Inorganic Chemistry

Article

pubs.acs.org/IC

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

Yulia Yu. Scaffidi-Domianello,[†] Anton A. Legin,[†,‡] Michael A. Jakupec,[†,‡] Alexander Roller,[†] Vadim Yu. Kukushkin,[§] Markus Galanski,*[,†] and Bernhard K. Keppler*[,†]

[†]Institute of Inorganic Chemistry and [‡]Research Platform "Translational Cancer Therapy Research", University of Vienna, Währinger Strasse 42, A-1090 Vienna, Austria

[§]Department of Chemistry, St. Petersburg State University, 198504 Stary Petergof, Russian Federation

Supporting Information

**ABSTRACT:** Novel platinum complexes of trans geometry $[PtCl_2\{(Z)\text{-}R(H)C{=}NOH\}_2]$ [R = Me (**1**), Et (**3**)] and $[PtCl_2\{(E)\text{-}R(H)C{=}NOH\}\{(Z)\text{-}R(H)C{=}NOH\}]$ [R = Me (**2**), Et (**4**)] as well as the classic *trans*-$[PtCl_2(R_2C{=}NOH)_2]$ [R = Me, Et] were reacted with an equivalent amount of silver acetate in acetone solution at ambient temperature, resulting in formation of unprecedented head-to-tail-oriented oximato-bridged dimers $[PtCl\{\mu\text{-}(Z)\text{-}R(H)C{=}NO\}\{(Z)\text{-}R(H)C{=}NOH\}]_2$ [R = Me (**5**), Et (**7**)], $[PtCl\{\mu\text{-}(Z)\text{-}R(H)C{=}NO\}\{(E)\text{-}R(H)C{=}NOH\}]_2$ [R = Me (**6**), Et (**8**)], and $[PtCl(\mu\text{-}R_2C{=}NO)(R_2C{=}NOH)]_2$ [R = Me (**9**), 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 ($^1$H, $^{13}$C, and $^{195}$Pt) 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 drawn. Thus, it was found that dimerization results in a substantial (up to 7-fold) improvement of $IC_{50}$ values of (aldoxime)$Pt^{II}$ compounds, whereas for the analogous complexes featuring ketoxime ligands the reverse trend was observed. Remarkably, the novel dimers yielded no cross-resistance with cisplatin in SW480 cells, exhibiting up to 2-fold enhanced cytotoxicity in comparison with the CH1 cell line and thereby possessing a promising potential to overcome resistance toward platinum anticancer drugs. The latter point was also confirmed by investigating the potency of apoptosis induction in the case of one monomer as well as one dimer; the investigated complexes proved to be strong apoptotic agents which could induce cell death even in the cisplatin-resistant SW480 cell line.

## 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 aromatic[19] 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 of the 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*-$[PtCl_2(Me_2C{=}NOH)(NH_2CHMe_2)]$, unlike its cis analogue, causes cell death via an apoptotic mechanism.[39] Furthermore, complexes $[PtX_2(R_2C{=}NOH)_2]$ (X = Cl, Br, I; R = Me, Et) exhibiting a 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

**Received:** January 19, 2012
**Published:** June 12, 2012

   dx.doi.org/10.1021/ic300148e | *Inorg. Chem.* 2012, 51, 7153−7163

antitumor agents clashing with the classic Cleare and Hoeschele's structure–activity rules.[1,2]

Conducting the project on the synthesis and antitumor activity of (oxime)$Pt^{II}$ complexes,[40−42] we attempted to elucidate the 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 $^1$H NMR titration. However, we were able to establish only p$K_{a1}$ (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 a 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 analogues. 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 focused on the development of new aldoxime analogues featuring (*E*)- or (*Z*)-R(H)C═NOH (R = Me, Et) ligands in the trans configuration. As expected, the novel agents exhibit considerably higher aqueous solubility as compared to their ketoxime congeners with ligated $R_2C$═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 general formula $[PtCl(\mu\text{-}RR'C{=}NO)(RR'C{=}NOH)]_2$ (R = Me, Et; R′ = H, Me, Et) and verified their cytotoxic properties. In addition, for R = R′ = Et a novel structure consisting of three platinum units linked in a linear fashion and bridged by oximato ligands was established.

**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 reaction of $K_2[PtCl_4]$ with 2 equiv of *E*/*Z*-R(H)C═NOH (R = Me, Et) in an aqueous solution at room temperature followed by thermal isomerization in $MeNO_2$ and column separation of isomers (yields are 31−35%). Complexes were characterized by elemental analysis, electrospray ionization mass spectrometry (ESI-MS), IR, and $^1$H, $^{13}$C{$^1$H}, and $^{195}$Pt NMR spectroscopy and also by X-ray crystallography (for **1** and **4**, Figure S1 and Table S1, Supporting Information). The trans configuration of the complexes and *E*/*Z* configuration of the ligands were established by X-ray diffraction. A detailed description of the syntheses and characterization of **1**−**4** is given in the Supporting Information, whereas synthetic experiments are reported in the Experimental Section.

**Synthesis, Characterization, and X-ray Structure Determination of Oximato-Bridged Platinum(II)**

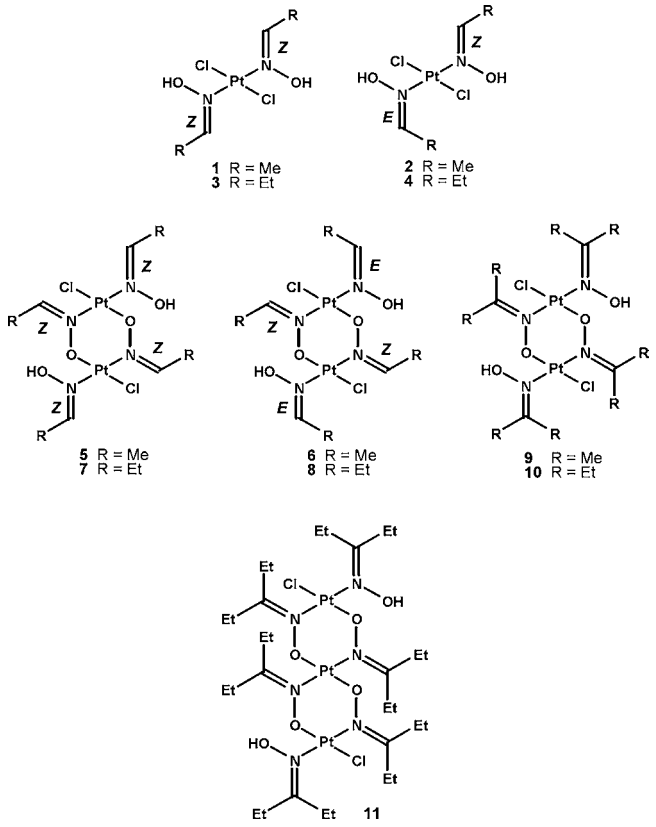


**Figure 1.** Platinum(II) oxime complexes used in this study.

**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*-$[PtCl_2(R_2C{=}NOH)_2]$ (R = Me,[46] Et[41]) 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%).

Interestingly, slow evaporation of a solution of complex **10** in a mixture of undried ethanol, ethylacetate, and acetone resulted in formation of trimer **11** (Figure 1). The obtained crystals were characterized by X-ray crystallography (see below) as well as ESI-MS spectrometry ($[M + Na]^+$ and $[M + K]^+$ peaks were detected). A plausible mechanism of trimer formation could be associated with a dimer−monomer equilibrium.

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 $\nu$(OH) [3143−3377 cm$^{-1}$] and $\nu$(NO) [958−970 cm$^{-1}$] stretching vibrations as well as two $\nu$(C═N) [1609−1625 and 1651−1681 cm$^{-1}$] bands of medium intensity. Expectedly, two sets of oxime proton signals were observed in the $^1$H 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 $[PtCl\{\mu\text{-}(Z)\text{-}R(H)C{=}NO\}\{(E)\text{-}R(H)C{=}NOH\}]_2$ (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


dx.doi.org/10.1021/ic300148e | Inorg. Chem. 2012, 51, 7153−7163

detected by NMR spectroscopy. Moreover, dissolution of **6** and **8** in $CD_3OD$ 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 might be explained by an equilibrium possibly established between a dimer and its corresponding monomer in solution, resulting in a mixture of dimers (Figure 2).

