 million deaths in 2030. These facts explain an extremely high importance of cancer research.

1. CLASSICAL PLATINUM-BASED ANTICANCER THERAPY

1.1. Cisplatin: discovery and mode of action

Cisplatin (**1**, **Figure 2**) is the first platinum-based anticancer agent, which became one of the most widely used antitumor drugs in cancer chemotherapy worldwide. The tumor-inhibiting

properties of cisplatin have been serendipitously revealed over 40 years ago by the physicist Barnett Rosenberg at the Biophysical Department of the Michigan State University (USA).³ Investigating the influence of an electric field on the growth of *Escherichia coli* cells, he observed unexpected filamentation of the bacteria. As it turned out, the fascinating biological effect was caused by compounds formed due to the chemical reaction: platinum from electrodes reacted with ammonium chloride from the culture medium under the influence of light. Thus, *cis*-[Pt^{II}Cl₂(NH₃)₂] (*cis*-diamminedichloridoplatinum(II), or cisplatin, **1**), and *cis*-[Pt^{IV}Cl₄(NH₃)₂] (*cis*-diamminetetracloridoplatinum(IV), **2**) were found to be responsible for this intriguing effect, whereas the corresponding *trans*-analogues (**3** and **4**) were proved to be significantly less active (**Figure 2**). The *cis*-complexes **1** and **2** were able to stop the cell division, leading to filamentous growth of bacteria; subsequent *in vivo* tests disclosed their antitumor activity.⁴ Finally, in 1978 the most active complex, cisplatin, was approved by the US Food and Drug Administration (FDA).

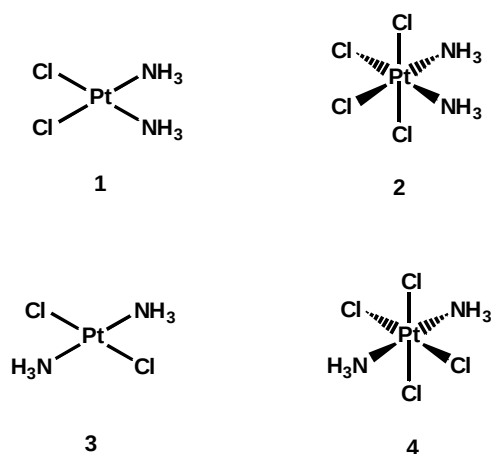


Figure 2. Structures of the *cis*-configured complexes responsible for complete cessation of cell division (**1** and **2**) as well as their nonactive *trans*-analogues (**3** and **4**).

Cisplatin is especially effective in the case of testicular cancer, exhibiting cure rates over 90%.⁵ Alone or in combination with other antitumor agents, it is applied for the treatment of ovarian, cervical, head and neck, esophageal and nonsmall-cell lung cancer.^{6–9} Cisplatin is administered intravenously. Due to a relatively high concentration of chloride ions in the blood (100 mM), the hydrolysis of cisplatin is suppressed and it stays in the intact neutral form. Cisplatin enters the cell basically by passive diffusion, but also by an active transport mechanism mediated by copper transport proteins (e.g. Ctr1) and/or organic cation transporters.^{10–12} The considerably diminished chloride concentration inside the cell (4–20 mM) favors the hydrolysis of the drug resulting in the formation of aqua- and hydroxido species (**Figure 3**).

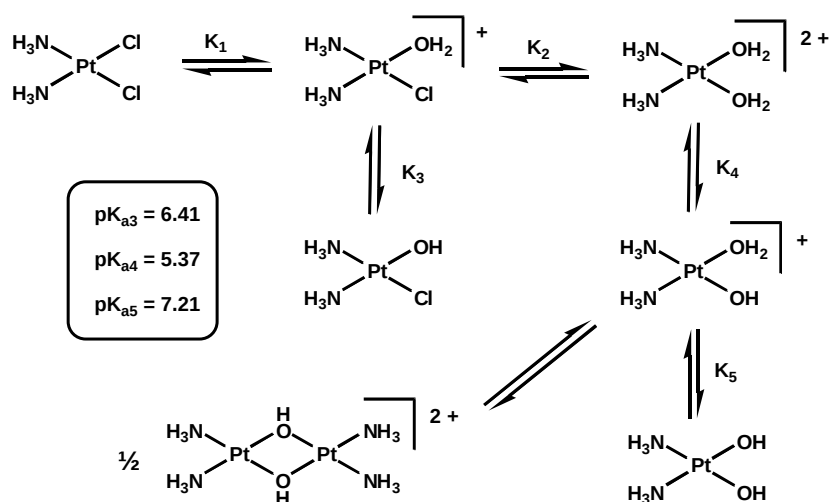


Figure 3. Behavior of cisplatin in aqueous solution.

There is strong evidence that genomic DNA is the primary target of cisplatin.^{13–15} Via exchange of chlorido by aqua ligand(s), the activation of the drug occurs, so that it can readily bind to N7 of the imidazole ring of guanine and to a significantly lesser extent of adenine. Cisplatin was shown to form 1,2-intrastrand cross-links involving adjacent guanine bases (*cis*-GG), comprising 47–50% of all adducts formed, or guanine and adenine bases (*cis*-AG), comprising another 23–28%. Additionally, 8–10% of 1,3-interstrand cross-links involving nonadjacent guanines were detected, as well as 2–3% of monofunctional adducts. It is worth mentioning that in all cases platinum proved to be bound to the N7-atom of a purine base. 1,2-Intrastrand cross-links were found to be the major DNA adducts formed by cisplatin and they are believed to be responsible for its anticancer activity.^{16,17} The binding of cisplatin to DNA distorts its structure by bending and unwinding, which prevents its replication and transcription, ultimately leading to apoptosis (programmed cell death). Additionally, the drug forms a high amount of adducts in mitochondrial DNA (6- and 4-fold higher proportion of adducts in mtDNA as compared with gDNA adducts was found). Considering the fact that mitochondria are incapable of nucleotide excision repair (NER), a major pathway to remove cisplatin-DNA adducts, mitochondrial DNA could be an important pharmacological target for cisplatin as well.¹⁵ Furthermore, it was observed that the complex is able to bind to RNA, which may play a significant role in its mode of action.¹⁸

DNA adducts formed by cisplatin are mainly repaired through the nucleotide excision repair (NER) mechanism,^{15,19} which involves recognition of DNA damage, binding of specific proteins (e.g. XPA, RPA) individually and selectively to the cisplatin-modified DNA, followed by excision of the damaged DNA fragment. Finally, the proteins dissociate and DNA polymerases and ligases fill in and close the gap.

Another significant repair mechanism proved to participate in processing of cisplatin-DNA adducts is the mismatch repair pathway (MMR).^{15,19} It is performed by MMR proteins,

recognizing mismatched or unmatched DNA base pairs and initiating the excision of the damaged area, subsequently resynthesized by DNA polymerases. It was shown that cisplatin-DNA adducts are effectively recognized by MMR proteins, interfering with normal MMR activity and preventing repair from being completed, which ultimately leads to apoptosis.²⁰ Further, a large family of high mobility group (HMG) domain proteins is worth to mention as an important class of proteins capable of specific binding to cisplatin-modified DNA adducts. Moreover, it was demonstrated that HMG-domain proteins selectively bind to 1,2-intrastrand adducts, but not to 1,3-interstrand or monofunctional adducts produced by cisplatin.^{21,22} There are two main hypotheses for how those proteins could influence the sensitivity of cells to cisplatin. Hence, cisplatin-DNA adducts could hijack the HMG-domain proteins from their normal binding sites, leading to the disruption of the cellular function. Alternatively, they could shield cisplatin-DNA adducts from damage recognition necessary for initiating the repair mechanisms, ultimately resulting in cell death. It should be noticed, that these two models do not exclude each other and could work jointly, affecting the biological activity of cisplatin.^{23,24} In spite of the high therapeutic potential of cisplatin, the treatment is accompanied by severe side effects such as nephrotoxicity, being a dose-limiting factor, as well as neurotoxicity, ototoxicity and myelosuppression. Nephrotoxicity, or reduced kidney function and damage, can be diminished through hydration therapy and diuresis.²⁵ Moreover, some tumors are characterized by intrinsic or acquired resistance to cisplatin, which are caused by (i) an insufficient amount of the drug binding to DNA owing to reduced cell accumulation,^{26,27} (ii) an increased level of intracellular thiols^{28,29} (e.g. glutathione, methionine or metallothionein), reacting with cisplatin and leading to its deactivation, as well as (iii) a failure of apoptosis induction after formation of platinum-DNA adducts due to enhanced DNA repair and tolerance.³⁰ Therefore, the search for novel anticancer agents capable of overcoming the drawbacks of cisplatin-based chemotherapy is indispensable.

1.2. Carboplatin and oxaliplatin – advantages and drawbacks

Followed by the discovery of cisplatin and its consequent approval by the FDA, thousands of platinum complexes have been synthesized and screened for their biological properties. However, nearly 40 of them have entered the clinical trials, resulting in the worldwide approval of carboplatin and oxaliplatin (**5** and **6**, **Figure 4**) in 1989 and 2004, respectively.

A second-generation platinum drug carboplatin (**5**), or *cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II), differs from the parent cisplatin solely by the leaving group, comprising the 1,1-cyclobutanedicarboxylato chelate instead of two chlorido ligands in cisplatin. Due to the bidentate coordination of the leaving ligand, carboplatin is kinetically more inert, resulting in substantially reduced side-effects, which enables application of the drug in significantly higher doses. Thus, it proved to be less toxic to the kidney and nervous system and caused less nausea and vomiting than cisplatin, providing an improved quality of

life to cancer patients. However, the spectrum of activity of carboplatin is identical to that of the parent drug, which is explainable by the same pattern of Pt-DNA adducts formed.³¹

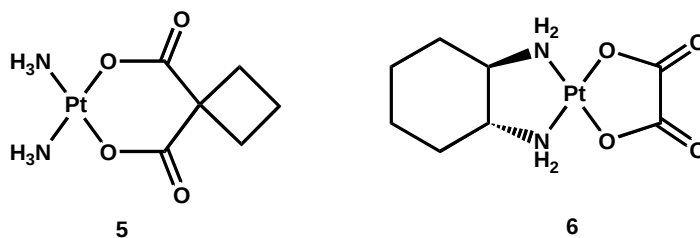


Figure 4. Structures of carboplatin (5) and oxaliplatin (6).

In the third generation platinum drug oxaliplatin (6), or {(1*R*, 2*R*)-cyclohexanediamine}oxalatoplatinum(II), the platinum center bears a bulky bidentate cyclohexanediamine carrier ligand (DACH) as well as a chelated oxalato leaving group. Oxaliplatin was proved to form the same type of interstrand and 1,2-intrastrand DNA cross-links between adjacent guanosine residues as *cis*- and carboplatin, whereas exhibiting a completely different spectrum of anticancer activity and a lack of cross-resistance with the classical anticancer agents.^{32,33} This is in accord with the fact that recognition of the oxaliplatin-DNA adducts by MMR and HMG1 proteins was found to be substantially less effective as compared to the DNA adducts produced by *cis*- and carboplatin.^{34,35}

Oxaliplatin in combination with 5-fluorouracil and leucovorin has been approved worldwide for the treatment of metastatic colorectal cancer,³⁶ while the investigation of its anticancer potential in other tumors is still ongoing. Besides neurotoxicity, which is the dose-limiting factor, side-effects of oxaliplatin include hematologic and gastrointestinal tract toxicity. As compared to cisplatin, it causes significantly less nausea and vomiting as well as milder nephrotoxicity, permitting administration of oxaliplatin without prehydration.³⁷ Interestingly, the following structure-activity relationships for the (cyclohexanediamine)oxalatoplatinum(II) steric isomers were observed: complexes containing the *trans*-1*R*,2*R*-DACH ligand were found to be the most cytotoxic, followed by species comprising coordinated *trans*-1*S*,2*S*-DACH, completing the sequence with the complex bearing *cis*-1*R*,2*S*-DACH being the least cytotoxic.^{38,39} A significance of chirality of the DACH carrier ligand in mediating interactions between oxaliplatin and DNA was additionally proved by X-ray crystallography.⁴⁰

1.3. Regionally approved platinum(II) antitumor drugs

Besides the three platinum-based drugs in worldwide clinical use, there are some complexes which received regionally limited approval for cancer treatment, *e.g.*, nedaplatin (Japan) (7), lobaplatin (China) (8) and heptaplatin (the Republic of Korea) (9) (Figure 5). Nedaplatin^{41,42} features two ammine carrier ligands along with glycolato leaving group. The complex was

selected based on the enhanced anticancer effect it showed in preclinical studies. In general, nedaplatin is less toxic as compared to cisplatin: it is characterized by reduced nephrotoxicity, but increased myelosuppression, being a dose-limiting factor. However, the platinum drug was proved to be cross-resistant with cisplatin in the L1210R leukemia model. In Japan, nedaplatin is approved for application against head and neck, testicular, lung, oesophagel, ovarian, and cervical cancer.

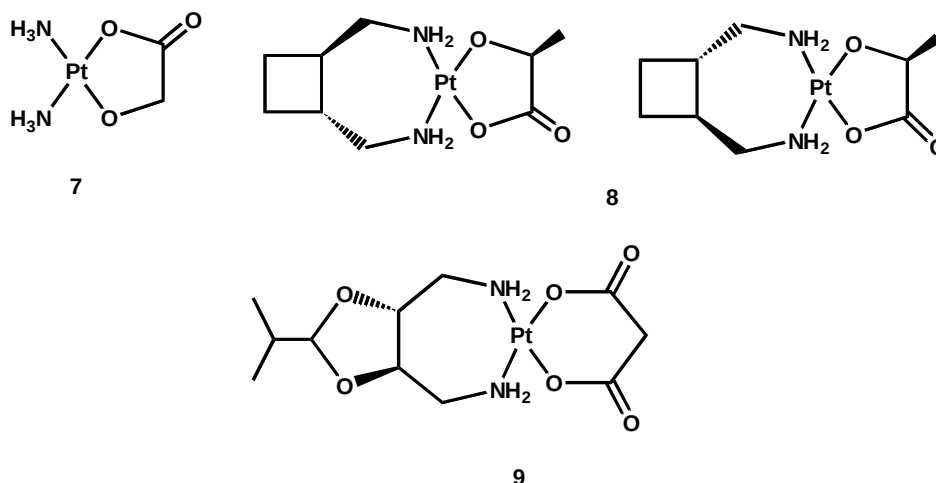


Figure 5. Structures of regionally approved nedaplatin (7), lobaplatin (8) and heptaplatin (9).

Lobaplatin^{42,43} represents an isomeric mixture of two diastereomers containing coordinated 1,2-bis(aminomethyl)cyclobutane and a lactato leaving group. The drug appeared to be similarly or even higher cytotoxic/active as compared to cisplatin both *in vitro* and *in vivo*, exhibiting a preclinical toxicity profile analogous to carboplatin. Thrombocytopenia was found to be dose-limiting. Furthermore, lobaplatin was observed to be noncross-resistant with cisplatin in a human embryonal cell line *in vitro* and in the P388 murine leukemia *in vivo*. The drug has been approved in China for the treatment of chronic myelogenous leukemia, inoperable metastatic breast cancer and small-cell lung cancer.⁴⁴

Heptaplatin^{45,46} features a (4*R*,5*R*)-bis(aminomethyl)-2-isopropyl-1,3-dioxolane carrier ligand and a bidentate malonato leaving group. It demonstrated similar to cisplatin cytotoxicity and antitumor activity, being significantly less toxic. Hepatotoxicity, nephrotoxicity and myelosuppression appeared to be dose-limiting factors. Moreover, heptaplatin was shown to be active against cisplatin-resistant L1210 leukemia cells. Currently the drug is applied in the Republic of Korea against advanced gastric and lung cancer.⁴⁷

2. NONCLASSICAL CYTOTOXIC PLATINUM COMPLEXES

2.1. Cisplatin and transplatin. Early structure-activity relationships

Inability of transplatin, or *trans*-diamminedichloridoplatinum(II), (**3**, **Figure 2**) to induce filamentation in *Escherichia coli* bacteria was revealed in the Barnett Rosenberg's experiments at the same time as the opposite effect of cisplatin was observed followed by elucidation of its anticancer activity.^{3,4} Differently, transplatin was recognized to lack the tumor-inhibiting properties *in vivo* and proved to be significantly less cytotoxic *in vitro* as compared to cisplatin.

Due to the general differences in the chemical structures of *cis*- and *trans*platin, the first aquation step is much faster in the case of *trans*-configuration. It can be explained by a higher *trans*-labilizing effect of the Cl situated *trans* to the leaving chlorido ligand in transplatin as compared with NH₃ ligand in cisplatin (**Figure 6**).

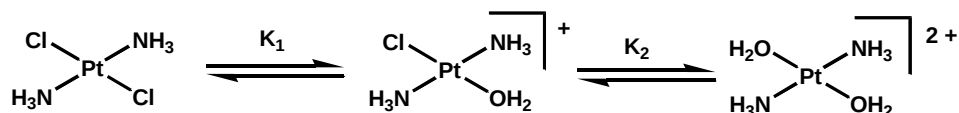


Figure 6. Stepwise hydrolysis of transplatin.

As a consequence, transplatin is more reactive compared to its *cis*-analogue, being involved into the undesired side-reactions on the way to its pharmacological target to a higher extent, which leads to its deactivation and results in a lower amount of the complex reaching DNA. On the contrary, the second step of hydrolysis of transplatin occurs extremely slowly due to a considerably weaker *trans*-effect of H₂O, being *trans*-located to the second leaving chlorido ligand, *versus* NH₃ in cisplatin. This is in accord with the lower rate of bisadducts formation found for transplatin.^{48,49} As far as transplatin-DNA monoadducts are concerned, they could be much easier repaired simply by a chemical pathway reacting with *trans*-labilizing nucleophiles present in the cell, e.g., thiourea, glutathione, whereas repair of the cisplatin-DNA conjugates might involve an enzymatic mechanism (**Figure 7**).

Owing to its stereochemistry, transplatin is not capable of forming 1,2-intrastrand cross-links, which predominate for cisplatin and are believed to determine its anticancer activity. Differently, it was shown to form mainly 1,3-intrastrand and interstrand cross-links proved to change less significantly the stability and structure of DNA.⁵⁰ The high mobility group protein HMG1, effectively recognizing cisplatin-DNA adducts and thereby altering the cellular sensitivity to the drug, was observed to be incapable of recognizing the adducts produced by transplatin.⁵¹ It correlates with the fact that the transplatin-DNA adducts are more rapidly repaired.

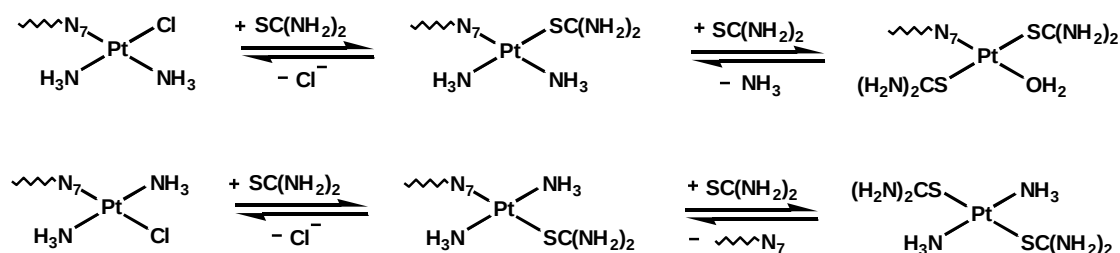


Figure 7. Reaction of cisplatin-(above) and transplatin-DNA monoadduct (below) with thiourea.

After the fascinating anticancer effect of cisplatin was disclosed, a wide variety of analogous platinum(II) complexes was tested for their tumor-inhibiting properties, resulting in some general structure-activity rules drawn by Cleare and Hoeschele.^{52,53} Hence, the following prerequisites for anticancer activity of platinum-based species were established: (i) the complex should have two leaving groups located in *cis*-position to each other; (ii) the compound has to be uncharged; (iii) the leaving groups should be bound moderately to the platinum center, since complexes exhibiting too labile leaving groups proved to be toxic whereas tightly bound leaving groups result in low activity; (iv) the complexes featuring fewer alkyl substituents should be more active.

2.2. Activation of *trans*-geometry

The early structure-activity relationships postulated inactivity of *trans*-configured species, leading to the consequence that platinum complexes of *trans*-geometry have been ignored for decades. However, over the last twenty years several classes of *trans* platinum compounds exhibiting cytotoxicity at least similar or even higher than their *cis*-isomers were published,^{54–56} breaking the classical structure-activity rules.^{52,53} A general interest for these complexes could be explained by their potential to overcome resistance towards the classical *cis*-configured platinum drugs: *trans*-configuration allows the formation of completely different Pt-DNA adducts, resulting in a new spectrum of activity which could be complementary to that of the cisplatin-like antitumor agents used in clinics. The cytotoxic platinum(II) species of *trans*-geometry can be classified into the following groups:

2.2.1. Platinum(II) complexes bearing planar amines

Replacement of one or two ammine ligands in transplatin by sterically demanding planar amine(s) led to complexes of the general formula *trans*-[PtCl₂(L)(L')] (L = NH₃, L' = pyridine, picoline, quinoline, isoquinoline, or thiazole; L = L' = pyridine, thiazole) (**10** and **11**, **Figure 8**).⁵⁶ The use of sterically hindered ligands resulted in reduced hydrolysis rates, decreasing deactivation of the drug before the pharmacological target is reached. Generally, the *trans*-

platinum planar amine (TPA) complexes exhibit cytotoxicity comparable with their *cis*-analogues and similar to cisplatin, whereas being substantially more cytotoxic than transplatin. Analysis of the novel class of platinum-based compounds in the NCI human tumor panel identified this group as having in comparison to the clinically used agents a different cytotoxicity profile and activity in cisplatin- and oxaliplatin-resistant cell-lines.⁵⁷ It was found that the TPA complexes form significantly more intrastrand cross-links in comparison with the “parent” compound transplatin. In general, the distribution of platinum-DNA adducts was carrier-ligand dependent, *e.g.*, in the particular case of thiazole the ratio of intra-, interstrand cross-links and monofunctional adducts was nearly equal.⁵⁸ Interestingly, the monofunctional adducts of TPA compounds were proved to be extremely stable, converting very slowly to a bifunctional mode.⁵⁹ Additionally, the complexes were found to produce single-stranded protein-associated DNA strand-breaks in cells, which offers an attractive potential for the design of selective DNA-protein cross-linkers.^{60, 61} Moreover, the anticancer activity of TPA complexes has been proved in an *in vivo* model: *trans*-[PtCl₂(NH₃)(tz)] demonstrated a moderate activity against P388 leukemia.⁶²

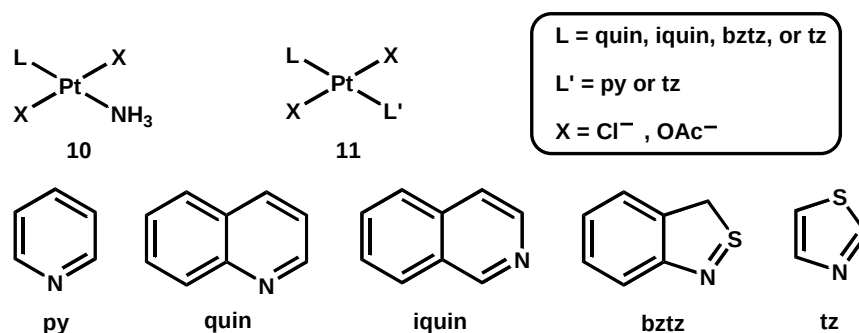


Figure 8. *Trans*-planar amine complexes (TPAs).

In order to increase the aqueous solubility of the TPA species, carboxylato leaving groups were introduced resulting in complexes of general formula *trans*-[Pt(O₂CR₂)(L)(L')].^{63,64} Indeed, the carboxylato derivatives were found to be very water-soluble and stable to hydrolysis, showing cytotoxicity similar to their dichlorido analogues. An increased cellular uptake even in cisplatin and oxaliplatin-resistant cells was observed.

2.2.2. Platinum(II) compounds with piperazine and piperidine

Positively charged *trans*-platinum(II) complexes with monodentately bound piperazine (pz) of general formula [PtCl₂(Am)(pz)] (Am = NH₃, 4-picoline, *n*-butanamine, isopropanamine) (**12**, **Figure 9**) exhibiting a good aqueous solubility were prepared by protecting one of the amines of pz by *tert*-butoxycarbonyl (BOC).⁶⁵ The cytotoxicity of the synthesized piperazine species

was screened on three pairs of cisplatin-sensitive and cisplatin-resistant tumor cell lines: A2780/A2780cisR, 41M/41McisR and CH1/CH1cisR, giving especially low IC₅₀ values in the cisplatin-resistant ovarian cancer cell line. Binding of the novel complexes to DNA occurs more rapidly with respect to cisplatin, while the reaction with cellular proteins, e.g., ubiquitin, myoglobin, proceeds much slower. Moreover, complexes containing piperazine and *n*-butanamine ligands (**12c**) as well as piperidino-piperazine and ammine (**13**) appeared to be the first platinum complexes of *trans*-configuration displaying antiparasitic activity, inducing cell-cycle arrest in G2/M and giving rise to cell death through apoptotic mechanism.⁶⁶

Further, neutral species containing the monofunctional piperidine ligand (**14**, **Figure 9**) were prepared and their cytotoxic potential was tested together with complex containing both piperazine and 4-picoline ligands (**12b**) in human ovarian carcinoma (OV-1063) and colon carcinoma cells (C-26). The latter compound proved to show the lowest IC₅₀ values from the group, however, still being 4-10-fold higher as compared to cisplatin.⁶⁷ These complexes were demonstrated to effectively platinate the cellular DNA, inducing apoptosis in ovarian, but not in colon cancer cells.^{68,69}

The interaction of the selected piperidine and piperazine complexes with DNA was investigated.⁷⁰ The formation of stable 1,3-interstrand cross-links between guanine bases to a significantly higher extent with respect to cis- and transplatin was detected. The produced Pt-DNA adducts were shown to be removed by NER mechanism less effectively than those of cisplatin.

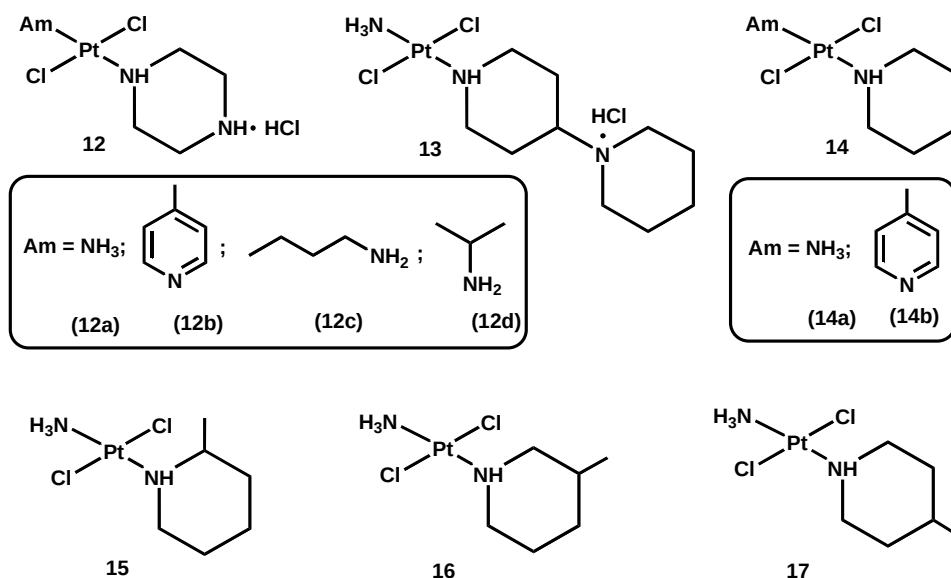


Figure 9. Platinum(II) complexes with piperazine (**12**), piperidino-piperazine (**13**), piperidine (**14**) and methylpiperidine (**15–17**).

Additionally, three isomeric complexes with methylpiperidine (Me-pip) ligands (**15–17**, **Figure 9**) were synthesized and *in vitro* evaluated in cisplatin-sensitive cells A2780, 41M, and CH1,

as well as in the corresponding cisplatin-resistant tumor cells A2780cisR, 41McisR and CH1cisR.⁷¹ Surprisingly, no considerable difference in IC₅₀ values of the isomers was observed; however, the compound containing 2-Me-pip reacted with glutathione and ubiquitin slower than its analogues probably due to steric reasons.

2.2.3. Platinum(II) complexes with aliphatic amines

Another intriguing class of *trans*-configured platinum(II) complexes demonstrating enhanced cytotoxicity with respect to their *cis*-analogues represent platinum(II) dichlorido complexes featuring asymmetric aliphatic amines *trans*-[PtCl₂(Am)(isopropylamine)] (Am = NH₃, *N,N*-dimethylamine, propanamine, butanamine) (**18**, Figure 10).^{72,73}

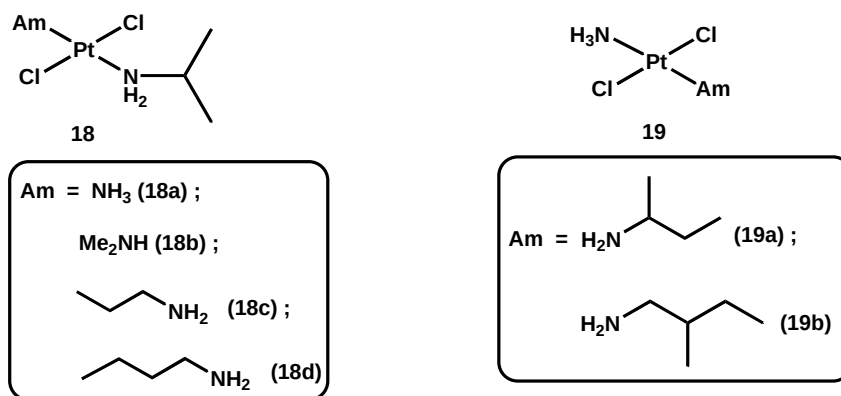


Figure 10. Platinum(II) complexes containing aliphatic amines

The complexes were screened *in vitro* in a series of tumor cell lines, exhibiting similar or even lower IC₅₀ values compared to cisplatin both against cisplatin-sensitive (Jurat, Hela, Vero) and -resistant (CH1cisR, HL-60, Pam212-*ras*) cancer cells. An apoptotic cell death initiation was confirmed in the case of *trans*-[PtCl₂(Me₂NH)(Me₂CHNH₂)] (**18b**) in Pam212-*ras* and in CH1cisR cells and for *trans*-[PtCl₂(*n*-BuNH₂)(Me₂CHNH₂)] (**18d**) both in Pam212-*ras* and A2780cisR cell lines. Moreover, complex **18b** demonstrated a lack of cross-resistance with cisplatin in both A2780cisR and CH1cisR cells and was shown to form mostly interstrand cross-links between complementary guanine and cytosine residues. However, no *in vivo* antitumor activity in human ovarian carcinoma xenografts in mice was detected, probably, due to a high binding rate towards plasma proteins.^{74,75}

Furthermore, *trans*-platinum(II) complexes containing an ammine ligand along with more sterically demanding branched amines *trans*-[PtCl₂(NH₃)(Am)] (Am = 2-methylbutanamine or *sec*-butanamine) (**19**, Figure 10), demonstrated outstanding cytotoxic properties, being more active than cisplatin both in cisplatin-sensitive and resistant cancer cell lines.⁷⁶ The

compounds were found to form mainly interstrand cross-links (40–50%) and monofunctional DNA lesions (30–40%).

2.2.4. Platinum(II) complexes with iminoether ligands

The following class of active platinum-based compounds of *trans*-geometry represent platinum(II) complexes featuring one or two iminoether ligands $\text{HN}=\text{C}(\text{OR})\text{Me}$ ($\text{R} = \text{Me}, \text{Et}$) reported by the group of Coluccia and Natile (**20–22**, **Figure 11**).^{77–79} Addition of an alcohol to a platinum-bound nitrile results in the formation of a coordinated *Z*-iminoether, which can be further converted into the corresponding *E*-analogue under basic conditions.⁸⁰

In general, the complexes are characterized by a considerably enhanced cellular accumulation as compared to that of cisplatin in ovarian cancer cells A2780 and A278cisR, probably due to their increased lipophilicity. Moreover, *trans*-iminoether complexes exhibit an increased degree of DNA platination. As opposed to *cis*- and *trans*platin, they demonstrated a low tendency to intrastrand DNA cross-linking, forming preferentially stable monofunctional adducts at guanine residues, which proved to be more resistant towards reactions with sulfur-donor ligands than the DNA adducts produced by *trans*platin.^{81, 82} Monofunctional DNA adducts formed by *trans*-(iminoether)platinum(II) complexes can readily react with proteins resulting in the formation of specific DNA-protein cross-links supposed to be responsible for the tumor-inhibiting properties of iminoether complexes.

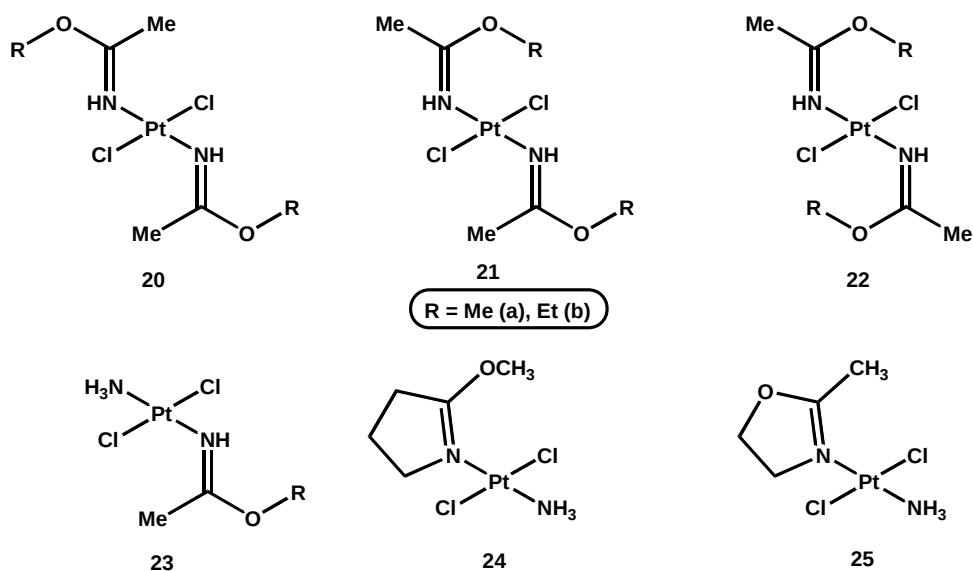


Figure 11. *Trans*-platinum(II) complexes comprising iminoethers (**20–23**) as well as cyclic iminoether-mimicking ligands (**24** and **25**).

A comparative *in vitro* study of *trans*- $[\text{PtCl}_2\{\text{HN}=\text{C}(\text{OMe})\text{Me}\}_2]$ isomers featuring *ZZ*-(**20a**), *EZ*-(**21a**) and *EE*-ligands (**22a**) elucidated that the former complex (**20a**) possessed the highest

cytotoxic potency. Furthermore, **20a** demonstrated a significant antitumor activity *in vivo* against P388 and P388cisR leukemia, being better tolerated by animals as compared to cisplatin.

Considering ethoxy analogues, *EE*-complex (**22b**) appeared to be more cytotoxic than its *ZZ*-isomer (**22a**) in a panel of human tumor cell lines (ovarian, colon, lung, and breast cancer), revealing opposite structure-activity relationships compared to the methoxy complexes.⁷⁸ Interestingly, both ethoxy compounds proved to be noncross-resistant with cisplatin in A2780cisR cells. However, *in vivo* tests carried out in the murine P388 leukemia system showed a slightly enhanced antitumor effect in case of the *ZZ*-isomer (**20b**).

Exchange of one ammine in transplatin for an iminoether ligand resulted in a dramatic increase of the cytotoxic potency (**23**, **Figure 11**), especially in the case of ovarian and colon tumor cells.⁷⁹ In addition, **23** appeared to be active *in vivo* against SK-OV-3 human cancer cell xenografts in nude mice and in the murine P388 leukemia system.

Finally, two compounds with cyclic ligands mimicking iminoethers were prepared in order to enable *E*↔*Z* isomerization (**24** and **25**, **Figure 11**).⁸³ The IC₅₀ values for both of the complexes were lower than those of **20–23** in a panel of human tumor cells (colon, ovary, lung, and breast). Interestingly, complex **25** was shown to be more cytotoxic as compared to **24**.

2.2.5. Acetonimine platinum(II) complexes

As next, platinum(II) complexes containing one or two acetonimine ligands have been prepared by condensation of platinum-coordinated ammine with acetone (**26–29**, **Figure 12**).⁸⁴

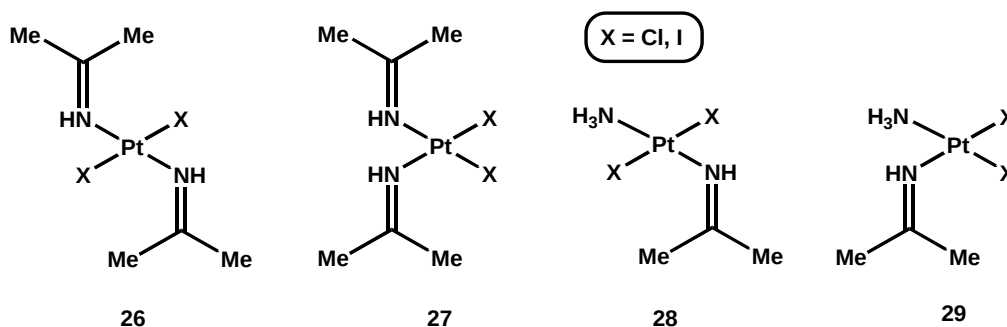


Figure 12. Platinum(II) complexes with acetonimine ligand(s).

A clear evidence for the activation of *trans*-geometry was demonstrated: while the cytotoxicity of the *cis*-configured acetonimine species (**27** and **29**) proved to be lower with respect to cisplatin in a panel of human ovarian, colon, lung, and breast cancer cells, the opposite dependence between the *trans*-isomers (**26** and **28**) and transplatin was observed.

Interestingly, in the case of *cis*-geometry the bisacetoneimine complex **27** appeared to be less active than ammine/acetonimine compound **29**, whereas the *trans*-bisacetoneimine species **26** exhibited enhanced cytotoxic properties compared to the corresponding *trans*-ammine/acetonimine complex **28**. Ability to circumvent either partially or completely the resistance to cisplatin was demonstrated for acetoneimine-containing complexes of *trans*-geometry.

2.2.6. Oxime platinum(II) complexes

Very recently a novel and intriguing class of cytotoxic *trans*-configured platinum species was published, namely, platinum(II) complexes containing oxime ligands. In the last few years two independent working groups reported on remarkable *in vitro* results for three acetone oxime containing complexes (**30–32**, **Figure 13**). Firstly, Navarro-Ranninger et al. comparatively examined the cytotoxicity and ability to induce cell death via either apoptotic or necrotic mechanism of *trans*-[PtCl₂(Me₂C=NOH)(Me₂CHNH₂)] complex (**30**) and the *cis*-configured counterpart (**31**) in rat renal tubular cells (NRK-52E) and human hepatoma cells (HepG2). Unlike *cis*-[PtCl₂(Me₂C=NOH)₂] (**31**), the complex of *trans*-geometry (**30**) was shown to cause apoptosis, although being less cytotoxic.⁸⁵

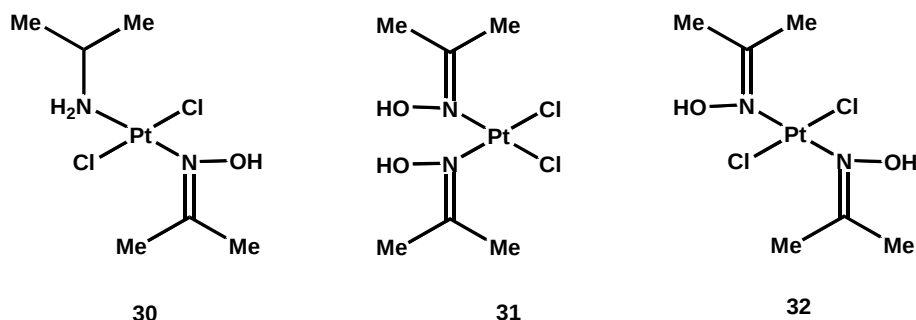


Figure 13. Structures of platinum(II) complexes bearing oxime ligands.

