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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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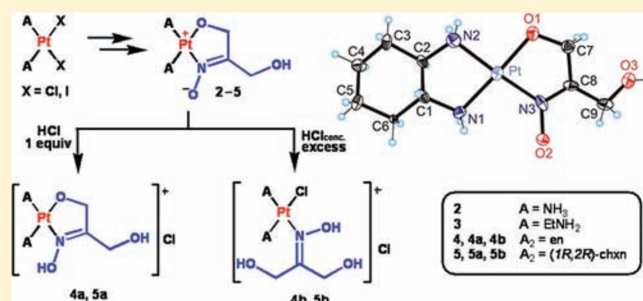
S Supporting Information

ABSTRACT: The reaction of 1,3-dihydroxyacetone oxime with diam(m)minediaquaplatinum(II) under basic conditions produced zwitterionic diam(m)mine(3-hydroxy-2-(oxidoimino)propan-1-olato-κ^2N,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 (^{1}H, ^{13}C, and ^{195}Pt) 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 IC_{50} values are in the medium or even low micromolar range, remarkable for platinum complexes having N_3O or N_3Cl coordination spheres. To study the solution behavior of the prepared complexes at physiologically relevant proton concentrations, time-dependent ^{1}H 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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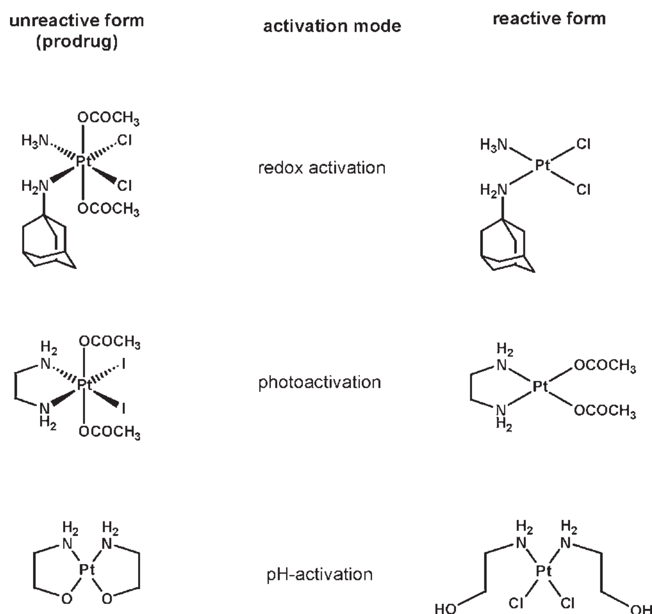


Figure 1. Strategies to activate rather unreactive platinum prodrugs in the tumor tissue.

(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

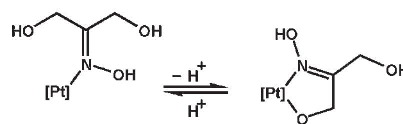


Figure 2. pH-Dependent intramolecular chelation and ring opening of the ligated 1,3-dihydroxyacetone oxime.

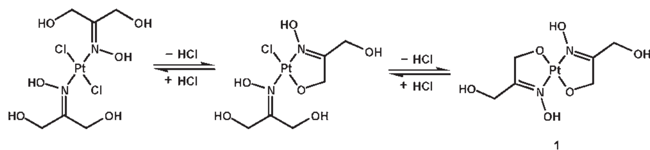


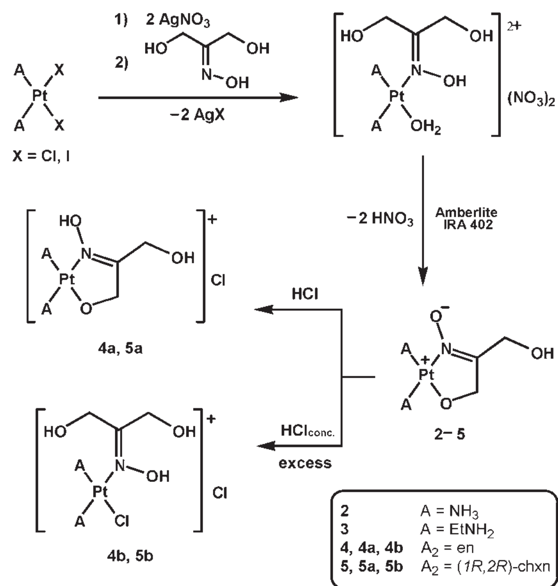
Figure 3. pH-Dependent equilibrium mixture of the opened-, mono-, and bischelated oximeplatinum species.