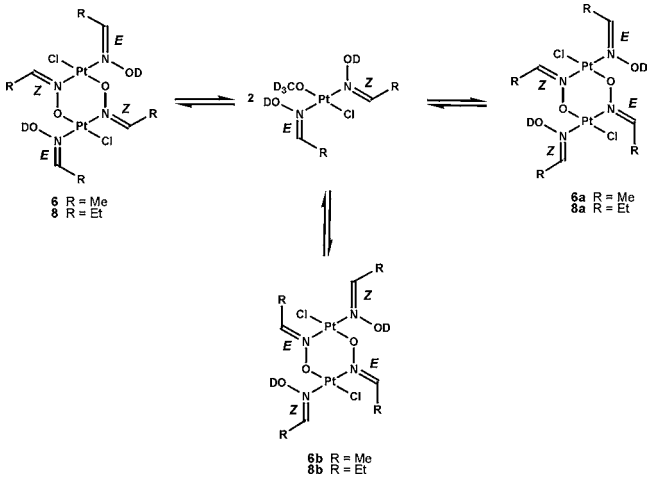


**Figure 2.** Equilibrium in $CD_3OD$ solution of **6** and **8**.

In the $^{195}$Pt NMR spectra, the platinum signals of aldoxime-containing dimers **5**−**8** appear in a narrow range between −208 and −251 ppm, while $\delta_{Pt}$ of the 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 3 and 4). 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 hydroxy 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 Pts in **11** is completed by the corresponding aldoxime (**6**) or ketoxime (**9** and **11**) and a chlorido ligand.

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), which is probably due to an increased steric effect exerted by the alkyl substituents of

**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) |

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

| | 1 | 4 | 6 | 9 | 11 |
|---|---|---|---|---|---|
| empirical formula | $C_4H_{10}Cl_2N_2O_2Pt$ | $C_6H_{14}Cl_2N_2O_2Pt$ | $C_8H_{18}Cl_2N_4O_4Pt_2$ | $C_{12}H_{26}Cl_2N_4O_4Pt_2$ | $C_{30}H_{62}Cl_2N_6O_6Pt_3$ |
| 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 |
| $\rho_{calcd}$, g cm$^{-3}$ | 2.778 | 2.409 | 2.931 | 2.471 | 2.156 |
| cryst 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 |

[a] $R1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$. [b] $wR2 = \{\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2}$. [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.


dx.doi.org/10.1021/ic300148e | Inorg. Chem. 2012, 51, 7153−7163

**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) |

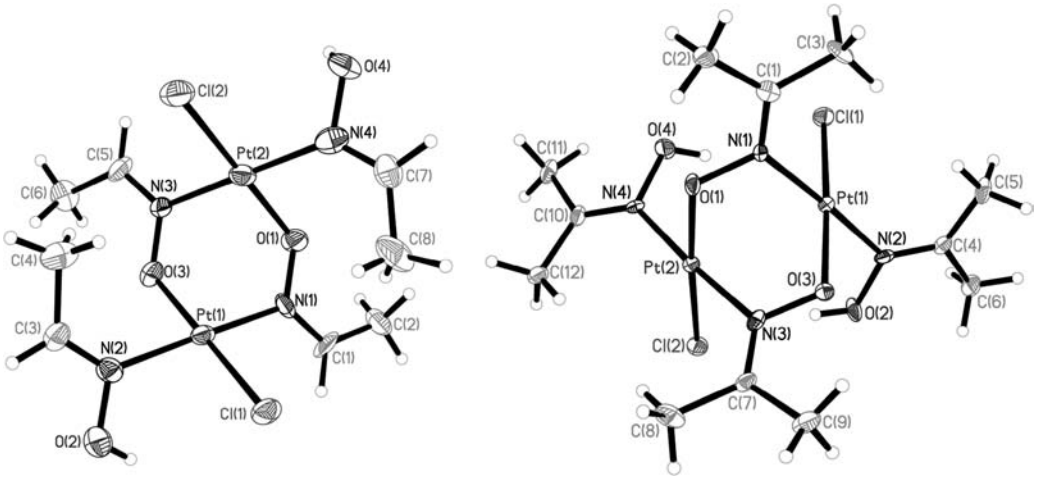


**Figure 3.** ORTEP view of **6** (left) and **9** (right) with atom-labeling scheme; thermal ellipsoids have been drawn at the 50% probability level.

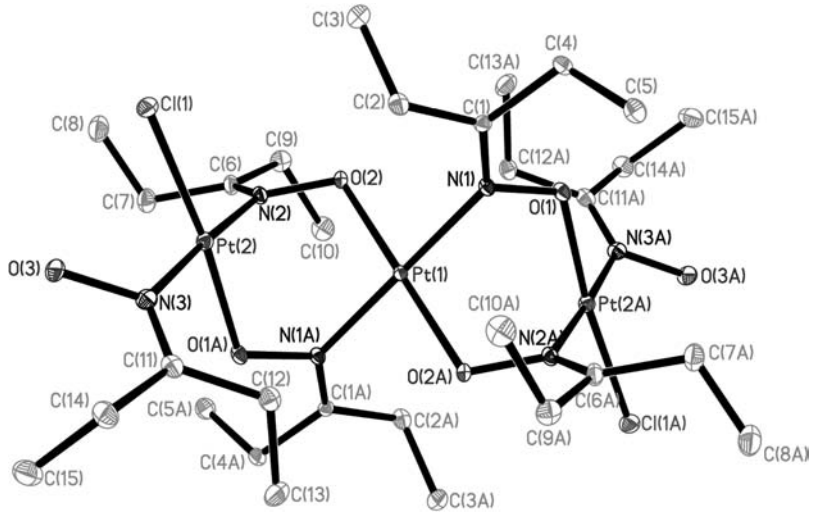


**Figure 4.** ORTEP view of **11** with atom-labeling scheme; thermal ellipsoids have been drawn at the 30% probability level.

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

| | dihedral angle,[a] deg | | | |
|---|---|---|---|---|
| | Pt···Pt, Å | $\tau$ | $\omega$ | Pt···Pt···Pt angle, deg |
| **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] $\tau$ is the tilt angle between the adjacent platinum coordination planes, and $\omega$ is the average torsion angle about the Pt(1)···Pt(2) vector.

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 the 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.

Generally, the distances between the adjacent platinum atoms in **6**, **9**, and **11** are shorter than a double van der Waals


dx.doi.org/10.1021/ic300148e | Inorg. Chem. 2012, 51, 7153−7163

radius for Pt [1.72 × 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-methylthyminate [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 ($\tau$) 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 $\tau$ value (57.2° versus 81.4° and 85.3° for **6** and **11**, respectively), additionally confirming the lowest repulsive interactions in the 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°, respectively), while in **11** the twist angle was proved to be substantially higher ($\omega$ = 19.2°), serving to minimize the steric interactions between the ethyl substituents of the bridging oximato and nonbridging oxime ligands.

The platinum ions in **6** and **9** and the terminal Pts 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) 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 the cytotoxic 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 the $IC_{50}$ 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 a similar response toward both monomeric (**1**−**4**) and dimeric (**5**−**9**) complexes, as also reported for (ketoxime)$Pt^{II}$ species.[41] However, in the 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. In contrast, the A549 cell line exhibited a somewhat lower sensitivity to the novel oxime complexes, yielding $IC_{50}$ 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 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 $IC_{50}$ values of dimers **5**−**8** (by a factor of 1.1−2.3). Interestingly, some influence of the *E*- and *Z*-configuration of the coordinated aldoximes on the $IC_{50}$ 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.

**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), 96 h | | |
|---|---|---|---|
| | 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 |
| *trans*-$[PtCl_2(Me_2C{=}NOH)_2]$[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 40.

