EurJIC
European Journal of Inorganic Chemistry

A Journal of
ChemPubSoc Europe

Accepted Article

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To be cited as: *Eur. J. Inorg. Chem.* 10.1002/ejic.201800055

Link to VoR: http://dx.doi.org/10.1002/ejic.201800055

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10.1002/ejic.201800055

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Synthesis and reactivity of cytotoxic platinum(II) complexes of bidentate oximes: a step towards the functionalization of bioactive complexes.

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Abstract: Two new complexes of platinum(II) bearing triphenylphosphine and bidentate oxime ligands [Pt(Cl)(PPh_3){(κ^2-N,O)-[1(C(R)=N(OH)-2(O)$C_{10}H_6$]}] (R = H, Me) were synthesized in good yields starting from *trans*-[PtCl(μ-Cl)(PPh_3)]$_2$. The structure of [Pt(Cl)(PPh_3){(κ^2-N,O)-[1(CH=N(OH)-2(O)$C_{10}H_6$]}] was determined by single crystal X-ray diffraction. Both complexes showed good antiproliferative properties *in vitro* against HeLa, A2780 and A2780cis cancer cell lines. They react cleanly with alkylating agents in the presence of aqueous bases under phase transfer catalysis conditions, affording the corresponding O-alkylation products [Pt(Cl)(PPh_3){(κ^2-N,O)-[1(HC=N(OR')-2(O)$C_{10}H_6$]}] (R' = CH_2CH_2Cl, CH_2Ph, $(CH_2)_4Br$) in good yields.

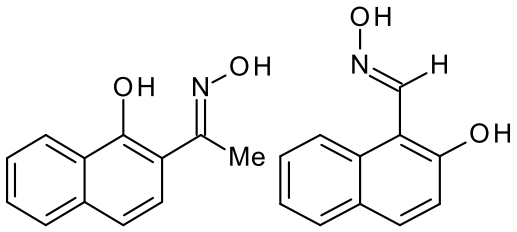
Introduction

After the discover and the approval of cisplatin [1] as powerful anticancer agent, every year hundreds of new platinum complexes are prepared and tested, in the continuous and challenging search for less toxic and more specific drugs. In this context, non-conventional analogues of cisplatin able to circumvent resistance and new synthetic approaches addressed to drug delivery are important research topics.[2] In this view, the presence of reactive functional groups can be of great help. Among unconventional platinum derivatives, those containing phosphane ligands [3] have often shown an interesting ability to circumvent cisplatin resistance, with mechanisms significantly different from cisplatin mode of action [3d,g,j,l] and with the phosphane ligand playing a crucial role in the intracellular drug uptake.[3k,l] In this context, we have recently been interested in oxime derivatives.[3a] In fact, the known acidity of oxime hydroxyl residue, which is greatly enhanced upon coordination to metals,[4] can be exploited both for the formation of non-bonding interactions and for synthetic modifications of the complexes. It is known, indeed, that some platinum oxime complexes possess interesting antiproliferative properties.[5] In our previous study [3a] concerning monodentate [$PtCl_2$(PPh_3)(oxime)] derivatives, we had observed an interesting reactivity of these systems with aqueous bases under phase transfer catalysis conditions, leading to the formation of rare dinuclear oximate complexes.[3a] Nevertheless, the scarce water solubility of the prepared compounds and their instability in DMSO had prevented us from testing them *in vitro* as antiproliferative agents. In this work, we report the synthesis and the *in vitro* antiproliferative properties of two stable, PPh_3, oxime Pt(II) chelated complexes and we show that the oxime hydroxyl group can be conveniently exploited to functionalize the compounds.

Results and Discussion

Synthesis of the complexes

Bidentate oxime ligands **1**-**2** (Figure 1) used in this work were prepared starting from the corresponding 2-hydroxyarylcarbonylic compounds, according to reported procedures.[6]