Almost at the same time, Keppler et al. reported on a *cis/trans* couple of platinum(II) complexes featuring two acetone oxime ligands (**31** and **32**).⁸⁶ Fascinatingly, *trans*-[PtCl₂(Me₂C=NOH)₂] (**32**) was found to be more cytotoxic than its *cis*-isomer (**31**) not only in cisplatin-sensitive ovarian carcinoma cell line (CH1), but also in cisplatin-resistant colon carcinoma cells (SW480), moreover, being by a factor of 15 more potent than cisplatin in SW480 cells. It was demonstrated that the novel complex of *trans*-geometry binds slowly to 5'-dGMP as compared to cisplatin ($\tau_{1/2}$ = 50 h versus 8.9 h, respectively), exhibits low reactivity towards DNA and influences insignificantly the secondary structure of DNA. Moreover, no Pt-DNA interstrand cross-links could be detected. All these facts indicate a completely different

mechanism of antitumor action of *trans*-[PtCl₂(Me₂C=NOH)₂] as compared with that characterizing the classical anticancer platinum drugs.

2.3. Synthesis of platinum(II) complexes with *trans*-configuration

There are several methods for the preparation of platinum(II) complexes exhibiting *trans*-geometry. The first pathway includes two steps and is widely used for the synthesis of symmetric ($Am_1 = Am_2$) and asymmetric diamine *trans*-[PtCl₂(Am₁)(Am₂)] species (**Figure 14**). Initially, the *cis*-isomer is converted into the cationic tetramine compound, followed by thermal decomposition or addition of concentrated HCl, which results in the target *trans*-dichlorido complex. At the second step, after one amine ligand is substituted for chloride, the *trans*-located amine is exchanged due to the higher *trans*-labilizing effect of Cl as compared to am(m)ines.⁸⁷ For instance, this pathway was applied to prepare *trans*-platinum(II) complexes with planar^{88,89} and aliphatic amines.⁷²

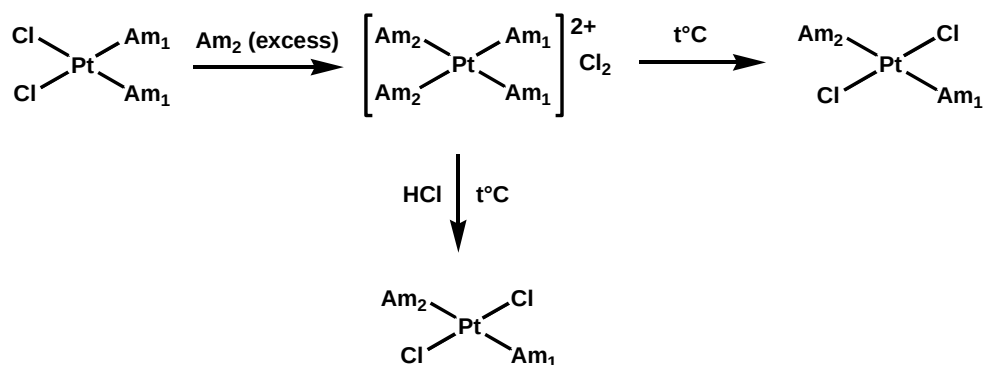


Figure 14.

Another possible way for the synthesis of *trans*-configured platinum(II) species is isomerization of the corresponding *cis*-complex into its *trans*-analogue carried out either in an appropriate solvent or in the solid state. The direction of the conversion is determined by a higher thermodynamic stability. For instance, dissolution of *cis*-[PtCl₂(cyclobutylamine)₂] in acetone leads to its conversion into the *trans*-isomer.⁹⁰ An additional example, *trans*-configured (oxime)platinum(II) complexes are prepared by thermal isomerization of their *cis*-analogues either in solution in nitromethane or in the solid state.⁹¹

Furthermore, *cis*-[PtCl₂(L)₂] is used as a starting complex as well, being firstly converted into the dichlorido-bridged dimer [Pt(μ-Cl)Cl(L)]₂ via reaction with platinum(II) chloride, followed by addition of the amine ligand L' to obtain the desired *trans*-[PtCl₂(L)(L')] complex (**Figure 15**). This pathway was applied for the synthesis of platinum(II) complexes with aliphatic amines⁷³ as well as various asymmetric compounds.^{92,93}

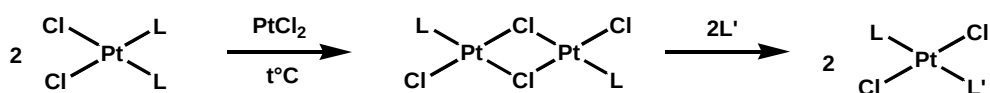


Figure 15.

For the generation of *trans*-diacetato complexes *trans*-[Pt(OAc)₂(L₁)(L₂)], the corresponding *trans*-dihalogenido species are treated with two equivalents of silver acetate either in DMF (for the low soluble dichlorido complexes) or in acetone (in the case of diiodido species) (**Figure 16**). This pathway was used for the preparation of *trans*-configured compounds featuring 3-picoline or 4-picoline ligands as well as for complexes containing pyridine, quinoline, or isoquinoline.^{63,64}

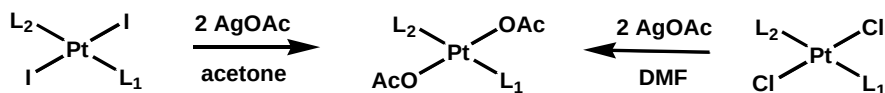


Figure 16.

2.4. Multinuclear platinum(II) compounds

Multinuclear platinum(II) complexes represent an individual class of platinum-based cytotoxic agents, differing from the classical compounds used in the clinics both by their structure and mode of biological action. A broad and the most extensively studied family consists of complexes comprising two or more platinum coordination units PtCl_n(NH₃)_{3-n} connected to each other in a chain manner by various linkers, *e.g.*, flexible NH₂(CH₂)_nNH₂ or more rigid 4,4'-dipyrazolylmethane.⁹⁴ Interestingly, the more flexibility the compounds displayed the higher activity they demonstrated.

The most promising representative appeared from this class which was already investigated in clinical trials is [*trans*-diamminechloridoplatinum(II)][(μ-*trans*-diamminedi-hexanediamine-*N,N'*)platinum(II)] nitrate, denominated as BBR3464 (**33**, **Figure 17**), a trinuclear compound with an overall charge of +4. The complex was shown to be taken up by both cisplatin-sensitive and resistant cancer cells much faster and in higher amounts as compared to cisplatin.^{95,96} Owing to the positive charge, BBR3464 is pre-associated on the polymer backbone of DNA, resulting in a faster DNA binding.⁹⁷⁻⁹⁹ The complex was shown to produce long-range intra- and interstrand DNA adducts, characterized by a high flexibility.¹⁰⁰⁻¹⁰² In *in vitro* studies, BBR3464 demonstrated IC₅₀ values up 1000-fold lower than those of cisplatin in

cisplatin-sensitive cells. Additionally, the compound appeared to be capable of overcoming cisplatin resistance in a broad spectrum of cancer cells (small-cell lung cancer (SCLC), nonsmall-cell lung cancer (NSCLC), melanoma, glioma, neuroblastoma, osteosarcoma, astrocytoma, cervical, ovarian and prostatic). However, BBR3464 demonstrated severe toxic effects, neutropenia and gastro-intestinal toxicity being the dose-limiting factors.¹⁰³ The complex reached clinical phase II for gastric, ovarian, SCLC and NSCLC but the trials were abandoned due to lack of activity.^{104,105} Phase II studies in locally advanced or metastatic pancreatic cancer are ongoing.

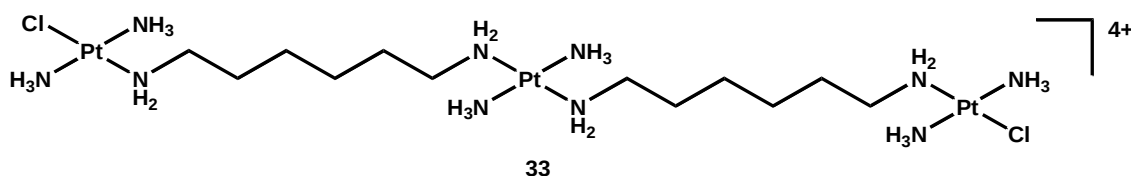


Figure 17. Structure of BBR3464.

Furthermore, several classes of multinuclear platinum(II) complexes displaying intriguing cytotoxic properties *in vitro* were reported in the literature. Firstly, dinuclear platinum-bisphosphonate complexes were prepared by Natile et al. in order to attain a specific accumulation of the drugs at the bone tissue (**34**, **Figure 18**).

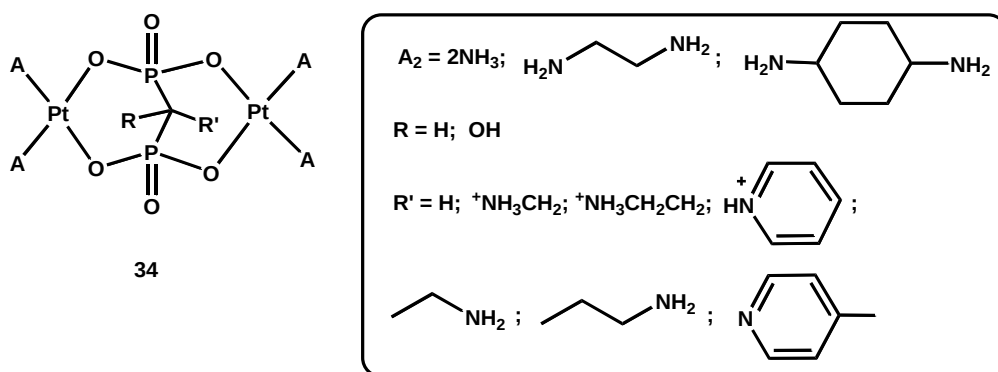


Figure 18. Dimeric platinum-bisphosphonate complexes.

Loading of the complexes comprising ethane-1,2-diamine into a silica-xerogel was performed with the aim to obtain a hybrid inorganic matrix releasing active platinum species for the treatment of bone tumors.¹⁰⁶ The presence of calcium in the matrix was found to influence the loading capacity, stability of the formulations and the nature of platinum complexes released. Further, complexes containing two ammine ligands or *cis*-cyclohexane-1,4-diamine along with $R' = ^+H_3NCH_2$ or $^+H_3NCH_2CH_2$ were prepared and evaluated in a set of 13 tumor cell lines of

multiple origin.¹⁰⁷ The new cationic compounds were found to be capable of overcoming cisplatin resistance in A431/Pt and C13* cells, in general exhibiting IC₅₀ values similar to those of carboplatin. Finally, diammine and *cis*-cyclohexane-1,4-diamine complexes featuring neutral amines as R' were synthesized, showing a modest cytotoxicity, which was explained by a slow and inadequate hydrolysis.¹⁰⁸

As next class, a series of hydroxo- and azolato-bridged platinum(II) complexes (**35**, **Figure 19**) has been prepared. OH⁻ acts as a leaving group, providing a bifunctional coordination to DNA.¹⁰⁹ The cytotoxic potential of the compounds featuring a bridging pyrazolate (pz) or 1,2,3-triazolate (1,2,3-trz) was investigated in several human tumor cell lines, e.g., melanoma, breast, colon, ovarian, renal, and nonsmall-cell lung cancer.¹¹⁰ The species featuring two ammine ligands were found to be up to 37 times higher cytotoxic than cisplatin, while those containing *trans*-cyclohexane-1,2-diamine were found to be moderately active. Further, a complex featuring a bridging 4-phenyl-1,2,3-triazolate (4-phe-1,2,3-trz) ligand along with the previously mentioned ammine-containing species was tested in both cisplatin-sensitive and resistant L1210 murine leukemia cell lines, exhibiting the ability to overcome acquired cisplatin-resistance in the cases of μ -1,2,3-trz or μ -4-phe-1,2,3-trz.¹¹¹ Additionally, two isomeric platinum(II) dimers containing bridging tetrazolato (ta) ligands coordinated to the platinum centers either *via* N1 and N2 or through N2 and N3 were synthesized (**35**, **Figure 19**).¹¹² The latter complex appeared to be double as cytotoxic as either cisplatin or its N1, N2-ligated isomer toward the H460 human NSCLC cell line. Moreover, it showed strikingly high antitumor efficacy in the pancreatic tumor PANC-1 xenograft, demonstrating markedly improved activity compared with gemcitabine, a front-line therapy for pancreatic cancer.

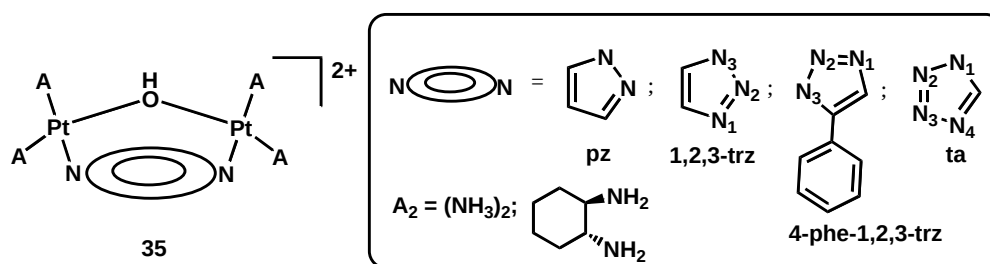


Figure 19. Platinum(II) dimers bridged by azolato ligands.

Additionally, isomeric azines, e.g., pyrazine (pzn), pyrimidine (pmn), and pyridazine (pdn) were applied as bridging ligands for novel dinuclear platinum(II) complexes (**36**, **Figure 20**).¹¹³ The compounds demonstrated high specificity for the IGROV and WIDR cell lines *in vitro*. The pyrazine-bridged complex showed the best results being as cytotoxic as cisplatin in L1210 leukemia cells and exhibiting the highest potential to circumvent acquired resistance to cisplatin in L1210cisR. Generally, the azine-bridged platinum(II) dimers (**36**) appeared to be

less cytotoxic as compared to the azolato-bridged complexes (**35**), which could be explained by a significantly slower reactivity of the latter species upon binding to nucleic acids.¹¹¹

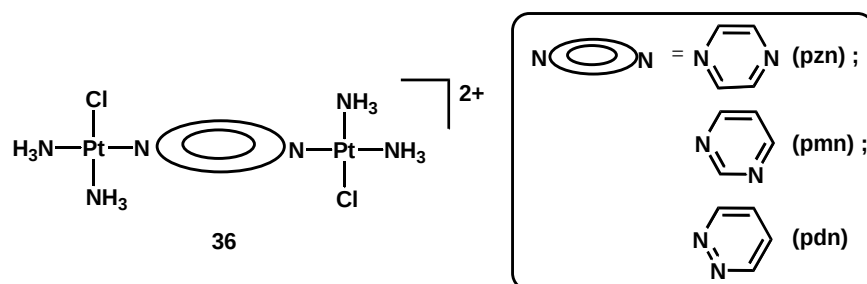


Figure 20. Azine-bridged dinuclear platinum(II) complexes.

Further, a dimeric platinum(II) complex with a bridging 4, 4'-dipyrazolylmethane ligand was prepared and *in vitro* evaluated in A2780 and A2780cisR human ovarian cancer cells (**37**, **Figure 21**),¹¹⁴ being significantly less cytotoxic than cisplatin with IC₅₀ values of 100 and 60 μM, respectively.

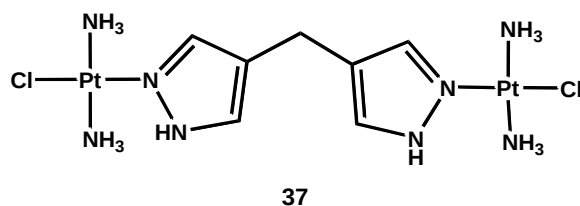


Figure 21.

An extraordinary example of a dinuclear platinum(II) compound represents a diplatinum(II) tetracarboxylate complex, having a dimeric carboplatin structure (**38**, **Figure 22**).¹¹⁵ The novel complex proved to be more cytotoxic than carboplatin in all malignant tumor cell lines tested (up to 8 times in HL60 cells), moreover, demonstrating *in vivo* a remarkably lower general toxicity in comparison with the parent clinical drug.

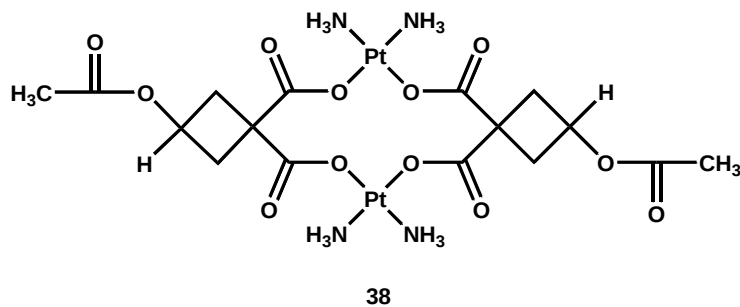


Figure 22. Dimeric carboplatin analogue.

2.5. Activation strategies of platinum-based prodrugs at the tumor site

Whereas the normal differentiated cells generate energy via mitochondrial oxidative phosphorylation, cancer cells were shown to rely on glycolysis (“the Warburg effect”, Otto Warburg, 1924).^{116,117} It results in an increased production of lactic acid, which cannot be always quickly removed due to the rapid growth and the poorly organized vasculature of the solid tumors. In the course of tumor growing, the oxygen concentration within the tumor decreases as compared to the normal tissue; the phenomenon is termed “hypoxia”^{118,119} and is explained by an insufficient tumor blood supply. For this reasons the shift to an anaerobic cellular metabolism is favored, leading to a significantly acidic extracellular pH (pH_e) (acidosis). Thus, pH_e of solid tumors was found in the range between 5.5 and 7.3,^{120–122} while in the case of the normal tissue pH_e is at approximately 7.4.

An acidic pH value as well as a reductive environment within solid tumors could be beneficially used: relatively inert anticancer agents (prodrugs) could be introduced being capable of activation at the tumor site either by reduction or protonation, leading to the formation of active species. This approach could help to significantly improve the therapeutic index defined as ratio of the drug efficacy to its toxicity.

2.5.1. Activation by reduction: platinum(IV) complexes

The ability of a platinum(IV) complex *cis*-[PtCl₄(NH₃)₂] (**2**, **Figure 1**) to induce the anticancer effect was disclosed simultaneously with the discovery of the tumor-inhibiting properties of cisplatin in Barnett Rosenberg’s experiments.^{3,4} However, the biological properties of **2** proved to be substantially weaker, resulting in a major focus on platinum(II) species. The crucial feature of platinum(IV) complexes is their greater kinetic inertness as compared to platinum(II) analogues leading to an extremely low rate of ligand-exchange reactions. Although the aquation of platinum(IV) species could be observed, it appeared to be much too slow to cause any biological consequences. To be activated, they have to be reduced into the corresponding platinum(II) analogues (so-called activation by reduction), playing thus a role of prodrugs (**Figure 23**).^{123,124}

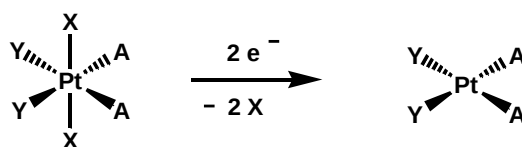


Figure 23. The two-electron reduction of octahedral platinum(IV) to square-planar platinum(II) complex, accompanied by a loss of axial ligands.

The rate of reduction in biological media was shown to be much higher than that of aquation.¹²⁵ This fact explains the primary importance of the reduction potential for platinum(IV) compounds, which is mainly determined by the nature of the axial ligands. Additionally, it is worth to mention that there are no structure-activity relationships for platinum(IV) anticancer agents except that the respective platinum(II) complex should be active.

To date, there are several platinum(IV) complexes that have entered the clinical trials, e.g., tetraplatin (**39**), iproplatin (**40**), JM216 or satraplatin (**41**) and LA-12 (**42**) (**Figure 24**). Tetraplatin ($[\text{PtCl}_4(\text{trans-cyclohexane-1,2-diamine})]$) (**39**) demonstrated promising results in preclinical studies, however, was found to cause severe peripheral neuropathy in treated patients and was abandoned after phase I trials.¹²⁶ Due to its high reduction potential owing to the axial chlorido ligands, tetraplatin is easily reduced being activated on the way to its pharmacological target, which results in a high general toxicity.

On the contrary, iproplatin ($\text{cis,trans,cis-}[\text{PtCl}_2(\text{OH})_2(\text{isopropylamine})_2]$) (**40**), featuring axial hydroxido ligands and therefore being characterized by a more negative reduction potential, stays mostly unchanged resulting in insufficient activity. The complex was abandoned after phase III clinical trials.^{127,128}

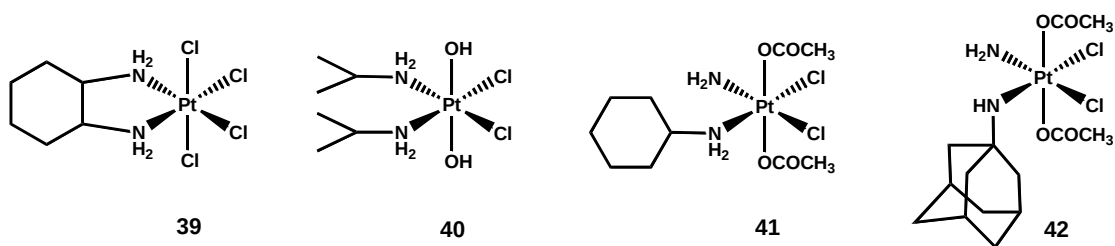


Figure 24. Platinum(IV) complexes entered clinical studies: tetraplatin (**39**), iproplatin (**40**), JM216 or satraplatin (**41**), and LA-12 (**42**).

JM-216 or satraplatin ($\text{cis,trans-}[\text{PtCl}_2(\text{OAc})_2(\text{NH}_3)(\text{cyclohexylamine})]$) (**41**), is a lipophilic complex which was rationally designed to allow oral administration.^{129, 130} Bearing axial acetato ligands, satraplatin exhibits an intermediate reduction potential, providing an optimal reduction rate in biological media. The drug was recently in phase III clinical trials but the trials were abandoned based on the lack of improvement over the anticancer agent already used in the clinics.

Lately, Kozubik et al. reported on a novel platinum(IV) drug candidate: LA-12 (**42**, **Figure 24**), featuring a bulky 1-adamantylamine ligand, is a highly lipophilic platinum(IV) complex, capable of overcoming acquired resistance to cisplatin *in vitro* in human ovarian A2780cisR cells.¹³¹ The complex demonstrated promising results in preclinical mouse studies: an oral activity was shown in a murine ADJ/PC6 plasmacytoma and human A2780 ovarian carcinoma tumor model.¹³² LA-12 entered phase I clinical trials.

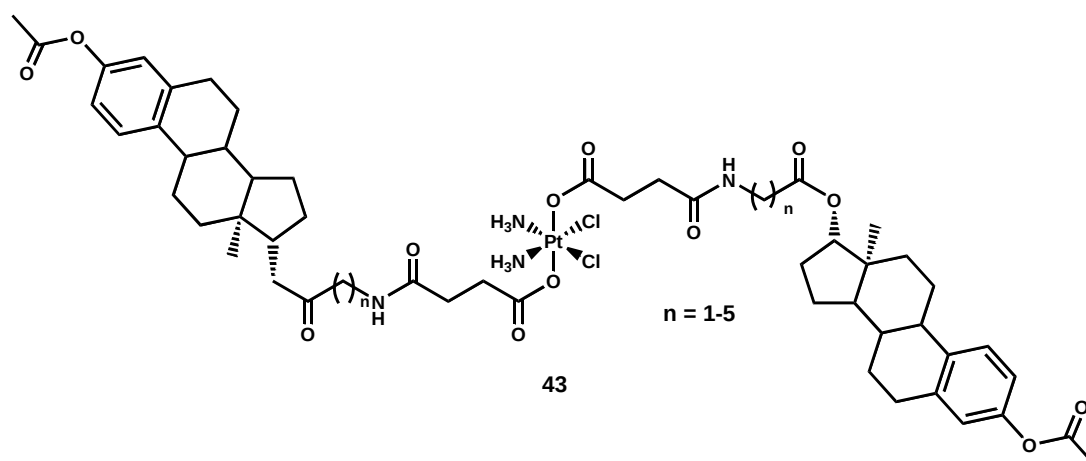


Figure 25.

Two works of particular interest in the field of antineoplastic platinum(IV) compounds are based on utilization of bioactive moieties in axial positions, targeting specific molecular features of a tumor and being released after reduction of Pt(IV) to Pt(II). For instance, in order to target platinum to estrogen receptor positive breast cancer cells, a conjugation of estradiol 3-benzoate ligands axially to a platinum core was performed by Lippard et al. (43, **Figure 25**).¹³³

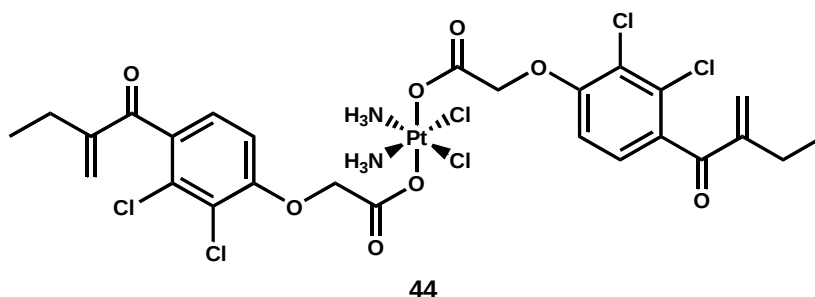


Figure 26.

Alternatively, attachment of ethacrylate to platinum(IV) was carried out by Dyson et al. with the aim to inhibit glutathione-S-transferase exhibiting increased levels in some cisplatin-resistant cancer cells (44, **Figure 26**).¹³⁴

Very recently, a novel class of platinum(IV) compounds was reported: three dihydroxido complexes of the general formula *trans, trans, trans*-[PtCl₂(OH)₂(Am₁)(Am₂)] exhibiting *trans*-configured equatorial amine ligands were published (45, **Figure 27**).^{135, 136} The cellular properties of *trans, trans, trans*-[PtCl₂(OH)₂(Me₂NH)(Me₂CHNH₂)] (45a) were evaluated in comparison with its platinum(II) counterpart in pairs of cisplatin-sensitive and -resistant cell lines (A2780/A2780cisR, CH1/CH1cisR, and 41M/41McisR).¹³⁵ It was demonstrated that

acquired cisplatin resistance was circumvented by platinum(IV) complex **45a** both in CH1cisR and 41McisR, but solely in CH1cisR cells by its platinum(II) counterpart. Moreover, only the platinum(IV) complex displayed activity *in vivo* in CH1 human ovarian carcinoma xenografts in mice, which was explained by its lower rate of binding to plasma proteins decreasing extracellular inactivation as compared with its platinum(II) analogue.

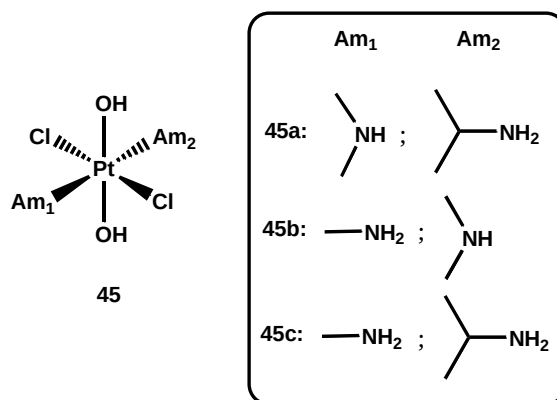


Figure 27. Platinum(IV) complexes of *trans*-geometry.

Later, two further *trans*-platinum(IV) complexes (**45b** and **45c**) were synthesized and their cytotoxic properties were examined.¹³⁶ Interestingly, *trans*-platinum(IV) compounds were demonstrated to be similarly or even higher cytotoxic than their platinum(II) congeners, although displaying considerably negative reduction potentials (between -666 and -770 mV), usually associated with resistance to reduction and high IC₅₀ values. The authors suggested that it could be related to the *trans*-location of the chlorido ligands, which are reduced much easier.

2.5.2. Activation under mildly acidic conditions

There are several classes of pH-sensitive platinum(II) complexes being capable of activation in the acidic microenvironment found in solid tumors.¹³⁷ Firstly, Schilling et al. reported on the synthesis and biological properties of alkyldithiocarbonatoplatinum(II) complexes (**46**, **Figure 28**) exhibiting enhanced cytotoxicity at pH 6.8 as compared to pH 7.4 due to opening of a rather kinetically inert chelate cycle leading to formation of an active form (**47**, **Figure 28**).^{138, 139} Thioplatin, or bis-(O-ethyldithiocarbonato)platinum(II) (**46**, R = Et), the most exhaustively studied representative of this class, was shown to platinate DNA with a substantially higher efficiency at pH 6.8 than at pH 7.4 as well as to dramatically improve its cytotoxic properties in slightly acidic milieu (IC₅₀ in HeLa cells: 5 μ M at pH 6.8 *versus* 51 μ M at pH 7.4). *In vivo* efficacy of thioplatin was evaluated in a human small-cell lung carcinoma (H16) and a colorectal carcinoma (SW707) xenograft in athymic nude mice, demonstrating

5–10 times better therapeutic index as compared to cisplatin.¹³⁸ To set up the structure-activity relationships, over 20 derivatives of thioplatin were prepared and their antitumor properties were investigated in a panel of six human tumor cell lines, demonstrating up to 7 or 25 times enhanced cytotoxicity as compared to thioplatin or cisplatin, correspondingly. Moreover, complexes featuring secondary xanthates ($R = \text{CH}(\text{R}_1)_2$ or CHR_1R_2) appeared to be significantly more active than those containing primary ligands ($R = \text{CH}_2\text{R}_1$).¹³⁹

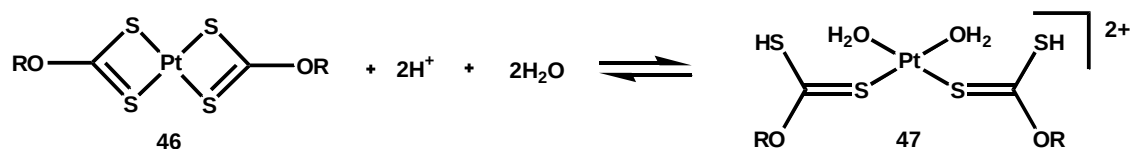


Figure 28. Activation of alkyldithiocarbonatoplatinum(II) complexes at acidic pH values.

Another fascinating class of pH-dependent platinum(II) species represent bis(aminoalcoholato)platinum(II) complexes (**48**, **Figure 29**).^{140 – 142} The compounds are capable of intramolecular ligand-exchange reactions, leading to the conversion of a relatively stable doubly ring-closed species (**48**) into the more reactive and consequently higher cytotoxic mono ring-closed (**49**) and ring-opened (**50**) forms.

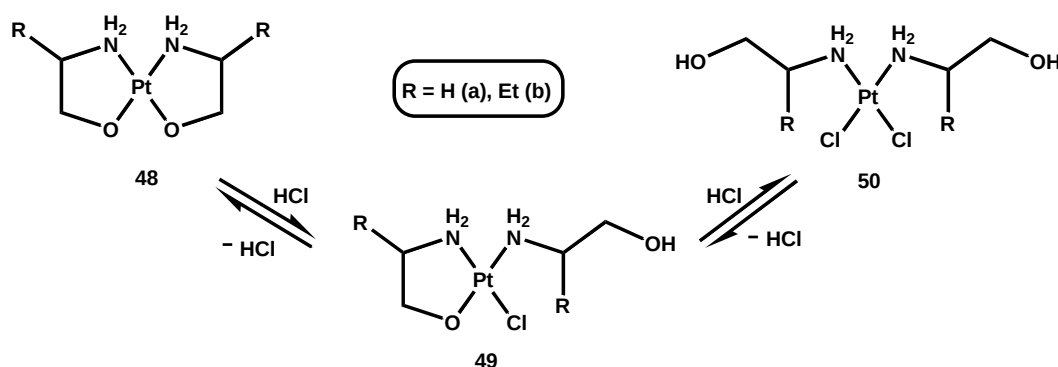


Figure 29. pH-dependent intramolecular ligand-exchange reaction of bis(aminoalcoholato)platinum(II) complexes.

Binding of the complexes to the DNA model nucleotide 5'-guanosine monophosphate (5'-GMP) at pH 7.4 and 6.0 was investigated both by NMR spectroscopy¹⁴³ and capillary electrophoresis,^{144 – 146} demonstrating a significantly higher reactivity under slightly acidic conditions (**Table 1**). For the corresponding doubly ring-closed (**48**) and ring-opened (**50**) complexes nearly identical half times were observed, being in good agreement with an adjustment of a pH-dependent equilibrium. Moreover, a comparative evaluation of the cytotoxic potency in the human nonsmall-cell lung cancer cell line (A549) at different proton

concentrations showed substantially improved IC₅₀ values at pH 6.0 as compared to those at pH 7.0 (**Table 1**).¹⁴⁷

Table 1. Half times for the reaction of platinum(II) complexes **48a**, **50a** and **48b** with 5'-GMP as well as their cytotoxicity in the human nonsmall-cell lung cancer cell line (A549).¹³⁷

	Half time of 5' GMP binding [h]		Cytotoxicity in A549 cells [μM]	
	pH 7.4	pH 6.0	pH 7.0	pH 6.0
48a	32.5	4.5	115	41.2
50a	28.5	4.5	110	45.6
48b	80.0	18.7	182	55.0

In addition, a new class of potential platinum(II)-based prodrugs able to be converted into the corresponding active species at lower pH values represent mixed-ligand complexes of *cis*-geometry featuring various amines along with aminoalcohol ligands (**51**, **Figure 30**).¹⁴⁸ The cytotoxic properties of the complexes were studied in human ovarian carcinoma cell lines sensitive (A2780) and resistant (A2780R) to cisplatin. Among the novel compounds, complexes comprising R₁ = Me, R₂ = H (**51a**) and R₁ = *i*-Pr, R₂ = H (**51b**) showed the most promising IC₅₀ values, being however cross-resistant with cisplatin in A2780R cells. Additionally, both **51a** and **51b** were tested in the mouse leukemia cell lines L1210 and L1210R, demonstrating slightly higher IC₅₀ values as compared to cisplatin and ability to partially overcome cisplatin resistance. However, no pH-dependent studies were reported.

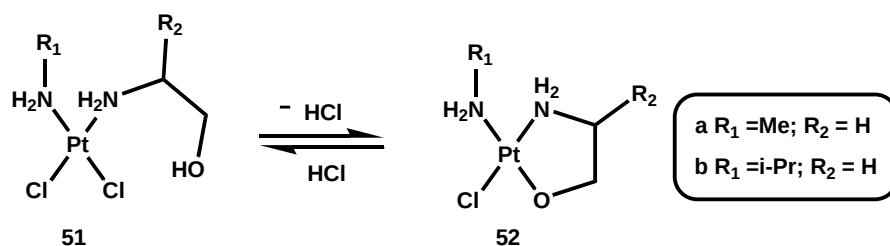


Figure 30. pH-sensitive mixed-ligand platinum(II) complexes.

Finally, a broad series of platinum(II) complexes with L-alanine and other α-amino acids was patented by Shaw et al. (**53**, **Figure 30**),^{149,150} shown to be more active under slightly acidic conditions that proved to accompany solid tumors and exhibiting lower activity at pH values found in normal tissues (pH 7.2–7.4).

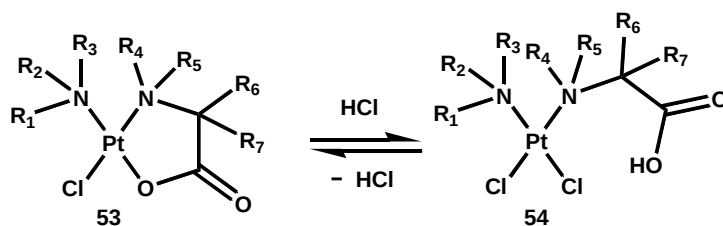


Figure 30. Activation of (aminocarboxylato)platinum(II) complexes.

3. FINAL REMARKS

In spite of incontestable achievements in the field of classical platinum-based anticancer therapy, there are enough shortcomings to be improved. Hence, there is still a need in developing novel antitumor agents characterized by a mode of action dissimilar to that of cisplatin. Thereby, a circumvention of resistance exhibited intrinsically by some tumors toward the antineoplastic platinum drugs used in the clinics or acquired in the course of treatment could be enabled. It would offer a solution of the main problem associated with the classical platinum-based anticancer therapy.

Platinum(II) acetone oxime complexes of *trans*-geometry reported recently represent promising candidates of nonclassic cytotoxic agents. In particular, *trans*-[PtCl₂(Me₂C=NOH)₂] was shown to display an enhanced cytotoxicity as compared both with *cis*-analogues and cisplatin as well as ability to overcome intrinsic cisplatin-resistance *in vitro* in SW480 cells. Deeper investigations of the complex, *e.g.*, binding to 5'-GMP, reactivity to DNA and influence on DNA secondary structure, point to a mode of action absolutely dissimilar to those platinum drugs used in the clinics. For this reason, it appears to be crucial to broaden the class of platinum(II)(oxime) compounds in order to draw and optimize structure-activity relationships. Moreover, it would help to obtain a deeper understanding of the mechanism behind their cytotoxic action.

This work contributes to the research on the synthesis, characterization, solution behavior, mode of action and cytotoxicity of novel platinum(II) oxime complexes.

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II. RESULTS

This PhD thesis is based on the following papers and manuscript, which are presented in the original format or as the submitted manuscript:

Novel Cis- and Trans-Configured Bis(oxime)platinum(II) Complexes: Synthesis, Characterization, and Cytotoxic Activity.

Scaffidi-Domianello, Yu. Yu.; Meelich, K.; Jakupec, M. A.; Arion, V. B.; Kukushkin, V. Yu.; Galanski, M.; Keppler, B. K. *Inorg Chem.* **2010**, *49*, 5669–5678.

Synthesis, Characterization and Cytotoxic Activity of Novel Potentially pH-Sensitive Nonclassical Platinum(II) Complexes Featuring 1,3-Dihydroxyacetone Oxime Ligands.

Scaffidi-Domianello, Yu. Yu.; Legin, A. A.; Jakupec, M. A.; Arion, V. B.; Kukushkin, V. Yu.; Galanski, M.; Keppler, B. K. *Inorg Chem.* **2011**, *50*, 10673–10681.

Novel Oximato-Bridged Platinum(II) Di- and Trimer(s): Synthetic, Structural, and in vitro Anticancer Activity Studies

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1. Novel Cis- and Trans-Configured Bis(oxime)platinum(II) Complexes: Synthesis, Characterization, and Cytotoxic Activity

Scaffidi-Domianello, Yu. Yu.; Meelich, K.; Jakupec, M. A.; Arion, V. B.; Kukushkin, V. Yu.; Galanski, M.; Keppler, B. K. *Inorg. Chem.* **2010**, *49*, 5669–5678.