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 $IC_{50}$ values in CH1 and SW480 cells, respectively, as compared to the corresponding monomer *trans*-$[PtCl_2(Me_2C{=}NOH)_2]$,[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 $IC_{50}$ 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 3.9−13). 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 $^1$H NMR measurements of monomer **1** and the respective dimer **5** were carried out in phosphate-buffered $D_2O$ solutions (10 mM, pH 7.4) containing NaCl (0.125 M) with concentrations of complexes being ca. 400 and 200 μM, respectively. Figure S2 (Supporting Information) shows a selected range of $^1$H NMR spectra (6−8 ppm) where the signals of the N═C*H* protons are detected. Keeping dimer **5** or monomer **1** under these conditions over 3 days resulted in a nearly identical peak pattern (spectra 2 and 4, respectively) consisting of five N═C*H* proton signals corresponding to the dimer, monomer, hydrolyzed monomer, and an additional so far unknown species. These results are in good agreement with $IC_{50}$ 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


dx.doi.org/10.1021/ic300148e | *Inorg. Chem.* 2012, 51, 7153−7163

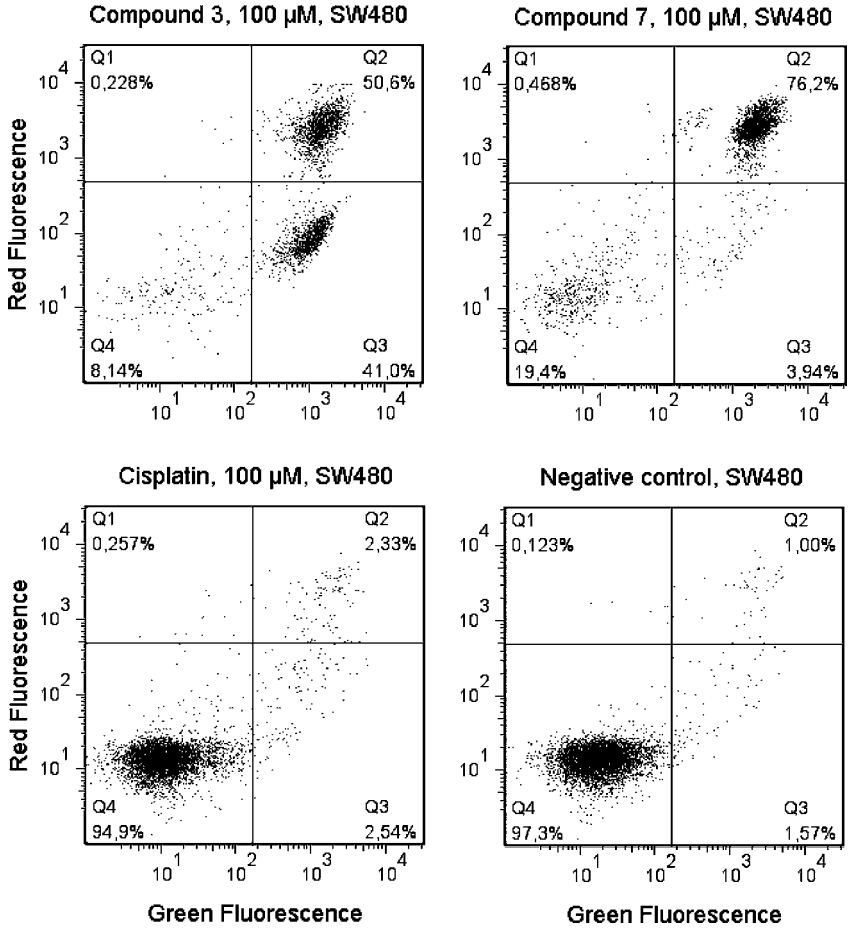


**Figure 5.** Apoptosis induction detected by annexin V-FITC and PI staining and FACS analysis in the cisplatin-resistant colon cancer cell line SW480 (24 h exposure with 100 $\mu$M of cisplatin, compounds **3** and **7**). Upper left quadrant (Q1) contains necrotic, lower right (Q3) early apoptotic, upper right (Q2) late apoptotic, and lower left (Q4) viable cell population.

$IC_{50}$(**1**)/$IC_{50}$(**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.

Spectra 5−7 (Figure S2, Supporting Information) 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 N=C*H* proton of the hydrolyzed species could be observed (spectrum 5). A total of 5 h after the experiment was started the dichlorido complex was completely converted into the aqua species (spectrum 6). In addition, small signals related to the dimeric complex were detected. Finally, 3 days later, the mixture of N=C*H* proton signals turned into one single peak (spectrum 7). With the intention to elucidate which species was formed as a result of the conversions taking place in aqueous solution, a $^{195}$Pt NMR spectrum was recorded. A signal at 316.7 ppm was observed, which correlates well with $\delta_{Pt}$ values for trans-configured platinum(II) species having a $[N_2O_2]$ ligand donor set reported in the literature in the range between 209 and 366 ppm.[62] Further, in the ESI-MS$^+$ spectrum a peak at *m*/*z* 956 was detected that could be attributed to a sodiated trimeric complex $[Pt_3(C_2H_4NO)_6 + Na]^+$ ($C_{12}H_{24}N_6O_6Pt_3Na$, calcd *m*/*z* 956.06). Due to the fact that only one set of N=C*H* proton signals was observed in the $^1$H NMR spectrum 7, a ring-shaped trimeric structure containing six equivalent oximato bridges can be suggested (Figure S3, Supporting Information).

**Apoptosis Assay.** In order to compare the capacities of inducing apoptosis and necrosis of compounds **3**, **7**, and cisplatin, growing SW480 and HL60 cells were treated with these drugs in equal concentrations for 24 h, then stained with annexin V-FITC/propidium iodide and analyzed by fluorescence-activated cell sorting (FACS). This method allows one to discriminate necrotic (stained by propidium iodide only, red fluorescence), early apoptotic (stained by annexin V-FITC only, green fluorescence), and late apoptotic (stained by both, green and red fluorescence) from viable (unstained, only self-fluorescence) cells. From the dot plots (Figures 5 and 6) we can infer that the apoptosis-inducing potency of compound **3** is much higher than that of cisplatin in SW480 cells but decreases relative to cisplatin in HL60 cells. The apoptosis-inducing potency of compound **7** exceeds that of cisplatin in SW480 cells and is comparable in HL60 cells. A concentration of 100 $\mu$M of compounds **3** and **7** reduces the amount of viable SW480 cells to below 20% by induction of both early and late apoptosis, while the same concentration of cisplatin has insignificant effects within the chosen exposure time. Summarized data (Figure S4 and Tables S2 and S3, Supporting Information) show that compounds **3** and **7** are strong apoptotic agents which can induce cell death even in a cisplatin-resistant cell line.