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 hydroxo 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 $K_2[PtCl_4]$ 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*-$[PtX_2A_2]$, where X = chloride or iodide and A_2 = $(\text{ammine})_2$, $(\text{ethylamine})_2$, 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 $K_2[PtX_4]$ with the corresponding am(m)ine or diamine by a reported method.[43,44] Further, halide abstraction using $AgNO_3$ 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 hydroxo group and resulting in the formation of zwitterionic oximato 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



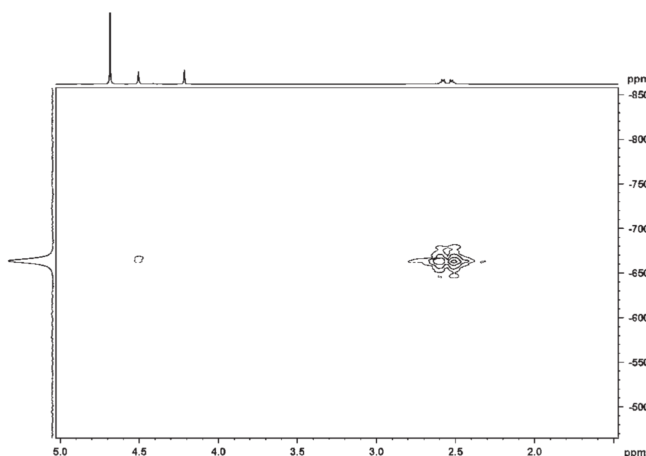


Figure 4. $^{1}H,^{195}Pt$ HMQC NMR spectrum of **4**.

Complexes **1**−**5** were characterized by elemental analyses; ^{1}H, $^{13}C\{^{1}H\}$, 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 $[M - H]^-$, while in the spectra of **2**−**5**, $[M + H]^+$ and $[M + Na]^+$ 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 ν(OH) and ν(NH) (for **2**−**5**) [2808−3474 cm^{-1}] and medium ν(C=N) [1602−1630 cm^{-1}] stretching vibrations. Chelation of the 1,3-dihydroxyacetone oxime ligand was confirmed (e.g., complex **4**) *via* acquisition of an $^{1}H,^{195}Pt$-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 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 [1.993−2.032 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**.

Table 1. Crystal Data and Details of Data Collection for $5 \cdot 2H_2O$

	$5 \cdot 2H_2O$
empirical formula	$C_9H_{23}N_3O_5Pt$
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 × 0.02 × 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] $R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$. [b] $wR_2 = \{\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2}$. [c] GOF $= \{\Sigma[w(F_o^2 - F_c^2)^2]/(n - p)\}^{1/2}$, where n is the number of reflections and p is the total number of parameters refined.

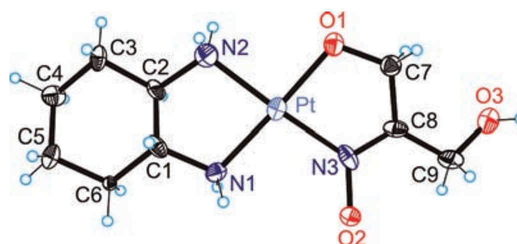


Figure 5. ORTEP view of **5** with the atom labeling scheme. The thermal ellipsoids have been drawn at the 50% probability level. Selected bond lengths (Å) and bond angles (deg): Pt−N1 = 2.040(11), Pt−N2 = 2.054(11), Pt−N3 = 1.997(13), Pt−O1 = 2.006(9); N1−Pt−N2 = 83.4(4), O1−Pt−N3 = 82.5(4).