Novel Cis- and Trans-Configured Bis(oxime)platinum(II) Complexes: Synthesis, Characterization, and Cytotoxic Activity

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Novel cis- and trans-configured bis(oxime)platinum(II) complexes have been synthesized and characterized by elemental analyses, IR, electrospray ionization mass spectrometry, multinuclear (¹H, ¹³C, and ¹⁹⁵Pt) NMR spectroscopy, and, in five cases, by X-ray diffraction. Their cytotoxicity was studied in the cisplatin-sensitive CH1 cell line as well as in inherently cisplatin-resistant SW480 cancer cells. Remarkably, every single dihalidobis(oxime)platinum(II) complex (with either a cis or trans configuration) shows a comparable cytotoxic potency in both cell lines, indicating a capacity of overcoming cisplatin resistance. Particularly strong cytotoxicities were observed in the case of *trans*-[PtCl₂(R₂C=NOH)₂] (R = Me, *n*-Pr, *i*-Pr) with IC₅₀ values in the high nanomolar concentration range in both CH1 and SW480 cancer cells. These complexes are as potent as cisplatin in CH1 cells and up to 20 times more potent than cisplatin in SW480 cells. In comparison to transplatin, the novel compounds are up to 90 (CH1) and 120 times (SW480) more cytotoxic. The previously reported observation that the trans geometry yields a more active complex in the case of [PtCl₂(Me₂C=NOH)₂] could be confirmed for at least two structural analogues.

Introduction

Over 40 years have passed since the serendipitous discovery of the biological activity of cisplatin,¹ which has become one of the most used anticancer drugs today. Since then, great efforts have been concentrated on the development of novel platinum drugs possessing therapeutic properties comparable to those of cisplatin but showing reduced side effects along with a broader spectrum of activity.^{2–4} The search for ever new drug candidates among the complexes complying with the classical structure–activity relationships^{5,6} led to the achievements of carboplatin and oxaliplatin but then seemed to become gradually exhausted. Therefore, nowadays

more investigators turn their attention toward nonclassic structures^{7–11} that may help to overcome the drawbacks of platinum-based chemotherapy. Moreover, the development of platinum compounds being structurally different from cisplatin and forming different types of Pt–DNA adducts may lead to a drug with a spectrum of clinical activity different from those of classic platinum drugs.

For decades, trans-configured platinum complexes attracted little interest because of the classic structure–activity relationships according to Cleare and Hoeschele,^{5,6} based on cisplatin/transplatin and their analogues. The presence of two monodentate or one bidentate exchangeable ligand(s) (leaving groups) coordinated in cis geometry was declared a requirement for antitumor activity. The significantly lower cytotoxicity of transplatin compared to that of cisplatin in vitro as well as its inactivity in vivo has been ascribed to its disfavored chelation reactions with DNA due to the trans-standing leaving groups. Consequently, the formation of 1,2-intrastrand cross-links, the predominant DNA adducts formed by cisplatin and believed to account for its cytotoxic activity, is greatly hindered.¹² It was shown that transplatin–DNA adducts are more rapidly repaired and cannot be

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recognized by the high-mobility group proteins HMG1, recognizing specifically 1,2-intrastrand cross-links and shielding them from repair. Moreover, the higher reactivity of transplatin compared to that of cisplatin may favor its deactivation, resulting in the lack of anticancer activity.

In the past few years, some exceptions from the classic rules have been reported. Several classes of trans complexes exhibiting higher cytotoxicity than or at least equal to that of the corresponding cis isomer are (i) platinum(II)^{13–16}/platinum(IV)¹⁷ complexes with pyridine-like ligands, (ii) platinum(II) complexes with iminoether ligands,^{18–21} (iii) platinum(II) complexes with nonplanar heterocyclic ligands, such as piperidine or piperazine,^{22–25} (iv) platinum(II)^{26–28}/platinum(IV)^{29,30} complexes with aliphatic amines, (v) platinum(II) carboxylato complexes with planar amines,^{31,32} azole and isopropylamine,³³ azole and ammine ligands,³⁴ (vi) platinum(II) complexes containing various phosphoric

groups,^{35,36} and (vii) platinum(II) complexes with acetimine ligands.³⁷ Interestingly, most of the listed complexes are similarly or even more cytotoxic than cisplatin and all of them proved to be non-cross-resistant to cisplatin in cellular models of acquired cisplatin resistance. Furthermore, for the first four of the above-mentioned classes, the antitumor activity was confirmed in an in vivo model. Moreover, some of the complexes even displayed a lack of cross-resistance to cisplatin in vivo.^{20,29}

Very recently, a new intriguing class of trans-configured platinum compounds exhibiting antitumor activity was reported. Almost simultaneously two independent groups of researcher published the fascinating results of in vitro biological investigations for two acetoxime-containing platinum(II) complexes, namely, *trans*-[PtCl₂(Me₂C=NOH)(NH₂-CH(CH₃)₂)]³⁸ as well as *trans*-[PtCl₂(Me₂C=NOH)]₂.³⁹ According to these investigations, the former complex, but not its cis isomer, causes cell death by apoptosis, even though the overall cytotoxicity was found to be higher for the cis-configured complex. Differently, *trans*-[PtCl₂(Me₂C=NOH)]₂ was found to be 16 times more cytotoxic than its cis isomer and as cytotoxic as cisplatin in cisplatin-sensitive ovarian carcinoma cells (CH1). Moreover, it appeared to be 15 times more cytotoxic than both cisplatin and its cis isomer in intrinsically cisplatin-resistant colon carcinoma cells (SW480).

In this context, it is worthwhile mentioning that oximes are attractive ligands in view of their potential to act as donors or acceptors for hydrogen bonds or even serve as weak acids (pK_a 3–8),⁴⁰ which could play an important role in the binding of platinum compounds to DNA.⁴¹ In addition, the biological relevance of oximes appreciably favors their use as ligands for potential metal-based drugs, which may yield increased cytotoxicity by virtue of synergistic effects between the platinum center and the coordinated oxime. For instance, it was reported that oxime complexes and other species bearing the oxime functional group caused biological effects such as endothelium-independent relaxation in blood vessels,⁴² an increase in the targeting of specific nuclear bases of DNA,⁴³ and oxidative DNA cleavage.⁴⁴

On the basis of the promising results obtained with *trans*-[PtCl₂(Me₂C=NOH)]₂ and with the aim to establish structure–activity relationships, we report on a series of novel dihalidobis(oxime)platinum(II) complexes, i.e., *trans*-[PtX₂(R₂C=NOH)]₂ (X = Cl, Br, I; R = Me, Et, *n*-Pr, *i*-Pr), of which at least two exhibit higher cytotoxicity than the corresponding cis species in both CH1 and SW480 cells. Moreover, the corresponding cis/trans couples of

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[Pt(NH₃)₂(R₂C=NOH)₂](NO₃)₂ (R = Me, Et, *n*-Pr) were synthesized and investigated. In accordance with the classic structure–activity relationships, stating that platinum complexes with four nitrogen donor ligands are devoid of cytotoxicity, the IC₅₀ values of the diamminebis(oxime) series were proven to be very high.

Experimental Section

Solvents were obtained from commercial sources and used as received. Acetone oxime was purchased from Fluka, while 3-pentanone, 4-heptanone, and 2,4-dimethyl-3-pentanone for the synthesis of the corresponding oximes were obtained from Aldrich. The complexes *cis*- and *trans*-[PtBr₂(Me₂C=NOH)]₂,⁵³ *cis*-[PtI₂(NH₃)₂]⁵⁴, and *trans*-[PtCl₂(NH₃)₂]⁵⁵ were prepared according to the published methods. For thin-layer chromatography (TLC), Merck UV 254 SiO₂ plates were used. Fluka silica gel 60 (220–440 mesh) was used for column chromatography. C, H, and N elemental analyses were carried out by the Microanalytical Laboratory of the University of Vienna and are within ±0.4% of the calculated values, confirming their ≥95% purity. Melting points were determined with a Büchi B-540 melting point apparatus and are uncorrected. Electrospray ionization mass spectrometry (ESI-MS) spectra were obtained with a Bruker Esquire 3000 instrument. Mid-infrared (MIR; 4000–400 cm^{−1}) spectra were recorded with a Perkin-Elmer Fourier transform infrared (FTIR) instrument in KBr pellets. ¹H, ¹³C, and ¹⁹⁵Pt NMR spectra were measured with a Bruker Avance III 500 MHz NMR spectrometer at 500.32 (¹H), 125.81 (¹³C), and 107.55 MHz (¹⁹⁵Pt), correspondingly, at 298 K. ¹⁹⁵Pt chemical shifts are given relative to external K₂[PtCl₄], and the half-height line width is given in parentheses.

Synthesis of Ligands. 3-Pentanone oxime, 4-heptanone oxime, and 2,4-dimethyl-3-pentanone oxime were synthesized by known procedures^{45–47} with some modifications: the corresponding ketones were treated with hydroxylamine hydrochloride in a MeOH/H₂O mixture (1:1), while K₂CO₃ was used for neutralization. The reaction mixture was stirred at 65–70 °C for 24 h, and then the solvent was removed and the product purified by column chromatography (1:4 ethyl acetate/hexane, first fraction). The yields were 87–92%.

Synthesis of Complexes. (**SP-4-2**)-Bis(acetone oxime-κN)dibromidoplatinum(II) (**2c**). The complex was prepared according to the published method from K₂[PtCl₄] (191 mg, 0.46 mmol), an excess of KBr (274 mg, 2.30 mmol), and 2 equiv of acetoxime (67 mg, 0.92 mmol) in water.⁵³ Yield: 138 mg (60%). Anal. (C₆H₁₄N₂Br₂O₂Pt) C, H, N. IR in KBr (selected bands): ν 1656 m (ν_{C=N}) cm^{−1}. ¹H NMR in D₂O: δ 2.69 (s, 3H, CH₃), 2.22 (s, 3H, CH₃) ppm. ESI-MS⁺ in MeOH: *m/z* 524 [M + Na]⁺, 560 [M + HCl + Na]⁺.

(**SP-4-1**)-Bis(acetone oxime-κN)dibromidoplatinum(II) (**2t**). The synthesis was performed by thermal isomerization of the corresponding *cis* complex.⁵³ **2c** (175 mg, 0.35 mmol) was kept at 140 °C for 6 h; the resulting gray powder was suspended in 2 mL of acetone and filtered through a small amount of SiO₂, which was washed three times with 2 mL of acetone. Then the solvent was partially evaporated under reduced pressure, and bright yellow crystals of the product were formed. The crystals were collected, washed with 0.5 mL of acetone, and dried in vacuo. Yield: 35 mg (20%). Anal. (C₆H₁₄N₂Br₂O₂Pt) C, H, N. IR in KBr (selected bands): ν 1664 m (ν_{C=N}) cm^{−1}. ¹H NMR in acetone-*d*₆: δ 2.62 (s, 3H, CH₃), 2.19 (s, 3H, CH₃) ppm. ESI-MS⁺ in MeOH: *m/z* 524 [M + Na]⁺, 560 [M + HCl + Na]⁺.

(**SP-4-2**)-Bis(acetone oxime-κN)diiodidoplatinum(II) (**3c**). Potassium iodide (406 mg, 2.45 mmol) was added to a solution of K₂[PtCl₄] (203 mg, 0.49 mmol) in 2 mL of H₂O, and the mixture was stirred at room temperature for 2 h. After the addition of acetone oxime (72 mg, 0.98 mmol), the reaction mixture was left to stand at the same temperature without stirring for 2 days, and the crystals of **3c** were filtered off, washed twice with 0.5 mL of ice-cold water, and dried in vacuo. Yield: 233 mg (80%). Anal. (C₆H₁₄N₂I₂O₂Pt) C, H, N. ESI-MS⁺ in acetone: *m/z* 545 [M – Me₂C=NOH + Na]⁺, 618 [M + Na]⁺, 634 [M + K]⁺. ESI-MS[−] in acetone: *m/z* 521 [M – Me₂C=NOH – H][−], 594 [M – H][−]. Mp 220 °C (dec). TLC [10:1 (v/v) CHCl₃/Me₂CO]: R_f = 0.52. IR in KBr (selected bands): ν 3242 s (ν_{OH}), 1653 m (ν_{C=N}) cm^{−1}. ¹H NMR in acetone-*d*₆: δ 2.65 (s, 3H, CH₃), 2.19 (s, 3H, CH₃) ppm. ¹³C{¹H} NMR in acetone-*d*₆: δ 164.6 (C=N), 24.6 (CH₃), 17.9 (CH₃) ppm. ¹⁹⁵Pt NMR in acetone-*d*₆: δ −1729 (600 Hz) ppm. Crystals for X-ray study were obtained by slow evaporation of an aqueous solution.

(**SP-4-1**)-Bis(acetone oxime-κN)diiodidoplatinum(II) (**3t**). The powder of **3c** (230 mg, 0.39 mmol) was refluxed in 5 mL of MeNO₂ at 95–100 °C for 3 h. A small amount of a brown precipitate was filtered off, and the yellow filtrate was reduced to 1.5 mL at 40 °C using a rotary evaporator, resulting in the precipitation of a yellow solid. The precipitate was collected by filtration, washed twice with 1 mL of MeNO₂, and dried in vacuo. Yield: 114 mg (50%). Anal. (C₆H₁₄N₂I₂O₂Pt) C, H, N. ESI-MS⁺ in acetone: *m/z* 618 [M + Na]⁺. Mp 200 °C (dec). TLC [15:1 (v/v) CHCl₃/Me₂CO]: R_f = 0.50. IR in KBr (selected bands): ν 3329 s (ν_{OH}), 1661 m (ν_{C=N}) cm^{−1}. ¹H NMR in DMF-*d*₇: δ 11.88 (s, br, 1H, OH^a), 11.71 (s, br, 1H, OH^b), 2.72 (s, 3H, CH₃^b), 2.68 (s, 3H, CH₃^a), 2.39 (s, 3H, CH₃^b), 2.32 (s, 3H, CH₃^a) ppm. ¹³C{¹H} NMR in DMF-*d*₇: δ 164.8 (C=N^b), 162.6 (C=N^a), 24.87 (CH₃^b), 24.86 (CH₃^a), 17.9 (CH₃^b), 17.5 (CH₃^a) ppm. ¹⁹⁵Pt NMR in DMF-*d*₇: δ −1800 (320) ppm. Crystals for X-ray study were obtained by slow evaporation of an acetone solution.

(**SP-4-2**)-Dichloridobis(3-pentanone oxime-κN)platinum(II) (**4c**) and (**SP-4-1**)-Dichloridobis(3-pentanone oxime-κN)platinum(II) (**4t**). 3-Pentanone oxime (113 mg, 1.12 mmol) was added to a solution of K₂[PtCl₄] (239 mg, 0.58 mmol) in 1.5 mL of H₂O, and the mixture was stirred at 70 °C for 0.5 h. Then, the reaction mixture was left to stand without stirring at room temperature for 2 days, resulting in the formation of pale-yellow crystals. The precipitate was filtered off, washed twice with 1 mL of ice-cold water, and dried at room temperature in air, whereupon isomers **4c** and **4t** were separated by column chromatography [8:1 (v/v) CHCl₃/Me₂C=O] and dried in vacuo. Yields: 148 mg (55%) and 108 mg (40%), respectively.

4c. Anal. (C₁₀H₂₂N₂Cl₂O₂Pt) C, H, N. ESI-MS⁺ in MeOH: *m/z* 491 [M + Na]⁺, 507 [M + K]⁺, 533 [M + Et₂C=NOH – Cl]⁺. ESI-MS[−] in MeOH: *m/z* 330 [M – Et₂C=NOH – HCl – H][−], 467 [M – H][−]. Mp 153 °C (dec). TLC [7:1 (v/v) CHCl₃/Me₂CO]: R_f = 0.46. IR in KBr (selected bands): ν 3255 s (ν_{OH}), 1649 m (ν_{C=N}) cm^{−1}. ¹H NMR in CDCl₃: δ 9.04 (s, br, 1H, OH), 3.18 (q, 2H, CH₂, ³J_{H,H} = 7.6 Hz), 3.60 (q, 2H, CH₂, ³J_{H,H} = 7.6 Hz), 1.26 (t, 3H, CH₃, ³J_{H,H} = 7.6 Hz), 1.09 (t, 3H, CH₃, ³J_{H,H} = 7.6 Hz) ppm. ¹³C{¹H} NMR in CDCl₃: δ 176.2 (C=N), 29.8 (CH₂), 23.4 (CH₂), 10.9 (CH₃), 10.3 (CH₃) ppm. ¹⁹⁵Pt NMR in CDCl₃: δ −441 (380 Hz) ppm. Crystals for X-ray study were obtained by slow evaporation of a chloroform/ethyl acetate solution.

4t. Anal. (C₁₀H₂₂N₂Cl₂O₂Pt) C, H, N. ESI-MS⁺ in MeOH: *m/z* 491 [M + Na]⁺. ESI-MS[−] in MeOH: *m/z* 330 [M – Et₂C=NOH – HCl – H][−], 366 [M – Et₂C=NOH – H][−]. Mp: 204 °C (dec). TLC [7:1 (v/v) CHCl₃/Me₂CO]: R_f = 0.54. IR in KBr (selected bands): ν 3254 s (ν_{OH}), 1658 m (ν_{C=N}) cm^{−1}. ¹H NMR in CDCl₃: δ 3.10 (q, 2H, CH₂, ³J_{H,H} = 7.6 Hz), 2.58 (q, 2H, CH₂, ³J_{H,H} = 7.6 Hz), 1.33 (t, 3H, CH₃, ³J_{H,H} = 7.6 Hz), 1.13 (t, 3H,

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CH_3 , $^3J_{\text{H,H}} = 7.6$ Hz) ppm. $^{13}\text{C}\{^1\text{H}\}$ NMR in CDCl_3 : δ 176.2 (C=N), 29.6 (CH_2), 23.5 (CH_2), 11.0 (CH_3), 10.3 (CH_3) ppm. ^{195}Pt NMR in CDCl_3 : δ -462 (450 Hz) ppm.

(SP-4-1)-Dichloridobis(4-heptanone oxime- κ N)platinum(II) (5t). 4-Heptanone oxime (182 mg, 1.41 mmol) was added to a solution of $\text{K}_2[\text{PtCl}_4]$ (292 mg, 0.70 mmol) in 2 mL of H_2O , and the mixture was stirred at 85 °C for 2 h. Then, the heating was switched off, and the reaction mixture was stirred at room temperature for 2 days, resulting in the formation of a large amount of lemon-yellow precipitate. The precipitate was filtered off, washed twice with 1 mL of H_2O , and dried at room temperature in air, whereupon it was purified by column chromatography [10:1 (v/v) $\text{CHCl}_3/\text{Me}_2\text{C}=\text{O}$] and then dried in vacuo. Yield: 240 mg (65%). Anal. ($\text{C}_{14}\text{H}_{30}\text{N}_2\text{Cl}_2\text{O}_2\text{Pt}$) C, H, N. ESI- MS^+ in MeOH: m/z 452 $[\text{M} - \text{HCl} - \text{Cl}]^+$, 525 $[\text{M} + \text{H}]^+$, 547 $[\text{M} + \text{Na}]^+$, 584 $[\text{M} + \text{HCl} + \text{Na}]^+$. ESI- MS^- in MeOH: m/z 358 $[\text{M} - \text{Pr}_2\text{C}=\text{NOH} - \text{HCl} - \text{H}]^-$, 394 $[\text{M} - \text{Pr}_2\text{C}=\text{NOH} - \text{H}]^-$. Mp: 173 °C (dec). TLC [10:1 (v/v) $\text{CHCl}_3/\text{Me}_2\text{CO}$]: $R_f = 0.48$. IR in KBr (selected bands): ν 3299 s (ν_{OH}), 1672 m ($\nu_{\text{C}=\text{N}}$) cm^{-1} . ^1H NMR in CDCl_3 : δ 7.42 (s, br, 1H, OH), 3.04 (m, 2H, CH_2), 2.54 (m, 2H, CH_2), 1.78 (m, 2H, CH_2CH_2), 1.58 (m, 2H, CH_2CH_2), 1.12 (t, 3H, CH_3 , $^3J_{\text{H,H}} = 7.3$ Hz), 0.98 (t, 3H, CH_3 , $^3J_{\text{H,H}} = 7.3$ Hz) ppm. $^{13}\text{C}\{^1\text{H}\}$ NMR in CDCl_3 : δ 173.8 (C=N), 38.4 (CH_2), 32.1 (CH_2), 20.2 (CH_2CH_2), 19.5 (CH_2CH_2), 14.3 (CH_3), 13.9 (CH_3) ppm. ^{195}Pt NMR in CDCl_3 : δ -453 (350 Hz) ppm. Crystals for X-ray study were obtained by slow evaporation of a chloroform/ethyl acetate solution.

(SP-4-1)-Dichloridobis(2,4-dimethyl-3-pentanone oxime- κ N)platinum(II) (6t). The synthesis and purification were carried out as described for 4t, using $\text{K}_2[\text{PtCl}_4]$ (153 mg, 0.37 mmol) and 2,4-dimethyl-3-pentanone oxime (95 mg, 0.74 mmol). Yield: 104 mg (60%). Anal. ($\text{C}_{14}\text{H}_{30}\text{N}_2\text{Cl}_2\text{O}_2\text{Pt}$) C, H, N. ESI- MS^+ in acetone: m/z 452 $[\text{M} - \text{HCl} - \text{Cl}]^+$, 510 $[\text{M} - \text{HCl} + \text{Na}]^+$, 547 $[\text{M} + \text{Pr}_2\text{C}=\text{NOH} - \text{Cl}]^+$. ESI- MS^- in acetone: m/z 358 $[\text{M} - \text{Pr}_2\text{C}=\text{NOH} - \text{HCl} - \text{H}]^-$, 394 $[\text{M} - \text{Pr}_2\text{C}=\text{NOH} - \text{H}]^-$, 487 $[\text{M} - \text{HCl} - \text{H}]^-$. Mp: 185 °C (dec). TLC [10:1 (v/v) $\text{CHCl}_3/\text{Me}_2\text{CO}$]: $R_f = 0.45$. IR in KBr (selected bands): ν 3330 s (ν_{OH}), 1662 m ($\nu_{\text{C}=\text{N}}$) cm^{-1} . ^1H NMR in CDCl_3 : δ 4.28 (m, 2H, 2CH), 2.67 (m, 2H, 2CH), 1.28 (d, 6H, 2CH₃, $^3J_{\text{H,H}} = 6.9$ Hz), 1.23 (d, 6H, 2CH₃, $^3J_{\text{H,H}} = 6.9$ Hz) ppm. $^{13}\text{C}\{^1\text{H}\}$ NMR in CDCl_3 : δ 180.7 (C=N), 37.1 (CH), 29.7 (CH), 18.9 (2CH₃), 18.5 (2CH₃) ppm. ^{195}Pt NMR in CDCl_3 : δ -441 (420 Hz) ppm.

(SP-4-2)-Bis(acetone oxime- κ N)diammineplatinum(II) Dinitrate (7c). *cis*- $[\text{PtI}_2(\text{NH}_3)_2]$ (72 mg, 0.15 mmol) and AgNO_3 (51 mg, 0.30 mmol) were suspended in 2 mL of H_2O , whereupon the mixture was stirred at room temperature overnight. The yellow precipitate of AgI was filtered off, and acetone oxime (22 mg, 0.30 mmol) was added. After the reaction mixture was stirred at the same temperature for 10 h, the clear colorless solution was reduced to 0.5 mL and 15 mL of acetone was added, inducing the precipitation of a white solid. The precipitate was filtered off, washed with acetone (2 $\times$ 1 mL), and dried in vacuo. Yield: 41 mg (55%). Anal. ($\text{C}_6\text{H}_{20}\text{N}_6\text{O}_8\text{Pt} \cdot \text{H}_2\text{O}$) C, H, N. ESI- MS^+ in $\text{H}_2\text{O}/\text{MeOH}$: m/z 357 $[\text{M} - \text{NH}_3 - \text{HNO}_3 - \text{NO}_3]^+$, 374 $[\text{M} - \text{HNO}_3 - \text{NO}_3]^+$, 696 $[\text{2M} - 3\text{NH}_3 - 3\text{HNO}_3 - \text{NO}_3]^+$, 713 $[\text{2M} - 2\text{NH}_3 - 3\text{HNO}_3 - \text{NO}_3]^+$, 730 $[\text{2M} - \text{NH}_3 - 3\text{HNO}_3 - \text{NO}_3]^+$, 747 $[\text{2M} - 3\text{HNO}_3 - \text{NO}_3]^+$. Mp: 147 °C (dec). IR in KBr (selected bands): ν 3194 s (ν_{OH}), 1666 m ($\nu_{\text{C}=\text{N}}$) cm^{-1} . ^1H NMR in D_2O : δ 2.52 (s, 3H, CH_3), 2.12 (s, 3H, CH_3) ppm. $^{13}\text{C}\{^1\text{H}\}$ NMR in D_2O : δ 172.5 (C=N), 23.8 (CH_3), 18.2 (CH_3) ppm. ^{195}Pt NMR in D_2O : δ -907 (1000 Hz) ppm.

(SP-4-1)-Bis(acetone oxime- κ N)diammineplatinum(II) Dinitrate (7t). *trans*- $[\text{PtCl}_2(\text{NH}_3)_2]$ (81 mg, 0.27 mmol) and AgNO_3 (92 mg, 0.54 mmol) were suspended in 2.5 mL of H_2O , whereupon the mixture was stirred at 60 °C overnight. The white precipitate of AgCl was filtered off, and acetone oxime (40 mg, 0.54 mmol) was added. After the reaction mixture was stirred at room temperature overnight, the clear yellowish solution was reduced to 0.5 mL and 10 mL of acetone was added, inducing the

precipitation of a white solid. The precipitate was filtered off, washed with acetone (2 $\times$ 1 mL) and ether (3 mL), and dried in vacuo. Yield: 88 mg (65%). Anal. ($\text{C}_6\text{H}_{20}\text{N}_6\text{O}_8\text{Pt}$) C, H, N. ESI- MS^+ in $\text{H}_2\text{O}/\text{MeOH}$: m/z 267 $[\text{M} - 2\text{NH}_3 - \text{Me}_2\text{C}=\text{NOH} - \text{HNO}_3 - \text{NO}_3]^+$, 340 $[\text{M} - 2\text{NH}_3 - \text{NO}_3 - \text{NO}_3]^+$, 357 $[\text{M} - \text{NH}_3 - \text{HNO}_3 - \text{NO}_3]^+$, 374 $[\text{M} - \text{HNO}_3 - \text{NO}_3]^+$, 639 $[\text{2M} - \text{Me}_2\text{C}=\text{NOH} - 2\text{NH}_3 - 3\text{HNO}_3 - \text{NO}_3]^+$, 657 $[\text{2M} - \text{Me}_2\text{C}=\text{NOH} - \text{NH}_3 - 3\text{HNO}_3 - \text{NO}_3]^+$, 696 $[\text{2M} - 3\text{NH}_3 - 3\text{HNO}_3 - \text{NO}_3]^+$, 713 $[\text{2M} - 2\text{NH}_3 - 3\text{HNO}_3 - \text{NO}_3]^+$, 747 $[\text{2M} - 3\text{HNO}_3 - \text{NO}_3]^+$. Mp: 234 °C (dec). IR in KBr (selected bands): ν 3211 s (ν_{OH}), 1667 m ($\nu_{\text{C}=\text{N}}$) cm^{-1} . ^1H NMR in D_2O : δ 4.27 (s, br, 3H, NH_3), 2.58 (s, 3H, CH_3), 2.19 (s, 3H, CH_3) ppm. $^{13}\text{C}\{^1\text{H}\}$ NMR in D_2O : δ 172.2 (C=N), 23.4 (CH_3), 18.0 (CH_3) ppm. ^{195}Pt NMR in D_2O : δ -907 (800 Hz) ppm. Crystals for X-ray study were obtained by slow evaporation of an aqueous solution.

(SP-4-2)-Diamminebis(3-pentanone oxime- κ N)platinum(II) Dinitrate (8c). *cis*- $[\text{PtI}_2(\text{NH}_3)_2]$ (109 mg, 0.23 mmol) and AgNO_3 (77 mg, 0.45 mmol) were suspended in 2 mL of H_2O , whereupon the mixture was stirred at room temperature overnight. The yellow precipitate of AgI was filtered off, and 3-pentanone oxime (50 μL , 0.45 mmol) in 2 mL of THF was added. After the reaction mixture was stirred at the same temperature for 2 days, the solvent was removed and the crude product was dried in vacuo. Then, the white solid was suspended in 10 mL of acetone, filtered off, washed with acetone (2 $\times$ 1 mL), and dried in vacuo. Yield: 53 mg (42%). Anal. ($\text{C}_{10}\text{H}_{28}\text{N}_6\text{O}_8\text{Pt}$) C, H, N. ESI- MS^+ in $\text{H}_2\text{O}/\text{MeOH}$: m/z 396 $[\text{M} - 2\text{NH}_3 - \text{HNO}_3 - \text{NO}_3]^+$, 413 $[\text{M} - \text{NH}_3 - \text{HNO}_3 - \text{NO}_3]^+$, 430 $[\text{M} - \text{HNO}_3 - \text{NO}_3]^+$, 606 $[\text{2M} - 2\text{Et}_2\text{C}=\text{NOH} - 3\text{NH}_3 - 3\text{HNO}_3 - \text{NO}_3]^+$, 690 $[\text{2M} - \text{Et}_2\text{C}=\text{NOH} - 4\text{NH}_3 - 3\text{HNO}_3 - \text{NO}_3]^+$, 724 $[\text{2M} - \text{Et}_2\text{C}=\text{NOH} - 2\text{NH}_3 - 3\text{HNO}_3 - \text{NO}_3]^+$, 808 $[\text{2M} - 3\text{NH}_3 - 3\text{HNO}_3 - \text{NO}_3]^+$, 825 $[\text{2M} - 2\text{NH}_3 - 3\text{HNO}_3 - \text{NO}_3]^+$, 843 $[\text{2M} - \text{NH}_3 - 3\text{HNO}_3 - \text{NO}_3]^+$, 859 $[\text{2M} - 3\text{HNO}_3 - \text{NO}_3]^+$. Mp: 158 °C (dec). IR in KBr (selected bands): ν 3194 s (ν_{OH}), 1666 m ($\nu_{\text{C}=\text{N}}$) cm^{-1} . ^1H NMR in D_2O : δ 3.00 (m, 2H, CH_2), 2.53 (m, 2H, CH_2), 1.12 (t, 3H, CH_3 , $^3J_{\text{H,H}} = 7.6$ Hz), 0.99 (t, 3H, CH_3 , $^3J_{\text{H,H}} = 7.6$ Hz) ppm. $^{13}\text{C}\{^1\text{H}\}$ NMR in D_2O : δ 178.9 (C=N), 29.5 (CH_2), 22.9 (CH_2), 9.9 (CH_3), 9.3 (CH_3) ppm. ^{195}Pt NMR in D_2O : δ -897 (1050 Hz) ppm.

(SP-4-1)-Diamminebis(pentanone oxime- κ N)platinum(II) Dinitrate (8t). *trans*- $[\text{PtCl}_2(\text{NH}_3)_2]$ (105 mg, 0.35 mmol) and AgNO_3 (118 mg, 0.70 mmol) were suspended in 4 mL of H_2O , whereupon the mixture was stirred at 60 °C overnight. The white precipitate of AgCl was filtered off, and 3-pentanone oxime (77 μL , 0.70 mmol) in 2 mL of THF was added. After the reaction mixture was stirred at room temperature overnight, the solvent was removed and the yellowish solid was suspended in 10 mL of acetone; the precipitate was then filtered off, washed with acetone (2 $\times$ 1 mL) and diethyl ether (3 mL), and dried in vacuo. Yield: 54 mg (45%). Anal. ($\text{C}_{10}\text{H}_{28}\text{N}_6\text{O}_8\text{Pt}$) C, H, N. ESI- MS^+ in $\text{H}_2\text{O}/\text{MeOH}$: m/z 396 $[\text{M} - 2\text{NH}_3 - \text{HNO}_3 - \text{NO}_3]^+$, 413 $[\text{M} - \text{NH}_3 - \text{HNO}_3 - \text{NO}_3]^+$, 430 $[\text{M} - \text{HNO}_3 - \text{NO}_3]^+$, 724 $[\text{2M} - \text{Et}_2\text{C}=\text{NOH} - 2\text{NH}_3 - 3\text{HNO}_3 - \text{NO}_3]^+$, 808 $[\text{2M} - 3\text{NH}_3 - 3\text{HNO}_3 - \text{NO}_3]^+$, 859 $[\text{2M} - 3\text{HNO}_3 - \text{NO}_3]^+$. Mp: 213 °C (dec). IR in KBr (selected bands): ν 3270 s (ν_{OH}), 1645 m ($\nu_{\text{C}=\text{N}}$) cm^{-1} . ^1H NMR in D_2O : δ 4.30 (s, br, 3H, NH_3), 3.02 (m, br, 2H, CH_2), 2.58 (m, br, 2H, CH_2), 1.17 (t, 3H, CH_3 , $^3J_{\text{H,H}} = 7.7$ Hz), 1.03 (t, 3H, CH_3 , $^3J_{\text{H,H}} = 7.6$ Hz) ppm. $^{13}\text{C}\{^1\text{H}\}$ NMR in D_2O : δ 180.2 (C=N), 29.3 (CH_2), 23.0 (CH_2), 10.0 (CH_3), 9.3 (CH_3) ppm. ^{195}Pt NMR in D_2O : δ -914 (1500 Hz) ppm.

(SP-4-2)-Diamminebis(4-heptanone oxime- κ N)platinum(II) dinitrate (9c). The synthesis was carried out as described for 7c, using 203 mg (0.42 mmol) of *cis*- $[\text{PtI}_2(\text{NH}_3)_2]$, 143 mg (0.84 mmol) of AgNO_3 , and 122 μL (0.84 mmol) of 4-heptanone oxime. Yield: 39 mg (15%). Anal. ($\text{C}_{14}\text{H}_{36}\text{N}_6\text{O}_8\text{Pt} \cdot \text{H}_2\text{O}$) C, H, N. ESI- MS^+ in $\text{H}_2\text{O}/\text{MeOH}$: m/z 340 $[\text{M} - \text{NH}_3 - n\text{-Pr}_2\text{C}=\text{NOH} - \text{HNO}_3 - \text{NO}_3]^+$, 452 $[\text{M} - 2\text{NH}_3 - \text{HNO}_3 - \text{NO}_3]^+$, 470 $[\text{M} - \text{NH}_3 - \text{HNO}_3 - \text{NO}_3]^+$, 486 $[\text{M} - \text{HNO}_3 - \text{NO}_3]^+$, 774 $[\text{2M} - n\text{-Pr}_2\text{C}=\text{NOH} - 4\text{NH}_3 - 3\text{HNO}_3 - \text{NO}_3]^+$, 791

Table 1. Crystal Data and Details of Data Collection for **3c**, **3t**, **4c**, **5t**, and **7t**

	3c	3t	4c	5t	7t
empirical formula	C ₆ H ₁₄ I ₂ N ₂ O ₂ Pt	C ₆ H ₁₄ I ₂ N ₂ O ₂ Pt	C ₁₀ H ₂₂ Cl ₂ N ₂ O ₂ Pt	C ₁₄ H ₃₀ Cl ₂ N ₂ O ₂ Pt	C ₆ H ₂₀ N ₆ O ₈ Pt
fw	595.08	595.08	468.29	524.39	499.37
space group	<i>P</i> 2 ₁ / <i>c</i>	<i>P</i> 2 ₁ 2 ₁ 2 ₁	<i>P</i> <i>T</i>	<i>P</i> <i>nma</i>	<i>P</i> <i>1</i>
<i>a</i> , Å	7.9315(2)	9.0055(4)	9.1808(2)	8.0268(6)	6.3713(4)
<i>b</i> , Å	17.4453(5)	9.0841(3)	9.4940(3)	16.3958(13)	7.6065(5)
<i>c</i> , Å	9.6186(2)	16.5477(5)	9.5723(3)	14.4299(12)	8.4282(6)
λ, deg			79.582(3)		91.570(4)
β, deg			79.141(2)		105.325(4)
γ, deg			72.957(2)		101.662(3)
<i>V</i> , Å ³	1303.00(6)	1353.72(9)	776.38(4)	1899.1(3)	384.39(4)
<i>Z</i>	4	4	2	4	1
α, Å	0.710 73	0.710 73	0.710 73	0.710 73	0.710 73
ρ _{calcd} , g cm ^{−3}	3.033	2.920	2.003	1.834	2.157
crystal size, mm	0.25 × 0.10 × 0.08	0.12 × 0.05 × 0.03	0.21 × 0.15 × 0.08	0.20 × 0.06 × 0.06	0.21 × 0.12 × 0.10
<i>T</i> , K	100	100	100	100	100
μ, cm ^{−1}	154.85	149.05	93.74	76.75	91.73
<i>R</i> 1 ^a	0.0250	0.0144	0.0174	0.0187	0.0195
<i>wR</i> 2 ^b	0.0543	0.0309	0.0354	0.0400	0.0385
GOF ^c	1.034	0.990	1.010	0.989	1.000

^a *R*1 = $\sum ||F_o| - |F_c|| / \sum |F_o|$. ^b *wR*2 = $\{\sum [w(F_o^2 - F_c^2)^2] / \sum [w(F_o^2)^2]\}^{1/2}$. ^c GOF = $\{\sum [w(F_o^2 - F_c^2)^2] / (n - p)\}^{1/2}$, where *n* is the number of reflections and *p* is the total number of parameters refined.

[2M − *n*-Pr₂C=NOH − 3NH₃ − 3HNO₃ − NO₃]⁺, 808 [2M − *n*-Pr₂C=NOH − 2NH₃ − 3HNO₃ − NO₃]⁺, 920 [2M − 3NH₃ − 3HNO₃ − NO₃]⁺, 937 [2M − 2NH₃ − 3HNO₃ − NO₃]⁺, 955 [2M − NH₃ − 3HNO₃ − NO₃]⁺, 972 [2M − 3HNO₃ − NO₃]⁺. Mp: 155 °C (dec). IR in KBr (selected bands): ν 3316 s (ν_{OH}), 1645 m (ν_{C=N}) cm^{−1}. ¹H NMR in D₂O: δ 2.94 (t, 2H, CH₂, ³*J*_{H,H} = 8.0 Hz), 2.51 (t, br, 2H, CH₂), 1.55 (m, br, 2H, CH₃CH₂), 1.45 (m, br, 2H, CH₃CH₂), 0.96 (t, 3H, CH₃, ³*J*_{H,H} = 7.4 Hz), 0.84 (t, 3H, CH₃, ³*J*_{H,H} = 7.2 Hz) ppm. ¹³C{¹H} NMR in D₂O: δ 177.8 (C=N), 38.3 (CH₂), 31.7 (CH₂), 19.5 (CH₃CH₂), 18.9 (CH₃CH₂), 13.34 (CH₃), 13.32 (CH₃) ppm. ¹⁹⁵Pt NMR in D₂O: δ −882 (1050 Hz) ppm.