1 **2**

Figure 1. Bidentate 2-hydroxyarylald- and ketoximes used in this work

Platinum complexes were prepared in refluxing 1,2-DCE

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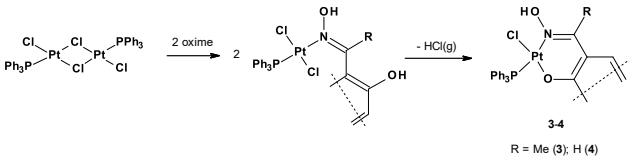
solution, starting from *trans*-[PtCl(μ-Cl)(PPh_3)]$_2$ [7] and following the reaction by ^{31}P-NMR. As expected, the bridge-splitting reaction is initially guided by the strong *trans* effect exerted by the PPh_3 group, leading to the formation of a monodentate *trans*-(Z) kinetic intermediate (Scheme 1). At variance with the analogous monodentate systems we studied earlier,[3a] the formation of the kinetic intermediate was not followed by any isomerization in solution. A slow elimination of HCl was instead observed, leading to single chelation products **3**-**4**, which were recovered by the usual work-up procedures and characterized by IR (ATR), ^{1}H-, ^{31}P-, ^{13}C- and ^{195}Pt-NMR (Figures SI1-SI8). Since the elimination of gaseous HCl (Scheme 1) took up to two weeks, with maximum isolated yields ranging from 30 to 47 %, the syntheses were repeated in the presence of silver acetate.



Scheme 1. Synthesis of chelated complexes **3** and **4**

In fact, while silver ion could facilitate the intermediate dehalogenation, the presence of acetate ion could help the deprotonation of phenolic residue on the oxime ligand. In these conditions the reactions were much faster (2h) and the isolated yield of the bidentate complexes could be improved (70-89%).
It is worth to note that deprotonation of the phenolic function was selective and the desired chelated complex was the only product observed. No deprotonation of oxime groups by silver acetate was observed in our system at variance with previous reports.[8] Complexes **3**-**4** are soluble in common chlorinated solvents and scarcely soluble in water or ethanol. They are soluble in DMSO and their stability was checked by ^{31}P NMR in a 20 mM DMSO solution containing H_2O (5 vol.-%). It is known, in fact, that, in some cases, the presence of water can favor the reactivity of platinum substrates toward DMSO.[9] In these conditions the solutions were stable up to 24h at r.t. (Figures SI9-SI10).Stock solutions of the complexes for the *in vitro* assays were thus prepared in DMSO and stocked at -18 °C.
For complex **4** the structure was confirmed by single crystal X-ray diffraction.
The structure of complex **4** is represented in Figure **2**, while the most significant bond lengths and angles are reported in Table **1**. Coordination around platinum center is square planar, with small deviations from ideal bond angles. The oxime chelates the metal through nitrogen atom and the deprotonated phenolic group on position 2 of the aromatic moiety. The platinum coordination sphere is completed by a PPh_3, in *trans* to the oxime nitrogen atom, and by a chloride ion. Pt-O and Pt-N bond lengths can be compared with those in the complex [Pt(o-OC_6H_4CH=NOH)$_2$][10]. Pt-O bond distance in **4** (1.981(3) Å) is quite close to the one reported by Pombeiro et al. (1.978(5) Å), while Pt-N bond in **4** (2.032(3) Å) is, as expected, slightly longer (2.063(4) Å *vs.* 1.974(6) Å), due to the presence of the phosphine ligand in trans position.

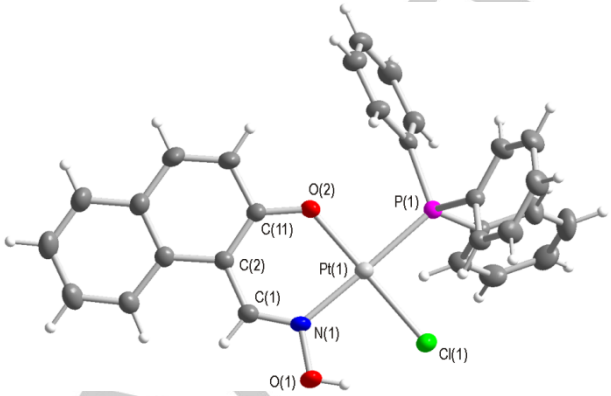
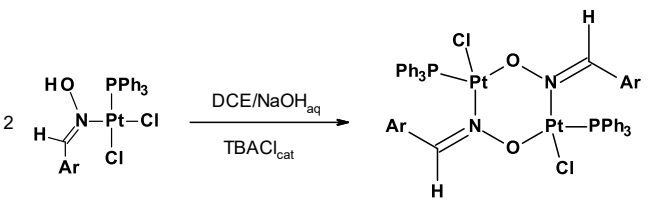


Figure 2. Molecular structure of complex **4**

Table 1. Bond lengths [Å] and angles [°] for complex **4**.