dx.doi.org/10.1021/ic300148e | *Inorg. Chem.* 2012, 51, 7153−7163

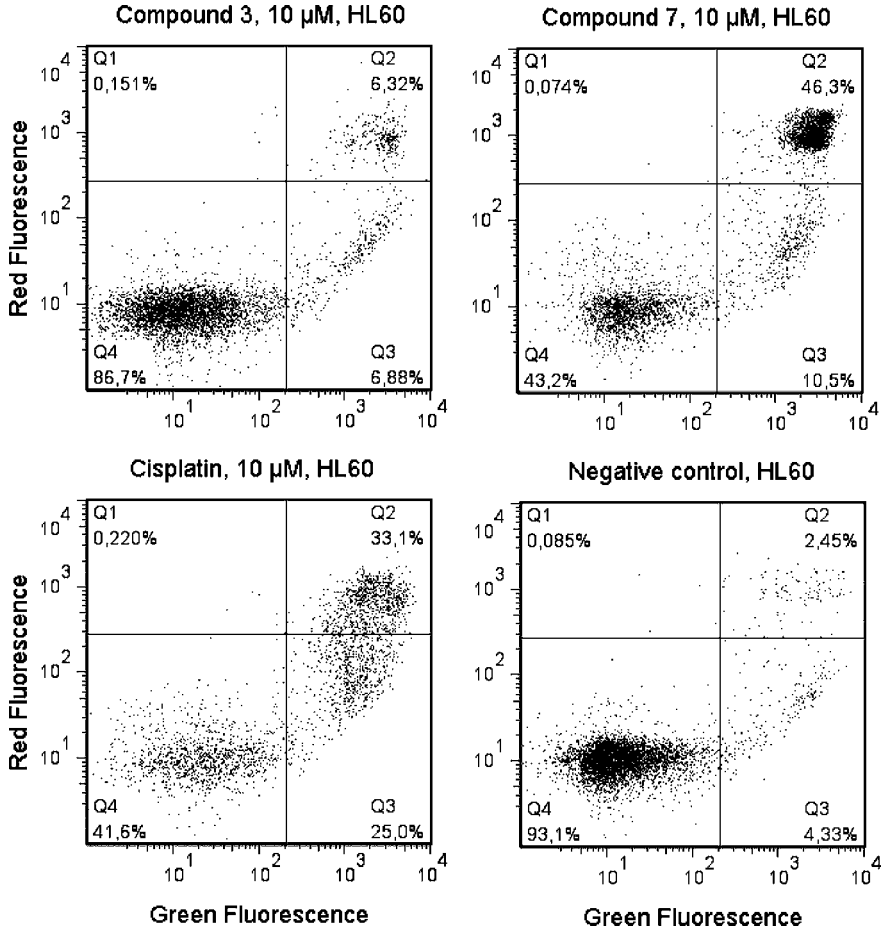


**Figure 6.** Apoptosis induction detected by annexin V-FITC and PI staining and FACS analysis in the cisplatin-sensitive human promyelocytic leukemia cell line HL60 (24 h exposure with 10 $\mu$M of cisplatin, compounds **3** and **7**). Upper left quadrant (Q1) contains necrotic, lower right (Q3) early apoptotic, upper right (Q2) late apoptotic, and lower left (Q4) viable cell population.

## CONCLUSIONS

The possibility of di- and trimerization of (oxime)$Pt^{II}$ complexes leading to 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 the case of two dimers. Additionally, the crystal structure of a novel oximato-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 Pd[69]) and partially for some representatives of group I (Cu[63,68,70−84]), II (Zn[63,64,85] Cd[85]), III (Gd[64]), IV (Ti[86] and Zr[86]), VI (Cr[64]), and VII (Mn[63,64,87−89]), there are no reports on oximato-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/oximato ligands coordinated to the platinum atom via oxygen.

In general, the di-/trimerization observed in this work at the kinetically inert platinum(II) center could give a possible rationale for various polymerizations involving oxime/oximato species based on more labile metals. Considering these results, previous work on the synthesis of polymeric oximato-bridged complexes should be thoroughly revisited and the possibility of the consecutive association of the monomeric oximato 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 oximato-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. The latter point was confirmed by investigating the ability of induction of apoptosis, demonstrating a strong apoptotic potential even in the cisplatin-resistant cell line.

## EXPERIMENTAL SECTION

**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 chemicals were used as received without further purification. 3-Pentanone oxime was synthesized from the corresponding ketone by a standard textbook procedure.[90,91] Complexes *trans*-$[PtCl_2(Me_2C{=}NOH)_2]$[46] and *trans*-$[PtCl_2(Et_2C{=}NOH)_2]$[41] were prepared according to the previously


dx.doi.org/10.1021/ic300148e | *Inorg. Chem.* 2012, 51, 7153−7163

published methods starting from $K_2[PtCl_4]$. 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 measured with a Bruker Esquire 3000 instrument. IR spectra were obtained using an ATR unit with a Perkin-Elmer 370 FTIR 2000 instrument (4000−400 $cm^{-1}$). $^1H$, $^{13}C\{^1H\}$, and $^{195}Pt$ 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 ($^{195}Pt$), respectively, at 298 K. $^{195}Pt$ chemical shifts are given relative to $K_2[PtCl_4]$, and the half-height line width is given in parentheses.

**Reaction of $K_2[PtCl_4]$ with *Z*/*E*-R(H)C═NOH (R = Me, Et).** $K_2[PtCl_4]$ (0.7 mmol) was dissolved in 15 mL of $H_2O$, and a solution of *Z*/*E*-R(H)C═NOH (1.4 mmol) in 2 mL of $H_2O$ 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 $H_2O$, and dried in vacuo. The filtrate was evaporated to dryness, and 10 mL of MeOH was added. A colorless precipitate of KCl was filtered off. Then MeOH was removed on a rotary evaporator, and 5 mL of $CHCl_3$ or $Et_2O$ (for R = Me or Et, respectively) was added, whereupon a yellow precipitate of $K[PtCl_3\{R(H)C═NOH\}]$ was filtered off, washed with $CHCl_3$ or $Et_2O$ (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 ($K[PtCl_3\{R(H)C═NOH\}]$, 0.16−0.18 mmol) in 10 mL of $H_2O$ 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 was 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 $MeNO_2$ was added, whereupon the reaction mixture was refluxed for 1 h. Afterward the solvent was removed and products **1** (first fraction) and **2** (second fraction) or **3** (first fraction) and **4** (second fraction) were separated by column chromatography ($CHCl_3$:$Me_2C═O$ 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-κ*N*)platinum(II) (1).** Anal. Calcd for $C_4H_{10}N_2Cl_2O_2Pt$: 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) $CHCl_3/Me_2C═O$): $R_f$ = 0.73. IR in KBr (selected bands), $cm^{-1}$: 3202 s ν(OH), 1667 m ν(C═N), 970 s ν(NO). $^1H$ NMR ($CD_3OD$), δ: 7.39 (q, 1*H*, C*H*, $^3J_{H,H}$ = 5.8 Hz), 2.05 (d, 3*H*, C$H_3$, $^3J_{H,H}$ = 5.7 Hz). $^{13}C\{^1H\}$ NMR ($CD_3OD$), δ: 156.4 (*C*═N), 12.7 ($CH_3$). $^{195}Pt$ NMR ($CD_3OD$), 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-κ*N*)((1*Z*)-*N*-hydroxyethanimine-κ*N*)platinum(II) (2).** Anal. Calcd for $C_4H_{10}N_2Cl_2O_2Pt$: 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) $CHCl_3/Me_2C═O$): $R_f$ = 0.52. IR in KBr (selected bands), $cm^{-1}$: 3215 s ν(OH), 1677 m ν(C═N), 980 s ν(NO). $^1H$ NMR ($CD_3OD$), δ: 7.51 (q, 1*H*, C*H*, $^3J_{H,H}$ = 6.1 Hz), 7.50 (q, 1*H*, C*H*, $^3J_{H,H}$ = 5.7 Hz), 2.49 (d, 3*H*, C$H_3$, $^3J_{H,H}$ = 6.1 Hz), 2.05 (d, 3*H*, C$H_3$, $^3J_{H,H}$ = 5.7 Hz). $^{13}C\{^1H\}$ NMR ($CD_3OD$), δ: 156.0 (*C*═N), 155.4 (*C*═N), 16.1 ($CH_3$), 12.7 ($CH_3$). $^{195}Pt$ NMR ($CD_3OD$), δ: −532 (550 Hz).

**(SP-4-1)-Dichloridobis((1*Z*)-*N*-hydroxypropane-1-imine-κ*N*)platinum(II) (3).** Anal. Calcd for $C_6H_{14}N_2Cl_2O_2Pt$: 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) $CHCl_3/Me_2C═O$): $R_f$ = 0.54. IR in KBr (selected bands), $cm^{-1}$: 3338 s ν(OH), 1671 m ν(C═N), 985 s ν(NO). $^1H$ NMR ($CD_3OD$), δ: 7.29 (t, 1*H*, C*H*, $^3J_{H,H}$ = 5.8 Hz), 2.52 (m, 2*H*, C$H_2$), 1.09 (t, 3*H*, C$H_3$, $^3J_{H,H}$ = 7.7 Hz). $^{13}C\{^1H\}$ NMR ($CD_3OD$), δ: 161.9 (*C*═N), 20.9 ($CH_2$), 8.6 ($CH_3$). $^{195}Pt$ NMR ($CD_3OD$), δ: −490 (510 Hz).