Protonation of the Oximato 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 (pH ≈ 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, $[M - Cl]^+$ and $[M + H]^+$ peaks were detected for **4a** and **5a**, whereas the ESI-MS spectra of **4b** and **5b** exhibited $[M - HCl - Cl]^+$ and $[M - Cl]^+$ signals. The IR spectra of the complexes displayed the characteristic ν(OH) and ν(NH) [2862−3394 cm^{-1}] bands of strong intensity, as well as medium-intensive ν(C═N) [1613−1675 cm^{-1}] bands. In the ^{1}H 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 (δ_{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 (δ_{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 ^{1}H,^{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 ^{1}H 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 ~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 ~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)

	IC_{50} [μM]		
compound	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 μM 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)

	GI_{50} [μM]			
	SW480		A549	
compound	pH 7.4	pH 6	pH 7.4	pH 6
4a	>640	590 ± 57	>640	373 ± 103
4b	231 ± 42	127 ± 22	438 ± 51	113 ± 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, IC_{50} 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 IC_{50} 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 IC_{50} 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 $[M_{\mathbf{5b}} - HCl - Cl]^+$ and 450 $[M_{\mathbf{5b}} - Cl]^+$) was reduced with time in comparison to the peak related to 5′-GMP (408 $[M_{5'\text{-GMP}} + 2Na - H]^+$; 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 $[M_{\mathbf{5b}} - HCl - Cl + GMP]^+$ ($C_{19}H_{34}N_8O_{11}PPt$, 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(*1R*,*2R*-chxn)Cl_2] 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(*1R*,*2R*-chxn)Cl_2] was reacted with 1 equiv of $AgNO_3$ 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 $[M_{[Pt(1R,2R\text{-chxn})Cl_2]} - Cl + GMP]^+$ ($C_{16}H_{28}ClN_7O_8PPt$, 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 $[M_{[Pt(1R,2R\text{-chxn})Cl_2]} - HCl - Cl + 2GMP]^+$ ($C_{26}H_{41}N_{12}O_{16}P_2Pt$, calcd m/z 1034.70) and 1056 $[M_{[Pt(1R,2R\text{-chxn})Cl_2]} - 2HCl + 2GMP + Na]^+$ ($C_{26}H_{40}N_{12}O_{16}P_2PtNa$, 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)Cl_2] 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 $[M_{5b} - 2Cl - \text{oxime} + 2GMP - 4H]^{2-}$ $C_{26}H_{38}N_{12}O_{16}P_2Pt$, calcd m/z 515.84), although to a significantly lesser extent compared to [Pt(1*R*,2*R*-chxn)Cl_2]. 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-κ^2N,O)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 IC_{50} 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 oximato 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*-[$PtI_2(NH_3)_2$], *cis*-[$PtI_2(EtNH_2)_2$], [$PtCl_2$(en)], and [$PtCl_2${(1*R*,2*R*)-chxn}] were prepared according to standard literature procedures starting from $K_2[PtCl_4]$.[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, $^{13}C\{1H\}$, ^{195}Pt, and two-dimensional NMR spectra were measured with a Bruker Avance III 500 MHz NMR spectrometer at 500.32 (^1H), 125.81 (^{13}C), and 107.55 MHz (^{195}Pt), correspondingly, at 298 K. ^{195}Pt chemical shifts are referenced relative to $K_2[PtCl_4]$, 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). $K_2[PtCl_4]$ (121.0 mg, 0.29 mmol) was dissolved in 2.5 mL of H_2O; 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 $C_6H_{12}N_2O_6Pt$: C, 17.87; H, 3.00; N, 6.95. Found: C, 18.02; H, 2.82; N, 6.89%. ESI-MS$^-$ (H_2O/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 (D_2O): δ 4.59 (s, br, OCH_2,), 4.28 (s, br, $HOCH_2$). $^{13}C\{^1H\}$ NMR (D_2O): δ 166.2 (*C*=N), 71.8 (OCH_2), 55.3 ($HOCH_2$). ^{195}Pt NMR (D_2O): δ −137 (780 Hz).