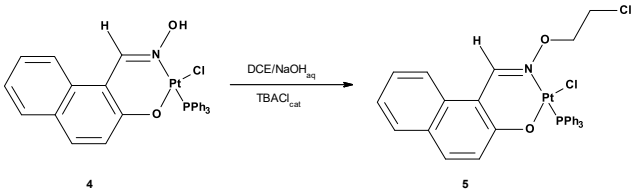
Bond lengths [Å]		Bond angles [°]	
Pt(1)-O(2)	1.981(3)	O(2)-Pt(1)-N(1)	89.23(11)
Pt(1)-N(1)	2.032(3)	O(2)-Pt(1)-P(1)	89.23(7)
Pt(1)-P(1)	2.2488(9)	N(1)-Pt(1)-P(1)	178.45(8)
Pt(1)-Cl(1)	2.3086(10)	O(2)-Pt(1)-Cl(1)	179.60(9)
N(1)-C(1)	1.290(5)	N(1)-Pt(1)-Cl(1)	90.68(8)
N(1)-O(1)	1.393(4)	P(1)-Pt(1)-Cl(1)	90.86(4)
		C(1)-N(1)-O(1)	112.1(3)
		C(1)-N(1)-Pt(1)	127.6(2)
		O(1)-N(1)-Pt(1)	120.3(2)

Reactivity
In our previous study [3a] concerning platinum complexes of monodentate oximes we found that aqueous bases, under phase transfer reaction conditions, deprotonate oxime hydroxyl group, affording rare dinuclear derivatives, where two oximate ligands bridge two platinum centres in a "head to tail" coordination mode (Scheme 2).



Scheme 2. Reactivity of complexes of monodentate oxime with aqueous bases

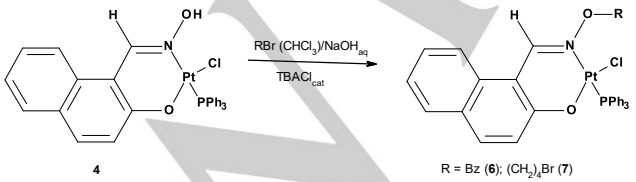
Complex **4** was tested for its reactivity in the same conditions. In a typical experiment, **4** was dissolved in 1,2-dichloroethane and the resulting solution was treated with an aqueous NaOH, in the presence of 5% of tetrabutyl ammonium chloride. The reaction progress was checked by ^{31}P-NMR on samples taken from the organic phase at different time spans. The spectroscopic analyses showed the gradual disappearing of the signal of **4** (6.38ppm, $^{1}J_{P\text{-}Pt}$ = 3821 Hz), in favor of a new one at 6.01ppm ($^{1}J_{P\text{-}Pt}$ = 3807 Hz). The conversion was complete in 24 h at 25 °C and a new, single product (**5**) was recovered from the organic phase after the usual work-up procedures. This complex was characterized spectroscopically. Unexpectedly, collected data showed that no dinuclear derivative had formed, but O-alkylation by the solvent had occurred (Scheme 3).



Scheme 3. O-alkylation of complex **4** by 1,2-DCE

Alkylation of the oxime hydroxyl residue was evident in the ^{1}H-NMR spectrum of isolated (53%) **5**, where oxime OH singlet at 10.53 ppm was absent and two triplet signals were observed at 4.70 and 3.82 ppm, both accounting for two hydrogen atoms and attributable to OCH_2 and CH_2Cl residues respectively. This interpretation is in agreement with the chemical shift and the coupling constant observed in the ^{31}P-NMR spectrum of **5**, which are both similar to those observed for the precursor. Finally, in the IR (ATR) spectrum, the broad absorption due to OH stretching in **4** had disappeared. The preparation of **5** is interesting, because 2-chloroethyl residues are typical of strong DNA alkylating agents and recently some Pt(II) and Pt(IV) complexes containing an analogous fragment were found active against pancreatic cancer cell lines.[11]

Interestingly, the observed reactivity was extended to alkylating agents other than 1,2-DCE. For instance, **4** was alkylated with benzyl bromide and with 1,4-dibromobutane under the same conditions, replacing 1,2-DCE with chloroform (Scheme 4).



Scheme 4. O-alkylation of complex **4** by RBr

In these cases, the reactions were much faster, being complete in 2-7 h and only a slight excess of alkylating agent was necessary. Complexes **6** and **7** were recovered in good yields. In all the cases studied, the O-alkylation process was chemoselective and no products arising from substitution at the metal centre were observed.