**(SP-4-1)-Dichlorido((1*E*)-*N*-hydroxypropane-1-imine-κ*N*)((1*Z*)-*N*-hydroxypropane-1-imine-κ*N*)platinum(II) (4).** Anal. Calcd for $C_6H_{14}N_2Cl_2O_2Pt$: 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) $CHCl_3/Me_2C═O$): $R_f$ = 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 ($CD_3OD$), δ: 7.42 (t, 1*H*, C*H*, $^3J_{H,H}$ = 6.9 Hz), 7.39 (t, 1*H*, C*H*, $^3J_{H,H}$ = 5.8 Hz), 3.04 (m, 2*H*, C$H_2$), 2.52 (m, 2*H*, C$H_2$), 1.21 (t, 3*H*, C$H_3$, $^3J_{H,H}$ = 7.6 Hz), 1.11 (d, 3*H*, C$H_3$, $^3J_{H,H}$ = 7.7 Hz). $^{13}C\{^1H\}$ NMR ($CD_3OD$), δ: 161.5 (*C*═N), 160.0 (*C*═N), 24.6 ($CH_2$), 20.9 ($CH_2$), 9.3 ($CH_3$), 8.6 ($CH_3$). $^{195}Pt$ NMR ($CD_3OD$), 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*-$[PtCl_2(R_2C═NOH)_2]$ (R = Me,[46] Et[41]) (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 column chromatography as the first fraction using a mixture of $CHCl_3$ and $Me_2C═O$ 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-κ*N*,κ*O*)bis[chlorido((1*Z*)-*N*-hydroxyethanimine-κ*N*)platinum(II)] (5).** Anal. Calcd for $C_8H_{18}N_4Cl_2O_4Pt_2$: 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) $CHCl_3/Me_2C═O$): $R_f$ = 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 ($CD_3OD$), δ: 7.59 (q, 1*H*, C*H*, $^3J_{H,H}$ = 5.8 Hz), 6.99 (q, 1*H*, C*H*, $^3J_{H,H}$ = 5.8 Hz), 2.07 (d, 3*H*, C$H_3$, $^3J_{H,H}$ = 5.7 Hz), 1.94 (d, 3*H*, C$H_3$, $^3J_{H,H}$ = 5.8 Hz). $^{13}C\{^1H\}$ NMR ($CD_3OD$), δ: 153.8 (*C*═N), 151.3 (*C*═N), 12.3 ($CH_3$), 12.1 ($CH_3$). $^{195}Pt$ NMR ($CD_3OD$), δ: −208 (480 Hz).

**(SP-4-2)-Di-μ-((1*Z*)-N-oxyethanimine-κ*N*,κ*O*)bis[chlorido((1*E*)-*N*-hydroxyethanimine-κ*N*)platinum(II)] (6).** Anal. Calcd for $C_8H_{18}N_4Cl_2O_4Pt_2$: 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) $CHCl_3/Me_2C═O$): $R_f$ = 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 ($CD_3OD$), δ: 7.78 (q, 1*H*, C*H*, $^3J_{H,H}$ = 5.8 Hz), 7.38 (q, 1*H*, C*H*, $^3J_{H,H}$ = 6.2 Hz), 2.33 (d, 3*H*, C$H_3$, $^3J_{H,H}$ = 6.2 Hz), 2.09 (d, 3*H*, C$H_3$, $^3J_{H,H}$ = 5.8 Hz). $^{13}C\{^1H\}$ NMR ($CD_3OD$), δ: 154.1 (*C*═N), 152.0 (*C*═N), 15.8 ($CH_3$), 12.4 ($CH_3$). $^{195}Pt$ NMR ($CD_3OD$), 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-κ*N*,κ*O*)bis[chlorido((1*Z*)-*N*-hydroxypropane-1-imine-κ*N*)platinum(II)] (7).** Anal. Calcd for $C_{12}H_{26}N_4Cl_2O_4Pt_2$: 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 $[M - 2Et(H)C═NOH + Na]^+$, 716 $[M - Cl]^+$, 752 $[M + H]^+$, 774 $[M + Na]^+$. TLC ($CHCl_3$): $R_f$ = 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 ($CD_3OD$), δ: 7.50 (t, 1H, C*H*, $^3J_{H,H}$ = 5.7 Hz), 6.88 (t, 1*H*, C*H*, $^3J_{H,H}$ = 5.8 Hz), 2.55 (m, 2*H*, C$H_2$), 2.43 (m, 2*H*, C$H_2$), 1.13 (t, 3*H*, C$H_3$, $^3J_{H,H}$ = 7.6 Hz), 1.06 (t, 3*H*, C$H_3$, $^3J_{H,H}$ = 7.7 Hz). $^{13}C\{^1H\}$ NMR ($CD_3OD$), δ: 160.0 (*C*═N), 157.9 (*C*═N), 20.6 ($CH_2$), 20.5 ($CH_2$), 8.9 ($CH_3$), 8.7 ($CH_3$). $^{195}Pt$ NMR ($CD_3OD$), δ: −212 (440 Hz).

**(SP-4-2)-Di-μ-((1*Z*)-*N*-oxypropane-1-imine-κ*N*,κ*O*)bis[chlorido((1*E*)-*N*-hydroxypropane-1-imine-κ*N*)platinum(II)] (8).** Anal. Calcd for $C_{12}H_{26}N_4Cl_2O_4Pt_2$: 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 $[M - 2Et(H)C═NOH + Na]^+$, 716 $[M - Cl]^+$, 752 $[M + H]^+$, 774 $[M + Na]^+$. TLC (20:1 (v/v) $CHCl_3/Me_2C═O$): $R_f$ = 0.71. IR in KBr


dx.doi.org/10.1021/ic300148e | *Inorg. Chem.* 2012, 51, 7153−7163

(selected bands), $cm^{-1}$: 3250 s $\nu$(OH), 1681 and 1624 m $\nu$(C═N), 958 s $\nu$(NO). $^1$H NMR ($CD_3OD$), $\delta$: 7.70 (t, 1*H*, C*H*, $^3J_{H,H}$ = 5.7 Hz), 7.31 (t, 1*H*, C*H*, $^3J_{H,H}$ = 6.8 Hz), 2.89 (m, 2*H*, C$H_2$), 2.58 (m, 2*H*, C$H_2$), 1.161 (t, 3*H*, C$H_3$, $^3J_{H,H}$ = 7.6 Hz), 1.160 (t, 3*H*, C$H_3$, $^3J_{H,H}$ = 7.7 Hz). $^{13}C\{^1H\}$ NMR ($CD_3OD$), $\delta$: 159.6 (C═N), 157.2 (C═N), 24.2 ($CH_3$), 20.7 ($CH_3$), 9.8 ($CH_3$), 8.6 ($CH_3$). $^{195}$Pt NMR ($CD_3OD$), $\delta$: −243 (450 Hz).

**(SP-4-2)-Di-$\mu$-(*N*-oxypropane-2-imine-$\kappa N,\kappa O$)bis[chlorido(*N*-hydroxypropane-2-imine-$\kappa N$))platinum(II)] (9).** Anal. Calcd for $C_{12}H_{26}N_4Cl_2O_4Pt_2$: 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) $CHCl_3/Me_2C$═O): $R_f$ = 0.53. IR in KBr (selected bands), $cm^{-1}$: 3143 s $\nu$(OH), 1666 and 1620 m $\nu$(C═N), 969 s $\nu$(NO). $^1$H NMR ($CDCl_3$), $\delta$: 8.67 (s, 2*H*, O*H*), 2.72 (s, 6*H*, C$H_3$), 2.55 (s, 6*H*, C$H_3$), 2.29 (s, 6*H*, C$H_3$), 2.09 (s, 6*H*, C$H_3$). $^{13}C\{^1H\}$ NMR ($CDCl_3$), $\delta$: 165.4 (*C*═N), 157.4 (*C*═N), 25.0 ($CH_3$), 23.7 ($CH_3$), 18.4 ($CH_3$), 17.7 ($CH_3$). $^{195}$Pt NMR ($CDCl_3$), $\delta$: −35 (400 Hz). Crystals for X-ray study were obtained by slow evaporation of an acetone solution at room temperature.