General Synthesis of (SP-4−3)-Diam(m)ine(3-hydroxy-2-(oxidoimino)propan-1-olato-κ²N,O)platinum(II). [PtX_2A_2] (0.50 mmol) and $AgNO_3$ (1.0 mmol) were mixed and suspended in 10 mL of H_2O, 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 H_2O 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 H_2O and subsequently mixed with approximately 3 g of a preconditioned basic anion exchange resign, 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 (H_2O/MeOH 98/2 for **2** and **4**, H_2O/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 H_2O, 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 $C_3H_{11}N_3O_3Pt\cdot 2H_2O$: C, 9.78; H, 4.11; N, 11.41. Found: C, 10.18; H, 3.77; N, 11.05%. ESI-MS$^+$ (H_2O/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 (D_2O): δ 4.51 (m, 2H, OCH_2), 4.23 (m, 2H, $HOCH_2$). $^{13}C\{^1H\}$ NMR (D_2O): δ 168.1 (C=N), 73.6 (OCH_2), 56 ($HOCH_2$). ^{195}Pt NMR (D_2O): δ −527 (800 Hz).

(SP-4−3)-Bis(ethylamine)(3-hydroxy-2-(oxidoimino)propan-1-olato-κ²N,O)platinum(II) (3). Anal. Calcd. for $C_7H_{19}N_3O_3Pt\cdot 1/2H_2O$: C, 21.16; H, 5.07; N, 10.58. Found: C, 20.90; H, 4.66; N, 10.21%. ESI-MS$^+$ (H_2O/MeOH), *m*/*z*: 344 $[M-EtNH_2+H]^+$, 389 $[M+H]^+$, 411 $[M+Na]^+$. ESI-MS$^-$ (H_2O/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 (D_2O): δ 4.57 (m, 2H, OCH_2), 4.30 (m, 2H, $HOCH_2$), 2.66 (m, 4H, $2CH_3CH_2$), 1.17 (t, 6H, $2CH_3$, $^3J_{H,H}$ = 6.9 Hz). $^{13}C\{^1H\}$ NMR (D_2O): δ 176.2 (*C*=N), 73.1 (OCH_2), 56.3 ($HOCH_2$), 41.9 (CH_3CH_2), 41.3 (CH_3CH_2), 15.6 ($2CH_3$). ^{195}Pt NMR (D_2O): 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 $C_5H_{13}N_3O_3Pt\cdot H_2O$: C, 16.35; H, 3.84; N, 11.44. Found: C, 16.68; H, 3.45; N, 11.14%. ESI-MS$^+$ (H_2O/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 (D_2O): δ 4.50 (m, 2H, OCH_2), 4.22 (m, 2H, $HOCH_2$), 2.55 (m, 4H, $2CH_2$). $^{13}C\{^1H\}$ NMR (D_2O): δ 168.3 (C=N), 73.6 (OCH_2), 55.9 ($HOCH_2$), 46.43 (CH_2), 46.36 (CH_2). ^{195}Pt NMR (D_2O): 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 $C_9H_{19}N_3O_3Pt\cdot H_2O$: C, 25.12; H, 4.92; N, 9.76. Found: C, 24.78; H, 4.71; N, 9.49%. ESI-MS$^+$ (H_2O/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, OCH_2), 4.34 (m, 2H, $HOCH_2$), 2.32 (m, 2H, 2C*H*), 2.03 (m, 2H, CH_2), 1.64 (m, 2H, CH_2), 1.33 (m, 2H, CH_2), 1.23 (m, 2H, CH_2). $^{13}C\{^1H\}$ NMR (MeOD): δ 167.5 (*C*=N), 74.2 (OCH_2), 61.2 (CH), 60.9 (CH), 56.5 ($HOCH_2$), 32.7 (CH_2), 32.5 (CH_2), 24.3 (CH_2), 24.2 (CH_2). ^{195}Pt 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) Oximato 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 H_2O, 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^2N,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 ν(OH) and ν(NH), 1675 m ν(C=N). ^{1}H NMR (D_2O): δ 5.16 (m, 4H, 2NH_2), 4.67 (s, 2H, OCH_2), 4.33 (s, 2H, HOCH_2), 2.55 (m, 4H, 2CH_2). ^{13}C{^{1}H} NMR (D_2O): δ 160.2 (C=N), 73.8 (OCH$_2$), 56.2 (HOCH$_2$), 46.7 (CH$_2$), 46.5 (CH$_2$). ^{195}Pt NMR (D_2O): δ −666 (960 Hz).