(SP-4-1)-Diamminebis(4-heptanone oxime-κN)platinum(II) Dinitrate (9t). *trans*-[PtCl₂(NH₃)₂] (200 mg, 0.67 mmol) and AgNO₃ (227 mg, 1.34 mmol) were suspended in 5 mL of H₂O, whereupon the mixture was stirred at 60 °C overnight. The white precipitate of AgCl was filtered off, and 4-heptanone oxime (173 mg, 1.34 mmol) in 1 mL of THF was added. After the reaction mixture was stirred at room temperature for 3 days, the yellowish solution was filtered from a small amount of a gray precipitate. Then the solvent was removed, and the solid was suspended in 10 mL of acetone; the white precipitate was filtered off and washed with acetone (2 × 1 mL) and ether (3 mL). After that, the solid was suspended in 5 mL of EtOH, filtered off, and then washed three times with 1 mL of EtOH. The filtrates were combined, the volume was reduced to 0.5 mL, and 5 mL of Et₂O was added, inducing the precipitation of a white solid. After filtration, the product was washed with 0.5 mL of Et₂O and dried in vacuo. Yield: 123 mg (30%). Anal. (C₁₄H₃₆N₆O₈Pt·H₂O) C, H, N. ESI-MS⁺ in H₂O/MeOH: *m/z* 452 [M − 2NH₃ − HNO₃ − NO₃]⁺, 469 [M − NH₃ − HNO₃ − NO₃]⁺, 486 [M − HNO₃ − NO₃]⁺. Mp: 211 °C (dec). IR in KBr (selected bands): ν 3202 s (ν_{OH}), 1657 m (ν_{C=N}) cm^{−1}. ¹H NMR in D₂O: δ 4.30 (s, br, 3H, NH₃), 2.96 (s, br, 2H, CH₂), 2.56 (t, 2H, CH₂, ³*J*_{H,H} = 8.0 Hz), 1.63 (m, br, 2H, CH₃CH₂), 1.49 (m, br, 2H, CH₃CH₂), 1.00 (t, 3H, CH₃, ³*J*_{H,H} = 7.3 Hz), 0.87 (t, 3H, CH₃, ³*J*_{H,H} = 7.4 Hz) ppm. ¹³C{¹H} NMR in D₂O: δ 178.2 (C=N), 38.1 (CH₂), 31.8 (CH₂), 19.6 (CH₃CH₂), 18.9 (CH₃CH₂), 13.4 (CH₃), 13.2 (CH₃) ppm. ¹⁹⁵Pt NMR in D₂O: δ −906, −914 (950 Hz) ppm.

Crystallographic Structure Determination. X-ray diffraction measurements were performed with a Bruker X8 APEX II CCD diffractometer at 100 K. Single crystals were positioned at 40, 40, 40, 40, and 35 mm from the detector, and 930, 2109, 1575, 804, and 1869 frames were measured, each for 5, 30, 30, 50, and

3 s over 1° scan width for **3c**, **3t**, **4c**, **5t**, and **7t**, respectively. The data were processed using the *SAINT* software package.⁴⁸ Crystal data, data collection parameters, and structure refinement details for **3c**, **3t**, **4c**, **5t**, and **7t** are given in Table 1. The structures were solved by direct methods and refined by full-matrix least-squares techniques. Non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were placed at calculated positions and refined as riding atoms in the subsequent least-squares model refinements. The isotropic thermal parameters were estimated to be 1.2 times the values of the equivalent isotropic thermal parameters of the non-hydrogen atoms to which the hydrogen atoms were bonded. *SHELXS-97*⁴⁹ was used for structure solution and *SHELXL-97*⁵⁰ for refinement, molecular diagrams were produced with ORTEP,⁵¹ and appropriate scattering factors were used.⁵²

Cell Lines and Culture Conditions. Human CH1 (ovarian carcinoma) and SW480 (colon carcinoma) cells were kindly provided by Lloyd R. Kelland (CRC Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, U.K.) and Brigitte Marian (Institute of Cancer Research, Department of Medicine I, Medical University of Vienna, Austria), respectively. Cells were grown in 75 cm² culture flasks (Iwaki/Asahi Technoglass, Gyouda, Japan) as adherent monolayer cultures in a complete culture medium, i.e., a minimal essential medium (MEM) supplemented with 10% heat-inactivated fetal bovine serum, 1 mM sodium pyruvate, and 4 mM L-glutamine (all purchased from Sigma-Aldrich, Vienna, Austria). Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO₂ and 95% air.

Cytotoxicity Tests in Cancer Cell Lines. Cytotoxicity was determined by means of a colorimetric microculture assay [MTT

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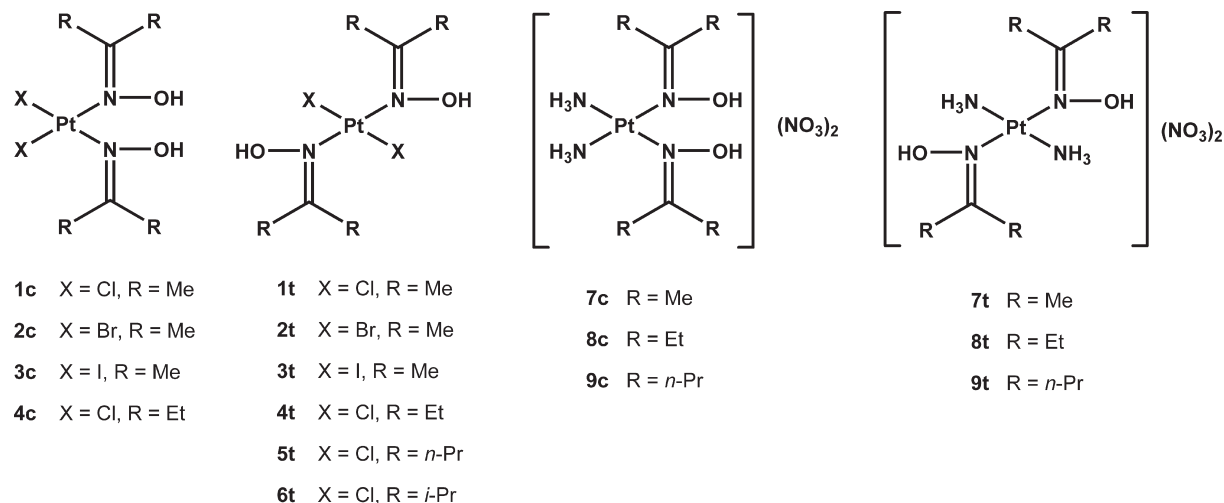


Figure 1. Platinum(II) oxime complexes under investigation.

assay, MTT = 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide]. For this purpose, CH1 and SW480 cells were harvested from culture flasks by trypsinization and seeded in 100 μ L aliquots in MEM supplemented with 10% heat-inactivated fetal bovine serum, 1 mM sodium pyruvate, 4 mM L-glutamine, and 1% nonessential amino acids (100 $\times$) (all purchased from Sigma-Aldrich) into 96-well microculture plates (Iwaki/Asahi Technoglass, Gyouda, Japan) in cell densities of 1.5×10^3 and 2.5×10^3 cells well⁻¹, respectively, in order to ensure exponential growth of untreated controls throughout drug exposure. For 24 h, cells were allowed to settle and resume exponential growth, followed by the addition of dilutions of the test compounds in aliquots of 100 μ L well⁻¹ in the same medium. Only for the less water-soluble compounds **5t** and **6t**, stock solutions had to be prepared in a dimethyl sulfoxide (DMSO)/H₂O mixture (the stability of the compounds in a DMSO solution was proven by ¹H NMR spectroscopy). The DMSO content in the actually tested dilutions did not exceed 0.5%. After continuous exposure for 96 h, drug solutions were replaced by a 130 μ L well⁻¹ RPMI 1640 culture medium (supplemented with 10% heat-inactivated fetal bovine serum and 4 mM L-glutamine) plus 20 μ L well⁻¹ MTT solution in phosphate-buffered saline (5 mg mL⁻¹). After incubation for 4 h, the medium/MTT mixtures were removed, and the formazan crystals formed by viable cells were dissolved in 150 μ L of DMSO per well. Optical densities at 550 nm were measured with a microplate reader (Tecan Spectra Classic), using a reference wavelength of 690 nm to correct for unspecific absorption. The quantity of vital cells was expressed in terms of *T/C* values by comparison to untreated control microcultures, and 50% inhibitory concentrations (IC₅₀) were calculated from concentration–effect curves by interpolation. Evaluation is based on means from at least three independent experiments, each comprising three replicates per concentration level.

Results and Discussion

Recently, we reported on the intriguing biological properties of an isomeric couple *cis/trans*-[PtCl₂(Me₂C=NOH)₂] (**1c/1t**).³⁹ In order to establish and learn more about their structure–activity relationships, a series of novel oximeplatinum(II) analogues were prepared: (i) *cis/trans* couples of [PtX₂(Me₂C=NOH)₂], where X = Br (**2c/2t**)⁵³ or I (**3c/3t**), with the aim of understanding the role of the ionic leaving ligands and the configuration of the complexes for their cytotoxic properties, (ii) [PtCl₂(R₂C=NOH)₂], where R = Et (**4c/4t**), *n*-Pr (**5t**), and *i*-Pr (**6t**), to elucidate the influence of increased lipophilicity and/or steric hindrance, and (iii)

complexes of the type [Pt(NH₃)₂(R₂C=NOH)₂](NO₃)₂, where R = Me (**7c/7t**), Et (**8c/8t**), and *n*-Pr (**9c/9t**), which were anticipated to be inactive (Figure 1).

Synthesis and Characterization of Dihalidobis(oxime)-platinum(II) Complexes. Synthesis of the target *cis*-dichloridoplatinum(II) complex **3c** was carried out in analogy to corresponding dibromido species **2c**.⁵³ The reaction between K₂[PtI₄], obtained in situ by the addition of KI to an aqueous solution of K₂[PtCl₄], and acetoxime proceeded in an aqueous solution at room temperature and led to precipitation of **3c**. Corresponding *trans* isomer **3t** was obtained via thermal isomerization of **3c** in a MeNO₂ solution at 95–100 °C for 3 h. The preparation of dichlorido species **4c** and **4t–6t** was performed by a one-step reaction of K₂[PtCl₄] and 2 equiv of the corresponding oxime in an aqueous solution at 75–85 °C; a higher temperature was needed to increase the solubility of the oximes in water. The formation of a *cis/trans* isomeric mixture was observed exclusively in the case of the *n*-pentanone oxime complex (**4c/4t**), and these isomers could be separated by column chromatography. The reactions with *n*-heptanone oxime as well as with 2,4-dimethyl-3-pentanone oxime resulted, because of steric reasons, solely in isomerically pure *trans* compounds **5t** and **6t**, respectively. The final products were obtained as pale-yellow solids and were isolated in moderate to good yields (40–65%).

Complexes **3c**, **4c**, and **3t–6t** were characterized by C, H, and N elemental analyses, ¹H, ¹³C{¹H}, and ¹⁹⁵Pt NMR and IR spectroscopy, ESI-MS, and also X-ray crystallography (for **3c**, **3t**, **4c**, and **5t**). All compounds gave satisfactory C, H, and N elemental analyses. ESI-MS spectra displayed the fragments [M + Na]⁺ and [M + K]⁺ in the positive mode as well as [M – oxime – H][–] and [M – H][–] in the negative mode, having isotopic patterns that were in good agreement with the calculated ones. The IR spectra of **3c**, **4c**, and **3t–6t** showed strong-intensity ν_{OH} (3242–3330 cm⁻¹) and medium-intensity $\nu_{C=N}$ (1649–1672 cm⁻¹) stretching vibrations. Interestingly, only in the case of the *trans*-configured complex **3t**, displaying sterically demanding iodo ligands, two sets of CH₃ signals were detected in the ¹H NMR spectrum. This circumstance is explainable by a hindered rotation of the oxime ligands around the Pt–N bonds as proven via

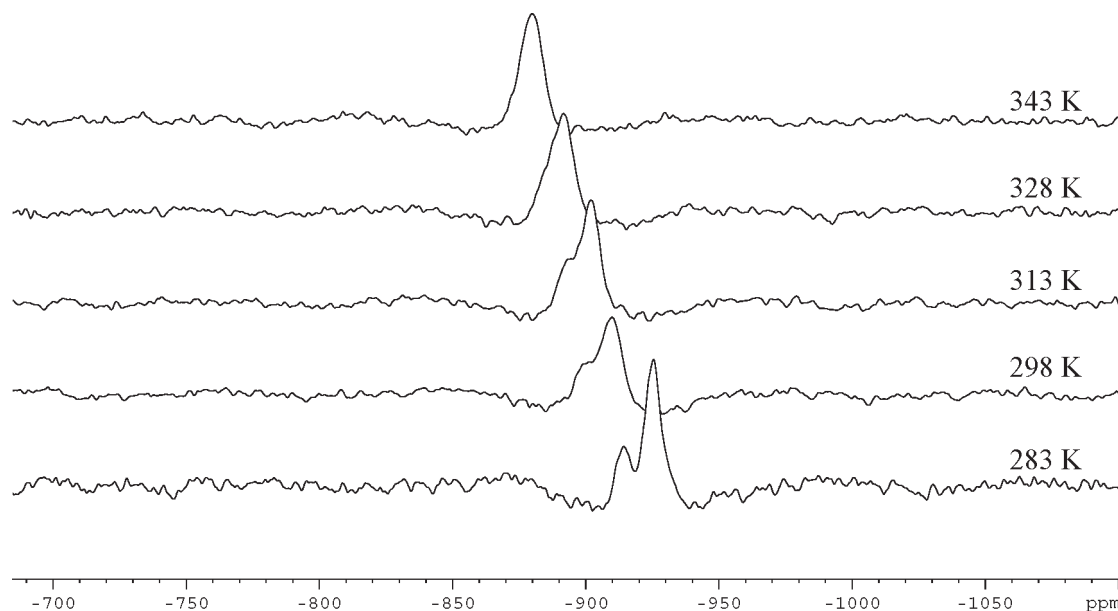


Figure 2. Temperature-dependent ^{195}Pt NMR spectra of **9t** (the region between -1100 and -700 ppm is shown). At low temperatures, the rotation of the ligands is slowed down, resulting in a splitting of the platinum resonances.

the acquisition of ^1H NMR spectra at different temperatures (278–335 K; Figure S1 in the Supporting Information).

Synthesis and Characterization of Diamminebis(oxime)platinum(II) Dinitrate Complexes. For the preparation of *cis*- and *trans*- $[\text{Pt}(\text{NH}_3)_2(\text{R}_2\text{C}=\text{NOH})_2](\text{NO}_3)_2$ (**7c/7t–9c/9t**), the well-known *cis*- $[\text{PtI}_2(\text{NH}_3)_2]^{54}$ and *trans*- $[\text{PtCl}_2(\text{NH}_3)_2]^{55}$ complexes were used as starting materials. First, halide abstraction of the starting complex by using AgNO_3 was carried out in an aqueous solution at room temperature in the case of the *cis* species and at 60°C for the corresponding *trans*-configured complexes. The elevated temperature in the latter case was necessary for the second chlorido ligand to be abstracted³¹ after the first chloride was already exchanged by a water molecule (aqua ligands are known for their extremely weak trans effect⁵⁶). After the formed AgX ($\text{X} = \text{Cl}, \text{I}$) precipitate was filtered off, 2 equiv of the corresponding oxime was added to the in situ generated *cis/trans*- $[\text{Pt}(\text{NH}_3)_2(\text{H}_2\text{O})_2]^{2+}$ species, leading to the formation of the desired complexes (**7c/7t–9c/9t**). The compounds were obtained in moderate to good yields (15–65%) via either precipitation (**7c**, **7t**, and **9c**; the addition of acetone to the aqueous solution) or solvent removal and suspension in acetone (**8c**, **8t**, and **9t**).

Complexes **7c/7t–9c/9t** gave satisfactory elemental analyses and expected molecular ion/fragmentation patterns in the ESI-MS spectra. The presence of a broad range of peaks was recognized in the positive mode (see Experimental Section). Complexes **7c/7t–9c/9t** were characterized by IR spectroscopy; characteristic ν_{OH} ($3194\text{--}3316\text{ cm}^{-1}$) and $\nu_{\text{C}=\text{N}}$ ($1645\text{--}1667\text{ cm}^{-1}$) bands were observed. With respect to the ^1H NMR spectra in D_2O of the *trans*- $[\text{Pt}(\text{NH}_3)_2(\text{R}_2\text{C}=\text{NOH})_2](\text{NO}_3)_2$ complexes **7t**, **8t**, and **9t**, it is worth mentioning that the proton signals become broader with the increased length of *R*. In turn, in the ^{195}Pt NMR spectra, the platinum signal of **8t**

was broader than that for **7t** (the half-height widths were 1500 and 800 Hz, respectively), turning into two signals (a broad signal with a shoulder) in the case of **9t**, which is most probably the result of a hindered rotation of the sterically demanding ligands. To clarify that fact, ^{195}Pt (Figure 2) and ^1H NMR (Figure S2 in the Supporting Information) spectra were measured at different temperatures for **9t**. At 283 K, two platinum signals at -914 and -926 ppm were observed; upon an increase in the temperature to 343 K, only one signal was detected, showing that the complex exists in temperature-dependent conformations.

An analogous behavior was monitored in the proton resonance spectra, measured at the same temperatures (Figure S2 in the Supporting Information). However, for the NMR spectra of the corresponding *cis*-configured $[\text{Pt}(\text{NH}_3)_2(\text{R}_2\text{C}=\text{NOH})_2](\text{NO}_3)_2$ complexes **7c**, **8c**, and **9c**, no such features were observed.

X-ray Crystal Structures. The structures of *cis*- $[\text{PtI}_2(\text{Me}_2\text{C}=\text{NOH})_2]$ (**3c**), *trans*- $[\text{PtI}_2(\text{Me}_2\text{C}=\text{NOH})_2]$ (**3t**), *cis*- $[\text{PtCl}_2(\text{Et}_2\text{C}=\text{NOH})_2]$ (**4c**), *trans*- $[\text{PtCl}_2(n\text{-Pr}_2\text{C}=\text{NOH})_2]$ (**5t**), and *trans*- $[\text{Pt}(\text{NH}_3)_2(\text{Me}_2\text{C}=\text{NOH})_2](\text{NO}_3)_2$ (**7t**) were determined by X-ray diffraction (Table 1 and Figures 3–5). In **3c**, **3t**, **4c**, and **5t**, the metal center is bound by two oximic nitrogen atoms and two halogenido ligands, being in the *cis* (**3c** and **4c**) or *trans* (**3t** and **5t**) positions. In **7t**, two ammonia and two oxime ligands are mutually *trans*. In the coordinated oximes, the $\text{C}=\text{N}$ bond lengths [$1.270(7)\text{--}1.292(7)\text{ \AA}$] are similar within 3σ and correspond to previously reported distances in metal-bound oximes [$1.268\text{--}1.324\text{ \AA}$].^{53,57–60} The platinum atom in the square-planar complex **3t** is slightly displaced out of the

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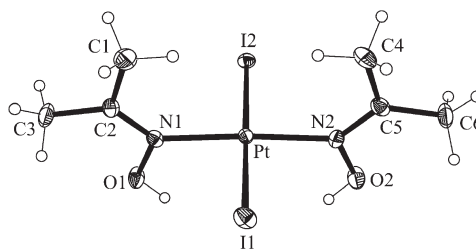
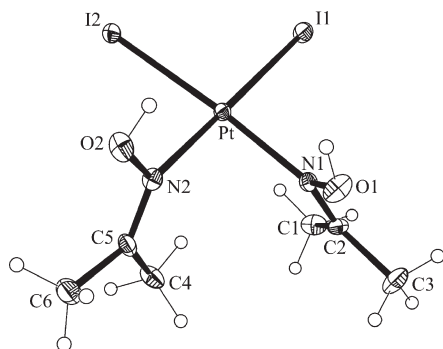


Figure 3. ORTEP diagram of **3c** (left) and **3t** (right) displaying thermal ellipsoids at 50% probability. Selected bond lengths (Å) and angles (deg) for **3c**: Pt–N1, 2.041(4); Pt–N2, 2.055(4); Pt–I1, 2.5819(4); Pt–I2, 2.5963(4); N1–C2, 1.292(7); N2–C5, 1.270(7); N1–Pt–N2, 89.03(17); N1–Pt–I1, 90.77(12); N1–Pt–I2, 176.08(12). Selected bond lengths (Å) and angles (deg) for **3t**: Pt–N1, 2.002(3); Pt–N2, 2.007(3); Pt–I1, 2.5938(3); Pt–I2, 2.6104(2); N1–C2, 1.285(4); N2–C5, 1.291(4); N1–Pt–N2, 174.39(11); N1–Pt–I1, 90.69(8); N1–Pt–I2, 89.63(8).

mean plane through N1–I1–N2–I2, by 0.01 Å, whereas in the case of **3c**, it lies ideally within the corresponding N1–I1–N2–I2 plane (Figure 3).

The Pt–I bond distances in complexes **3c** and **3t** were found in the range of 2.5819(4)–2.6104(2) Å. These distances agree well with those of previously published platinum(II) iodide compounds.^{61–63} The Pt–N distances in the case of cis-configured **3c** at 2.041(4) and 2.055(4) Å for Pt–N1 and Pt–N2, respectively, are significantly longer than those found in **3t** [Pt–N1, 2.002(3) Å; Pt–N2, 2.007(3) Å] because of the more pronounced trans influence of the coordinated iodide.⁵⁶

In dichloridoplatinum(II) complexes **4c** and **5t** (Figures 4 and 5), deviation of the platinum atom from the mean planes through N1–Cl1–N2–Cl2 and N1–Cl1–N1–I1–Cl12 is not larger than 0.04 Å. The Pt–Cl bond distances in **4c** and **5t** vary from 2.2821(9) to 2.3208(8) Å, which falls into the range of typical Pt–Cl distances.^{64,65} The Pt–N1 bond length in **4c** at 2.0049(19) Å is in the expected range for platinum complexes with a cis configuration.^{53,57} Of note is the relatively long distance Pt–N2 at 2.0249(19) Å, displaying a 7σ difference from the Pt–N1 bond length. The N1–Pt–N2 angle at 92.08(8)° in **4c** is larger than that in **3c** [N1–Pt–N2, 89.03(17)°], depending on the coordinated halogenido ligand (chlorido versus iodido).

The Pt–N1 bond of trans-configured **5t** at 2.006(2) Å is well comparable with Pt–N distances found in **3t** and **7t** at 2.002(3) and 2.006(2) Å, respectively. In contrast, the Pt–N2 distance (coordinated ammine ligand) in **7t** at 2.045(2) Å is significantly longer.

Cytotoxicity and Structure–Activity Relationships. The cytotoxic potencies of the halogenido oxime complexes **2c–4c** and **2t–6t**, as well as the diamminebis(oxime) compounds **7c/7t–9c/9t**, were compared to those of cisplatin and transplatin in the cisplatin-sensitive ovarian carcinoma

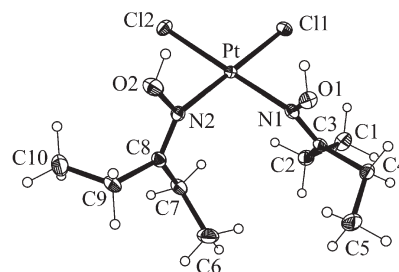


Figure 4. ORTEP diagram of **4c** displaying thermal ellipsoids at 50% probability. Selected bond lengths (Å) and angles (deg): Pt–N1, 2.0049(19); Pt–N2, 2.0249(19); Pt–Cl1, 2.2977(6); Pt–Cl2, 2.2957(6); N1–C3, 1.290(3); N2–C8, 1.287(3); N1–Pt–N2, 92.08(8); N1–Pt–Cl1, 88.35(6); N1–Pt–Cl2, 178.14(5).

cell line CH1 and the intrinsically cisplatin-resistant colon carcinoma cell line SW480 by means of the colorimetric MTT assay. IC₅₀ values are listed in Table 2. In general, both cell lines are similarly sensitive to the halogenido oxime compounds **2c–4c** and **2t–6t**, while CH1 cancer cells are considerably more sensitive to the cationic diamminebis(oxime) complexes **7c–9c** as well as **7t** and **9t** than SW480 cells; complex **8t** showed no antiproliferative effect at all.

Specific structural modifications of the previously reported *cis/trans*-[PtCl₂(Me₂C=NOH)₂] complexes **1c** and **1t**³⁹ were made in order to investigate the structure–activity relationships with respect to the effect of (i) the leaving group, (ii) the carbon chain length of coordinated oximes in the case of the chlorido complexes, as well as (iii) an increasing oxime carbon chain length in cationic diamminebis(oxime) complexes.

i. Effect of the Leaving Group. Substitution of the chlorido ligands in **1c/1t** for bromides, yielding **2c/2t**, dramatically decreases the cytotoxic potencies by factors of 9–39. These results were expected and can be explained by the lower reactivity of bromido complexes compared to chlorido analogues.⁶ Still, the trans isomer **2t** is more cytotoxic than its cis analogue **2c**. However, this trend is not continued in the iodido counterparts. **3c** and **3t** proved to be more cytotoxic than, or at least similarly cytotoxic to the corresponding bromido species **2c** and **2t**, despite a higher stability [hard and soft acids and bases (HSAB) principle]. This might be caused by the higher lipophilicity of **3c** and **3t**, facilitating cellular accumulation of the complexes. Thus, the order of cytotoxicity of

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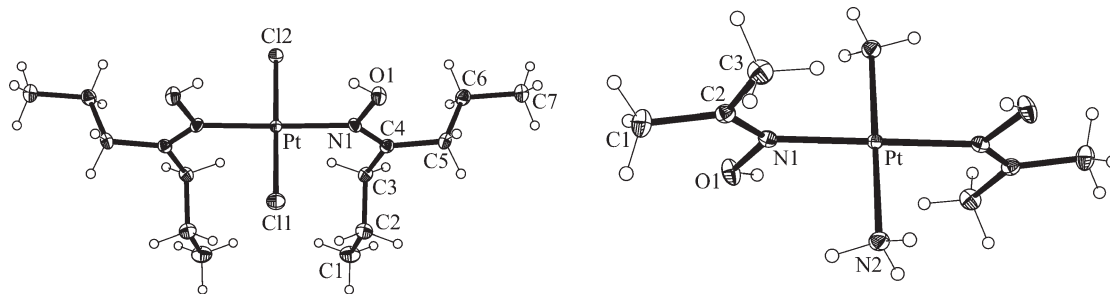


Figure 5. ORTEP diagram of **5t** (left) and **7t** (right) displaying thermal ellipsoids at 50% probability. Selected bond lengths (Å) and angles (deg) for **5t**: Pt–N1, 2.006(2); Pt–Cl1, 2.2821(9); Pt–Cl2, 2.3208(8); N1–C4, 1.277(3); N1–Pt–N1#1, 174.82(11); N1–Pt–Cl1, 89.09(6); N1–Pt–Cl2, 90.90(6). Selected bond lengths (Å) and angles (deg) for **7t**: Pt–N1, 2.006(2); Pt–N2, 2.045(2); N1–C2, 1.288(3); N1–Pt–N2, 89.66(9); N1–Pt–N2i1, 90.34(9); N1–Pt–N1i1, 180.0(11).

Table 2. Cytotoxicity of Platinum(II) Oxime Complexes in CH1 and SW480 Cancer Cells (Exposure Time 96 h)

cis configuration			trans configuration		
compound	IC ₅₀ , μM		compound	IC ₅₀ , μM	
	CH1	SW480		CH1	SW480
1c	2.7 ± 0.7	3.4 ± 0.3	1t	0.17 ± 0.09	0.22 ± 0.05
2c	25 ± 4	45 ± 6	2t	6.7 ± 0.2	2.5 ± 0.4
3c	1.6 ± 0.2	1.8 ± 0.5	3t	3.0 ± 0.5	2.8 ± 0.3
4c	11 ± 1	15 ± 1	4t	1.4 ± 0.3	1.9 ± 0.6
			5t	0.18 ± 0.03	0.16 ± 0.02
			6t	0.19 ± 0.03	0.21 ± 0.03
7c	21 ± 7	567 ± 39	7t	468 ± 180	> 1000
8c	717 ± 46	> 1000	8t	> 1000	> 1000
9c	162 ± 46	> 1000	9t	29 ± 5	350 ± 30
cisplatin	0.14 ± 0.03	3.3 ± 0.4	transplatin	15 ± 2	19 ± 3

[PtX₂(Me₂C=NOH)₂]-type agents is dependent on the halido ligand X as follows: I > Cl > Br (cis geometry) and Cl > I ≥ Br (trans geometry). Contrary to the chlorido (**1c**/**1t**) and bromido (**2c**/**2t**) complexes, the cis isomer **3c** is slightly more cytotoxic than trans-configured **3t**, reversing the structure–activity relationships to a more cis/transplatin-like behavior.

ii. Effect of the Oxime Carbon Chain in Dichlorido Complexes. A series of complexes with chlorido ligands and a systematically increased carbon chain of the oxime ligand were investigated. Using *n*-pentanone oxime in **4c**/**4t** instead of acetoxime in **1c**/**1t** resulted in a 4–8.6-fold decrease of cytotoxicity. Remarkably, the trans-configured complex **4t** is 8 times more cytotoxic than **4c**. Further increasing the length of the oxime carbon chain of the trans complexes by either *n*-propyl (**5t**) or isopropyl (**6t**) residues results in similarly enhanced cytotoxicity with IC₅₀ values in the high nanomolar concentration range in both the cisplatin-sensitive CH1 and cisplatin-resistant SW480 cells. Hence, the cytotoxicity of the *trans*-[PtCl₂(R₂C=NOH)₂]-type complexes depends on the residues R as follows: Me ≈ *n*-Pr ≈ *i*-Pr > Et.

iii. Effect of the Oxime Carbon Chain Length in Cationic Diamminebis(oxime) Complexes. All six cationic diamminedioxime complexes (**7c**/**7t**–**9c**/**9t**) displayed either a low or a negligible cytotoxicity. This is in accordance with the classic structure–activity relationships, according to which complexes with four nitrogen donor ligands

(virtually no leaving groups⁶⁶) or charged compounds (low cellular accumulation but increased renal elimination⁶) are generally considered to be noncytotoxic. The reason for the observed (albeit low) cytotoxicity of **7c** and **9t**, especially in CH1 cells, is yet unclear.

In comparison to cisplatin, the cis-configured halido complexes **1c**–**4c** are considerably (up to 70 times) less potent in the cisplatin-sensitive cell line CH1 but almost completely retain their cytotoxicity in the cisplatin-resistant SW480 cells, as indicated by a comparison of the resistance factors (RF = IC₅₀ in the cisplatin-resistant cell line/IC₅₀ in the cisplatin-sensitive cell line) of **1c**–**4c** (RF = 1.1–1.8; Table S1 in the Supporting Information) with that of cisplatin (RF = 24).

In the case of *trans*-halido compounds **1t**–**6t**, the resistant factors of 0.4–1.4 show that the complexes are not cross-resistant with cisplatin either. **1t**, **5t**, and **6t** are similarly cytotoxic as cisplatin in CH1 cells and up to 20 times more cytotoxic than cisplatin in SW480 cells. In comparison to transplatin, **1t**–**6t** are up to 90 (CH1) and 120 (SW480) times more cytotoxic.

Conclusions

A series of novel bis(oxime)platinum(II) complexes with either cis or trans configuration have been synthesized, characterized, and investigated in two human cancer cell lines. Structure–activity relationships could be inferred from these results. The observation that the trans geometry yields a more active complex in the case of the prototypic oxime complex [PtCl₂(Me₂C=NOH)₂] could be confirmed for at least two further structural analogues, [PtBr₂(Me₂C=NOH)₂] and [PtCl₂(Et₂C=NOH)₂]. Every single cis- or trans-configured halido bis(oxime) complex showed comparable cytotoxic potency in both cell lines, the cisplatin-sensitive CH1 and the inherently cisplatin-resistant SW480 cancer cells. Particularly high cytotoxicities were revealed for the three trans compounds with chlorido ligands, with IC₅₀ values as low as 0.16–0.22 μM. Given these encouraging results, their mechanism of action should be studied in more detail in order to gain an understanding, which, in turn, may help to optimize their anticancer activity.

Acknowledgment. The authors are indebted to the FFG–Austrian Research Promotion Agency (811591), the Austrian Council for Research and Technology Development (IS526001), the FWF (Austrian Science Fund), COST D39, and Russian Fund for Basic Research (Grant

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09-03-12173-ofim). We thank Nikolay V. Kukushkin (St. Petersburg State University) for experimental assistance.

Supporting Information Available: Figures with temperature-dependent ^1H NMR spectra of **3t** and **9t**, tables with resistance factors and elemental analyses of platinum oxime complexes, and X-ray crystallographic data in CIF format for **3c**, **3t**, **4c**, **5t**,

and **7t**. Crystallographic data have been deposited with the Cambridge Crystallographic Data Center as CCDC 767592–767596 for **4c**, **5t**, **3c**, **3t**, and **7t**, respectively. Copies of data can be obtained, free of charge, upon application to CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (deposit@ccdc.com.ac.uk). This material is available free of charge via the Internet at <http://pubs.acs.org>.

Supporting Information

Novel *cis*- and *trans*-configured bis(oxime)platinum(II) complexes – synthesis, characterization, and cytotoxic activity

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page S3	Table S1 with resistance factors of platinum oxime complexes.
page S4	Table S2 with elemental analyses of platinum oxime complexes.

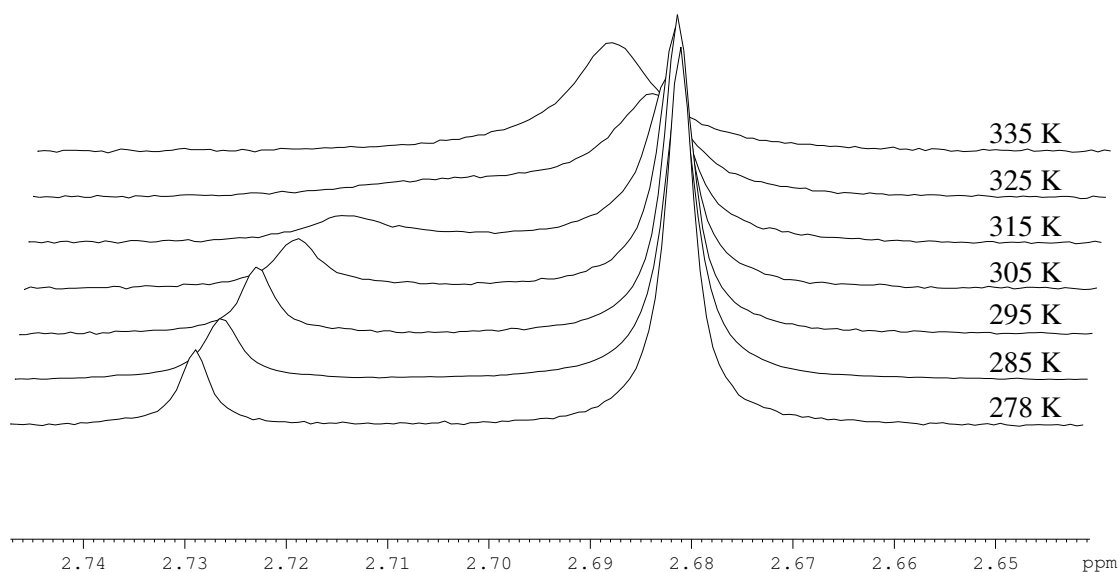


Figure S1. Temperature dependent ^1H NMR spectra of **3t** (region between 2.65 and 2.74 ppm is shown). At 278 K, two separated signals in a ratio of about 1:5 were detected. Upon heating up to 335 K, only one broad signal was observed.

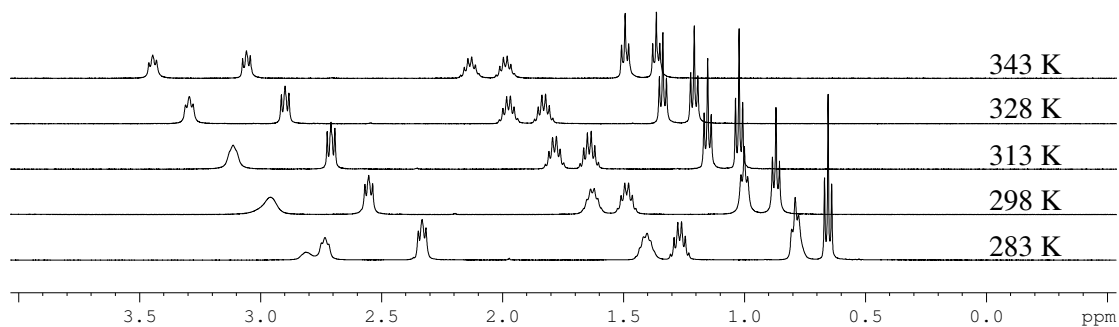


Figure S2. Temperature dependent ^1H NMR spectra of **9t** (region between -0.50 and 4.00 ppm is shown). At 343 K, a doubled set of CH_2 and CH_3 resonances was detected, displaying a good resolution of each signal. During cooling down to 283 K, one set of signals became broader and at 283 K, the methylene protons $\text{CH}_2\text{-CN}$ additionally were split into two clearly separated signals.

Table S1. Resistance Factors of Platinum Oxime Complexes, IC₅₀ (SW480, Cisplatin Resistant Cell Line) / IC₅₀ (CH1, Cisplatin Sensitive Cell Line)

<i>cis</i> -configuration		<i>trans</i> -configuration	
compound	resistance factor	compound	resistance factor
1c	1.3	1t	1.3
2c	1.8	2t	0.4
3c	1.1	3t	0.9
4c	1.4	4t	1.4
---	---	5t	0.9
---	---	6t	1.1
7c	27	7t	---
8c	---	8t	---
9c	---	9t	12
cisplatin	24	transplatin	1.3

Table S2. Elemental Analyses of Platinum Oxime Complexes

complex	Calculated, %			Found, %		
	C	H	N	C	H	N
2c	14.38	2.82	5.59	14.76	2.80	5.36
2t	14.38	2.82	5.59	14.67	2.70	5.53
3c	12.11	2.37	4.71	12.03	2.18	4.66
3t	12.11	2.37	4.71	12.14	2.36	4.79
4c	25.65	4.74	5.98	25.84	4.47	5.90
4t	25.65	4.74	5.98	25.73	4.61	5.75
5t	32.07	5.77	5.34	32.41	5.76	5.32
6t	32.07	5.77	5.34	32.11	5.62	5.27
7c·H₂O	13.93	4.29	16.24	13.93	4.04	16.27
7t	14.43	4.04	16.83	14.30	3.84	16.81
8c	21.62	5.08	15.13	21.45	4.78	14.94
8t	21.62	5.08	15.13	21.41	4.88	15.14
9c·H₂O	26.71	6.08	13.35	26.90	5.69	13.61
9t·H₂O	26.71	6.08	13.35	26.94	5.67	13.71

2. Synthesis, Characterization and Cytotoxic Activity of Novel Potentially pH-Sensitive Nonclassical Platinum(II) Complexes Featuring 1,3-Dihydroxyacetone Oxime Ligands

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Synthesis, Characterization, and Cytotoxic Activity of Novel Potentially pH-Sensitive Nonclassical Platinum(II) Complexes Featuring 1,3-Dihydroxyacetone Oxime Ligands

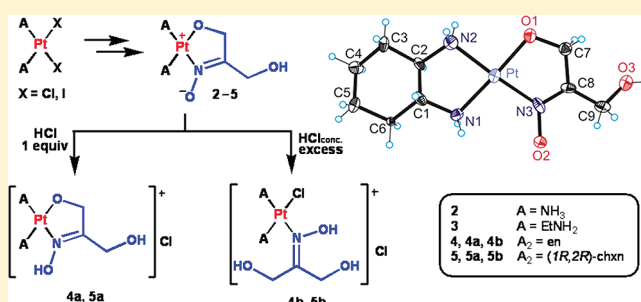
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S Supporting Information

ABSTRACT: The reaction of 1,3-dihydroxyacetone oxime with diam(m)inediaquaplatinum(II) under basic conditions produced zwitterionic diam(m)mine(3-hydroxy-2-(oxidoimino)propan-1-olato- κ^2 N,O)platinum(II) complexes featuring the N,O-chelating ligand. Upon reaction with hydrochloric acid, it was possible to isolate either the singly protonated species still exhibiting the intact N,O-chelate or the open-chain chlorido complex. All complexes were characterized in detail with multinuclear (¹H, ¹³C, and ¹⁹⁵Pt) NMR spectroscopy, ESI mass spectrometry, and in one case X-ray diffraction. Cytotoxicity was investigated in three human cancer cell lines (CH1, SW480, and A549). The obtained IC₅₀ values are in the medium or even low micromolar range, remarkable for platinum complexes having N₃O or N₃Cl coordination spheres. To study the solution behavior of the prepared complexes at physiologically relevant proton concentrations, time-dependent ¹H NMR measurements were performed for the ethane-1,2-diamine-containing series at pH values of 7.4, 6.0, and exemplarily 5.0. While the zwitterionic complex proved to be stable at both pH 7.4 and 6.0, the protonated species were deprotonated at pH 7.4, tending toward ring opening in slightly acidic environments, as characteristic for many solid tumors. Finally, the open-chain form stayed intact at pH 6.0, being completely converted into its chelated analogue at pH 7.4. A pH-dependent evaluation of antiproliferative effects of the two latter complexes at pH 7.4 and pH 6.0 revealed an activation under slightly acidic conditions, which might be of interest for further *in vivo* studies.