**(SP-4-2)-Di-$\mu$-(*N*-oxypentane-3-imine-$\kappa N,\kappa O$)bis[chlorido(*N*-hydroxypentane-3-imine-$\kappa N$))platinum(II)] (10).** Anal. Calcd. for $C_{20}H_{42}N_4Cl_2O_4Pt_2$: 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) $CHCl_3/Me_2C$═O): $R_f$ = 0.43. IR in KBr (selected bands), $cm^{-1}$: 3377 s $\nu$(OH), 1651 and 1609 m $\nu$(C═N), 966 s $\nu$(NO). $^1$H NMR ($CD_3OD$), $\delta$: 3.59 (m, 2*H*, 2CH*H*), 3.36 (m, 2*H*, 2CH*H*), 3.22 (m, 2*H*, 2CH*H*), 3.11 (m, 2*H*, 2CH*H*), 2.65 (m, 4*H*, 4CH*H*), 2.52 (m, 2*H*, 2CH*H*), 2.36 (m, 2*H*, 2CH*H*), 1.34 (t, 6*H*, 2C$H_3$, $^3J_{H,H}$ = 7.6 Hz), 1.21 (t, 6*H*, 2C$H_3$, $^3J_{H,H}$ = 7.6 Hz), 1.16 (t, 6*H*, 2C$H_3$, $^3J_{H,H}$ = 7.6 Hz), 1.00 (t, 6*H*, 2C$H_3$, $^3J_{H,H}$ = 7.6 Hz). $^{13}C\{^1H\}$ NMR ($CD_3OD$), $\delta$: 173.2 (2*C*═N), 168.5 (2*C*═N), 29.1 ($CH_2$), 28.9 ($CH_2$), 28.3 ($CH_2$), 28.1 ($CH_2$), 22.4 (2$CH_2$), 22.1 (2$CH_2$), 10.3 (4$CH_3$), 9.17 (2$CH_3$), 9.0 (2$CH_3$). $^{195}$Pt NMR ($CD_3OD$), $\delta$: −152 (510 Hz). Crystals of the respective 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. Data were processed using the 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. 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-97[93] was used for structure solution and SHELXL-97[94] 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, U.K.) and 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 experiments. Cells were grown in 75 $cm^2$ 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, 4 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% $CO_2$.

**Cytotoxicity 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-2*H*-tetrazolium bromide). A549, CH1, and SW480 cells were harvested from culture flasks by trypsinization and seeded in 100 $\mu$L aliquots in MEM into 96-well plates (Iwaki/Asahi Technoglass, Gyouda, Japan) in cell densities of $3 \times 10^3$, $1 \times 10^3$, and $2.5 \times 10^3$ cells per well, respectively. After 24 h preincubation of the cells, the test compounds were dissolvedand serially diluted in MEM, and 100 $\mu$L 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 $\mu$L of RPMI 1640 culture medium (supplemented with 10% heat-inactivated fetal bovine serum and 2 mM L-glutamine) plus 20 $\mu$L of 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 $\mu$L 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 ($IC_{50}$) 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.

**Solution Behavior.** For investigation of the behavior of monomer **1** and respective dimer **5** in aqueous solution, time-dependent $^1$H NMR measurements were carried out in a 10 mM phosphate-buffered $D_2O$ solution at pH of 7.4 containing 0.125 M NaCl over 3 days. The concentrations of complexes were ca. 400 and 200 $\mu$M, 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.

**Apoptosis Assay.** Cell death induction was analyzed by fluorescence-activated cell sorting (FACS) using FITC-conjugated annexin V (BioVision, USA) and propidium iodide (PI; Fluka) staining. Colon carcinoma (SW480) and human promyelocytic leukemia (HL60) cells were seeded into 12-well plates (CytoOne, Starlab, U.K.) in amounts of $1 \times 10^5$ cells per well in complete medium (MEM) and allowed to settle for 24 h. The cells were exposed to cisplatin and compounds **3** and **7** for 24 h at 37 °C. After incubation, SW480 cells were gently trypsinized and both cell lines were collected, washed with PBS, and resuspended with FITC-conjugated annexin V (0.25 $\mu$g/mL) in binding buffer (10 mM HEPES/NaOH pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$) at room temperature for 15 min. PI (1 $\mu$g/mL) was added shortly before measurement. Stained cells were analyzed with Guava 8HT EasyCyte Flow cytometer (Merk Milipore, Guava, USA) using InCyte software. At least three independent experiments were conducted, and 5000 cells were counted per analysis.

## ■ 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 and angles for complexes **1** and **4**; figure with $^1$H NMR spectra of monomer **1** and corresponding dimer **5** under different conditions; geometry optimization of a ring-shaped trimer; figure with the optimized structure of the ring-shaped trimer; figure and tables with results of the induction of apoptosis in SW480 and HL60 cancer cells; X-ray crystallographic file in CIF format for **1**, **4**, **6**, **9**, and **11**. This material is available free of charge via the Internet at http://pubs.acs.org.


dx.doi.org/10.1021/ic300148e | *Inorg. Chem.* 2012, 51, 7153−7163

## AUTHOR INFORMATION


### Corresponding Author

*Phone: +43-1-4277-52600. Fax: +43-1-4277-52680. E-mail: markus.galanski@univie.ac.at; bernhard.keppler@univie.ac.at.


### Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS


The authors are indebted to the FFG-Austrian Research Promotion Agency, the Austrian Council for Research and Technology Development, and COST D39. V.Y.K. is grateful to RFBR for grant 11-03-90417. We are thankful to Mag. H. Varbanov for geometry optimization of the ring-shaped trimeric structure.