(SP-4−3)-{(R,R)-Cyclohexane-1,2-diamine}(3-hydroxy-2-(hydroxyimino)propan-1-olato-κ^2N,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 ν(OH) and ν(NH), 1613 m ν(C=N). ^{1}H NMR (D_2O): δ 4.60 (s, 2H, OCH_2), 4.28 (s, 2H, HOCH_2), 2.32 (m, 2H, 2CH), 1.97 (m, 2H, CH_2), 1.51 (m, 2H, CH_2), 1.22 (m, 2H, CH_2), 1.09 (m, 2H, CH_2). ^{13}C{^{1}H} NMR (D_2O): δ 174.9 (C=N), 73.8 (OCH$_2$), 61.1 (CH), 61.0 (CH), 56.1 (HOCH$_2$), 32.3 (CH$_2$), 32.2 (CH$_2$), 24.0 (CH$_2$), 23.9 (CH$_2$). ^{195}Pt 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 H_2O, 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-dihydroxoacetone Oxime-κN)-(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 ν(OH) and ν(NH), 1647 m ν(C=N). ^{1}H NMR (D_2O): δ 5.57 (m, 2H, 2NH_2), 5.37 (m, 2H, 2NH_2), 4.97 (s, 2H, HOCH_2), 4.60 (s, 2H, HOCH_2), 2.61 (m, 4H, 2CH_2). ^{13}C{^{1}H} NMR (D_2O): δ 169.2 (C=N), 60.6 (HOCH$_2$), 57.3 (HOCH$_2$), 48.2 (CH$_2$), 47.7 (CH$_2$). ^{195}Pt NMR (D_2O): δ −978 (670 Hz).

(SP-4−3)-Chlorido{(R,R)-cyclohexane-1,2-diamine}(1,3-dihydroxyacetone Oxime-κN)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 ν(OH) and ν(NH), 1647 m ν(C=N). ^{1}H NMR (D_2O): δ 5.91 (m, 1H, NHH), 5.62 (m, 1H, NHH), 5.04 (m, 2H, 2NHH), 4.98 (s, 2H, HOCH_2), 4.60 (s, 2H, HOCH_2), 2.46 (m, 1H, CH), 2.38 (m, 1H, CH), 1.99 (m, 2H, CH_2), 1.53 (m, 2H, CH_2), 1.26 (m, 2H, CH_2), 1.09 (m, 2H, CH_2). ^{13}C{^{1}H} NMR (D_2O): δ 169.1 (C=N), 62.8 (HOCH$_2$), 62.2 (HOCH$_2$), 60.7 (CH), 57.3 (CH), 32.0 (CH$_2$), 31.8 (CH$_2$), 23.8 (CH$_2$), 23.7 (CH$_2$). ^{195}Pt NMR (D_2O): δ −945 (670 Hz).

^{1}H 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, HOCH_2), 2.54 (m, 2H, 2CH), 2.10 (m, 2H, CH_2), 1.57 (m, 2H, CH_2), 1.49 (m, 2H, CH_2), 1.15 (m, 2H, CH_2). ^{13}C{^{1}H} NMR (DMF-d_6): δ 170.5 (C=N), 62.8 (HOCH$_2$), 62.3 (HOCH$_2$), 60.8 (CH), 57.1 (CH), 32.3 (CH$_2$), 31.7 (CH$_2$), 24.5 (CH$_2$), 24.3 (CH$_2$). ^{15}N NMR (DMF-d_6): δ −22.6 (NH$_2$), −10.7 (NH$_2$). ^{195}Pt NMR (DMF-d_6): δ −933 (500 Hz).

Crystal Structure Determination. X-ray diffraction measurement for $5\cdot 2H_2O$ 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-97*[52] 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 cm^2 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% CO_2 (standard conditions) or 8% CO_2 (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 μL 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 μL per well. After continuous exposure for 96 h, drug solutions were replaced with 100 μL of RPMI 1640 medium (supplemented with 10% heat-inactivated fetal bovine serum and 2 mM L-glutamine) plus 20 μL 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 μL 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 (IC_{50}) 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 μL 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 μL per well) for another 24 h in a moist atmosphere containing 8% CO_2 at 37 °C (for drugs dissolved in DMEM pH 6) and 5% CO_2 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(*1R,2R*-chxn)Cl_2] 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 negaive ion mode.

■ ASSOCIATED CONTENT

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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