INTRODUCTION

Currently, there are three platinum-based anticancer drugs in worldwide clinical use, viz., cisplatin and carboplatin—mainly but not exclusively applied in the treatment of tumors of the urogenital tract—and oxaliplatin, which is used in colorectal cancer.^{1–5} In spite of important achievements of platinum-based chemotherapy, one cannot deny a number of serious drawbacks of platinum medicines, especially severe side effects caused by their unselective reaction with biomolecules. Therefore, development of antitumor complexes attributed with an enhanced selectivity for tumor tissue or increased reactivity at the tumor site is a crucial goal of current cancer research.

Preferably, novel platinum drugs should attack exclusively cancerous cells without affecting the normal ones. However, this bar is almost certainly unreachable in the case of such a complex disease as cancer. Nevertheless, there is a possibility of approximating the ideal situation, developing platinum-based prodrugs almost innocent in the administered form and being activated under certain conditions by conversion within the tumor tissue to their cytotoxic/reactive analogues. Ideally, only the latter form destroys cancer cells.

Several strategies for the design and application of platinum-based drugs which can be activated in the tumor tissue have been developed so far (Figure 1). The first and most abundant class of platinum-based anticancer prodrugs are kinetically inert and unreactive platinum(IV) complexes, which can be converted to more reactive platinum(II) species, taking advantage of the reducing environment in solid tumors (activation by reduction).^{2,6–10} The second promising platinum prodrug strategy pursues the development of platinum(IV) species which can be photoactivated, implying that they are inactive in the dark but can be photoreduced to cytotoxic platinum(II) species by visible light,^{11–19} with the advantage of external initiation and control of the activation. Third, an intriguing class of platinum-based antitumor prodrugs represents platinum complexes that are inert at physiological pH but which can be activated under slightly acidic pH values, converting platinum species to their cytotoxic/activated form.^{20–29}

As far as the latter strategy is concerned, it is worth mentioning that many solid tumors are characterized by a significant acidity

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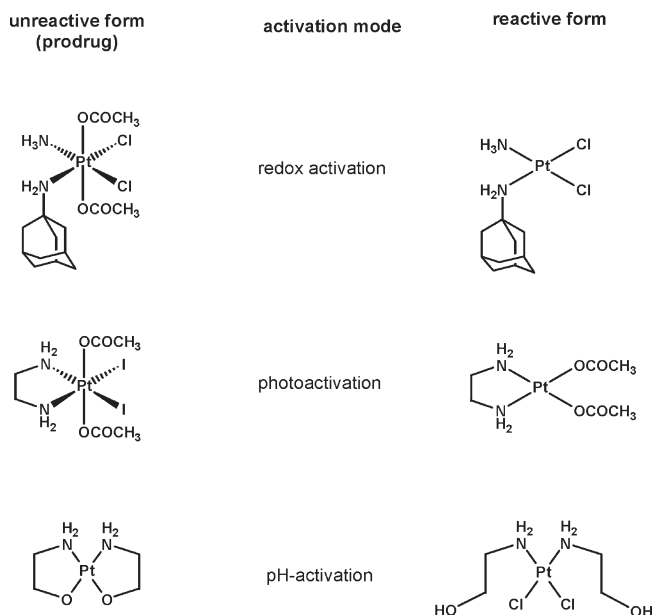


Figure 1. Strategies to activate rather unreactive platinum prodrugs in the tumor tissue.

(pH 5.6–6.8),³⁰ compared to the normal tissue (pH 7.4), due to their poor blood perfusion, resulting in low oxygen availability.³¹ This promotes an increased metabolism of glucose and the production of metabolic acids, such as lactic acid.³² An acidic milieu within solid tumors could be beneficially used for the application of pH sensitive anticancer platinum drugs kinetically inert and nearly inactive under physiological conditions (the unreactive or prodrug form) and displaying increased cytotoxicity under mildly acidic pH values in tumoral tissues (the activated or reactive form, Figure 1).

Up to now, only two small groups of platinum-based anticancer compounds displaying pH-dependent antitumor activity have been reported, viz., platinum(II) complexes bearing two chelating O-alkyldithiocarbonato^{20,21} or aminoalcoholato ligands.^{22–29} Both of them are, as many chelates, rather inert but can be activated under acidic conditions *via* protonation and subsequent opening of rather thermodynamically stable five-membered rings.

In view of our current interest in platinum(II) oxime complexes^{33–37} and their recently reported outstanding cytotoxic properties, appreciably enhanced in the case of *trans*-geometry,^{38–40} we focused our efforts on the development of novel oxime-based pH-sensitive platinum prodrugs. The scenario of this work was the following: (i) to synthesize and characterize kinetically inert platinum(II) complexes bearing a chelating oxime ligand; (ii) to study their pH-dependent behavior, especially at physiologically relevant pH values 7.4 (normal tissue) and 6.0 (tumor tissue); and (iii) to evaluate the cytotoxic properties of these compounds in three human tumor cell lines originating from ovarian carcinoma (CH1), colon carcinoma (SW480), and nonsmall cell lung cancer (A549), including a comparative investigation under standard pH screening conditions (pH 7.4) and in an acidified medium (pH 6.0) to figure out whether the concept of pH-dependent activation also works for this novel class of compounds.

RESULTS AND DISCUSSION

Inspired by intriguing cytotoxic properties of (oxime)Pt^{II} complexes,^{38–40} we turned our attention to a commercially

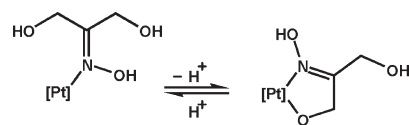


Figure 2. pH-Dependent intramolecular chelation and ring opening of the ligated 1,3-dihydroxyacetone oxime.

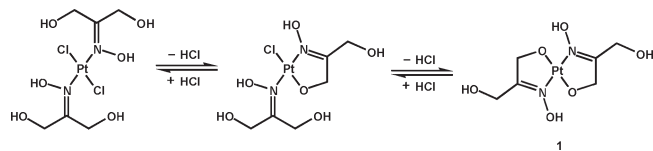


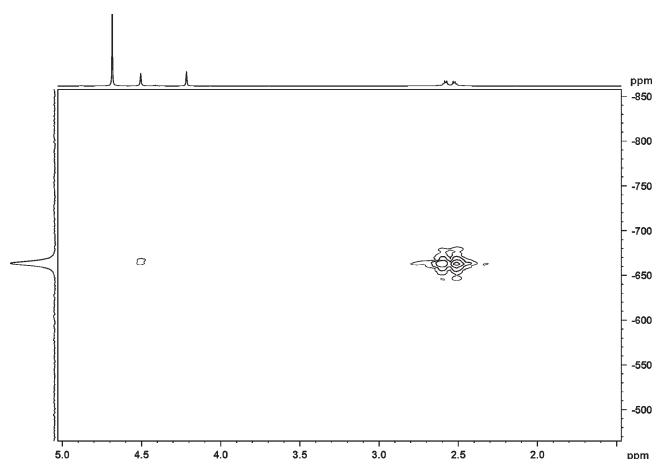
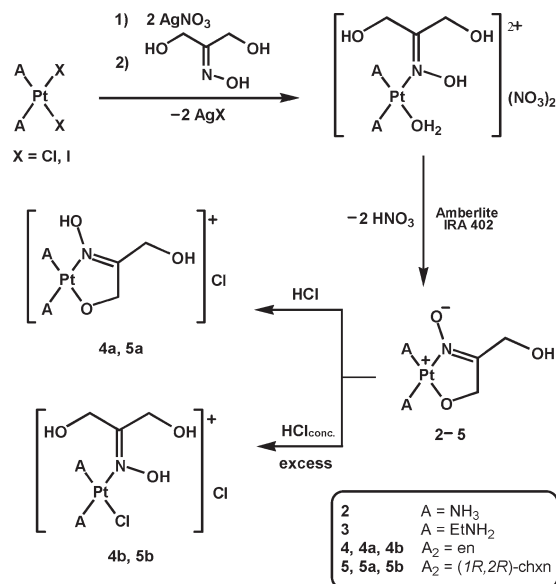
Figure 3. pH-Dependent equilibrium mixture of the opened-, mono-, and bischelated oximeplatinum species.

available 1,3-dihydroxyacetone oxime (IUPAC name:⁴¹ 1,3-dihydroxypropan-2-one oxime) as a promising ligand for the development of novel potentially tumor-inhibiting platinum complexes that are rather inert but which can be activated at acidic pH values. Owing to the presence of three hydroxy groups, the ligand is able to form multiple hydrogen bonds, being of great importance for the binding of platinum compounds to DNA.⁴² Moreover, the 1,3-dihydroxyacetone oxime ligand is particularly interesting because it is capable of a pH-dependent intramolecular chelation and ring opening (Figure 2). This reaction could be crucial in view of the lower pH value in solid tumors. Thus, the kinetically inert chelated compound might be applied as a prodrug, being converted into the more (re)active open-chain form at lower pH values in tumor tissues.

Synthesis, Characterization, and X-Ray Structure Determination of Platinum(II) Species with Chelated 1,3-Dihydroxyacetone Oxime. Synthesis of bischelate **1** proceeded in a one-step reaction through the addition of 2 equiv of 1,3-dihydroxyacetone oxime to an aqueous solution of K₂[PtCl₄] at room temperature. As a consequence, an equilibrium mixture of the opened, mono-, and bischelated complexes was formed (Figure 3). The latter species, notable for the lowest solubility, precipitated from the aqueous solution and was isolated (45%) by filtration. An additional portion of the product was formed after the addition of 1M NaOH solution to the filtrate, resulting in a change of pH value from 2.0 to 5.5 and giving an overall yield of 62%.

As starting materials for the preparation of the diam(m)-ineplatinum(II) compounds bearing the 3-hydroxy-2-(oxidoimino)-propan-1-olato ligand, we addressed the readily accessible complexes *cis*-[PtX₂A₂], where X = chloride or iodide and A₂ = (amine)₂, (ethylamine)₂, ethane-1,2-diamine (en), or (*R,R*)-cyclohexane-1,2-diamine [(1*R*,2*R*)-chxn]. The precursors of target species **2–5** (Scheme 1) were prepared by a one-step reaction of K₂[PtX₄] with the corresponding am(m)ine or diamine by a reported method.^{43,44} Further, halide abstraction using AgNO₃ was carried out in an aqueous solution at room temperature, whereupon one equivalent of 1,3-dihydroxyacetone oxime was slowly added in order to avoid/minimize the formation of a bis(oxime)-platinum(II) species. As a next step, the reaction mixture was treated with a basic ion exchanger, leading to deprotonation of both the side-chain and oximic hydroxy group and resulting in the formation of zwitterionic oximate species **2–5**. Finally, **2–4** were purified by semipreparative HPLC yielding 20–58% of the desired products, while **5** was isolated in 32% yield as a colorless precipitate after concentration of the reaction mixture.

Scheme 1

Figure 4. ^1H , ^{195}Pt HMQC NMR spectrum of **4**.

Complexes **1–5** were characterized by elemental analyses; ^1H , $^{13}\text{C}\{^1\text{H}\}$, and ^{195}Pt NMR and IR spectroscopy; electrospray ionization mass spectrometry (ESI-MS); and also X-ray crystallography (in the case of **5**). All compounds gave satisfactory C, H, and N elemental analyses. The ESI-MS spectrum of **1** exhibited the fragment $[\text{M} - \text{H}]^-$, while in the spectra of **2–5**, $[\text{M} + \text{H}]^+$ and $[\text{M} + \text{Na}]^+$ peaks were found. In all cases, the observed and calculated isotopic patterns agreed well with each other.

The IR spectra of **1–5** displayed strong to medium $\nu(\text{OH})$ and $\nu(\text{NH})$ (for **2–5**) $[2808\text{--}3474\text{ cm}^{-1}]$ and medium $\nu(\text{C}=\text{N})$ $[1602\text{--}1630\text{ cm}^{-1}]$ stretching vibrations. Chelation of the 1,3-dihydroxyacetone oxime ligand was confirmed (e.g., complex **4**) via acquisition of an ^1H , ^{195}Pt -HMQC NMR spectrum (Figure 4). Thus, for compound **4**, three correlation peaks were detected, derived from the coupling of the ^{195}Pt nucleus with the protons of the coordinated ethane-1,2-diamine as well as with one of the oxime CH_2 groups located within the chelate ring. The presence of the latter cross-peak undoubtedly proved the fact of the ring closure.

The structure of **5** was determined by X-ray diffraction (Table 1 and Figure 5). The platinum(II) atom has a slightly

Table 1. Crystal Data and Details of Data Collection for $\text{5} \cdot 2\text{H}_2\text{O}$

	$\text{5} \cdot 2\text{H}_2\text{O}$
empirical formula	$\text{C}_9\text{H}_{23}\text{N}_3\text{O}_5\text{Pt}$
fw	448.39
space group	$P2_12_12_1$
a [Å]	7.3783(15)
b [Å]	8.3748(19)
c [Å]	22.125(5)
V [Å ³]	1367.1(5)
Z	4
λ [Å]	0.71073
ρ_{calcd} [g cm ⁻³]	2.178
cryst size [mm ³]	$0.40 \times 0.02 \times 0.01$
T [K]	100
μ [mm ⁻¹]	10.281
R_1^a	0.0556
wR_2^b	0.1145
GOF ^c	1.009

^a $R_1 = \sum ||F_o| - |F_c|| / \sum |F_o|$. ^b $wR_2 = \{ \sum [w(F_o^2 - F_c^2)^2] / \sum [w(F_o^2)^2] \}^{1/2}$. ^c $\text{GOF} = \{ \sum [w(F_o^2 - F_c^2)^2] / (n - p) \}^{1/2}$, where n is the number of reflections and p is the total number of parameters refined.

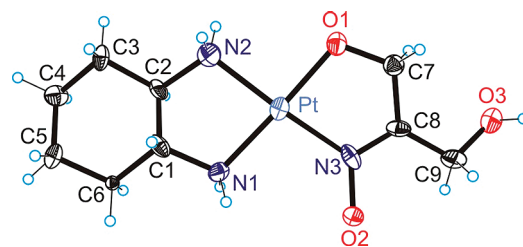


Figure 5. ORTEP view of **5** with the atom labeling scheme. The thermal ellipsoids have been drawn at the 50% probability level. Selected bond lengths (Å) and bond angles (deg): Pt–N1 = 2.040(11), Pt–N2 = 2.054(11), Pt–N3 = 1.997(13), Pt–O1 = 2.006(9); N1–Pt–N2 = 83.4(4), O1–Pt–N3 = 82.5(4).

distorted square-planar coordination geometry. It is coordinated by two ligands, bidentate (*R,R*)-cyclohexane-1,2-diamine and a doubly deprotonated 1,3-dihydroxyacetone oxime ligand acting as a bidentate as well. Deviation of the platinum atom from the mean plane through N1, N2, O1, N3 is at 0.011 Å. The bond lengths Pt–N1 = 2.040(11) and Pt–N2 = 2.054(11) Å are in a good agreement with those of oxaliplatin, (SP-4-2)-((*R,R*)-cyclohexane-1,2-diamine)oxalato-platinum(II) [Pt–N1 = 2.06(2) Å, Pt–N2 = 2.04(2) Å].⁴⁵ The Pt–N3 distance is 1.997(13) Å and correlates well with Pt–N bond lengths in previously reported (oxime)Pt(II) complexes displaying an *N*-bound ligand in the *trans* position to an oxime $[1.993\text{--}2.032\text{ Å}]$.^{46–48} It is worth mentioning that no nitrate counteranions were detected in the structure of **5**, providing additional evidence for the double deprotonation of the 1,3-dihydroxyacetone oxime. The atoms O1 and O2 seem to be deprotonated, acting as proton acceptors in hydrogen bonding interactions with cocrystallized water molecules and the O3–H group of the oxime ligand in the adjacent molecule of **5**.

Protonation of the Oximate Group and Opening of the Oxime Chelate Ring. Above described complexes **4** and **5** were

converted into the corresponding cationic species by the addition of an equivalent amount of hydrochloric acid ($\text{pH} \approx 2.5$; Scheme 1), whereupon the reaction mixture was immediately frozen and lyophilized in order to avoid further protonation of the ligated oxime and opening of the chelate ring. The compounds **4a** and **5a** were isolated with nearly quantitative yields.

Opening of the oxime chelate ring was performed in an analogous way, adding an excess of HCl_{conc} (about 4 equivs) until a pH of 1 was reached to an aqueous solution of **4** or **5** and stirring the mixture for 24 h at room temperature for completion of the reaction (Scheme 1). Finally, products were isolated *via* lyophilization, resulting in pale-yellow hygroscopic species **4b** and **5b**, obtained in 65% and 72% yield, respectively. Unfortunately, HCl addition to complexes **1–3** led to the formation of highly unstable species which could not be isolated.

Complexes **4a**, **4b**, **5a**, and **5b** gave satisfactory elemental analyses and the expected molecular ion/fragmentation patterns in ESI-MS spectra. Thus, $[\text{M} - \text{Cl}]^+$ and $[\text{M} + \text{H}]^+$ peaks were detected for **4a** and **5a**, whereas the ESI-MS spectra of **4b** and **5b** exhibited $[\text{M} - \text{HCl} - \text{Cl}]^+$ and $[\text{M} - \text{Cl}]^+$ signals. The IR spectra of the complexes displayed the characteristic $\nu(\text{OH})$ and $\nu(\text{NH})$ $[2862\text{--}3394\text{ cm}^{-1}]$ bands of strong intensity, as well as medium-intensity $\nu(\text{C}=\text{N})$ $[1613\text{--}1675\text{ cm}^{-1}]$ bands. In the ^1H NMR spectra, the downfield shifting of the oxime CH_2 group signals as a result of oximato group protonation and consequent chelate ring opening could be clearly observed. Thus, the above-mentioned proton signals appeared at 4.50 and 4.22 ppm for chelated oximato species **4** and **5**, changing to 4.60 and 4.28 ppm for protonated cationic complexes **4a** and **5a**, and finally resonating at 4.98 and 4.60 ppm for open-chain compounds **4b** and **5b**. As expected, the oximato group protonation hardly influenced the chemical shift of the platinum signal in ^{195}Pt NMR spectra ($\delta_{\text{Pt}} = -663$ ppm for **4** vs -666 ppm for **4a**). In contrast, the oxime ring opening resulted in a significant shift of the platinum signal ($\delta_{\text{Pt}} = -978$ ppm for **4b**) due to the change in the ligand sphere (Cl vs O). Additionally, the presence of the oxime chelate ring for complexes **4a** and **5a** was confirmed by ^1H , ^{195}Pt -HMQC NMR.

Time-Dependent NMR Studies Under Physiological and Slightly Acidic Conditions. To elucidate the solution behavior of the prepared complexes at physiologically relevant proton concentrations, time-dependent ^1H NMR measurements at pH values of 7.4, 6.0, and in one case 5.0 were carried out with the most water-soluble ethane-1,2-diamine complexes **4**, **4a**, and **4b**. For that purpose, a phosphate-buffered solution (pH 7.4 and pH 6.0) or a solution of DNO_3 in D_2O (pH 5.0) was used.

The zwitterionic complex **4** proved to be stable at both pH 7.4 and 6.0. Thus, no changes in the NMR spectra were observed during a measurement time of 24 h at ambient temperature. In contrast to **4**, compound **4a**, featuring the protonated oximic group, was immediately deprotonated at pH 7.4 and stayed unchanged for the next 24 h. Expectedly, at pH 6.0, no deprotonation of **4a** took place, but a partial opening of the oximic chelate ring was observed. Hence, small signals of the open-chain form **4b** were visible already 5 min after dissolution and increased with time, 17 h later reaching an equilibrium ratio of 10 to 1 (**4a** versus **4b**), or in other words $\sim 9\%$ of conversion. This different behavior of **4a** in comparison to **4** at a given pH value is at first sight astonishing but might be explainable due to the presence of chloride ions in the case of **4a**, having an influence on the ring-opening reaction. Additional time-dependent measurements were carried out also at pH 5.0, resulting in $\sim 12\%$ ring opening after 8 h.

Table 2. Cytotoxicity of the (1,3-Dihydroxyacetone Oxime)Platinum(II) Complexes in CH1, SW480, and A549 Cancer Cells (Exposure Time 96 h)

compound	IC_{50} [μM]		
	CH1	SW480	A549
1	190 ± 20	571 ± 47	>640
2	3.9 ± 1.1	130 ± 23	302 ± 7
3	16 ± 5	277 ± 23	575 ± 86
4	33 ± 9	350 ± 34	>640
4a	15 ± 2	307 ± 6	>320
4b	1.0 ± 0.1	37 ± 9	63 ± 19
5	37 ± 10	33 ± 2	217 ± 36
5a	8.3 ± 3.2	12 ± 3	56 ± 13
5b	1.1 ± 0.2	0.97 ± 0.30	5.6 ± 1.6
cisplatin	0.14 ± 0.3	3.3 ± 0.4	1.3 ± 0.4
oxaliplatin ^a	0.33 ± 0.09	0.30 ± 0.08	

^a Data taken from ref 49.

As far as complex **4b** is concerned, chelate ring closure takes place while keeping it at a pH of 7.4. Thus, the first signals of **4** appeared within 5 min after dissolution, whereas the complete conversion of **4b** into **4** occurred 25 h later, proving the reversibility of the ring-opening process. As expected, at pH 6.0, no chelation was observed, and the open-chain complex **4b** stayed intact during the measurement time (24 h).

Cytotoxic Activity at Physiologically Relevant Proton Concentrations. The *in vitro* cytotoxicity of complexes **1–5**, **4a**, **4b**, **5a**, and **5b** was evaluated in three human cancer cell lines (CH1, SW480, and A549), using the MTT assay (Table 2 and Figures S1–S3, Supporting Information) under standard screening conditions at a pH of 7.4. Of all compounds, the bis(1,3-dihydroxyacetone oxime) complex **1** displayed the lowest antiproliferative potency. For example, in CH1 cells, the IC_{50} value of **1** was 190 times higher compared to the most potent complex in this series. Among zwitterionic ring-closed compounds **2–5** (Figure S1, Supporting Information), the nonleaving am(m)ine ligands have a distinct influence on cytotoxic properties. Complexes with monodentate amine ligands (NH_3 in complex **2** or EtNH_2 in complex **3**) yielded lower IC_{50} values in the CH1 cell line as compared to complexes **4** or **5** featuring bidentate ethane-1,2-diamine or (*R,R*)-cyclohexane-1,2-diamine ligands. However, this cannot be generalized for other, inherently cisplatin-resistant cell lines (such as SW480 and A549), as the (*R,R*)-cyclohexane-1,2-diamine ligand (in complex **5**) results in a completely different sensitivity profile analogous to oxaliplatin.

Comparing analogues **4**, **4a**, and **4b** (Figure S2, Supporting Information) or **5**, **5a**, and **5b** (Figures S3, Supporting Information), oxidoimino complexes **4** and **5** showed the lowest cytotoxic potency. A comparable or by trend slightly improved antiproliferative behavior was found in the case of protonated counterparts **4a** and **5a**, respectively, a circumstance which is not explainable at the moment. Ring opening and formation of the chloridoplatinum(II) species **4b** and **5b** was accompanied by a noteworthy lowering of the IC_{50} values (increase in cytotoxicity) down to $1\text{ }\mu\text{M}$ in CH1 cells. The antiproliferative potency is significantly lower compared to cisplatin. However, these cytotoxicities in the low micromolar range are remarkable, taking into account that **4b** and **5b** display three coordinated nitrogen donor atoms (nonclassic platinum complexes). Similar structure–activity relationships were found in SW480 and A549 cells.

Table 3. Comparison of the Cytotoxicities of **4a** and **4b** in SW480 and A549 Cancer Cells at pH 7.4 and 6.0 (Drug Exposure Time 24 h)

compound	GI ₅₀ [μ M]			
	SW480		A549	
	pH 7.4	pH 6	pH 7.4	pH 6
4a	>640	590 $\pm$ 57	>640	373 $\pm$ 103
4b	231 $\pm$ 42	127 $\pm$ 22	438 $\pm$ 51	113 $\pm$ 31

Not unexpectedly for complexes **1**–**4**, the cytotoxic potency was highest in the cisplatin sensitive cell line CH1 but decreased significantly in inherently cisplatin-resistant SW480 and A549 cells. Contrarily, IC₅₀ values for the (*R,R*)-cyclohexane-1,2-diamine derivatives **5**, **5a**, and **5b**, were comparable in CH1 (ovarian carcinoma) and SW480 (colon carcinoma) cells. As mentioned above, this pattern is analogous to oxaliplatin, which is clinically approved for the treatment of colorectal cancer (compare with IC₅₀ values of oxaliplatin, Table 2).

Protonation and/or ring opening of complexes **2**–**5** in an acidic environment (as observed in many solid tumors) should be accompanied by an enhanced cytotoxic effect. Therefore, ethane-1,2-diamine compound **4a** and its open-chain analog **4b** were exemplarily investigated in SW480 and A549 cells under standard screening conditions at a pH of 7.4 and in an acidified cell culture medium at a pH of 6.0 (Table 3). Note that the drug exposure time was only 24 h, followed by incubation in a drug-free medium at a pH of 7.4 for a further 72 h; consequently, the IC₅₀ values are higher in comparison to experiments with 96 h of exposure. Expectedly, antiproliferative activity was improved under acidic conditions in both cell lines for **4a** as well as for **4b**.

Binding of Complex **5b to 5'-GMP.** Monodentately bound oxime in **4b** and **5b** may act as a leaving or nonleaving group, leading to either bis- or monoadduct formation with DNA. In order to judge the reactivity, complex **5b** (0.5 mM) was incubated with a 10-fold excess of the model nucleotide 5'-guanosine monophosphate (5'-GMP, 5 mM) in a phosphate buffer (10 mM, pH 6) at 25 °C. The reaction was monitored by electrospray mass spectrometry (ESI-MS).

During the course of incubation, the intensity of the peaks corresponding to **5b** (413 [M_{5b} – HCl – Cl]⁺ and 450 [M_{5b} – Cl]⁺) was reduced with time in comparison to the peak related to 5'-GMP (408 [M_{5'-GMP} + 2Na – H]⁺; Figure S4, Supporting Information). A total of 8 h after the experiment was started, a peak at 776 corresponding to the mono-5'-GMP adduct was detected and attributed to the positively charged species [M_{5b} – HCl – Cl + GMP]⁺ (C₁₉H₃₄N₈O₁₁PPt, calcd *m/z* 776.57; Figure S5, Supporting Information). Signals in the range where bisconjugates of **5b** with 5'-GMP would be expected were not visible in the positive ion mode. With the aim of proving that bifunctional adducts could be detected under these conditions and for reasons of comparison, an experiment was performed with [Pt(1*R*,2*R*-chxn)Cl₂] in parallel. The latter complex is an analogue to **5b**, in which the monodentate oxime ligand was exchanged for chloride, now featuring two chlorido leaving groups [Pt(1*R*,2*R*-chxn)Cl₂] was reacted with 1 equiv of AgNO₃ in order to increase the aqueous solubility of the complex). As could be expected, a peak related to the Pt-5'-GMP monoadduct at 708 [M_{[Pt(1*R*,2*R*-chxn)Cl₂] – Cl + GMP]⁺ (C₁₆H₂₈ClN₇O₈PPt, calcd *m/z* 707.94) was detected 25 min after the beginning of the incubation, whereupon two peaks}

corresponding to Pt-5'-GMP bisadducts were observed at 1034 [M_{[Pt(1*R*,2*R*-chxn)Cl₂] – HCl – Cl + 2GMP]⁺ (C₂₆H₄₁N₁₂O₁₆P₂Pt, calcd *m/z* 1034.70) and 1056 [M_{[Pt(1*R*,2*R*-chxn)Cl₂] – 2HCl + 2GMP + Na]⁺ (C₂₆H₄₀N₁₂O₁₆P₂PtNa, calcd *m/z* 1056.68) after 24 h, increasing with time.}}

A second experiment was performed with complex **5b** and [Pt(1*R*,2*R*-chxn)Cl₂] under the same conditions, but at 37 °C and with MS detection both in the positive and in the negative ion mode (Figures S6 and S7, Supporting Information). In the positive ion mode, bisconjugates of **5b** with 5'-GMP were not visible. However, bisadduct formation of **5b** after 1 week of incubation with 5'-GMP could be proven by detection of a doubly negatively charged peak at 515.6 corresponding to [M_{5b} – 2Cl – oxime + 2GMP – 4H]²⁻ (C₂₆H₃₈N₁₂O₁₆P₂Pt, calcd *m/z* 515.84), although to a significantly lesser extent compared to [Pt(1*R*,2*R*-chxn)Cl₂]. On the basis of these results, the oxime group may be classified as a leaving ligand. However, whether such a ligand is easily exchangeable in a physiologically relevant time frame cannot be answered yet.

CONCLUSIONS

The results of this work may be considered from two perspectives. In a narrow sense, four novel (3-hydroxy-2-(oxidoimino)propan-1-olato-κ²N₂O)platinum(II) complexes were prepared and completely characterized, along with the protonated and open-chain species in the case of two complexes featuring a bidentate ethane-1,2-diamine or (*R,R*)-cyclohexane-1,2-diamine ligand. The cytotoxicity of the investigated compounds in human cancer cells was found in the medium or even low micromolar range, which is remarkable for nonclassic platinum complexes with three coordinated N donor atoms. Interestingly, similar structure–activity relationships were found for two series of complexes featuring ethane-1,2-diamine and (*R,R*)-cyclohexane-1,2-diamine ligands. Chelate ring opening improved the IC₅₀ values 8 and 15 times, respectively, in CH1 ovarian cancer cells. Time-dependent NMR studies at physiologically relevant pH values, performed for the most water-soluble ethane-1,2-diamine-containing series, showed that zwitterionic oximate complex **4** is stable toward ring opening both at pH 7.4 and at pH 6.0. Differently, protonated form **4a** is being deprotonated to analogue **4** at pH 7.4, while tending to chelate ring-opening in an acidic medium. As discussed above, this different behavior of complex **4a** in comparison to **4** at a given pH value is most probably the result of the presence of one equivalent of chloride ions in the case of **4a**. In contrast, open-chain species **4b**, stable at pH 6.0, was completely converted into the ring-closed form **4** at pH 7.4. Comparative investigation of the cytotoxic potency of complexes **4a** and **4b** under standard cell line screening conditions (pH 7.4) and in an acidified cell culture medium (pH 6.0) showed a significant increase of antiproliferative activity under slightly acidic conditions. Whether this pH-dependent activation is of relevance for the *in vivo* situation will be the subject of our future research program.

In a wider sense, this research is an important contribution to the development of inert and unreactive complexes (prodrugs) which can be activated in the tumor tissue. From this point of view, a number of novel oxime-based pH-sensitive potential platinum prodrugs have been prepared. Moreover, in this work, we have demonstrated the possibility of reversible pH-dependent intramolecular chelation and ring-opening reaction for the novel complexes as well as an increase of their cytotoxic potency under the

slightly acidic conditions characteristic for many solid tumor tissues. However, the prepared 3-hydroxy-2-(oxidoimino)propan-1-olato species proved to be less accessible to protonation at slightly acidic pH values than, for instance, the previously reported aminoalcoholato or O-alkyldithiocarbonato complexes. A promising strategy to facilitate the chelate ring opening under acidic conditions could be introducing, e.g., an alkyl substituent into the 1,3-dihydroxyacetone oxime ligand, which should result in a decrease of acidity of the side-chain hydroxo group due to its positive inductive effect. As a consequence, complexes of a weaker acid are probably easier to protonate, which should favor the formation of the open-chain species. Investigation of these aspects is ongoing.

■ EXPERIMENTAL SECTION

General Procedures. Potassium tetrachloridoplatinate and 1,3-dihydroxyacetone oxime were supplied by Johnson Matthey and TCI, respectively. Ammonium hydroxide solution (25% in water), ethylamine solution (70% in water), and (*R,R*)-cyclohexane-1,2-diamine [(1*R*,2*R*)-chxn] were purchased from Fluka, while ethane-1,2-diamine (en) was obtained from Aldrich. All of these chemicals were used as received without further purification. The complexes *cis*-[PtI₂(NH₃)₂], *cis*-[PtI₂(EtNH₂)₂], [PtCl₂(en)], and [PtCl₂{(1*R*,2*R*)-chxn}] were prepared according to standard literature procedures starting from K₂[PtCl₄].^{43,44} Bidistilled water was used. The synthetic procedures were carried out in a light-protected environment due to the known photosensitivity of some platinum species.⁵⁰ For HPLC separation, a semipreparative 250 × 20 mm Kromasil 100 Å C18 10 μm column was used. C, H, and N elemental analyses were carried out by the elemental analyses laboratory of the University of Vienna using a Perkin-Elmer 2400 CHN elemental analyzer. ESI-MS spectra were obtained with a Bruker Esquire 3000 instrument. IR spectra were obtained by using an ATR unit with a Perkin-Elmer 370 FTIR 2000 instrument (4000–400 cm⁻¹). ¹H, ¹³C{¹H}, ¹⁹⁵Pt, and two-dimensional NMR spectra were measured with a Bruker Avance III 500 MHz NMR spectrometer at 500.32 (1H), 125.81 (13C), and 107.55 MHz (195Pt), correspondingly, at 298 K. ¹⁹⁵Pt chemical shifts are referenced relative to K₂[PtCl₄], and the half-height line width is given in parentheses.

Synthesis of the Platinum(II) Complexes with Chelated 1,3-Dihydroxyacetone Oxime. (SP-4–1)-Bis(3-hydroxy-2-(hydroxyimino)propan-1-olato-κ²N,O)platinum(II) (1). K₂[PtCl₄] (121.0 mg, 0.29 mmol) was dissolved in 2.5 mL of H₂O; then 1,3-dihydroxyacetone oxime (61.0 mg, 0.58 mmol) was added. The reaction mixture was stirred at room temperature overnight. Formation of a colorless precipitate was observed, which was filtered off. Then, 1M NaOH solution (160 μL, 0.16 mmol) was added to the yellow filtrate (pH value changed from 2.0 to 5.5), and the reaction mixture was stirred at room temperature for 24 h. An additional portion of the colorless precipitate formed was filtered off, combined with the first portion, washed twice with 0.5 mL of ice-cold water, and dried *in vacuo* at room temperature. Yield: 73 mg (62%). Anal. Calcd. for C₆H₁₂N₂O₆Pt: C, 17.87; H, 3.00; N, 6.95. Found: C, 18.02; H, 2.82; N, 6.89%. ESI-MS⁺ (H₂O/MeOH), *m/z*: 402 [M – H]⁺. IR in KBr (selected bands), cm⁻¹: 3474 s, 3422 s ν(OH), 1615 m ν(C=N). ¹H NMR (D₂O): δ 4.59 (s, br, OCH₂), 4.28 (s, br, HOCH₂). ¹³C{¹H} NMR (D₂O): δ 166.2 (C=N), 71.8 (OCH₂), 55.3 (HOCH₂). ¹⁹⁵Pt NMR (D₂O): δ –137 (780 Hz).

General Synthesis of (SP-4–3)-Diam(m)ine(3-hydroxy-2-(oxidoimino)propan-1-olato-κ²N,O)platinum(II). [PtX₂A₂] (0.50 mmol) and AgNO₃ (1.0 mmol) were mixed and suspended in 10 mL of H₂O, whereupon the mixture was stirred at room temperature overnight. The precipitate of AgX was filtered off, and a solution of 1,3-dihydroxyacetone oxime (0.50 mmol) in 2 mL of H₂O was added slowly (dropwise within 1–2 h). After the reaction mixture was stirred at the same temperature for 12 h, it was diluted with 50 mL of H₂O and

subsequently mixed with approximately 3 g of a preconditioned basic anion exchange resin, Amberlite IRA 402 (the commercially available chloride form was treated with 30 mL of 2M NaOH for 30 min and washed with deionized water until a pH of 7), and the mixture was stirred for several minutes. After the ion-exchanger turned from slightly yellow to dark-gray (pH ~9–10), it was filtered off, and the fine precipitate of platinum black (formed in small amount) was removed by filtration through a Glass Microfibre 696. In the case of 2–4, the resulting yellow solution was evaporated to dryness, and 15 mL of acetone was added. The yellow solid formed was filtered off, washed with acetone (2 × 1 mL), and dried *in vacuo*. After that, the crude product was purified through semipreparative HPLC (H₂O/MeOH 98/2 for 2 and 4, H₂O/MeOH 95/5 for 3) and lyophilized. In the case of 5, the yellowish filtrate was reduced to 0.5 mL and placed into the fridge (+5 °C) for 2 h. The colorless precipitate formed was filtered off, washed twice with a few drops of ice-cold H₂O, and dried *in vacuo*. The yields were 25% (2), 20% (3), 58% (4), and 32% (5).

(SP-4–3)-Diammine(3-hydroxy-2-(oxidoimino)propan-1-olato-κ²N,O)platinum(II) (2). Anal. Calcd. for C₃H₁₁N₃O₃Pt · 2 H₂O: C, 9.78; H, 4.11; N, 11.41. Found: C, 10.18; H, 3.77; N, 11.05%. ESI-MS⁺ (H₂O/MeOH), *m/z*: 333 [M + H]⁺, 355 [M + Na]⁺. IR in KBr (selected bands), cm⁻¹: 3092 s, 2987 s, 2808 m ν(OH) and ν(NH), 1630 m ν(C=N). ¹H NMR (D₂O): δ 4.51 (m, 2H, OCH₂), 4.23 (m, 2H, HOCH₂). ¹³C{¹H} NMR (D₂O): δ 168.1 (C=N), 73.6 (OCH₂), 56 (HOCH₂). ¹⁹⁵Pt NMR (D₂O): δ –527 (800 Hz).