In vitro assays

Complexes **3** and **4** were tested *in vitro* for their antiproliferative activity against three human tumor cell lines, HeLa (cervix adenocarcinoma), A2780 (ovarian carcinoma) and A2780cis (ovarian carcinoma cisplatin resistant). Data, expressed as GI_{50} (μM), are reported in Table 2.

Table 2. Cell growth inhibition values in the presence of examined complexes and cisplatin as reference.

Complex	Cell Line GI_{50}[a] (μM)		
	HeLa	A2780	A2780cis
3	3.53±0.99	0.62±0.12	2.73±0.21
4	3.67±0.30	0.94±0.28	1.40±0.06
cisplatin	1.42±0.32	0.80±0.06	7.01±0.26

[a] Values are the mean ± SD of at least three independent experiments

Complexes **3** and **4** are able to exert a significant antiproliferative activity on both HeLa and A2780 cell lines. In particular, on HeLa cells the cytotoxicity induced by the new complexes appear quite lower with respect to that observed for cisplatin, while on A2780 cells comparable GI_{50} values were obtained. Notably, notwithstanding the similar effect on A2780 cells, sensitive toward cisplatin, on the corresponding resistant cell line, A2780cis, the cytotoxic capacity of **3** and **4** is significantly higher with respect to that of the drug, suggesting the possibility for these new Pt(II)-based structures to overcome the resistance phenomenon. In this connection, **4** shows the most interesting behavior with a GI_{50} value on A2780cis comparable to that obtained on A2780 cells. These results point to the new platinum(II) complexes bearing triphenylphosphine and bidentate oxime ligand as a lead moiety worth to be further developed for the synthesis of more efficacious Pt(II) complexes.

Conclusions

Two new platinum(II) complexes bearing PPh_3 and bidentate oxime ligands were prepared in good yields from *trans*-[PtCl(μ-Cl)(PPh_3)]$_2$ in the presence of silver acetate. Both complexes showed antiproliferative properties when tested against HeLa, A2780 and A2780cis cancer cell lines. In particular, they were able to circumvent resistance towards A2780cis cells, with **4** showing the best results. The mechanism of action is currently under investigation. The synthesized complexes reacted cleanly with alkylating agents in the presence of bases under PTC

conditions, affording only the O-alkylation products. The reaction appears to be general, allowing the functionalization of the complex without affecting the coordination sphere of the bioactive platinum centre. This reactivity could be exploited to link the platinum center to fragments capable to bind to particular receptors, bioactive moieties or suitable nanoparticles, thus opening the way toward cooperation effects and drug delivery.

Experimental Section

Materials and general methods

All manipulations were performed under a dinitrogen atmosphere, if not otherwise stated. Solvents and liquid reagents were dried according to reported procedures[12]. ^{1}H-, ^{13}C-, ^{31}P- and ^{195}Pt NMR spectra were recorded with a Bruker ''Avance DRX400'' spectrometer, in $CDCl_3$ solution if not otherwise stated. Chemical shifts were measured in ppm (δ) from TMS by residual solvent peaks for ^{1}H and ^{13}C, from aqueous (D_2O) H_3PO_4 (85 %) for ^{31}P and from aqueous (D_2O) hexachloroplatinic acid for ^{195}Pt. A sealed capillary containing C_6D_6 was introduced in the NMR tube to lock the spectrometer to the deuterium signal when non-deuterated solvents were used. FTIR spectra in solid phase were recorded with a Perkin–Elmer ''Spectrum One'' spectrometer, equipped with an ATR accessory. Elemental analyses (C, H, N) were performed at Dipartimento di Scienze e Tecnologie Chimiche, Università di Udine and at Dipartimento di Chimica e Chimica Industriale, Università di Pisa. *Trans*-[Pt(μ-Cl)Cl(PPh_3)]$_2$ [7] was prepared according to a reported procedure. Aldoxime ligands **1**-**2** (Figure 1) were prepared by a slight modification of a described procedure.[6] Hydroxylamine hydrochloride (NH_2OH·HCl) (99 %, Carlo Erba) and silver acetate (98 %, Sigma Aldrich) were used without further purification.

In the text, the following abbreviations were used: 1,2-dichloroethane (1,2-DCE), tetrabutylammonium chloride (TBACl).