## REFERENCES


(1) Cleare, M. J.; Hoeschele, J. D. *Platinum Met. Rev.* **1973**, *17*, 2−13.
(2) Cleare, M. J.; Hoeschele, J. D. *Bioinorg. Chem.* **1973**, *2*, 187−210.
(3) Rosenberg, B.; Van Kamp, L.; Crigas, T. *Nature* **1965**, *205*, 698−699.
(4) Jakupec, M. A.; Galanski, M.; Keppler, B. K. *Rev. Physiol. Biochem. Pharmacol.* **2003**, *146*, 1−54.
(5) Galanski, M.; Jakupec, M. A.; Keppler, B. K. *Curr. Med. Chem.* **2005**, *12*, 2075−2094.
(6) Galanski, M.; Arion, V. B.; Jakupec, M. A.; Keppler, B. K. *Curr. Pharm. Des.* **2003**, *9*, 2078−2089.
(7) Farrell, N. *PCT Int. Appl.* **1991**, 1−31.
(8) Farrell, N.; Qu, Y.; Hoeschele, J. D. *PCT Int. Appl.* **1994**, 1−36.
(9) Kraker, A. J.; Hoeschele, J. D.; Elliott, W. L.; Showalter, H. D.; Hollis, L. S.; Sercel, A. D.; Farrell, N. P. *J. Med. Chem.* **1992**, *35*, 4526−4532.
(10) Wu, P. K.; Qu, Y.; Van Houten, B.; Farrell, N. *J. Inorg. Biochem.* **1994**, *54*, 207−220.
(11) Farrell, N.; Appleton, T. G.; Qu, Y.; Roberts, J. D.; Fontes, A. P. S.; Skov, K. A.; Wu, P.; Zou, Y. *Biochemistry* **1995**, *34*, 15480−15486.
(12) Qu, Y.; Bloemink, M. J.; Reedijk, J.; Hambley, T. W.; Farrell, N. *J. Am. Chem. Soc.* **1996**, *118*, 9307−9313.
(13) Farrell, N.; Menta, E.; Valsecchi, M.; Di Domenico, R.; Da Re, G.; Manzotti, C.; Pezzoni, G.; Giuliani, F. C.; Spinelli, S *J. Inorg. Biochem.* **1997**, *67*, 173.
(14) Qu, Y.; Farrell, N.; Kasparkova, J.; Brabec, V. *J. Inorg. Biochem.* **1997**, *67*, 174.
(15) Perego, P.; Caserini, C.; Gatti, L.; Carenini, N.; Romanelli, S.; Supino, R.; Colangelo, D.; Viano, I.; Leone, R.; Spinelli, S.; Pezzoni, G.; Manzotti, C.; Farrell, N.; Zunino, F. *Mol. Pharmacol.* **1999**, *55*, 528−534.
(16) Kloster, M. B. G.; Hannis, J. C.; Muddiman, D. C.; Farrell, N. *Biochemistry* **1999**, *38*, 14731−14737.
(17) Qu, Y.; Rauter, H.; Fontes, A. P. S.; Bandarage, R.; Kelland, L. R.; Farrell, N. *J. Med. Chem.* **2000**, *43*, 3189−3192.
(18) Cox, J. W.; Berners-Price, S. J.; Davies, M. S.; Qu, Y.; Farrell, N *J. Am. Chem. Soc.* **2001**, *123*, 1316−1326.
(19) Fan, D.; Yang, X.; Wang, X.; Zhang, S.; Mao, J.; Ding, J.; Lin, L.; Guo, Z. *J. Biol. Inorg. Chem.* **2007**, *12*, 655−665.
(20) McGregor, T. D.; Hegmans, A.; Kasparkova, J.; Neplechova, K.; Novakova, O.; Penazova, H.; Vrana, O.; Brabec, V.; Farrell, N. *J. Biol. Inorg. Chem.* **2002**, *7*, 397−404.
(21) Kasparkova, J.; Zehnulova, J.; Farrell, N.; Brabec, V. *J. Biol. Chem.* **2002**, *277*, 48076−48086.
(22) Hegmans, A.; Berners-Price, S. J.; Davies, M. S.; Thomas, D. S.; Humphreys, A. S.; Farrell, N. *J. Am. Chem. Soc.* **2004**, *126*, 2166−2180.
(23) Kasparkova, J.; Vrana, O.; Farrell, N.; Brabec, V. *J. Inorg. Biochem.* **2004**, *98*, 1560−1569.
(24) Harris, A. L.; Ryan, J. J.; Farrell, N. *Mol. Pharmacol.* **2006**, *69*, 666−672.
(25) Kabolizadeh, P.; Ryan, J.; Farrell, N. *Biochem. Pharmacol.* **2007**, *73*, 1270−1279.
(26) Malina, J.; Farrell, N. P.; Brabec, V. *Chem.−Asian J.* **2011**, *6*, 1566−1574.
(27) Wheate, N. J.; Collins, J. G. *J. Inorg. Biochem.* **2000**, *78*, 313−320.
(28) Wheate, N. J.; Cutts, S. M.; Phillips, D. R.; Aldrich-Wright, J. R.; Collins, J. G. *J. Inorg. Biochem.* **2001**, *84*, 119−127.
(29) Wheate, N. J.; Evison, B. J.; Herlt, A. J.; Phillips, D. R.; Collins, J. G. *Dalton Trans.* **2003**, *18*, 3486−3492.
(30) Collins, J. G.; Wheate, N. J. *J. Inorg. Biochem.* **2004**, *98*, 1578−1584.
(31) Chikuma, M.; Hirai, M.; Komeda, S.; Kimura, Y.; Kumakura, K. *J. Inorg. Biochem.* **1997**, *67*, 348.
(32) Komeda, S.; Lutz, M.; Spek, A. L.; Chikuma, M.; Reedijk, J. *Inorg. Chem.* **2000**, *39*, 4230−4236.
(33) Komeda, S.; Lutz, M.; Spek, A. L.; Yamanaka, Y.; Sato, T.; Chikuma, M.; Reedijk, J. *J. Am. Chem. Soc.* **2002**, *124*, 4738−4746.
(34) Komeda, S.; Kalayda, G. V.; Lutz, M.; Spek, A. L.; Yamanaka, Y.; Sato, T.; Chikuma, M.; Reedijk, J. *J. Med. Chem.* **2003**, *46*, 1210−1219.
(35) Komeda, S.; Lin, Y.-L.; Chikuma, M. *Chem. Med. Chem.* **2011**, *6*, 987−990.
(36) Margiotta, N.; Ostuni, R.; Teoli, D.; Morpurgo, M.; Realdon, N.; Palazzo, B.; Natile, G. *Dalton Trans.* **2007**, *29*, 3131−3139.
(37) Margiotta, N.; Ostuni, R.; Piccinonna, S.; Natile, G.; Zanellato, I.; Boidi, C. D.; Bonarrigo, I.; Osella, D. *J. Inorg. Biochem.* **2011**, *105*, 548−557.
(38) Xie, M.; Liu, W.; Lou, L.; Chen, X.; Ye, Q.; Yu, Y.; Chang, Q.; Hou, S. *Inorg. Chem.* **2010**, *49*, 5792−5794.
(39) Quiroga, A. G.; Cubo, L.; de Blas, E.; Aller, P.; Navarro-Ranninger, C. *J. Inorg. Biochem.* **2007**, *101*, 104−110.
(40) Zorbas-Seifried, S.; Jakupec, M. A.; Kukushkin, N. V.; Groessl, M.; Hartinger, C. G.; Semenova, O.; Zorbas, H.; Kukushkin, V. Yu.; Keppler, B. K. *Mol. Pharmacol.* **2007**, *71*, 357−365.
(41) 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.
(42) Bartel, C.; Bytzek, A. K.; Scaffidi-Domianello, Yu. Yu.; Grabmann, G.; Jakupec, M. A.; Hartinger, C. G.; Galanski, M.; Keppler, B. K. *J. Biol. Inorg. Chem.* **2012**, *17*, 465−474.
(43) Grabmann, G.; Meier, S. M.; Scaffidi-Domianello, Yu. Yu.; Galanski, M.; Keppler; B. K.; Hartinger, C. G. Manuscript in preparation.
(44) Kukushkin, V. Yu.; Belsky, V. K.; Aleksandrova, E. A.; Konovalov, V. E.; Kirakosyan, G. A. *Inorg. Chem.* **1992**, *31*, 3836−3840.
(45) Kukushkin, V. Yu.; Tudela, D.; Izotova, Y. A.; Belsky, V. K.; Stash, A. I. *Polyhedron* **1998**, *17*, 2455−2461.
(46) Kukushkin, V. Yu.; Izotova, Y. A.; Tudela, D. *Inorg. Synth.* **2004**, *34*, 81−85.
(47) Wells, A. F. *Structural Inorganic Chemistry*, 5th ed.; Oxford University Press: Oxford, U.K., 1986; pp 357, 692.
(48) Hollis, L. S.; Lippard, S. J. *J. Am. Chem. Soc.* **1981**, *103*, 1230−1232.
(49) Peilert, M.; Erxleben, A.; Lippert, B. *Z. Anorg. Allg. Chem.* **1996**, *622*, 267−274.
(50) Liu, F.; Chen, W.; Wang, D. *Dalton Trans.* **2006**, 3445−3453.
(51) Cornacchia, D.; Pellicani, R. Z.; Intini, F. P.; Pacifico, C.; Natile, G. *Inorg. Chem.* **2009**, *48*, 10800−10810.
(52) Sakai, K.; Takeshita, M.; Tanaka, Y.; Ue, T.; Yanagisawa, M.; Kosaka, M.; Tsubomura, T.; Ato, M.; Nakano, T. *J. Am. Chem. Soc.* **1998**, *120*, 11353−11363.
(53) Hollis, L. S.; Lippard, S. J. *Inorg. Chem.* **1983**, *22*, 2600−2604.
(54) O'Halloran, T. V.; Roberts, M. M.; Lippard, S. J. *Inorg. Chem.* **1986**, *25*, 957−964.
(55) Crespo, M.; Font-Bardia, M.; Granell, J.; Martinez, M.; Solans, X. *Dalton Trans.* **2003**, 3763−3769.
(56) Vicente, J.; Chicote, M.-T.; Lagunas, M.-C. *Inorg. Chem.* **1995**, *34*, 5441−5445.
(57) Marino, N.; Fazen, C. H.; Blakemore, J. D.; Incarvito, C. D.; Hazari, N.; Doyle, R. P. *Inorg. Chem.* **2011**, *50*, 2507−2520.