(SP-4–3)-Bis(ethylamine)(3-hydroxy-2-(oxidoimino)propan-1-olato-κ²N,O)platinum(II) (3). Anal. Calcd. for C₇H₁₉N₃O₃Pt · 1/2 H₂O: C, 21.16; H, 5.07; N, 10.58. Found: C, 20.90; H, 4.66; N, 10.21%. ESI-MS⁺ (H₂O/MeOH), *m/z*: 344 [M – EtNH₂ + H]⁺, 389 [M + H]⁺, 411 [M + Na]⁺. ESI-MS[–] (H₂O/MeOH), *m/z*: 433 [M – Cl][–]. IR in KBr (selected bands), cm⁻¹: 3200 s, 3128 s, 3068 s ν(OH) and ν(NH), 1606 ν(C=N). ¹H NMR (D₂O): δ 4.57 (m, 2H, OCH₂), 4.30 (m, 2H, HOCH₂), 2.66 (m, 4H, 2CH₃CH₂), 1.17 (t, 6H, 2CH₃, ³J_{H,H} = 6.9 Hz). ¹³C{¹H} NMR (D₂O): δ 176.2 (C=N), 73.1 (OCH₂), 56.3 (HOCH₂), 41.9 (CH₃CH₂), 41.3 (CH₃CH₂), 15.6 (2CH₃). ¹⁹⁵Pt NMR (D₂O): δ –589 (720 Hz).

(SP-4–3)-(Ethane-1,2-diamine)(3-hydroxy-2-(oxidoimino)propan-1-olato-κ²N,O)platinum(II) (4). Anal. Calcd. for C₅H₁₃N₃O₃Pt · H₂O: C, 16.35; H, 3.84; N, 11.44. Found: C, 16.68; H, 3.45; N, 11.14%. ESI-MS⁺ (H₂O/MeOH), *m/z*: 359 [M + H]⁺. IR in KBr (selected bands), cm⁻¹: 3177 m, 2935 s, 2889 s ν(OH) and ν(NH), 1618 m ν(C=N). ¹H NMR (D₂O): δ 4.50 (m, 2H, OCH₂), 4.22 (m, 2H, HOCH₂), 2.55 (m, 4H, 2CH₂). ¹³C{¹H} NMR (D₂O): δ 168.3 (C=N), 73.6 (OCH₂), 55.9 (HOCH₂), 46.43 (CH₂), 46.36 (CH₂). ¹⁹⁵Pt NMR (D₂O): δ –663 (600 Hz).

(SP-4–3)-{(R,R)-Cyclohexane-1,2-diamine}(3-hydroxy-2-(oxidoimino)propan-1-olato-κ²N,O)platinum(II) (5). Anal. Calcd. for C₉H₁₉N₃O₃Pt · H₂O: C, 25.12; H, 4.92; N, 9.76. Found: C, 24.78; H, 4.71; N, 9.49%. ESI-MS⁺ (H₂O/MeOH), *m/z*: 413 [M + H]⁺. IR in KBr (selected bands), cm⁻¹: 3184 s, 3100 s, 2937 m ν(OH) and ν(NH), 1602 m ν(C=N). ¹H NMR (MeOD): δ 4.66 (m, 2H, OCH₂), 4.34 (m, 2H, HOCH₂), 2.32 (m, 2H, 2CH), 2.03 (m, 2H, CH₂), 1.64 (m, 2H, CH₂), 1.33 (m, 2H, CH₂), 1.23 (m, 2H, CH₂). ¹³C{¹H} NMR (MeOD): δ 167.5 (C=N), 74.2 (OCH₂), 61.2 (CH), 60.9 (CH), 56.5 (HOCH₂), 32.7 (CH₂), 32.5 (CH₂), 24.3 (CH₂), 24.2 (CH₂). ¹⁹⁵Pt NMR (MeOD): δ –610 (630 Hz). Crystals for X-ray study were obtained by slow evaporation of a water solution at room temperature.

Protonation of the Chelated Platinum(II) Oximate Species. In a typical experiment, 1 equiv of 1.2M HCl was added to a solution/suspension of 4 or 5 (0.1 mmol) in 10 mL of H₂O, resulting in the change of the solution color from pale- to bright-yellow. The reaction mixture was immediately frozen and lyophilized. The products 4a and 5a were isolated as colorless solids in almost quantitative yield.

(SP-4-3)-(Ethane-1,2-diamine)(3-hydroxy-2-(hydroxyimino)propan-1-olato- κ^2 N,O)platinum(II) Chloride (4a). Anal. Calcd. for $C_5H_{14}N_3O_3ClPt \cdot 1/2H_2O$: C, 14.87; H, 3.74; N, 10.41. Found: C, 15.06; H, 3.78; N, 9.98%. ESI-MS⁺ ($H_2O/MeOH$), m/z : 359 [M - Cl]⁺, 396 [M + H]⁺. ESI-MS⁻ ($H_2O/MeOH$), m/z : 394 [M - H]⁻, 430 [M + Cl]⁻. IR (selected bands), cm⁻¹: 3353 m, 3224 s, 3055 s, 2966 s $\nu(OH)$ and $\nu(NH)$, 1675 m $\nu(C=N)$. ¹H NMR (D_2O): δ 5.16 (m, 4H, 2NH₂), 4.67 (s, 2H, OCH₂), 4.33 (s, 2H, HOCH₂), 2.55 (m, 4H, 2CH₂). ¹³C{¹H} NMR (D_2O): δ 160.2 (C=N), 73.8 (OCH₂), 56.2 (HOCH₂), 46.7 (CH₂), 46.5 (CH₂). ¹⁹⁵Pt NMR (D_2O): δ -666 (960 Hz).

(SP-4-3)-{(R,R)-Cyclohexane-1,2-diamine}(3-hydroxy-2-(hydroxyimino)propan-1-olato- κ^2 N,O)platinum(II) Chloride (5a). Anal. Calcd. for $C_9H_{20}N_3O_3ClPt \cdot 1/2H_2O$: C, 23.61; H, 4.62; N, 9.18. Found: C, 23.57; H, 4.49; N, 8.91%. ESI-MS⁺ ($H_2O/MeOH$), m/z : 413 [M - Cl]⁺, 435 [M - HCl + Na]⁺, 450 [M + H]⁺. IR in KBr (selected bands), cm⁻¹: 3394 s, 3191 s, 3067 s, 2938 s $\nu(OH)$ and $\nu(NH)$, 1613 m $\nu(C=N)$. ¹H NMR (D_2O): δ 4.60 (s, 2H, OCH₂), 4.28 (s, 2H, HOCH₂), 2.32 (m, 2H, 2CH), 1.97 (m, 2H, CH₂), 1.51 (m, 2H, CH₂), 1.22 (m, 2H, CH₂), 1.09 (m, 2H, CH₂). ¹³C{¹H} NMR (D_2O): δ 174.9 (C=N), 73.8 (OCH₂), 61.1 (CH), 61.0 (CH), 56.1 (HOCH₂), 32.3 (CH₂), 32.2 (CH₂), 24.0 (CH₂), 23.9 (CH₂). ¹⁹⁵Pt NMR (D_2O): δ -636 (870 Hz).

Oxime Chelate Ring Opening. In a typical experiment, 4 equiv of HCl_{conc} was added to a solution/suspension of **4** or **5** (0.2 mmol) in 5 mL of H₂O, and the reaction mixture was stirred for 24 h at room temperature. A small amount of a brownish-yellow solid formed was filtered off through a Glass Microfibre 696. The resulting clear yellow solution was lyophilized, giving hygroscopic pale-yellow solids of **4b** and **5b**. Yields of **4b** and **5b** were 65% and 72%, respectively.

(SP-4-3)-Chlorido(1,3-dihydroxyacetone Oxime- κ N)-(Ethane-1,2-diamine)platinum(II) Chloride (4b). Anal. Calcd. for $C_5H_{15}N_3O_3Cl_2Pt \cdot H_2O$: C, 13.37; H, 3.81; N, 9.35. Found: C, 13.74; H, 3.59; N, 9.04%. ESI-MS⁺ ($H_2O/MeOH$), m/z : 359 [M - HCl - Cl]⁺, 396 [M - Cl]⁺. IR (selected bands), cm⁻¹: 3380 s, 3249 s, 3193 s, 3094 s, 2994 m $\nu(OH)$ and $\nu(NH)$, 1647 m $\nu(C=N)$. ¹H NMR (D_2O): δ 5.57 (m, 2H, 2NH₂), 5.37 (m, 2H, 2NH₂), 4.97 (s, 2H, HOCH₂), 4.60 (s, 2H, HOCH₂), 2.61 (m, 4H, 2CH₂). ¹³C{¹H} NMR (D_2O): δ 169.2 (C=N), 60.6 (HOCH₂), 57.3 (HOCH₂), 48.2 (CH₂), 47.7 (CH₂). ¹⁹⁵Pt NMR (D_2O): δ -978 (670 Hz).

(SP-4-3)-Chlorido{(R,R)-cyclohexane-1,2-diamine}(1,3-dihydroxyacetone Oxime- κ N)platinum(II) Chloride (5b). Anal. Calcd. for $C_9H_{21}N_3Cl_2O_3Pt$: C, 22.28; H, 4.36; N, 8.66. Found: C, 22.10; H, 4.23; N, 8.26%. ESI-MS⁺ ($H_2O/MeOH$), m/z : 413 [M - HCl - Cl]⁺, 450 [M - Cl]⁺. IR in KBr (selected bands), cm⁻¹: 3183 s, 3083 s, 2937 s, 2862 m $\nu(OH)$ and $\nu(NH)$, 1647 m $\nu(C=N)$. ¹H NMR (D_2O): δ 5.91 (m, 1H, NHH), 5.62 (m, 1H, NHH), 5.04 (m, 2H, 2NHH), 4.98 (s, 2H, HOCH₂), 4.60 (s, 2H, HOCH₂), 2.46 (m, 1H, CH), 2.38 (m, 1H, CH), 1.99 (m, 2H, CH₂), 1.53 (m, 2H, CH₂), 1.26 (m, 2H, CH₂), 1.09 (m, 2H, CH₂). ¹³C{¹H} NMR (D_2O): δ 169.1 (C=N), 62.8 (HOCH₂), 62.2 (HOCH₂), 60.7 (CH), 57.3 (CH), 32.0 (CH₂), 31.8 (CH₂), 23.8 (CH₂), 23.7 (CH₂). ¹⁹⁵Pt NMR (D_2O): δ -945 (670 Hz).

¹H NMR (DMF-*d*₆): δ 12.44 (s, 1H, HON), 6.91 (m, 1H, NHH), 6.22 (m, 1H, NHH), 5.99 (m, 1H, NHH), 5.57 (m, 1H, NHH), 5.08 (d, 1H, HOCHH, ³J_{H,H} = 12.8 Hz), 5.00 (d, 1H, HOCHH, ³J_{H,H} = 12.8 Hz), 4.60 (s, 2H, HOCH₂), 2.54 (m, 2H, 2CH), 2.10 (m, 2H, CH₂), 1.57 (m, 2H, CH₂), 1.49 (m, 2H, CH₂), 1.15 (m, 2H, CH₂). ¹³C{¹H} NMR (DMF-*d*₆): δ 170.5 (C=N), 62.8 (HOCH₂), 62.3 (HOCH₂), 60.8 (CH), 57.1 (CH), 32.3 (CH₂), 31.7 (CH₂), 24.5 (CH₂), 24.3 (CH₂). ¹⁵N NMR (DMF-*d*₆): δ -22.6 (NH₂), -10.7 (NH₂). ¹⁹⁵Pt NMR (DMF-*d*₆): δ -933 (500 Hz).

Crystal Structure Determination. X-ray diffraction measurement for **5**·2H₂O was performed on a Bruker X8 APEXII CCD diffractometer. A single crystal was positioned at 35 mm from the detector, and

1330 frames were measured, each for 60 s over 1° scan width. The data were processed using SAINT software.⁵¹ Crystal data, data collection parameters, and structure refinement details are given in Table 1. The structure was solved by direct methods and refined by full-matrix least-squares techniques. Non-H atoms were refined with anisotropic displacement parameters. H atoms were inserted in calculated positions and refined with a riding model. The structure solution was achieved with SHELXS-97⁵² and refinement with SHELXL-97,⁵³ and graphics were produced with ORTEP-3.⁵⁴

Cell Lines and Culture Conditions. The human cancer cell lines CH1 (ovarian carcinoma), kindly provided by Lloyd R. Kelland (CRC Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, UK), as well as A549 (nonsmall cell lung cancer) and SW480 (colon carcinoma), both provided by Brigitte Marian (Institute of Cancer Research, Department of Medicine I, Medical University of Vienna, Austria), were used for the experiment. Cells were grown in 75 cm² culture flasks (Iwaki/Asahi Technoglass, Gyouda, Japan) as adherent monolayer cultures in a complete medium [i.e., Minimal Essential Medium (MEM) supplemented with 10% heat-inactivated fetal bovine serum, 1 mM sodium pyruvate, 2 mM L-glutamine, and 1% nonessential amino acids for the standard condition experiments and bicarbonate-free Dulbecco's Modified Eagles Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1 mM sodium pyruvate, 4 mM L-glutamine, 1% nonessential amino acids, and 8.8 mM (pH 6.0) or 39.6 mM (pH 7.4) sodium bicarbonate for pH-dependent experiments (all purchased from Sigma-Aldrich, Austria)]. Cell cultures were incubated at 37 °C in a moist atmosphere containing 5% CO₂ (standard conditions) or 8% CO₂ (pH-dependent experiments).

Cytotoxicity Tests in Cancer Cell Lines. The cytotoxic activity of the compounds was determined by means of a colorimetric microculture assay (MTT assay, MTT = 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide). A549, CH1, and SW480 cells were harvested from culture flasks by trypsinization and seeded in 100 μ L aliquots in MEM into 96-well plates (Iwaki/Asahi Technoglass, Gyouda, Japan) in cell densities of 3×10^3 , 1×10^3 , and 2.5×10^3 cells per well, respectively. After 24 h of preincubation of the cells, the test compounds were dissolved in a complete medium and then added in aliquots of 100 μ L per well. After continuous exposure for 96 h, drug solutions were replaced with 100 μ L of RPMI 1640 medium (supplemented with 10% heat-inactivated fetal bovine serum and 2 mM L-glutamine) plus 20 μ L of MTT solution in phosphate-buffered saline (5 mg/mL). After incubation for 4 h, the medium/MTT mixtures were removed, and the formazan crystals formed by viable cells were dissolved in 150 μ L of DMSO per well. Optical densities at 550 nm were measured with a microplate reader (Tecan Spectra Classic), using a reference wavelength of 690 nm to correct for unspecific absorption. The quantity of viable cells was expressed in terms of T/C values by comparison to untreated control microcultures, and 50% inhibitory concentrations (IC₅₀) were calculated from concentration-effect curves by interpolation. Evaluation is based on means from at least three independent experiments, each comprising three replicates per concentration level. For pH-dependent experiments, A549 and SW480 cells were harvested from culture flasks by trypsinization and seeded in 100 μ L aliquots in DMEM (pH 7.4) into 96-well plates in cell densities of 3×10^3 and 4×10^3 cells per well, respectively. After 24 h, the medium from adherent microcultures was removed, and cells were exposed to the drug solutions (200 μ L per well) for another 24 h in a moist atmosphere containing 8% CO₂ at 37 °C (for drugs dissolved in DMEM pH 6) and 5% CO₂ at 37 °C (for drugs dissolved in DMEM pH 7.4), followed by incubation in drug-free DMEM (pH 7.4) for a further 72 h. Further incubation and measurements were performed as described above.

Binding of Complex 5b to 5'-GMP. For investigating the time-dependent binding of 5'-GMP to complex **5b**, the complex (0.5 mM) was incubated with 5'-GMP (5 mM) in a phosphate buffer (10 mM, pH 6)

at 25 °C. In parallel, an experiment was performed with [Pt(1*R*,2*R*-chxn)Cl₂] under the same conditions. A second experiment was performed with complex **5b** under the same conditions, but at 37 °C. The progress of the reaction was monitored by electrospray mass spectrometry (ESI-MS) in the positive as well as in the negative ion mode.

■ ASSOCIATED CONTENT

S Supporting Information. Figures with concentration-effect curves of complexes **1–5**, **4a**, **4b**, **5a**, and **5b** in CH1 cells. Figures with ESI-MS spectra of **5b** incubated with 5'-GMP. X-ray crystallographic file in CIF format for **5**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Supporting Information

Synthesis, characterization and cytotoxic activity of novel potentially pH-sensitive non-classical platinum(II) complexes featuring 1,3-dihydroxyacetone oxime ligands

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Figure S1. Concentration-effect curves of complexes **1–5** in CH1 cells.

Figure S2. Concentration-effect curves of complexes **4**, **4a** and **4b** in CH1 cells.

Figure S3. Concentration-effect curves of complexes **5**, **5a** and **5b** in CH1 cells.

Figure S4. ESI-MS⁺ spectra (m/z range 380 – 480) of **5b** incubated with 5'-GMP

Figure S5. ESI-MS⁺ spectra (m/z range 750 – 840) of **5b** incubated with 5'-GMP

Figure S6. ESI-MS⁺ spectrum (m/z range 700 – 1200) of **5b** incubated with 5'-GMP

Figure S7. ESI-MS[–] spectrum (m/z range 500–530) of [Pt(*1R,2R*-chxn)Cl₂] and **5b** incubated with 5'-GMP

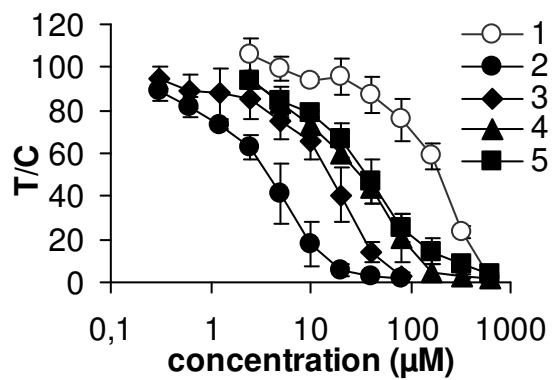


Figure S1. Concentration-effect curves of complexes **1–5** in CH1 cells, obtained by the MTT assay (96 h exposure).

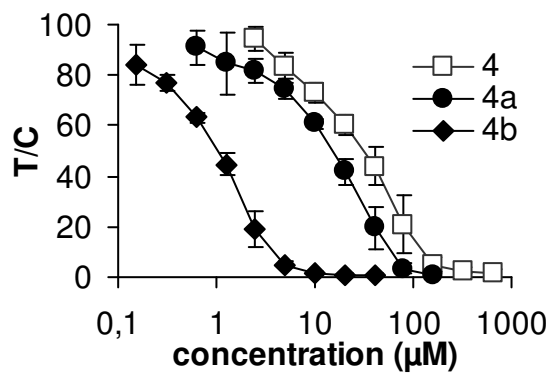


Figure S2. Concentration-effect curves of complexes **4**, **4a** and **4b** in CH1 cells, obtained by the MTT assay (96 h exposure).

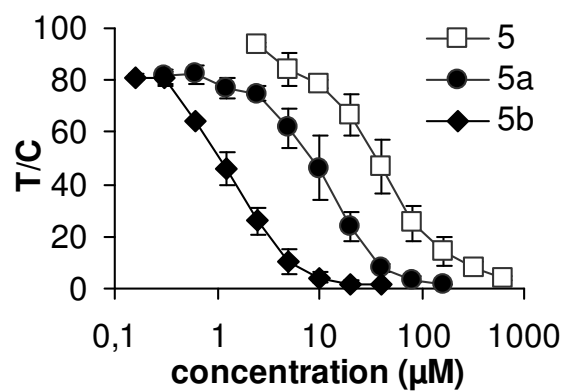


Figure S3. Concentration-effect curves of complexes **5**, **5a** and **5b** in CH1 cells, obtained by the MTT assay (96 h exposure).

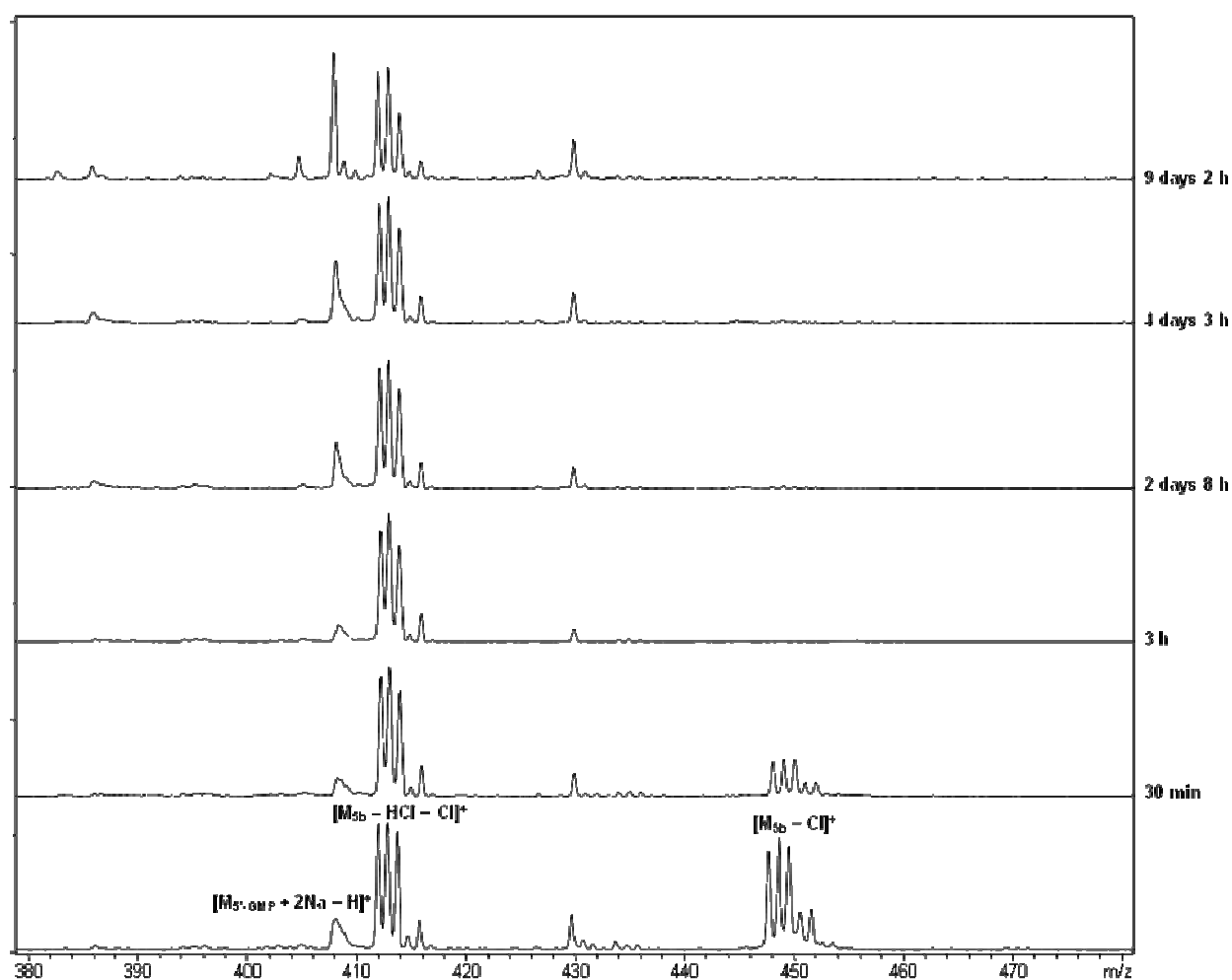


Figure S4. ESI-MS⁺ spectra (*m/z* range 380 –480) of **5b** incubated with 5'-GMP in the molar ratio of 1:10 at pH 6.0 and 25°C recorded over a period of *ca.* 9 days.

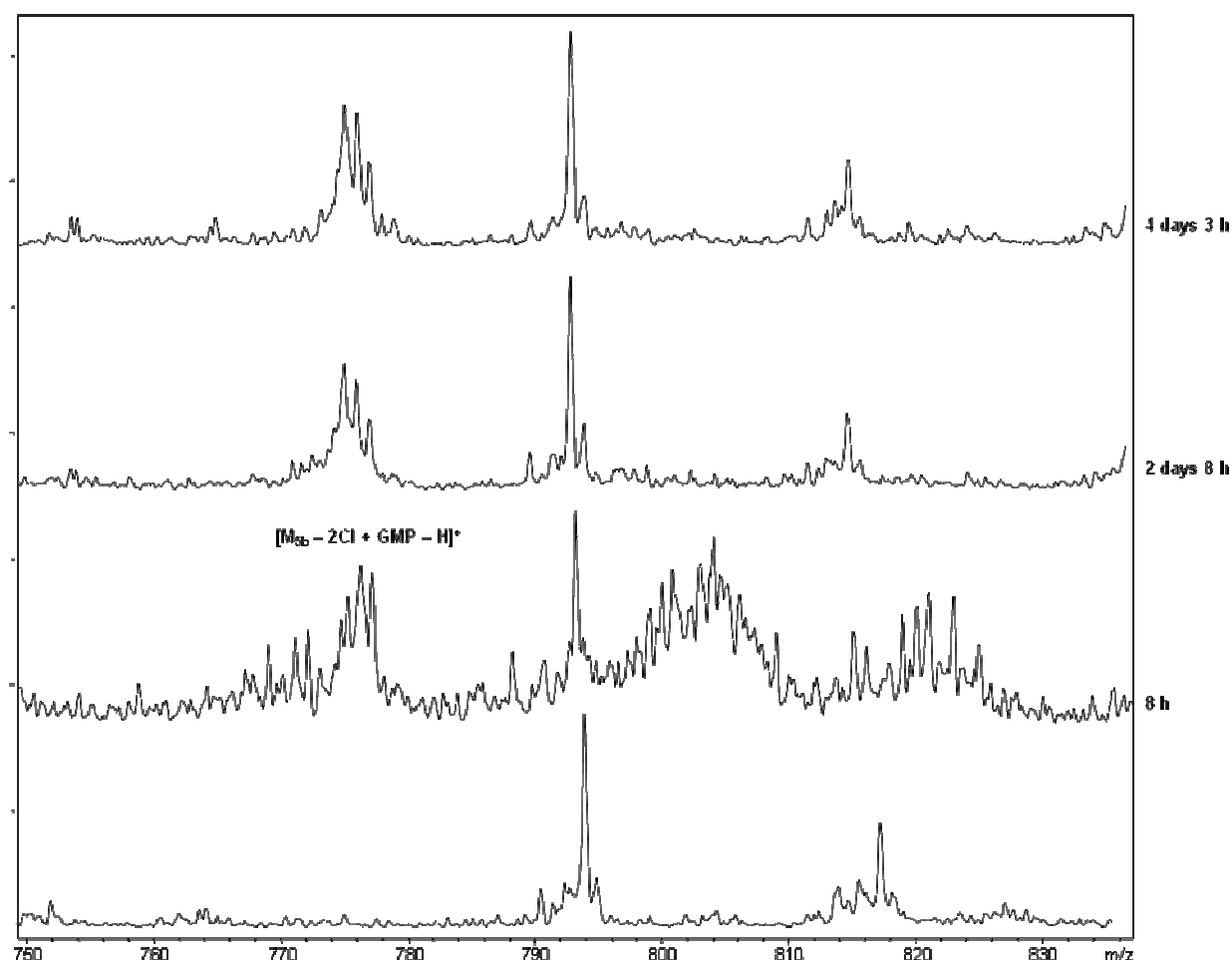


Figure S5. ESI-MS⁺ spectra (m/z range 750 –840) of **5b** incubated with 5'-GMP in the molar ratio of 1:10 at pH 6.0 and 25°C recorded over a period of *ca.* 9 days (after the 4th day no change was observed).

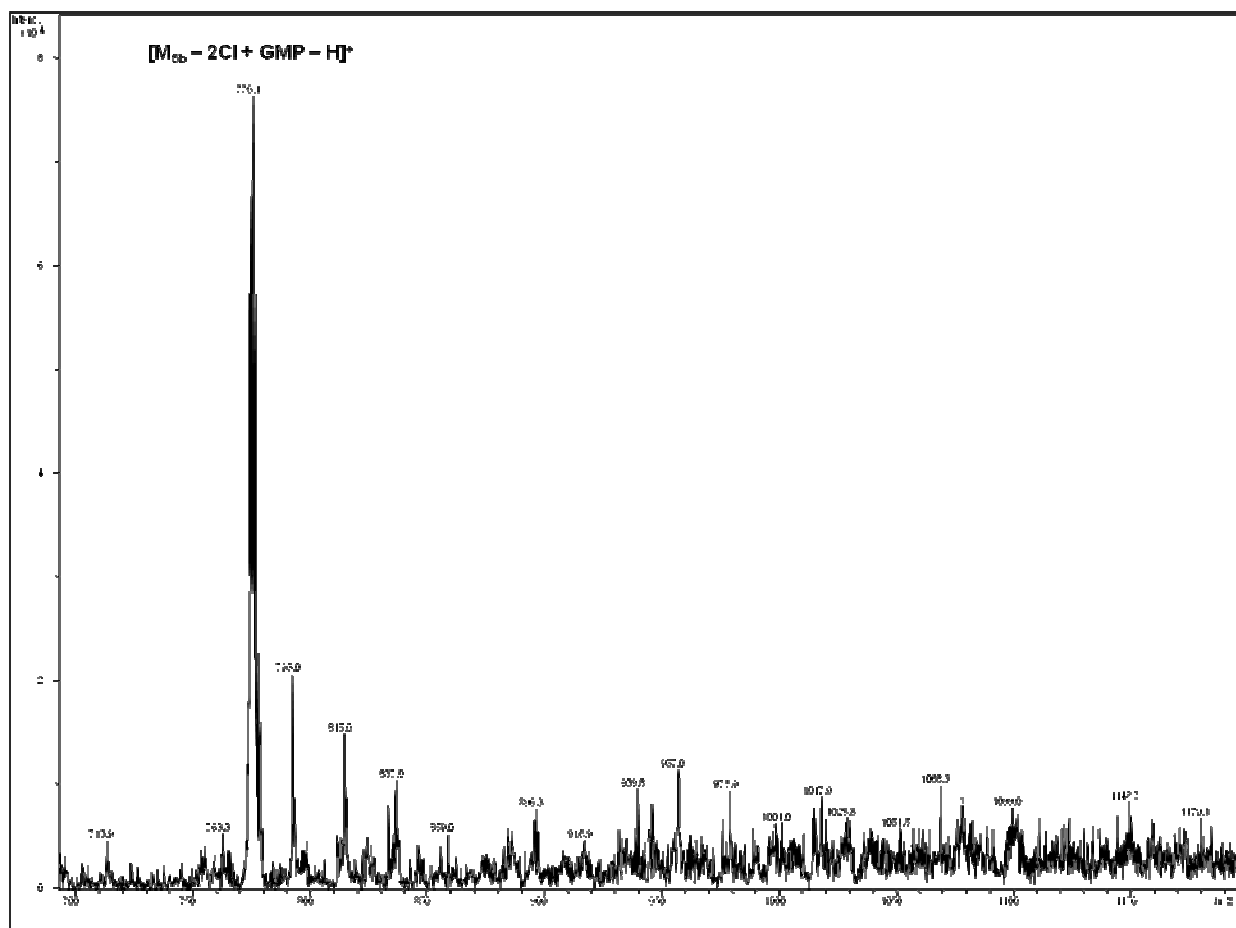


Figure S6. ESI-MS⁺ spectrum (m/z range 700 –1200) of **5b** incubated with 5'-GMP in the molar ratio of 1:10 at pH 6.0 and 37°C recorded after 7 days incubation.

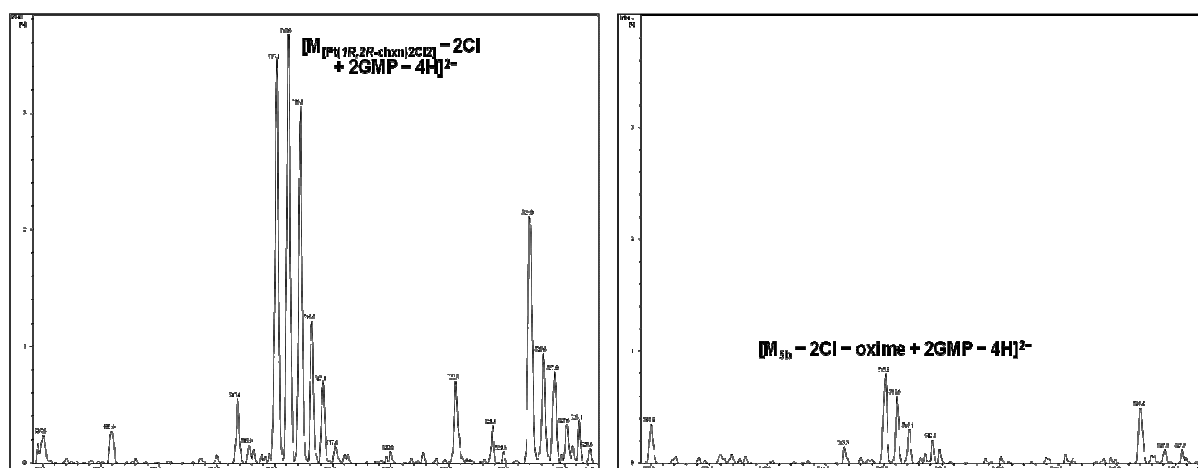


Figure S7. ESI-MS⁻ spectrum (m/z range 500–530) of [Pt(1R,2R-chxn)Cl₂] (left) and **5b** (right) incubated with 5'-GMP in the molar ratio of 1:10 at pH 6.0 and 37°C recorded after 7 days incubation. The intensity of Pt-5'-GMP bisadduct ([PtC₂₆H₃₈N₁₂O₁₆P₂]²⁻, calcd. 1031.68) is expressed in percent relative to the intensity of a peak corresponding to deprotonated 5'-GMP ([C₁₀H₁₃N₅O₈P]⁻, 362.21).

3. Novel Oximato-Bridged Platinum(II) Di- and Trimer(s): Synthetic, Structural, and in vitro Anticancer Activity Studies

Scaffidi-Domianello, Yu. Yu.; Legin, A. A.; Jakupiec, M. A.; Roller, A.; Kukushkin, V. Yu.; Galanski, M.; Keppler, B. K. *Inorg. Chem.*, submitted.

Novel Oximato-Bridged Platinum(II) Di- and Trimer(s): Synthetic, Structural, and *in vitro* Anticancer Activity Studies

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Supporting Information Placeholder

ABSTRACT: Novel platinum complexes of *trans*-geometry [PtCl₂[(Z)-R(H)C=NOH]₂] [R = Me (1), Et (3)] and [PtCl₂[(E)-R(H)C=NOH][(Z)-R(H)C=NOH]] [R = Me (2), Et (4)] as well as the classic *trans*-[PtCl₂(R₂C=NOH)₂] [R = Me, Et] were reacted with an equivalent amount of silver acetate in acetone solution at ambient temperature, resulting in the formation of unprecedented head-to-tail oriented oximato-bridged dimers [PtCl(μ-(Z)-R(H)C=NO)[(Z)-R(H)C=NOH]₂] [R = Me (5), R = Et (7)], [PtCl(μ-(Z)-R(H)C=NO)[(E)-R(H)C=NOH]₂] [R = Me (6), R = Et (8)], and [PtCl(μ-R₂C=NO)(R₂C=NOH)₂] [R = Me (9), R = Et (10)], correspondingly. The dimeric species feature a unique six-membered diplatinacycle and represent the first example of oxime ligands coordinated to platinum *via* the oxygen atom. All complexes were characterized by elemental analyses, electrospray ionization mass spectrometry, IR and multinuclear (¹H, ¹³C, and ¹⁹⁵Pt) NMR spectroscopy, as well as X-ray diffraction in the cases of dimers **6** and **9**. Furthermore, the crystal and molecular structures of a trimeric oximato-bridged complex **11** comprising three platinum units connected in a chain way were established. The cytotoxicity of both dimers and the respective monomers was comparatively evaluated in three human cancer cell lines: cisplatin-sensitive CH1 cells as well as cisplatin-resistant SW480 and A549 cells, whereupon structure-activity relationships were established. Thus, it was found that dimerization results in a substantial (up to 7-fold) improvement of IC₅₀ values of (aldoxime)Pt^{II} compounds, whereas for the analogous complexes featuring ketoxime ligands the reverse trend was observed. Remarkably, the novel dimers proved to be not cross-resistant with cisplatin in SW480 cells, exhibiting up to two-fold enhanced cytotoxicity in comparison with the CH1 cell line and thereby possessing a promising potential to overcome resistance toward platinum anticancer drugs.

INTRODUCTION

Great efforts have been undertaken to develop novel anticancer platinum-based drugs breaking the classic structure-activity relationships.^{1,2} This is being done with the expectation that the novel species would be capable of forming DNA adducts completely dissimilar to those deriving from clinically approved platinum agents.^{3–6} Moreover, others may show a radically different mode of action, e.g., with respect to alternative cellular targets. Among reported nonclassic platinum-based complexes exhibiting extraordinary cytotoxic properties, there are a few

classes of multinuclear platinum(II) compounds, *viz.*, (i) di- and triplatinum(II) complexes with bridging aliphatic^{7–18} and aromatic¹⁹ diamine ligands, where the platinum units are linked in a linear fashion (with trinuclear complex BBR3464^{20–26} representing the most promising anticancer agent that reached phase II clinical trials); (ii) azole-bridged platinum(II) dimers, yielding high uptake levels as well as a high extent of interstrand cross-links;^{27–35} (iii) dinuclear platinum(II)-bisphosphonate complexes, featuring medium cytotoxicity but capable of specific accumulation in the bone;^{36,37} and (iv) a dimeric carboplatin analogue, showing greater *in vitro* antitumor activity and lower toxicity in mice than carboplatin.³⁸ All above listed species are able to circumvent cisplatin resistance *in vitro*.

Recently, some fascinating results concerning a novel class of nonclassic platinum(II) complexes, *i.e.*, ketoxime-containing platinum(II) species, were reported. It was demonstrated that *trans*-[PtCl₂(Me₂C=NOH)(NH₂CHMe₂)], unlike its *cis*-analogue, causes cell death *via* an apoptotic mechanism.³⁹ Furthermore, the complexes [PtX₂(R₂C=NOH)₂] (X = Cl, Br, I; R = Me, Et) exhibiting *trans*-configuration were shown to possess a higher cytotoxicity,^{40,41} enhanced cellular accumulation, and elevated DNA platination, compared to their *cis*-isomers.⁴² Hence, (ketoxime)Pt^{II} complexes of *trans*-geometry represent a new and promising class of platinum-based antitumor agents clashing the classic Cleare&Hoeschele's structure-activity rules.^{1,2}

Conducting the project on synthesis and antitumor activity of (oxime)Pt^{II} complexes,^{40–42} we attempted to elucidate a solution behavior of these species in the course of the hydrolysis studies insofar as the displacement of the so-called leaving groups (mostly chlorido or carboxylato ligands) by water molecules is believed to be a prerequisite for anticancer activity of the platinum-based drugs. As a starting point, we determined their acidity constants by ¹H NMR titration. However, we were able to establish only pK_{a1} (ca. 7.2;⁴³ the acid-base equilibrium is reversible), whereas further titration at pH over 8 indicated a partial irreversibility of the system due to a chemical reaction. Being interested in deep understanding of possible conversions of the oxime complexes in aqueous solutions, we carried out a synthetic experiment and isolated novel-type oximato-bridged di- and trinuclear species generated as a result of association of the oximato complexes.

Herein, we report on the synthesis and characterization of unprecedented di- and triplatinum(II) oximato-bridged species derived from classic *trans*-configured ketoxime platinum(II) complexes^{40,41} as well as from newly prepared aldoxime analo-

gues. Furthermore, the cytotoxicity of the novel monomeric and dimeric compounds was comparatively evaluated in three human tumor cell lines originating from cisplatin-sensitive (CH1) or resistant (SW480 and A549) cancer cells.

RESULTS AND DISCUSSION

Recently, we reported on the promising cytotoxic properties of *trans*-configured platinum(II) ketoxime complexes.^{40–42} In line with these results, we now have focused on the development of new aldoxime analogues featuring (*E*)- or (*Z*)-R(H)C=NOH (R = Me, Et) ligands in *trans*-configuration. As expected, the novel agents exhibit considerably higher aqueous solubility as compared to their ketoxime congeners with ligated R₂C=NOH (R = Me, Et). As a next step of the project, we performed dimerization of both aldoxime- and ketoxime-containing complexes, yielding head-to-tail oriented oximato-bridged dimers of the general formula [PtCl(μ-RR'C=NO)(RR'C=NOH)]₂ (R = Me, Et; R' = H, Me, Et) and verified their cytotoxic properties. In addition, for R = R' = Et a novel structure consisting of three linked in a linear fashion platinum units bridged by oximato ligands was established.

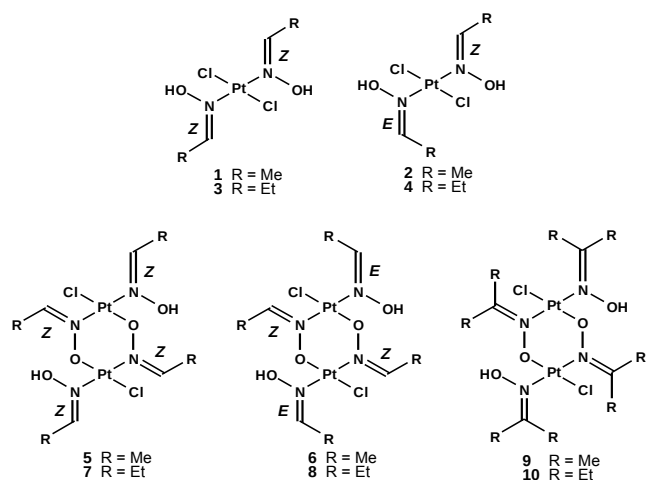


Figure 1. Platinum(II) oxime complexes used in this study.

Synthesis, Characterization and X-ray Structure Determination of *trans*-Bis(aldoxime)platinum(II) Complexes. Generation of 1–4 (Figure 1) was carried out by a conventional route^{44–46} via the reaction of K₂[PtCl₄] with 2 equivs of a *E*/*Z*-R(H)C=NOH (R = Me, Et) in an aqueous solution at room temperature followed by thermal isomerization in MeNO₂ and column separation of isomers (yields are 31–35%). The complexes were characterized by elemental analyses, electrospray ionization mass spectrometry (ESI-MS), IR, and ¹H, ¹³C{¹H}, and ¹⁹⁵Pt NMR spectroscopy, and also by X-ray crystallography (for 1 and 4, Figure S1, Table S1). *Trans*-configuration of the complexes and *E*/*Z*-configurations of the ligands were established by X-ray diffraction. Detailed description of syntheses and characterizations of 1–4 is given in the Supporting Information, whereas synthetic experiments are reported in the Experimental Section.

Synthesis, Characterization, and X-ray Structure Determinations of Oximato-Bridged Platinum(II) Dimers/Trimer. The

corresponding dimeric species derived from 1–4 which were obtained in this work as well as originated from the previously reported *trans*-[PtCl₂(R₂C=NOH)]₂ (R = Me,⁴⁶ Et⁴¹) were prepared by abstraction of a chlorido ligand with 1 equiv of silver acetate. The reaction was carried out in acetone at room temperature for a limited time (1–3 h) in order to minimize the probability of acetato complex formation; final products 5–10 (Figure 1) were purified by column chromatography, and isolated in moderate yields (31–36%).

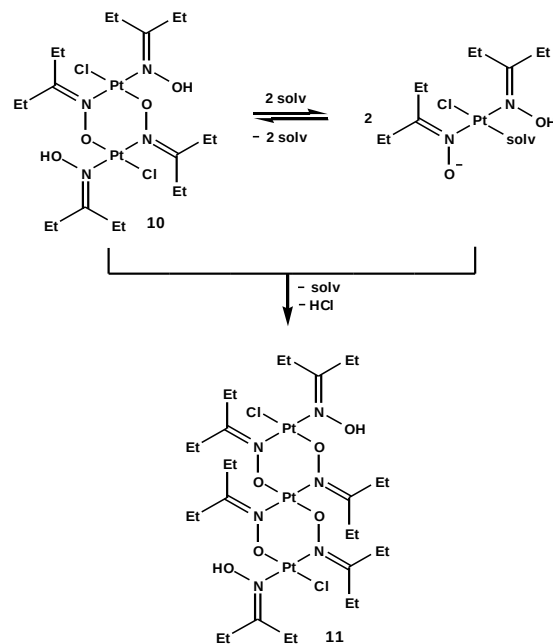


Figure 2. Plausible route for generation of the trimer.

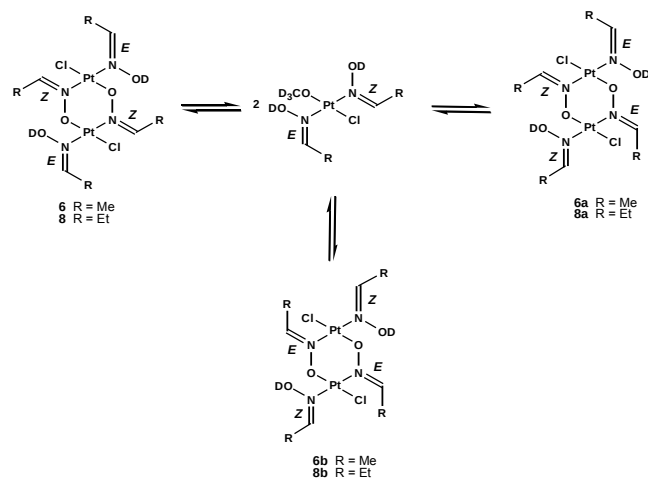
Interestingly, slow evaporation of a solution of complex 10 in a mixture of undried ethanol, ethylacetate, and acetone resulted in formation of trimer 11. The obtained crystals were characterized by X-ray crystallography (see below), as well as by ESI-MS spectrometry ([M + Na]⁺ and [M + K]⁺ peaks were detected). A plausible mechanism of the trimer formation could be associated with a dimer-monomer equilibrium (Figure 2).

Complexes 5–10 gave satisfactory elemental analyses and appropriate molecular ion/fragmentation patterns in the ESI-MS⁺ spectra ([M + H]⁺ and [M + Na]⁺ peaks were found). Furthermore, 5–10 were characterized by IR spectroscopy, exhibiting characteristic strong ν(OH) [3143–3377 cm^{−1}] and ν(NO) [958–970 cm^{−1}] stretching vibrations, as well as two ν(C=N) [1609–1625 and 1651–1681 cm^{−1}] bands of medium intensity. Expectedly, two sets of oxime proton signals were observed in the ¹H NMR spectra of all prepared dimers, corresponding to the bridging and nonbridging ligated oximes. Starting with *Z*/*E* monomeric complexes 2 and 4, the respective dimers [PtCl(μ-(*Z*)-R(H)C=NO)(*E*)-R(H)C=NOH]₂ (R = Me (6), Et (8)), featuring bridging *Z*-oximato ligands, were formed as major products. However, traces of *Z*,*E*- (6a/8a) and *E*,*E*-oximato bridged dimers (6b/8b) were detected by NMR spectroscopy. Moreover, dissolution of 6 and 8 in CD₃OD led to an increase of the signals of the latter species with time, resulting in the following equilibrium ratios: 6 : 6a : 6b ≈ 7 : 2 : 1 and 8 : 8a : 8b ≈ 3 : 2 : 1. This fact could be explained by equilibrium possibly established

Table 1. Crystal Data and Details of Data Collection for 1, 4, 6, 9, and 11

	1	4	6	9	11
empirical formula	C ₄ H ₁₀ Cl ₂ N ₂ O ₂ Pt	C ₆ H ₁₄ Cl ₂ N ₂ O ₂ Pt	C ₈ H ₁₈ Cl ₂ N ₄ O ₄ Pt ₂	C ₁₂ H ₂₆ Cl ₂ N ₄ O ₄ Pt ₂	C ₃₀ H ₆₂ Cl ₂ N ₆ O ₆ Pt ₃
fw	384.13	412.18	695.34	751.45	1259.03
space group	P-1	Pn	P2 ₁ /c	P-1	P-1
a, Å	4.2101(3)	4.3305(3)	11.1533(6)	8.4994(8)	9.2330(3)
b, Å	6.5803(6)	10.0784(6)	10.7869(6)	9.7473(10)	9.5490(3)
c, Å	8.7596(7)	13.1600(7)	13.1609(7)	13.5101(15)	11.2825(4)
α, deg	75.388(4)			79.033(3)	85.637(3)
β, deg	82.707(5)	98.440(4)	95.596(3)	79.998(3)	82.020(2)
γ, deg	78.825(4)			67.676(2)	80.387(2)
V, Å ³	229.59(3)	568.14(6)	1575.84(15)	1010.11(18)	969.84(6)
Z	1	2	4	2	1
λ, Å	0.71073	0.71073	0.71073	0.71073	0.71073
ρ _{calcd} , g cm ⁻³	2.778	2.409	2.931	2.471	2.156
crystal size, mm	0.50 × 0.04 × 0.01	0.30 × 0.05 × 0.05	0.13 × 0.13 × 0.04	0.20 × 0.20 × 0.15	0.20 × 0.10 × 0.05
T, K	100	100	100	100	100
μ, cm ⁻¹	158.16	127.92	180.90	141.21	10.976
R1 ^a	0.0185	0.0336	0.0369	0.0287	0.0302
wR2 ^b	0.0407	0.0659	0.0951	0.0719	0.0736
GOF ^c	1.033	1.024	1.005	1.081	1.031

^aR1 = $\sum ||F_o| - |F_c|| / \sum |F_o|$. ^bwR2 = $\{\sum [w(F_o^2 - F_c^2)^2] / \sum [w(F_o^2)^2]\}^{1/2}$. ^cGOF = $\{\sum [w(F_o^2 - F_c^2)^2] / (n - p)\}^{1/2}$, where n is the number of reflections and p is the total number of parameters refined.

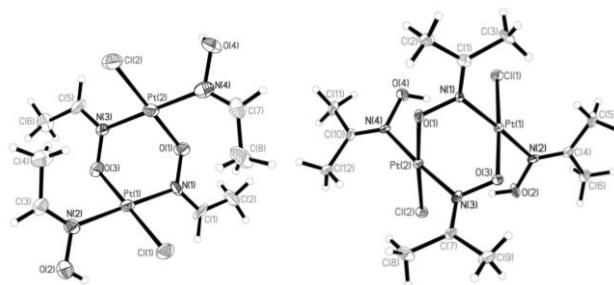

Figure 3. Equilibrium in CD₃OD solution of **6** and **8**.

between a dimer and its corresponding monomer in solution, resulting in a mixture of dimers (Figure 3).

In the ¹⁹⁵Pt NMR spectra, the platinum signals of aldoxime-containing dimers **5–8** appear in a narrow range between –208 and –251 ppm, while δ_{Pt} of corresponding ketoxime complexes **9** and **10** resonate at a lower field (–35 and –152 ppm, respectively).

The crystal structures of dimers **6** and **9**, and trimer **11** were determined by X-ray diffraction (Tables 1, 2 and 3, Figures 4 and 5). The three compounds feature pairs of head-to-tail oriented oximato ligands, which bind the platinum centers *via* the oximic nitrogen and the oxygen of the deprotonated hydroxo group. In **11**, three platinum atoms are connected to

each other *via* bridging ligands in a chain manner. Apart from the bridging ligands, the coordination sphere of each platinum center in **6** and **9** and the terminal Pt's in **11** is completed by the corresponding aldoxime (**6**) or ketoxime (**9** and **11**) and a chlorido ligand.


Figure 4. ORTEP view of **6** (left) and **9** (right) with the atom labeling scheme; the thermal ellipsoids have been drawn at the 50% probability level.

A comparison of selected geometric features of oximato-bridged platinum complexes **6**, **9**, and **11** is presented in Table 4. Interestingly, the Pt···Pt distances in **6** and **11** (3.1872(6) and 3.2094(2) Å, respectively) are considerably longer compared to that in **9** (2.8668(5) Å), that is probably due to an increased steric effect exerted by the alkyl substituents of the nonbridging oxime ligand directed to the platinum-platinum core. Contrary to **6** and **11**, in **9**, the methyl substituents of the coordinated acetoxime are turned away from the bisoximato bridge. Moreover, the two hydrogen bonds between the oximic hydroxo group of one platinum unit and chlorido ligand of the second one could favor the approaching of two platinum centers and result in an additional stabilization of the dimeric structure of **9**. The

O4...Cl1 and O2...Cl2 distances are at 3.301 and 3.303 Å, respectively. These distances fall into the range of typical O...Cl contacts when OH...Cl hydrogen bonding takes place [2.95–3.40 Å],⁴⁷ representing relatively weak interactions compared to the reported values. The angles O4–H4...Cl1 and O2–H2...Cl2 are 145.3 and 162.3°, correspondingly.

Table 2. Selected Bond Lengths (Å) and Angles (deg) for Complexes **6** and **9**

	6	9
Pt(1)–N(1)	1.992(9)	2.009(6)
Pt(1)–N(2)	2.032(9)	2.019(7)
Pt(1)–Cl(1)	2.282(2)	2.2962(19)
Pt(1)–O(3)	2.023(7)	2.030(5)
Pt(2)–N(3)	1.974(8)	2.012(6)
Pt(2)–N(4)	2.024(9)	2.026(6)
Pt(2)–Cl(2)	2.291(3)	2.2981(19)
Pt(2)–O(1)	2.036(8)	2.016(5)
N(1)–C(1)	1.260(14)	1.294(10)
N(2)–C(3)	1.264(14)	
N(3)–C(5)	1.278(14)	
N(4)–C(7)	1.268(15)	
N(2)–C(4)		1.273(10)
N(3)–C(7)		1.289(10)
N(4)–C(10)		1.272(10)
N(1)–Pt(1)–N(2)	176.2(3)	177.0(3)
N(1)–Pt(1)–Cl(1)	91.6(3)	90.88(19)
N(2)–Pt(1)–Cl(1)	89.8(2)	91.09(18)
N(3)–Pt(2)–N(4)	177.5(4)	176.0(3)
N(3)–Pt(2)–Cl(2)	91.0(3)	90.26(19)
N(4)–Pt(2)–Cl(2)	90.4(3)	92.04(19)

Generally, the distances between the adjacent platinum atoms in **6**, **9**, and **11** are shorter than a double van der Waals radius for Pt [$1.72 \times 2 = 3.44$ Å]. Compared to the reported Pt...Pt distances in the platinum(II) dimeric complexes featuring two bridging ligands, e.g., (i) α -pyridonate [2.88–2.90 Å],⁴⁸ (ii) 1-methyluracilate or 1-methylthymine [2.927–2.986 Å],⁴⁹ (iii) amidate [2.993–3.060 Å],⁵⁰ (iv) amidinate [3.381 Å],⁵¹ and (v) carboxylate [2.952–2.990 Å],⁵² the Pt...Pt distances in **6** and **11** correspond to a rather weak nonbonded interaction between the platinum centers. On the contrary, the Pt...Pt distance in **9** is one of the shortest known for platinum(II) dimers. Being in good agreement with literature data,^{53,54} the tilt angle (τ) between the neighboring platinum coordination planes is directly related to the Pt...Pt distance. Thus, the shortest Pt...Pt distance in **9** corresponds to the lowest τ value (57.2° versus 81.4° and 85.3° for **6** and **11**, respectively), additionally confirming the lowest repulsive interactions in case of the acetone oxime-containing platinum(II) dimer. The coordination planes in **6**

and **9** are twisted only slightly ($\omega = 9.9$ and 2.5° , correspondingly), while in **11** the twist angle was proved to be substantially higher ($\omega = 19.2^\circ$), serving to minimize the steric interactions between the ethyl substituents of the bridging oximato and nonbridging oxime ligands.

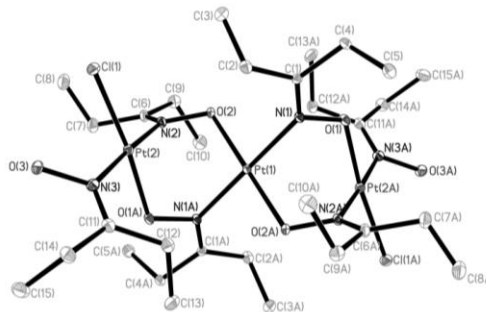


Figure 5. ORTEP view of **11** with the atom labeling scheme; the thermal ellipsoids have been drawn at the 30% probability level.

The platinum ions in **6**, **9**, and the terminal Pt's in **11** nearly adopt a square-planar geometry, being slightly displaced out of the coordination plane by 0.02–0.06 Å, whereas Pt1 in **11** lies ideally within the corresponding N1–O2–N1'–O2' plane. The Pt–Cl bond lengths [2.282(2)–2.3169(13) Å] correspond to previously reported distances for platinum(II) chlorido compounds.^{55,56} The Pt–N and Pt–O bond lengths appear in the range normally found in related platinum(II) complexes,^{51,57,58} namely: 1.974(8)–2.032(9) Å and 2.005(3)–2.047(3) Å, respectively. The values of C=N distances [1.260(14)–1.294(10) Å] are similar within 3σ and correspond to previously published distances of C=N double bonds in metal-bound oximes.^{59–61}

Comparative Studies of the Cytotoxic Activity of Novel Platinum(II) Oxime Complexes and Corresponding Oximato-Bridged Dimers. In view of our previous findings on antitumor activity of *trans*-(oxime)Pt^{II} complexes,^{40,41} the cytotoxicity of **1–9** was evaluated in comparison with cisplatin and transplatin by means of a colorimetric microculture assay (MTT) in three human cancer cell lines: cisplatin-sensitive ovarian carcinoma (CH1), as well as intrinsically cisplatin-resistant colon carcinoma (SW480) and nonsmall cell lung cancer (A549) cell lines, yielding IC₅₀ values listed in Table 5. The cytotoxic potency of dimer **10** could not be examined because of its insufficient aqueous solubility.

Generally, CH1 and SW480 cancer cell lines showed similar response toward both monomeric (**1–4**) and dimeric (**5–9**) complexes, as it was also reported for (ketoxime)Pt^{II} species.⁴¹ However, in case of **1** and **3** nearly 2-fold improvement of the cytotoxic potency in cisplatin-resistant SW480 cells compared to cisplatin-sensitive CH1 cells could be observed. Differently, the A549 cell line exhibited a somewhat lower sensitivity to the novel oxime complexes, yielding IC₅₀ values increased by factors of 2.2–4.0 for monomers **1–4** and 4.3–12 for dimers **5–9** in comparison with those in the CH1 cell line.

Unexpectedly, an enlargement of the alkyl chain length of the oxime ligand by using propionaldehyde oxime (in **3**, **4**, **7**, and **8**) instead of acetaldehyde oxime (in **1**, **2**, **5**, and **6**) did not result in an increase of cytotoxicity in the case of monomers **1–4** and only slightly reduced IC₅₀ values of dimers **5–8** (by factors 1.1–2.3). Interestingly, some influence of *E*- and *Z*-configurati-

Table 3. Selected Bond Lengths (Å) and Angles (deg) for Complex 11

Pt(1)–N(1)	2.028(4)	Pt(2)–O(1A)	2.005(3)
Pt(1)–O(2)	2.047(3)	N(1)–C(1)	1.290(6)
Pt(2)–N(2)	1.997(4)	N(2)–C(6)	1.280(6)
Pt(2)–N(3)	2.020(4)	N(3)–C(11)	1.284(6)
Pt(2)–Cl(1)	2.3169(13)		
N(1A)–Pt(1)–N(1)	180.000(1)	N(2)–Pt(2)–N(3)	177.00(16)
N(1)–Pt(1)–O(2)	87.66(14)	N(2)–Pt(2)–Cl(1)	93.46(12)
N(1A)–Pt(1)–O(2)	92.34(14)	N(3)–Pt(2)–Cl(1)	89.46(12)

Table 4. Selected Geometric Parameters of the Oximato-Bridged Platinum Complexes

	Pt...Pt, Å	dihedral deg	angle, ^a deg	Pt...Pt...Pt deg	angle, deg
		τ	ω		
6	3.1872(6)	81.4	9.9	–	
9	2.8668(5)	57.2	2.5	–	
11	3.2094(2)	85.3	19.2	180.0	

^a τ is the tilt angle between the adjacent platinum coordination planes, and ω is the average torsion angle about the Pt(1)···Pt(2) vector.

on of the coordinated aldoximes on the IC₅₀ values was observed. Thus, monomeric complexes **2** and **4** featuring both *E*- and *Z*-aldoximes proved to be by a factor of 2 more cytotoxic in CH1 cells compared to their isomers **1** and **3**, containing two *Z*-ligands.

Comparing aldoxime-containing monomeric complexes **1–4** and the corresponding dimers **5–8**, it should be pointed out that dimerization results in 1.8- to 6.7-fold enhancement of the cytotoxic potency in CH1 cells. However, dimer **9** featuring a ketoxime ligand exhibited 13 and 11 times higher IC₅₀ values in CH1 and SW480 cells, respectively, as compared to the corresponding monomer *trans*-[PtCl₂(Me₂C=NOH)₂],⁴⁰ reversing the structure-activity relationships valid for (aldoxime)Pt^{II} complexes.

With respect to cisplatin, monomeric species **1–4** proved to be significantly less cytotoxic in CH1 (24–47 times) and A549 (10–16 times) cell lines, while in SW480 cells the IC₅₀ values were similar to that of cisplatin. Remarkably, dimers **5–9** exhibited 1.3–3.0-fold improved cytotoxicity in SW480 cells compared to cisplatin, being however less potent both in CH1 and A549 cell lines (by factors of 3.9 to 13). In comparison to transplatin, novel (oxime)Pt^{II} complexes **1–9** were found to display up to 17 times enhanced cytotoxic potency.

Behavior of Monomer 1 and Dimer 5 in Aqueous Solution under Physiological Conditions. In order to reveal the behavior of the prepared monomeric and dimeric species in aqueous solutions under the conditions used for cell culture experiments, time-dependent ¹H NMR measurements of monomer **1** and the respective dimer **5** were carried out in phosphate buf-

Table 5. Cytotoxicity of Monomeric (1–4) and Dimeric (5–9) (oxime)Pt^{II} Complexes in CH1, SW480, and A549 Cancer Cells (Exposure Time 96 h)

Compound	IC ₅₀ (μM), 96h		
	CH1	SW480	A549
1	6.6 ± 0.2	3.5 ± 0.1	14 ± 1
2	3.3 ± 0.1	3.3 ± 0.1	13 ± 1
3	6.2 ± 0.3	3.6 ± 0.1	21 ± 3
4	3.4 ± 0.2	3.3 ± 0.04	13 ± 1
5	0.95 ± 0.1	1.2 ± 0.1	12 ± 0.4
6	1.8 ± 0.2	1.9 ± 0.1	12 ± 1
7	0.94 ± 0.09	1.0 ± 0.1	5.1 ± 0.5
8	0.96 ± 0.09	1.3 ± 0.1	5.7 ± 0.3
9	2.2 ± 0.4	2.5 ± 0.3	9.5 ± 1.6
<i>trans</i> -[PtCl ₂ (Me ₂ C=NOH) ₂] ^a	0.17 ± 0.09	0.22 ± 0.05	
cisplatin	0.14 ± 0.03	3.3 ± 0.4	1.3 ± 0.4
transplatin ^a	15 ± 2	19 ± 3	

^a Data taken from ref. 44

fered D₂O solutions (10 mM, pH 7.4) containing NaCl (0.125 M), with concentrations of complexes being ca. 400 and 200 μM, correspondingly. Figure 6 shows a selected range of ¹H NMR spectra (6–8 ppm) where the signals of N=CH protons are detected. Keeping dimer **5** or monomer **1** under these conditions over three days resulted in a nearly identical peak pattern (spectra (2) and (4), respectively), consisting of five N=CH proton signals corresponding to the dimer (black), the monomer (red), the hydrolyzed monomer (green), and an additional so far unknown species (blue).

These results are in good agreement with IC₅₀ values of **1** and **5** in SW480 cell line, showing a 2-fold improvement of the cytotoxicity in the case of dimer **5** as compared to monomer **1**. However, the ratio IC₅₀(**1**)/IC₅₀(**5**) in two other cell lines used in this work deviate from a factor of 2, being 6.6 in CH1 and 1.25 in A549 cells, indicating a slightly different behavior in these cell lines.

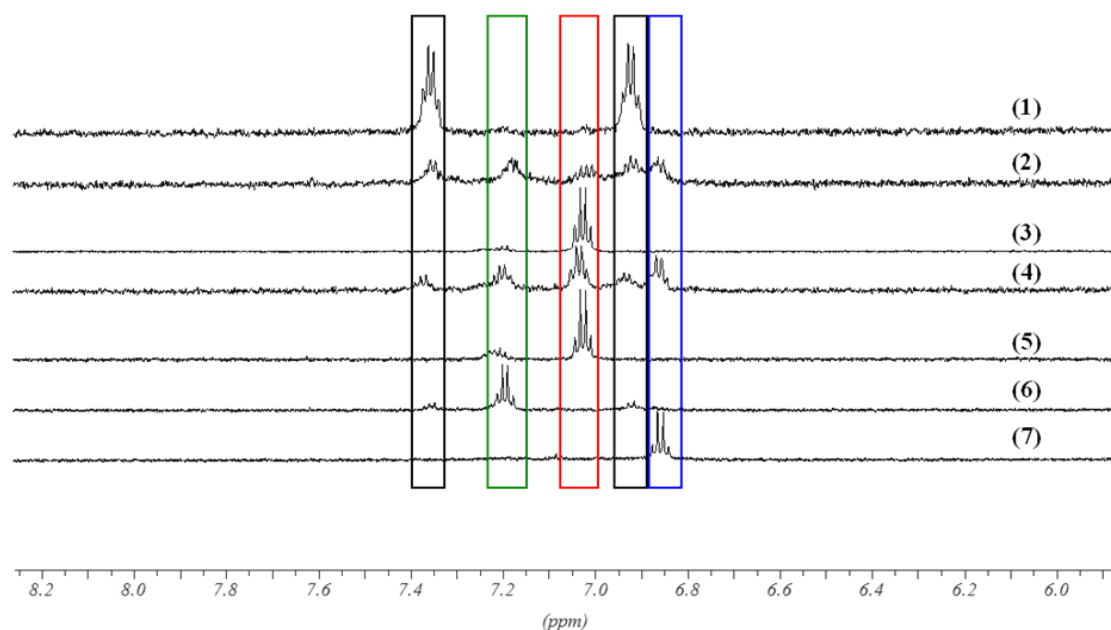


Figure 6. The ^1H NMR spectra of monomer **1** and corresponding dimer **5** in the range 6–8 ppm: dimer **5** five min (1) and *ca.* three days (2) after dissolution in 10 mM phosphate buffered D_2O solution (pH 7.4), containing 0.125 M NaCl; monomer **1** five min (3) and *ca.* three days (4) after dissolution in 10 mM phosphate buffered D_2O solution (pH 7.4), containing 0.125 M NaCl; monomer **1** five min (5), five h (6) and *ca.* three days (7) after dissolution in 10 mM phosphate buffered D_2O solution (pH 7.4) in the absence of NaCl.

Spectra (5)–(7) (Figure 6) additionally represent time-dependent hydrolysis studies of complex **1**. For this purpose no sodium chloride was added to the phosphate buffered D_2O solution of **1** (pH 7.4), resulting in an enhanced aquation of the complex. Five minutes after dissolution of the compound, a peak corresponding to the $\text{N}=\text{CH}$ proton of the hydrolyzed species (green) could be observed (spectrum (5)). A total of 5 h after the experiment was started, the dichlorido complex (red) was completely converted into the aqua species (green) (spectrum (6)). In addition, small signals related to the dimeric species (black) were detected. Finally, three days later, the mixture of $\text{N}=\text{CH}$ proton signals turned into one single peak (blue) (spectrum (7)). With the intention to elucidate which species was formed as a result of the conversions taking place in the aqueous solution, ^{195}Pt NMR spectrum was recorded. A signal at 316.7 ppm was observed, which correlate well with the δ_{Pt} values for *trans*-configured platinum(II) species having $[\text{N}_2\text{O}_2]$ ligand donor set reported in the literature in the range between 209 and 366 ppm.⁶² Further, in ESI- MS^+ spectrum a peak at m/z 956 was detected that could be attributed to a sodiated trimeric complex $[\text{Pt}_3(\text{C}_2\text{H}_4\text{NO})_6 + \text{Na}]^+$ ($\text{C}_{12}\text{H}_{24}\text{N}_6\text{O}_6\text{Pt}_3\text{Na}$, calcd m/z 956.06). Due to the fact, that only one set of $\text{N}=\text{CH}$ proton signals was observed in the ^1H NMR spectrum (7), a ring-shaped trimeric structure containing six equivalent oximato bridges can be suggested (Figure 7).

Geometry Optimization. Full geometry optimization for a ring-shaped *Z*-acetaldoximato-bridged trimer was performed at the DFT B3LYP theoretical level. In the optimized structure (Figure 7), three platinum atoms display square-planar geometries deviating only slightly (by 0.075–0.080 Å) from their respective N–O–N–O coordination planes. The optimized Pt–O

[2.068–2.075 Å] and Pt–N [2.041–2.048 Å] distances appeared to be somewhat longer as compared to those bond lengths observed in the X-ray structures of dimers **6** and **9**, and trimer **11** (Pt–O [2.005(3)–2.047(3) Å] and Pt–N [1.974(8)–2.032(9) Å]). As the Pt···Pt distances are concerned, they were found between 3.019 and 3.033 Å in the optimized structure, correlating well with the values measured for **6**, **9**, and **11** [2.8668(5)–3.2094(2) Å].

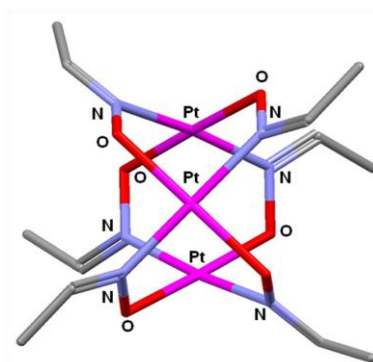


Figure 7. An optimized structure of a ring-shaped trimer.

CONCLUSIONS

A possibility of di- and trimerization of (oxime) Pt^{II} complexes leading to the formation of a six-membered diplatinacycle has been demonstrated. The oximato-bridged platinum(II) dimers were isolated and completely characterized, including X-ray

diffraction in case of two dimers. Additionally, a crystal structure of a novel oximate-bridged platinum(II) trimer featuring platinum units connected to each other in a chain manner was determined.

Although the bridging properties of oximic ligands have been extensively studied, e.g., nearly exhaustively in the case of group VIII metals (Fe,^{63,64} Ru,⁶⁵ Os,⁶⁶ Co,^{67,64} Ni,^{63,64,68–70} and Pd⁶⁹), and partially for some representatives of group I (Cu^{63,68,70–84}), II (Zn,^{63,64,85} Cd⁸⁵), III (Gd⁶⁴), IV (Ti,⁸⁶ Zr⁸⁶), VI (Cr⁶⁴) and VII (Mn^{63,64,87–89}), there are no reports on oximate-bridged platinum complexes. Moreover, to the best of our knowledge the novel di-/trimer(s) exhibit a unique six-membered diplatinacycle as well as represent the first example of oxime/oximate ligands coordinated to the platinum atom *via* the oxygen.

In general, the di-/trimerization observed in this work on the kinetically inert platinum(II) center could give a possible rationale for various polymerizations involving oxime/oximate species based on more labile metals. Considering these results, previous work on the synthesis of polymeric oximate-bridged complexes should be thoroughly revisited and the possibility of the consecutive association of the monomeric oximate units should be taken into account in further studies.

Additionally, the cytotoxic properties of the novel dimers were examined in three human cancer cell lines (CH1, SW480, and A549) in comparison with the corresponding monomeric complexes of *trans*-geometry and structure-activity relationships were established. It was found that dimerization improves significantly the cytotoxic potency of the aldoxime-containing complexes, whereas the dimer bearing ketoxime ligands proved to be considerably less potent than the respective monomer. Notably, the novel oximate-bridged dimers display appreciably enhanced cytotoxicity in the intrinsically cisplatin-resistant SW480 cell line as compared to cisplatin, indicating in perspective a promising potential to overcome cisplatin resistance.

EXPERIMENTAL

General Procedures. Potassium tetrachloridoplatinate and 2-propanone oxime were supplied by Johnson Matthey and Fluka, respectively. 3-Pentanone, acetaldehyde oxime (a mixture of *syn* and *anti* forms) and propionaldehyde oxime (a mixture of *syn* and *anti* forms) were purchased from Aldrich. All these chemicals were used as received without further purification. 3-Pentanone oxime was synthesized from the corresponding ketone by a standard text-book procedure.^{90,91} Complexes *trans*-[PtCl₂(Me₂C=NOH)₂]⁴⁶ and *trans*-[PtCl₂(Et₂C=NOH)₂]⁴¹ were prepared according to the previously published methods starting from K₂[PtCl₄]. Synthetic procedures were carried out in a light-protected environment when platinum was involved. Fluka silica gel 60 (220–440 mesh) was used for column chromatography. C, H, and N elemental analyses were carried out by the elemental analyses laboratory of the University of Vienna using a Perkin-Elmer 2400 CHN elemental analyzer. ESI-MS spectra were obtained with a Bruker Esquire 3000 instrument. IR spectra were obtained by using an ATR unit with a Perkin-Elmer 370 FTIR 2000 instrument (4000–400 cm⁻¹). ¹H, ¹³C{¹H}, and ¹⁹⁵Pt NMR spectra were measured with a Bruker Avance III 500 MHz NMR spectrometer at 500.32 (¹H), 125.81 (¹³C), and 107.55 MHz (¹⁹⁵Pt), correspondingly, at 298 K. ¹⁹⁵Pt chemical shifts are given relative to K₂[PtCl₄], and the half-height line width is given in parentheses.

Reaction of K₂[PtCl₄] with *Z/E*R(H)C=NOH (R = Me, Et). K₂[PtCl₄] (0.7 mmol) was dissolved in 15 mL of H₂O and a solution of *Z/E*R(H)C=NOH (1.4 mmol) in 2 mL of H₂O was added dropwise. The reaction mixture was stirred for 24 h at room temperature. A small amount of undefined reddish precipitate formed was filtered off, washed with H₂O and dried *in vacuo*. The filtrate was evaporated to dryness and 10 mL of MeOH added. A colorless precipitate of KCl was filtered off. Then MeOH was removed on a rotary evaporator and 5 mL of CHCl₃ or Et₂O (for R = Me or Et, respectively) was added, whereupon a yellow precipitate of K[PtCl₃{R(H)C=NOH}] was filtered off, washed with CHCl₃ or Et₂O (2×2 mL) and dried *in vacuo* (yield was 23–26%). The filtrate was evaporated to dryness and the crude product was stored for purification with column chromatography, see below.

To a solution (K[PtCl₃{R(H)C=NOH}]), 0.16–0.18 mmol) in 10 mL of H₂O an equivalent amount of *Z/E*R(H)C=NOH was added and the reaction mixture was stirred at room temperature for 24 h. The solvent was removed, 10 mL of MeOH were added and a colorless precipitate of KCl was filtered off, whereupon MeOH was removed on a rotary evaporator, the solid residue was combined with the crude products synthesized previously (see above) and 15 mL of MeNO₂ was added, whereupon the reaction mixture was refluxed for 1 h. After that the solvent was removed and products 1 (1st fraction) and 2 (2nd fraction), or 3 (1st fraction) and 4 (2nd fraction) were separated by column chromatography (CHCl₃ : Me₂C=O 8:1 (v/v) for R = Me and 10:1 (v/v) for R = Et). Yields of 1–4: 31–35%.

(SP-4-1)-Dichloridobis((1*Z*-N-hydroxyethanimine-κN)platinum(II) (1). Anal. Calcd. for C₄H₁₀N₂Cl₂O₂Pt: C, 12.51; H, 2.62; N, 7.29. Found: C, 12.90; H, 2.46; N, 7.03%. ESI-MS⁺ (MeOH), *m/z*: 407 [M + Na]⁺, 423 [M + K]⁺. TLC (8:1 (v/v) CHCl₃/Me₂C=O): R_f = 0.73. IR in KBr (selected bands), cm⁻¹: 3202 s ν(OH), 1667 m ν(C=N), 970 s ν(NO). ¹H NMR (CD₃OD), δ: 7.39 (q, 1H, CH, ³J_{H,H} = 5.8 Hz), 2.05 (d, 3H, CH₃, ³J_{H,H} = 5.7 Hz). ¹³C{¹H} NMR (CD₃OD), δ: 156.4 (C=N), 12.7 (CH₃). ¹⁹⁵Pt NMR (CD₃OD), δ: -479 (500 Hz). Crystals for X-ray study were obtained by slow evaporation of an acetone/toluene solution.

(SP-4-1)-Dichlorido((1*E*-N-hydroxyethanimine-κN)/((1*Z*-N-hydroxyethanimine-κN)platinum(II) (2). Anal. Calcd. for C₄H₁₀N₂Cl₂O₂Pt: C, 12.51; H, 2.62; N, 7.29. Found: C, 12.79; H, 2.60; N, 7.22%. ESI-MS⁺ (MeOH), *m/z*: 407 [M + Na]⁺, 466 [M + Me(H)C=NOH + Na]⁺, 482 [M + Me(H)C=NOH + K]⁺. ESI-MS⁻ (MeOH), *m/z*: 287 [M - Me(H)C=NOH - HCl - H]⁻. TLC (8:1 (v/v) CHCl₃/Me₂C=O): R_f = 0.52. IR in KBr (selected bands), cm⁻¹: 3215 s ν(OH), 1677 m ν(C=N), 980 s ν(NO). ¹H NMR (CD₃OD), δ: 7.51 (q, 1H, CH, ³J_{H,H} = 6.1 Hz), 7.50 (q, 1H, CH, ³J_{H,H} = 5.7 Hz), 2.49 (d, 3H, CH₃, ³J_{H,H} = 6.1 Hz), 2.05 (d, 3H, CH₃, ³J_{H,H} = 5.7 Hz). ¹³C{¹H} NMR (CD₃OD), δ: 156.0 (C=N), 155.4 (C=N), 16.1 (CH₃), 12.7 (CH₃). ¹⁹⁵Pt NMR (CD₃OD), δ: -532 (550 Hz).

(SP-4-1)-Dichloridobis((1*Z*-N-hydroxypropane-1-imine-κN)platinum(II) (3). Anal. Calcd. for C₆H₁₄N₂Cl₂O₂Pt: C, 17.48; H, 3.42; N, 6.80. Found: C, 17.77; H, 3.11; N, 6.65%. ESI-MS⁺ (MeOH), *m/z*: 435 [M + Na]⁺, 451 [M + K]⁺. TLC (10:1 (v/v) CHCl₃/Me₂C=O): R_f = 0.54. IR in KBr (selected bands), cm⁻¹: 3338 s ν(OH), 1671 m ν(C=N), 985 s ν(NO). ¹H NMR (CD₃OD), δ: 7.29 (t, 1H, CH, ³J_{H,H} = 5.8 Hz), 2.52 (m, 2H, CH₂), 1.09 (t, 3H, CH₃, ³J_{H,H} = 7.7 Hz). ¹³C{¹H} NMR (CD₃OD),

δ : 161.9 (C=N), 20.9 (CH₂), 8.6 (CH₃). ¹⁹⁵Pt NMR (CD₃OD), δ : -490 (510 Hz).