(58) Wang, K.-W.; Chen, J.-L.; Cheng, Y.-M.; Chung, M.-W.; Hsieh, C.-C.; Lee, G.-H.; Chou, P.-T.; Chen, K.; Chi, Y. *Inorg. Chem.* **2010**, *49*, 1372−1383.
(59) Kukushkin, V. Yu.; Belsky, V. K.; Tudela, D. *Inorg. Chem.* **1996**, *35*, 510−513.
(60) Scaffidi-Domianello, Yu. Yu.; Haukka, M.; Kelly, P. F.; Galanski, M.; Keppler, B. K.; Kukushkin, V. Yu. *Z. Krystallogr. NCS* **2006**, *221*, 226−228.
(61) Kaplan, S. F.; Kukushkin, V. Yu.; Shova, S.; Suwinska, K.; Wagner, G.; Pombeiro, A. J. L. *Eur. J. Inorg. Chem.* **2001**, 1031−1038.
(62) Appleton, T. G.; Bailey, A. J.; Barnham, K. J.; Hall, J. R. *Inorg. Chem.* **1992**, *31*, 3077−3082.
(63) Biswas, B.; Salunke-Gawali, S.; Weyhermüller, T.; Bachler, V.; Bill, E.; Chaudhuri, P. *Inorg. Chem.* **2010**, *49*, 626−641.
(64) Costes, J.-P.; Dahan, F.; Dupuis, A.; Laurent, J.-P. *Inorg. Chem.* **2000**, *39*, 169−173.
(65) Langenbahn, M.; Stoeckli-Evans, H.; Suess-Fink, G. *J. Organomet. Chem.* **1990**, *397*, 347−358.
(66) Chao, M.-H.; Kumaresan, S.; Wen, Y.-S.; Lin, S.-C.; Hwu, J. R.; Lu, K.-L. *Organomet.* **2000**, *19*, 714−717.
(67) Dreos, R.; Tauzher, G.; Trendafilova, D. H.; Nardin, G.; Randaccio, L. *Inorg. Chem.* **1996**, *35*, 2715−2716.
(68) Kanderal, O. M.; Kozłowski, H.; Dobosz, A.; Swiatek-Kozlowska, J.; Meyerd, F.; Fritsky, I. O. *Dalton Trans.* **2005**, 1428−1437.
(69) Audhya, A.; Bhattacharya, K.; Maity, M.; Chaudhury, M. *Inorg. Chem.* **2010**, *49*, 5009−5015.
(70) Kanderal, O. M.; Kozłowski, H.; Dobosz, A.; Swiatek-Kozlowska, J.; Meyerd, F.; Fritsky, I. O. *Dalton Trans.* **2005**, 1428−1437.
(71) Ross, P. F.; Murmann, R. K.; Schlemper, E. O. *Acta Crystallogr.* **1974**, *B30*, 1120−1123.
(72) Bencini, A.; Costes, J.-P.; Dahan, F.; Dupuis, A.; Garcia-Tojal, J.; Gatteschi, D.; Totti, F. *Inorg. Chim. Acta* **2004**, *357*, 2150−2156.
(73) Mathrubootham, V.; Addison, A. W. K.; Holman, T.; Laurence, E. S.; Thompson, K. *Dalton Trans.* **2009**, 8111−8116.
(74) Cano, J.; Rodríguez-Fortea, A.; Alemany, P.; Alvarez, S.; Ruiz, E. *Chem.—Eur. J.* **2000**, *6*, 327−333.
(75) Liu, X.-W.; Chu, S.; Wang, X.-Q.; Zhang, Y.; Wang, R.; Shen, G.-Q.; Shen, D.-Z. *Inorg. Chem. Commun.* **2002**, *5*, 1086−1089.
(76) Dominguez-Vera, J. M.; Colacio, E.; Escuer, A.; Klinga, M.; Kivekäs, R.; Romerosa, A. *Polyhedron* **1996**, *16*, 281−289.
(77) Escuer, A.; Vlahopoulou, G.; Perlepes, S. P.; Font-Bardia, M.; Calvet, T. *Dalton Trans.* **2011**, *40*, 225−233.
(78) Datta, D.; Mascharak, P. K.; Chakravorty, A. *Inorg. Chem.* **1981**, *20*, 1673−1679.
(79) Akagi, F.; Michihiro, Y.; Nakao, Y.; Matsumoto, K.; Sato, T.; Mori, W. *Inorg. Chim. Acta* **2004**, *357*, 684−688.
(80) Koumousi, E. S.; Raptopoulou, C. P.; Perlepes, S. P.; Escuer, A.; Stamatatos, T. C. *Polyhedron* **2010**, *29*, 204−211.
(81) Ruiz, R.; Lloret, F.; Julve, M.; Faus, J. M.; Mufioz, C.; Solans, X. *Inorg. Chim. Acta* **1998**, *268*, 263−269.
(82) Maekawa, M.; Kitagawa, S.; Nakao, Y.; Sakamoto, S.; Yatani, A.; Mori, W.; Kashino, S.; Munakata, M. *Inorg. Chim. Acta* **1999**, *293*, 20−29.
(83) Cervera, B.; Ruiz, R.; Lloret, F.; Julve, M.; Cano, J.; Faus, J.; Bois, C.; Mrozinski, J. *Dalton Trans.* **1997**, 395−401.
(84) Birkelbach, F.; Winter, M.; Flöke, U.; Haupt, H.-J.; Butzlaff, C.; Lengen, M.; Bill, E.; Trautwein, A. X.; Wieghardt, K.; Chaudhuri, P. *Inorg. Chem.* **1994**, *33*, 3990−4001.
(85) Salonen, M. *J. Coord. Chem.* **2010**, *63*, 3127−3136.
(86) Buyuktas, B. S.; Aktas, O. *Transition Met. Chem.* **2006**, *31*, 56−61.
(87) Stoumpos, C. C.; Inglis, R.; Roubeau, O.; Sartzi, H.; Kitos, A. A.; Milios, C. J.; Aromi, G.; Tasiopoulos, A. J.; Nastopoulos, V.; Brechin, E. K.; Perlepes, S. P. *Inorg. Chem.* **2010**, *49*, 4388−4390.
(88) Milios, C. J.; Wood, P. A.; Parsons, S.; Foguet-Albiol, D.; Lampropoulos, C.; Christou, G.; Perlepes, S. P.; Brechin, E. K. *Inorg. Chim. Acta* **2007**, *360*, 3932−3940.
(89) Cremades, E.; Cano, J.; Ruiz, E.; Rajaraman, G.; Milios, C. J.; Brechin, E. K. *Inorg. Chem.* **2009**, *48*, 8012−8019.
(90) Portela-Cubillo, F.; Lymer, J.; Scanlan, E. M.; Scott, J. S.; Walton, J. C. *Tetrahedron* **2008**, *64*, 11908−11916.
(91) Wang, K.; Fu, X.; Liu, J.; Liang, Y.; Dong, D. *Org. Lett.* **2009**, *11*, 1015−1018.
(92) *SAINT-Plus*, version 7.06a and APEX2; Bruker-Nonius AXS Inc.: Madison, WI, 2004.
(93) Sheldrick, G. M. *Acta Crystallogr.* **1990**, *A46*, 467−473.
(94) Sheldrick, G. M. *Acta Crystallogr.* **2008**, *A64*, 112−122.
(95) Burnett, M. N.; Johnson, G. K. *ORTEP III*, Report ORNL-6895; Oak Ridge National Laboratory: Oak Ridge, TN, 1996.