(SP-4-1)-Dichlorido((1*E*)-N-hydroxypropane-1-imine-κN)(1*Z*-N-hydroxypropane-1-imine-κN)platinum(II) (4). Anal. Calcd. for C₆H₁₄N₂Cl₂O₂Pt: C, 17.48; H, 3.42; N, 6.80. Found: C, 17.73; H, 3.13; N, 6.66%. ESI-MS⁺ (MeOH), m/z : 435 [M + Na]⁺, 451 [M + K]⁺. ESI-MS⁻ (MeOH), m/z : 301 [M - Et(H)C=NOH - HCl - H]⁻. TLC (10:1 (v/v) CHCl₃/Me₂C=O): R_f = 0.41. IR in KBr (selected bands), cm⁻¹: 3246 s ν(OH), 3204 s ν(OH), 1676 m ν(C=N), 1666 m ν(C=N), 983 s ν(NO). ¹H NMR (CD₃OD), δ : 7.42 (t, 1H, CH, ³J_{H,H} = 6.9 Hz), 7.39 (t, 1H, CH, ³J_{H,H} = 5.8 Hz), 3.04 (m, 2H, CH₂), 2.52 (m, 2H, CH₂), 1.21 (t, 3H, CH₃, ³J_{H,H} = 7.6 Hz), 1.11 (d, 3H, CH₃, ³J_{H,H} = 7.7 Hz). ¹³C{¹H} NMR (CD₃OD), δ : 161.5 (C=N), 160.0 (C=N), 24.6 (CH₂), 20.9 (CH₂), 9.3 (CH₃), 8.6 (CH₃). ¹⁹⁵Pt NMR (CD₃OD), δ : -529 (500 Hz). Crystals for X-ray study were obtained by slow evaporation of a methanol/toluene solution.

Dimerization of *trans*-Bis(aldoxime)- and *trans*-Bis(ketoxime)platinum(II) Complexes. Monomers 1–4 and *trans*-[PtCl₂(R₂C=NOH)₂] (R = Me⁴⁶, Et⁴¹) (0.1 mmol) were dissolved in 2–3 mL of acetone and an equivalent amount of AgOAc (0.1 mmol) was added. The reaction mixture was stirred at room temperature for 1–3 h (followed by TLC), then the precipitate of AgCl was filtered off and the resulting yellow solution was reduced to dryness. The product was isolated by a column chromatography as the first fraction using a mixture of CHCl₃ and Me₂C=O as mobile phase with a ratio of 20:1 for 5, 8:1 for 6 and 9, 30:1 for 7, and 10:1 for 8 and 10. Yields were 31–36%.

(SP-4-2)-Di-μ((1*Z*)-N-oxyethanimine-κN,κO)bis[chlorido((1*Z*)-N-hydroxyethanimine-κN)platinum(II)] (5). Anal. Calcd. for C₈H₁₈N₄Cl₂O₄Pt₂: C, 13.82; H, 2.61; N, 8.06. Found: C, 13.87; H, 2.36; N, 7.65%. ESI-MS⁺ (MeOH), m/z : 542 [M - 2 Me(H)C=NOH - Cl]⁺, 600 [M - 2 Me(H)C=NOH + Na]⁺, 718 [M + Na]⁺, 734 [M + K]⁺. TLC (20:1 (v/v) CHCl₃/Me₂C=O): R_f = 0.58. IR in KBr (selected bands), cm⁻¹: 3247 s ν(OH), 1678 and 1619 m ν(C=N), 970 s ν(NO). ¹H NMR (CD₃OD), δ : 7.59 (q, 1H, CH, ³J_{H,H} = 5.8 Hz), 6.99 (q, 1H, CH, ³J_{H,H} = 5.8 Hz), 2.07 (d, 3H, CH₃, ³J_{H,H} = 5.7 Hz), 1.94 (d, 3H, CH₃, ³J_{H,H} = 5.8 Hz). ¹³C{¹H} NMR (CD₃OD), δ : 153.8 (C=N), 151.3 (C=N), 12.3 (CH₃), 12.1 (CH₃). ¹⁹⁵Pt NMR (CD₃OD), δ : -208 (480 Hz).

(SP-4-2)-Di-μ((1*Z*)-N-oxyethanimine-κN,κO)bis[chlorido((1*E*)-N-hydroxyethanimine-κN)platinum(II)] (6). Anal. Calcd. for C₈H₁₈N₄Cl₂O₄Pt₂: C, 13.82; H, 2.61; N, 8.06. Found: C, 14.02; H, 2.42; N, 7.72%. ESI-MS⁺ (MeOH), m/z : 600 [M - Me(H)C=NOH - Cl]⁺, 676 [M - 2 Me(H)C=NOH + K]⁺, 718 [M + Na]⁺, 734 [M + K]⁺. TLC (8:1 (v/v) CHCl₃/Me₂C=O): R_f = 0.48. IR in KBr (selected bands), cm⁻¹: 3248 s ν(OH), 1676 and 1618 m ν(C=N), 964 s ν(NO). ¹H NMR (CD₃OD), δ : 7.78 (q, 1H, CH, ³J_{H,H} = 5.8 Hz), 7.38 (q, 1H, CH, ³J_{H,H} = 6.2 Hz), 2.33 (d, 3H, CH₃, ³J_{H,H} = 6.2 Hz), 2.09 (d, 3H, CH₃, ³J_{H,H} = 5.8 Hz). ¹³C{¹H} NMR (CD₃OD), δ : 154.1 (C=N), 152.0 (C=N), 15.8 (CH₃), 12.4 (CH₃). ¹⁹⁵Pt NMR (CD₃OD), δ : -251 (490 Hz). Crystals for X-ray study were obtained by slow evaporation of a methanol/toluene solution at room temperature.

(SP-4-2)-Di-μ((1*Z*)-N-oxypropane-1-imine-κN,κO)bis[chlorido((1*Z*)-N-hydroxypropane-1-imine-κN)platinum(II)] (7). Anal. Calcd. for C₁₂H₂₆N₄Cl₂O₄Pt₂: C, 19.18; H, 3.49; N, 7.46. Found: C, 19.11; H, 3.25; N, 7.22%. ESI-MS⁺ (MeOH), m/z : 628 [M - 2 Et(H)C=NOH + Na]⁺, 716

[M - Cl]⁺, 752 [M + H]⁺, 774 [M + Na]⁺. TLC (CHCl₃): R_f = 0.59. IR in KBr (selected bands), cm⁻¹: 3232 s ν(OH), 1660 and 1625 m ν(C=N), 961 s ν(NO). ¹H NMR (CD₃OD), δ : 7.50 (t, 1H, CH, ³J_{H,H} = 5.7 Hz), 6.88 (t, 1H, CH, ³J_{H,H} = 5.8 Hz), 2.55 (m, 2H, CH₂), 2.43 (m, 2H, CH₂), 1.13 (t, 3H, CH₃, ³J_{H,H} = 7.6 Hz), 1.06 (t, 3H, CH₃, ³J_{H,H} = 7.7 Hz). ¹³C{¹H} NMR (CD₃OD), δ : 160.0 (C=N), 157.9 (C=N), 20.6 (CH₂), 20.5 (CH₂), 8.9 (CH₃), 8.7 (CH₃). ¹⁹⁵Pt NMR (CD₃OD), δ : -212 (440 Hz).

(SP-4-2)-Di-μ((1*Z*)-N-oxypropane-1-imine-κN,κO)bis[chlorido((1*E*)-N-hydroxypropane-1-imine-κN)platinum(II)] (8). Anal. Calcd. for C₁₂H₂₆N₄Cl₂O₄Pt₂: C, 19.18; H, 3.49; N, 7.46. Found: C, 19.21; H, 3.43; N, 7.16%. ESI-MS⁺ (MeOH), m/z : 628 [M - 2 Et(H)C=NOH + Na]⁺, 716 [M - Cl]⁺, 752 [M + H]⁺, 774 [M + Na]⁺. TLC (20:1 (v/v) CHCl₃/Me₂C=O): R_f = 0.71. IR in KBr (selected bands), cm⁻¹: 3250 s ν(OH), 1681 and 1624 m ν(C=N), 958 s ν(NO). ¹H NMR (CD₃OD), δ : 7.70 (t, 1H, CH, ³J_{H,H} = 5.7 Hz), 7.31 (t, 1H, CH, ³J_{H,H} = 6.8 Hz), 2.89 (m, 2H, CH₂), 2.58 (m, 2H, CH₂), 1.161 (t, 3H, CH₃, ³J_{H,H} = 7.6 Hz), 1.160 (t, 3H, CH₃, ³J_{H,H} = 7.7 Hz). ¹³C{¹H} NMR (CD₃OD), δ : 159.6 (C=N), 157.2 (C=N), 24.2 (CH₃), 20.7 (CH₃), 9.8 (CH₃), 8.6 (CH₃). ¹⁹⁵Pt NMR (CD₃OD), δ : -243 (450 Hz).

(SP-4-2)-Di-μ(N-oxypropane-2-imine-κN,κO)bis[chlorido(N-hydroxypropane-2-imine-κN)platinum(II)] (9). Anal. Calcd. for C₁₂H₂₆N₄Cl₂O₄Pt₂: C, 19.36; H, 3.27; N, 7.06. Found: C, 19.54; H, 3.29; N, 7.43%. ESI-MS⁺ (MeOH), m/z : 752 [M + H]⁺, 774 [M + Na]⁺. TLC (8:1 (v/v) CHCl₃/Me₂C=O): R_f = 0.53. IR in KBr (selected bands), cm⁻¹: 3143 s ν(OH), 1666 and 1620 m ν(C=N), 969 s ν(NO). ¹H NMR (CDCl₃), δ : 8.67 (s, 2H, OH), 2.72 (s, 6H, CH₃), 2.55 (s, 6H, CH₃), 2.29 (s, 6H, CH₃), 2.09 (s, 6H, CH₃). ¹³C{¹H} NMR (CDCl₃), δ : 165.4 (C=N), 157.4 (C=N), 25.0 (CH₃), 23.7 (CH₃), 18.4 (CH₃), 17.7 (CH₃). ¹⁹⁵Pt NMR (CDCl₃), δ : -35 (400 Hz). Crystals for X-ray study were obtained by slow evaporation of an acetone solution at room temperature.

(SP-4-2)-Di-μ(N-oxypropane-3-imine-κN,κO)bis[chlorido(N-hydroxypentane-3-imine-κN)platinum(II)] (10). Anal. Calcd. for C₂₀H₄₂N₄Cl₂O₄Pt₂: C, 27.81; H, 4.90; N, 6.49. Found: C, 27.42; H, 4.73; N, 6.27%. ESI-MS⁺ (MeOH), m/z : 828 [M - Cl]⁺, 864 [M + H]⁺, 886 [M + Na]⁺. TLC (20:1 (v/v) CHCl₃/Me₂C=O): R_f = 0.43. IR in KBr (selected bands), cm⁻¹: 3377 s ν(OH), 1651 and 1609 m ν(C=N), 966 s ν(NO). ¹H NMR (CD₃OD), δ : 3.59 (m, 2H, 2CHH), 3.36 (m, 2H, 2CHH), 3.22 (m, 2H, 2CHH), 3.11 (m, 2H, 2CHH), 2.65 (m, 4H, 4CHH), 2.52 (m, 2H, 2CHH), 2.36 (m, 2H, 2CHH), 1.34 (t, 6H, 2CH₃, ³J_{H,H} = 7.6 Hz), 1.21 (t, 6H, 2CH₃, ³J_{H,H} = 7.6 Hz), 1.16 (t, 6H, 2CH₃, ³J_{H,H} = 7.6 Hz), 1.00 (t, 6H, 2CH₃, ³J_{H,H} = 7.6 Hz). ¹³C{¹H} NMR (CD₃OD), δ : 173.2 (2C=N), 168.5 (2C=N), 29.1 (CH₂), 28.9 (CH₂), 28.3 (CH₂), 28.1 (CH₂), 22.4 (2CH₂), 22.1 (2CH₂), 10.3 (4CH₃), 9.17 (2CH₃), 9.0 (2CH₃). ¹⁹⁵Pt NMR (CD₃OD), δ : -152 (510 Hz). Crystals of the trimer for X-ray study were obtained by slow evaporation of an EtOH, EtOAc and acetone mixture solution at room temperature.

Crystal Structure Determinations. X-ray diffraction measurements were performed with Bruker X8 APEX II CCD diffractometer at 100 K. Single crystals were positioned at 35 mm from the detector, and 1557, 1865, 1853, 3003, and 1194 frames were measured, each for 60, 30, 5, 20, and 50 s over 1° scan width for 1, 4, 6, 9, and 11, respectively. The data were processed using SAINT software package.⁹² Crystal data, data

collection parameters, and structure refinement details for **1**, **4**, **6**, **9**, and **11** are given in Table 1. The structures were solved by direct methods and refined by full-matrix least-squares techniques. Non-hydrogen atoms were refined with anisotropic displacement parameters. H atoms were placed at calculated positions and refined as riding atoms in the subsequent least-squares model refinements. The isotropic thermal parameters were estimated to be 1.2 times the values of the equivalent isotropic thermal parameters of the non-hydrogen atoms to which hydrogen atoms were bonded. SHELXS-97⁹³ was used for structure solution and SHELXL-97⁹⁴ for refinement, molecular diagrams were produced with ORTEP.⁹⁵

Cell Lines and Culture Conditions. Human cancer cell lines: CH1 (ovarian carcinoma) kindly provided by Lloyd R. Kelland (CRC Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, UK), A549 (non-small cell lung cancer) and SW480 (colon carcinoma), both provided by Brigitte Marian (Institute of Cancer Research, Department of Medicine I, Medical University of Vienna, Austria) were used for the experiment. Cells were grown in 75 cm² culture flasks (Iwaki/Asahi Technoglass, Gyouda, Japan) as adherent monolayer cultures in complete medium [i.e., Minimal Essential Medium (MEM) supplemented with 10% heat-inactivated fetal bovine serum, 1 mM sodium pyruvate, 2 mM L-glutamine, and 1% nonessential amino acids (all purchased from Sigma-Aldrich, Austria)]. Cell cultures were incubated at 37 °C in a moist atmosphere containing 5% CO₂.

Cytotoxicity in Cancer Cell Lines. The cytotoxic activity of the compounds was determined by means of colorimetric microculture assay (MTT assay, MTT = 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide). A549, CH1 and SW480 cells were harvested from culture flasks by trypsinization and seeded in 100 µL aliquots in MEM into 96-well plates (Iwaki/Asahi Technoglass, Gyouda, Japan) in cell densities of 3×10³, 1×10³ and 2.5×10³ cells per well, respectively. After 24 h preincubation of the cells, the test compounds were dissolved in MEM, and 100 µL of solution was added per well. Compounds **5–9**, which are insufficiently soluble in medium, were dissolved in dimethyl sulfoxide and then diluted in MEM not to exceed the concentration of 0.5% DMSO in the applied solution. After continuous exposure for 96 h, drug solutions were replaced with 100 µL RPMI 1640 culture medium (supplemented with 10% heat-inactivated fetal bovine serum and 2 mM L-glutamine) plus 20 µL MTT solution in phosphate-buffered saline (5 mg/ml). After incubation for 4 h, the RPMI/MTT mixtures were removed, and the formazan crystals formed in viable cells were dissolved in 150 µL of DMSO per well. Optical densities were measured at 550 nm with a microplate reader (Tecan Spectra Classic), using a reference wavelength of 690 nm to correct for unspecific absorption. The quantity of viable cells was expressed in terms of T/C values by comparison to untreated control microcultures, and 50% inhibitory concentrations (IC₅₀) were calculated from concentration–effect curves by interpolation. Evaluation is based on means from at least three independent experiments, each comprising three replicates per concentration level.

Theoretical Calculations. All calculations were performed using the Gaussian 09 software package.⁹⁶ Geometry optimization and frequency calculations of the complex were carried out using the hybrid DFT method using B3LYP functional^{97,99} combined with LANL2DZ basis set. No solvation models were

employed: the results described are in the gas phase. Frequency calculation verified the absence of imaginary values.

Solution Behavior. For investigation of the behavior of monomer **1** and respective dimer **5** in aqueous solution, time-dependent ¹H NMR measurements were carried out in a 10 mM phosphate buffered D₂O solution at pH of 7.4 containing 0.125 M NaCl over three days. The concentrations of complexes were ca. 400 and 200 µM, correspondingly. Additionally, the hydrolysis experiment for **1** was performed at analogous conditions in the absence of NaCl. All NMR measurements were performed at ambient temperature.

ASSOCIATED CONTENT

Supporting Information. Description of synthesis and characterization of *trans*-bis(aldoxime)platinum(II) complexes, as well as X-ray structure determination for **1** and **4**. ORTEP view of **1** and **4**. Table with selected bond lengths (Å) and angles (deg) for complexes **1** and **4**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Supporting Information

Novel Oximato-Bridged Platinum(II) Di- and Trimer(s): Synthetic, Structural, and *in vitro* Anticancer Activity Studies

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1. Synthesis and Characterization of *trans*-Bis(aldoxime)platinum(II) Complexes.

2. X-Ray Structure Determination for Complexes **1** and **4**.

Figure S1. ORTEP view of **1** and **4**.

Table S1. Selected Bond Lengths (Å) and Angles (deg) for Complexes **1** and **4**.

1. Synthesis and Characterization of *trans*-Bis(aldoxime)platinum(II) Complexes.

Generation of the target bis(aldoxime)dichloridoplatinum(II) complexes of *trans*-configuration (**1–4**, Figure 1) was carried out by a reaction of potassium tetrachloridoplatinate with two equivalents of *E/Z*-R(H)C=NOH (R = Me, Et) in aqueous solution at room temperature. When the reaction was complete, a small amount of reddish undefined precipitate insoluble in common solvents was filtered off, water was removed and the mixture was suspended in chloroform (for R = Me) or diethyl ether (in case of R = Et), whereupon K[PtCl₃{R(H)C=NOH}] (23–26%) was isolated by filtration. Then, the filtrate was evaporated to dryness and the residue (a mixture of *cis*- and *trans*-[PtCl₂{R(H)C=NOH}₂]) was refluxed in nitromethane for 1 h in order to convert the *cis*-species into the *trans*-analogues. Although the reported thermal isomerization of acetoxime-containing platinum(II) complexes was performed in aqueous solution,^{1,2} MeNO₂ was used as solvent due to its inertness in order to avoid possible side-reactions/decomposition. As a result, a mixture of *trans*-[PtCl₂{(Z)-R(H)C=NOH}₂] and *trans*-[PtCl₂{(E)-R(H)C=NOH}{(Z)-R(H)C=NOH}] was obtained and the isomers were then separated by column chromatography. An additional amount of target species **1–4** was isolated after the reaction of K[PtCl₃{R(H)C=NOH}] with a further equiv of the corresponding aldoxime in aqueous solution, resulting in overall yields of 31–35% for complexes **1–4**.

Compounds **1–4** were characterized by elemental analyses, ¹H, ¹³C{¹H}, and ¹⁹⁵Pt NMR and IR spectroscopy, electrospray ionization mass spectrometry (ESI-MS), and also by X-ray crystallography (for **1** and **4**). C, H, and N elemental analyses were satisfactory for all complexes. ESI-MS spectra displayed in the positive ion mode the fragments [M + Na]⁺ and [M + K]⁺ having isotopic patterns that were in agreement with calculated ones. In the IR spectra of **1–4** strong ν(OH) [3202–3338 cm⁻¹], medium ν(C=N) [1666–1677 cm⁻¹] and strong ν(NO) [970–985 cm⁻¹]

¹ Kukushkin, V. Yu.; Belsky, V. K.; Aleksandrova, E. A.; Konovalov, V. E.; Kirakosyan, G. A. *Inorg. Chem.* **1992**, *31*, 3836–3840.

² Kukushkin, V. Yu.; Tudela, D.; Izotova, Y. A.; Belsky, V. K.; Stash, A. I. *Polyhedron* **1998**, *17*, 2455–2461.

stretching vibrations were detected. As expected, in the ^1H NMR spectra one set of oxime proton signals was observed for symmetric complexes **1** and **3**, while for complexes **2** and **4**, bearing two isomeric oxime ligands, two sets of corresponding signals were detected. It is worth mentioning that the signals related to the *E*-ligands were shifted to lower field as compared to those of the coordinated *Z*-isomers. Concerning ^{195}Pt NMR spectra, a significant difference in chemical shifts was observed between symmetric species **1** and **3**, containing two *Z*-ligands ($\delta_{\text{Pt}} = -479$ and -490 ppm, respectively) and their isomers **2** and **4**, featuring both *E*- and *Z*-aldoximes ($\delta_{\text{Pt}} = -532$ and -529 ppm, correspondingly).

2. X-ray Structure Determination for Complexes 1 and 4. The structures of *trans*-[PtCl₂{(Z)-Me(H)C=NOH}₂] (**1**) and *trans*-[PtCl₂{(E)-Et(H)C=NOH}{(Z)-Et(H)C=NOH}] (**4**) were determined by X-ray diffraction (Tables 1 and 1S, Figure 1S). The coordination geometries of the two complexes are slightly distorted square-planar. The deviation of the platinum atom in complex **4** from the mean plane through N1–Cl1–N1–Cl1 is 0.02 Å, while **1** lies ideally within the corresponding N1–Cl1–N2–Cl2 plane. In both compounds, the platinum center is coordinated by two oximic nitrogen atoms and two chlorido ligands, exhibiting *trans* configuration. The Pt–Cl bond distances are in the range from 2.3046(19) to 2.307(2) Å, and these distances are in a good agreement with the reported dichlorido platinum(II) species of *trans* geometry [2.282–2.321 Å].^{3,4,5} The Pt–N bond lengths [2.002(3)–2.019(6) Å] correlate well with the corresponding bond distances in previously characterized *trans*-bis(oxime)platinum(II) complexes [1.974–2.016 Å].^{2,6} In the coordinated aldoximes, the values of C=N bond lengths are between 1.277(9) and 1.281(4)

³ Scaffidi-Domianello, Yu. Yu.; Meelich, K.; Jakupc, M. A.; Arion, V. B.; Kukushkin, V. Yu.; Galanski, M.; Keppler, B. K. *Inorg. Chem.* **2010**, *49*, 5669–5678.

⁴ Scaffidi-Domianello, Yu. Yu.; Nazarov, A. A.; Haukka, M.; Galanski, M.; Keppler, B. K.; Schneider, J.; Du, P.; Eisenberg, R.; Kukushkin, V. Yu. *Inorg. Chem.* **2007**, *46*, 4469–4482.

⁵ Scaffidi-Domianello, Yu. Yu.; Haukka, M.; Kelly, P.F.; Galanski, M.; Keppler, B. K.; Kukushkin, V. Yu. *Z. Kristallogr. NCS* **2006**, *221*, 226–228.

⁶ Kaplan, S. F.; Kukushkin, V. Yu.; Shova, S.; Suwinska, K.; Wagner, G.; Pombeiro, A. J. L. *Eur. J. Inorg. Chem.* **2001**, 1031–1038.

Å being in good agreement with distances of the C=N double bond in platinum(II)-bound oxime ligands [1.268–1.302 Å] reported recently.^{2,5,6}

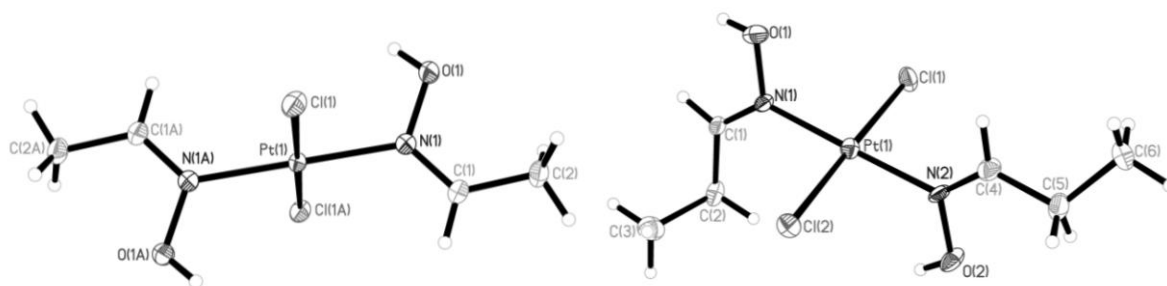


Figure S1. ORTEP view of **1** (left) and **4** (right) with the atom labeling scheme; the thermal ellipsoids have been drawn at the 50% probability level.

Table S1. Selected Bond Lengths (Å) and Angles (deg) for Complexes **1** and **4**

	1	4
Pt(1)–N(1)	2.002(3)	2.019(6)
Pt(1)–N(2)		2.006(6)
Pt(1)–Cl(1)	2.3066(8)	2.307(2)
Pt(1)–Cl(2)		2.3046(19)
N(1)–C(1)	1.281(4)	1.277(9)
N(2)–C(4)		1.279(10)
N(1)–Pt(1)–Cl(1)	89.53(8)	90.28(18)
N(1)–Pt(1)–N(1A)	180.0	
N(1)–Pt(1)–Cl(1A)	90.47(8)	
N(2)–Pt(1)–N(1)		178.6(3)
N(2)–Pt(1)–Cl(1)		89.55(19)

III. CONCLUSIONS

In this thesis a series of novel platinum(II) complexes featuring diverse oxime ligands was prepared, characterized and their biological activity was investigated *in vitro*.

In the first project, various structural modifications of *cis*- and *trans*-platinum(II) compounds with coordinated acetone oxime were carried out. With the aim of setting up and optimizing structure-activity relationships for platinum(II)(oxime) compounds, *cis*-/*trans*-[PtX₂(R₂C=NOH)₂] [X = Cl, Br, I; R = Me, Et, *n*-Pr, *i*-Pr] as well as *cis*-/*trans*-[Pt(NH₃)₂(R₂C=NOH)₂](NO₃)₂ [R = Me, Et, *n*-Pr] were generated and characterized by elemental analysis, IR and electrospray mass spectrometry (ESI-MS), ¹H, ¹³C, and ¹⁹⁵Pt NMR spectroscopy and, in five cases, by X-ray crystallography.

Interestingly, two of the prepared complexes, namely, *trans*-[PtI₂(Me₂C=NOH)₂] and *trans*-[Pt(NH₃)₂(*n*-Pr₂C=NOH)₂](NO₃)₂, were proved to exist in solution as a mixture of two temperature-dependent conformations. This phenomenon could be explained by a hindered rotation of the coordinated oximes around the Pt–N bond due to the sterically demanding iodo ligands or *n*-propyl groups, respectively.

All synthesized platinum(II)(oxime) complexes were evaluated *in vitro* in cisplatin-sensitive ovarian carcinoma (CH1) and intrinsically cisplatin-resistant colon carcinoma (SW480) cell lines. Unlike cisplatin itself, *cis*-/*trans*-[PtX₂(R₂C=NOH)₂] complexes showed comparable IC₅₀ values in both cell lines, which indicates a powerful potential to circumvent cisplatin resistance. As expected, the lower reactive bromido species were found to be less cytotoxic than the chlorido analogues. Differently, the iodo compounds exhibited enhanced cytotoxicity as compared to the bromido species, probably based on a higher lipophilicity favoring cellular uptake of the complexes. Considering *cis*-/*trans*-[PtCl₂(R₂C=NOH)₂], the complexes containing acetone oxime, 4-heptanone oxime and 2,4-dimethyl-3-pentanone oxime ligands proved to be similarly cytotoxic, whereas the pentanone oxime species were significantly less active. As the cationic complexes *cis*-/*trans*-[Pt(NH₃)₂(R₂C=NOH)₂](NO₃)₂ are concerned, no considerable cytotoxicity was observed, as it could be expected for the charged species exhibiting four N-donor ligands. Importantly, a behavior clashing the early structure-activity rules postulated by Cleare and Hoeschele, namely, a superior cytotoxicity of *trans*-complexes as compared to their *cis*-analogues, was observed for two additional *cis*-/*trans*-oximeplatinum(II) couples.

In the second part of the thesis, four zwitterionic diam(m)ine(3-hydroxy-2-(oxidoimino)propan-1-olato-κ²N,O)platinum(II) complexes [diam(m)ine = (ammonia)₂, (ethylamine)₂, ethane-1,2-diamine, and (*R,R*)-cyclohexane-1,2-diamine] were prepared as potential prodrugs activatable under slightly acidic conditions, as characteristic for many solid tumors. Further, the oximato group in the compounds comprising ethane-1,2-diamine or (*R,R*)-cyclohexane-1,2-diamine ligands was protonated by a reaction with hydrochloric acid and consequently a chelate ring opening was performed.

All complexes were characterized by elemental analysis, IR and electrospray mass spectrometry (ESI-MS), and multinuclear (^1H , ^{13}C , and ^{195}Pt) NMR spectroscopy. Additionally, ^1H , ^{195}Pt -HMQC NMR spectra of the zwitterionic species were recorded, proving the *N,O*-chelate formation. Furthermore, the crystal structure of $(\text{SP-4-3})\text{-}\{(R,R)\text{-cyclohexane-1,2-diamine}\}(3\text{-hydroxy-2-(oxidoimino)propan-1-olato-}\kappa^2\text{N,O})\text{platinum(II)}$ was determined by X-ray diffraction, providing a further confirmation for the double deprotonation of the 1,3-dihydroxiacetone oxime ligand.

Cytotoxic activity of the prepared complexes was evaluated by means of the MTT assay in three human cancer cell lines: ovarian carcinoma (CH1), colon carcinoma (SW480), and nonsmall-cell lung cancer (A549). Among the zwitterionic chelated species, compounds featuring two monodentate amine ligands proved to be more cytotoxic in CH1 cells as compared to those comprising a bidentate diamine, showing IC_{50} values in the medium or even low micromolar range. An analogous tendency was observed in inherently cisplatin-resistant SW480 and A549 cells, with exception of an enhanced cytotoxicity of $(\text{SP-4-3})\text{-}\{(R,R)\text{-cyclohexane-1,2-diamine}\}(3\text{-hydroxy-2-(oxidoimino)propan-1-olato-}\kappa^2\text{N,O})\text{platinum(II)}$.

The latter complex showed a completely different activity profile reminding that of oxaliplatin.

The solution behavior of compounds featuring the ethane-1,2-diamine ligand was investigated *via* acquisition of time-dependent ^1H NMR spectra at physiologically relevant proton concentrations. It was found that the zwitterionic complex stayed unchanged both at pH 7.4 and 6.0, whereas the protonated species were deprotonated at pH 7.4 and partially converted to an open-chain complex in a slightly acidic milieu (pH 6.0). As the open-chain compound is concerned, it was found to stay unchanged at pH 6.0, while at pH 7.4 a complete ring-closure was observed.

Evaluation of the pH dependent cytotoxic potency for two latter complexes showed up to 4-fold improvement of the IC_{50} values at pH 6.0 as compared to those obtained at pH 7.4.

In order to reveal if the monodentately bound 1,3-dihydroxiacetone oxime could act as a leaving group producing bisadducts with DNA, binding of $(\text{SP-4-3})\text{-chlorido}\{(R,R)\text{-cyclohexane-1,2-diamine}\}(1,3\text{-dihydroxiacetone oxime-}\kappa\text{N})\text{platinum(II) chloride}$ towards a model nucleotide 5'-GMP was investigated using ESI-MS. Formation of the Pt-5'-GMP bisadduct was proven, although it was produced to a significantly lower extend as compared to bifunctional $[\text{PtCl}_2\{(R,R)\text{-cyclohexane-1,2-diamine}\}]$ used as a control. Thereby, the oxime ligand could be rated as a leaving group.

In the third project, platinum(II)(aldoxime) complexes *trans*- $[\text{PtCl}_2\{(Z)\text{-R(H)C=NOH}\}_2]$ and *trans*- $[\text{PtCl}_2\{(E)\text{-R(H)C=NOH}\}\{(Z)\text{-R(H)C=NOH}\}]$ [$\text{R} = \text{Me, Et}$] featuring an enhanced aqueous solubility as compared to the ketoxime analogues were prepared by a reaction of $\text{K}_2[\text{PtCl}_4]$ with *E/Z*-isomeric mixture of the corresponding aldoximes, followed by a thermal *cis*→*trans*-isomerization in MeNO_2 solution and column separation of the isomers.

Further, oximato-bridged dimers of the novel platinum(II)(aldoxime) species and *trans*- $[\text{PtCl}_2(\text{R}_2\text{C=NOH})_2]$ complexes of general formula $[\text{PtCl}(\mu\text{-RR'C=NO})(\text{RR'C=NOH})]_2$ [$\text{R} = \text{Me, Et}$; $\text{R}' = \text{H, Me, Et}$] were generated by addition of an equivalent amount of silver acetate to the respective monomer. To the best of our knowledge, the dimeric complexes represent the first example of oxime/oximato ligands coordinated to

platinum *via* the oxygen atom and feature a unique six-membered diplatinacycle. No oximato-bridged platinum complexes have been reported by now.

Monomeric and dimeric species were completely characterized by elemental analysis, IR and electrospray mass spectrometry (ESI-MS), ^1H , ^{13}C , and ^{195}Pt NMR spectroscopy and, in four cases, by X-ray crystallography. Additionally, the crystal structure of a pentanone oximato-bridged trimer comprising three platinum coordination units connected in a chain manner was determined.

Behavior of *trans*- $[\text{PtCl}_2\{(\text{Z})\text{-Me(H)C=NOH}\}_2]$ and its corresponding dimer in aqueous solution was investigated under simulated physiological conditions (pH 7.4, 0.125 M NaCl) by ^1H NMR spectroscopy, leading to the formation of practically identical equilibrium mixtures, including the monomer, its hydrolyzed product, the dimer, and an additional species, supposed to have a ring-shaped trimeric structure.

Hydrolysis of *trans*- $[\text{PtCl}_2\{(\text{Z})\text{-Me(H)C=NOH}\}_2]$ was studied in aqueous solution at pH 7.4 in the absence of NaCl, leading firstly to formation of the hydrolyzed product, followed by a partial dimerization, finally being completely converted into the previously mentioned trimeric compound.

Structure optimization for the ring-shaped acetaldoximato-bridged trimer was performed, demonstrating only a slight deviation of the geometric parameters from those in oximato-bridged dimeric structures determined by X-ray diffraction.

The cytotoxic activity of the prepared monomers and dimers was investigated *in vitro* in three human cancer cell lines (CH1, SW480, and A549), showing significantly lower IC_{50} values (up to 6.6 times) for the dimers as compared to the corresponding monomers. All complexes exhibited similar cytotoxicity in cisplatin-sensitive CH1 and intrinsically cisplatin-resistant SW480 cell lines, indicating capability to circumvent cisplatin resistance.

IV. CURRICULUM VITAE

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Winter term 2006	Research work at the Institute of Inorganic Chemistry, University of Vienna
9.2005	Master of Science (University of Vienna), First Class with Distinction "Sulfoxide Platinum(II) Complexes with Imines and Heteroimines: Synthesis and Reactivity"
Summer term 2005	University of Vienna, Faculty of Chemistry
May 15, 2005	Master of Science (St.-Petersburg State University),

First Class with Honor

“Sulfoxide Platinum(II) Complexes with Imines and Heteroimines:
Synthesis and Reactivity”

2000 - 2005

St.-Petersburg State University, Faculty of Chemistry

1989 – 2000

Elementary and Grammar School, Russia

PROFESSIONAL SKILLS AND RESEARCH INTERESTS

Biological Inorganic Chemistry, Coordination and Organometallic Chemistry, Medicinal Chemistry,
Analytical Chemistry (HPLC, Mass Spectrometry)

AWARDS AND FELLOWSHIPS

01.01.08 – 31.12.08

Research Fellowship of the University of Vienna (Forschungsstipendium)
“New Potentially Antitumor-Active Platinum(II) Oxime Complexes”

LANGUAGE SKILLS

German	fluently
English	fluently
Russian	native language

PUBLICATIONS IN SCIENTIFIC JOURNALS

Yu. Yu. Scaffidi-Domianello, A. Legin, M. A. Jakupec, A. Roller, V. Yu. Kukushkin, M. Galanski, B. K. Keppler **Novel Oximato-Bridged Platinum(II) Di- and Trimer(s): Synthetic, Structural, and in vitro Anticancer Activity Studies** *Inorg Chem.*, submitted.

C. Bartel, A. K. Bytze, Yu. Yu. Scaffidi-Domianello, G. Grabmann, M. A. Jakupec, C. G. Hartinger, V. Yu. Kukushkin, M. Galanski, B. K. Keppler **Cellular Accumulation and DNA Interaction Studies of Cytotoxic Trans-Platinum Anticancer Compounds** *J. Biol. Inorg. Chem.*, accepted.

Yu. Yu. Scaffidi-Domianello, A. Legin, M. A. Jakupec, V. B. Arion, V. Yu. Kukushkin, M. Galanski, B. K. Keppler **Synthesis, Characterization, and Cytotoxic Activity of Novel Potentially pH-Sensitive Nonclassical Platinum(II) Complexes Featuring 1,3-Dihydroxyacetone Oxime Ligands** *Inorg. Chem.*, **2011**, 50, 10673–10681.

Yu. Yu. Scaffidi-Domianello, K. Meelich, M. A. Jakupec, V. B. Arion, V. Yu. Kukushkin, M. Galanski, B. K. Keppler **Novel Cis- and Trans-Configured Bis(oxime)platinum(II) Complexes: Synthesis, Characterization, and Cytotoxic Activity** *Inorg Chem.*, **2010**, 49, 5669–5678.

Yu. Yu. Scaffidi-Domianello, A. A. Nazarov, M. Haukka, M. Galanski, B. K. Keppler, J. Schneider, P. Du, R. Eisenberg, and V. Yu. Kukushkin **First Example of the Solid-State Thermal Cyclometalation of Ligated Benzophenone Imine Giving Novel Luminescent Platinum(II) Species** *Inorg Chem.*, **2007**, 46, 4469–4482.

Yu. Yu. Scaffidi-Domianello, M. Haukka, P. F. Kelly, M. Galanski, B. K. Keppler, V. Yu. Kukushkin **Crystal Structure of Trans-Dichloro(dimethylsulfoxide)-(diphenylsulfimide)platinum(II) toluene hemisolvate, $\text{PtCl}_2(\text{C}_2\text{H}_6\text{SO})(\text{C}_{12}\text{H}_{10}\text{SNH}) \cdot \frac{1}{2}\text{C}_7\text{H}_8$** *Z. Kristallogr. NCS*, **2006**, 221, 226–228.

Yu. Yu. Novoselova, B. K. Keppler, M. Galanski, V. Yu. Kukushkin **Different Behaviour of the Coordinated Imines in Platinum(II) Complexes upon Heating Them in the Solid State**, *Transactions of the XI International Conference of Students and PhD Students on Fundamental Sciences "Lomonosov-2004"*, Moscow, MGU, **2004**, 58.

Yu. Yu. Novoselova, V. Yu. Kukushkin, B. K. Keppler, M. Galanski **Sulfoxide Platinum(II) Complexes with Imines and Heteroimines. Synthesis and Reactivity**, *Proceedings of the III^d Scientific Session UNCH*, St. Petersburg, NIIH, **2004**, 209.

PRESENTATIONS AT CONFERENCES

Yu. Yu. Novoselova, V. Yu. Kukushkin, B. K. Keppler, M. Galanski **Sulfoxide platinum(II) complexes with imines and heteroimines. Synthesis and reactivity** III^d Scientific Session UNCH, St. Petersburg, Russia **2004**. Poster presentation.

Yu. Yu. Novoselova, B. K. Keppler, M. Galansky, V. Yu. Kukushkin **Different behaviour of the coordinated imines in platinum(II) complexes upon heating them in the solid state** XIth International Conference of Students and PhD Students on Fundamental Sciences "Lomonosov-2004", Moscow, MGU, Russia **2004**. Oral Communication.