General procedure for the synthesis of bidentate oxime complexes

A Schlenk tube equipped with a magnetic stirrer was charged with [Pt(μ-Cl)Cl(PPh_3)]$_2$ (0.300-0.500 g), the suitable bidentate oxime (oxime/Pt = 1.03 molar ratio), silver acetate (Ag(OAc)/oxime = 1.00 molar ratio) and 1,2-DCE. The mixture was shielded from light and stirred at 25 °C. A yellow solution was obtained and a colorless solid formed almost immediately. The suspension was stirred until the maximum conversion was reached (^{31}P-NMR, 2 h), then was filtered on a short package of celite. The liquid phase was concentrated under vacuum up to one third of the original volume and then treated with heptane. The yellow solid precipitated was filtered, washed with heptane and dried under vacuum.

For each of the bidentate complexes, the oxime used, the % isolated yield and the characterization are reported.

*[Pt(Cl)(PPh_3){(κ^2-N,O)-[1(O)-2(C(Me)N(OH)$C_{10}H_6$]}] (**3**)*

Oxime **1**; 70 %. $C_{30}H_{25}ClNO_2PPt\cdot1.5H_2O$ Anal. Calc.: C 50.0, H 3.9, N 1.9%. Exp.: C 50.0, H 4.0, N 1.7 %. IR (ATR, $\tilde{\nu}$, cm^{-1}): 3257; 3053; 2960; 1625; 1593; 1570; 1544; 1501; 1481; 1456; 1435; 1418; 1398; 1372; 1338; 1260; 1245; 1188; 1133; 1099; 1021; 998; 975; 900; 868; 796; 747; 710. ^{1}H NMR: 10.81 (s, 1H, OH); 7.87-7.82 (m, 6H, H_{arom} PPh_3); 7.76-7.71 (m, 2H, H_{arom}); 7.54-7.44 (m, 9H, H_{arom} PPh_3), 7.35-7.31 (m, 1H, H_{arom}); 7.08 (d, 1H, J=8.7 Hz, H_{arom}); 6.82-6.79 (m, 1H, H_{arom}); 6.59 (d, 1H, J=8.7 Hz, H_{arom}); 2.60 (s, 3H, CH_3). ^{13}C NMR: 158.3; 134.8 (d, J=10.7 Hz); 131.1; 128.5 (d, J=11.2 Hz); 128.4; 128.3; 128.2; 127.7; 127.5 125.9 (d, J=74.4 Hz); 125.5; 124.3; 116.9; 116.4; 77.2; 15.06. ^{31}P NMR: 6.73 (J_{P-Pt}=3891Hz). ^{195}Pt NMR:-2938 (J_{P-Pt}=3891 Hz).

*[Pt(Cl)(PPh_3){(κ^2-N,O)-[1(CHN(OH)-2(O)$C_{10}H_6$]}] (**4**)*

Oxime **2**; 89%. $C_{29}H_{23}ClNO_2PPt\cdot H_2O$. Anal. Calc.: C 50.0, H 3.6, N 2.0 %; Exp.: C 49.8, H 3.2, N 2.1 %. IR (ATR, $\tilde{\nu}$, cm^{-1}): 3237; 3055; 2963; 2903; 2860; 1628; 1599; 1548; 1515; 1482; 1455; 1435; 1424; 1405; 1354; 1332; 1298; 1260; 1189; 1092; 1017; 947; 865; 797; 745; 707; 692. ^{1}H NMR: 10.53 (s, 1H, OH); 9.21 (d, 1H, J=9.6 Hz, CHN); 7.92 (d, 1H, J=9.0 Hz, H_{arom}); 7.84-7.79 (m, 7H, H_{arom}+ H_{arom} PPh_3); 7.64 (d, 1H, J=7.9 Hz, H_{arom}); 7.57-7.48 (m, 11H, H_{arom} + H_{arom} PPh_3); 6.41 (d, 1H, J=9.0 Hz, H_{arom}). ^{13}C NMR: 149.2; 134.9 (d, J=10.6 Hz); 133.1; 131.2; 131.1; 128.7; 128.2 (d, J=11.3 Hz); 127.9; 127.8; 127.5 (d, J=67 Hz); 127.4; 127.3; 123.0; 122.7; 119.5. ^{31}P NMR: 7.28 (J_{P-Pt}=3829 Hz). ^{195}Pt NMR: -2909 (J_{P-Pt}=3829 Hz).

O-Alkylation of bidentate oxime complexes

[Pt(Cl)(PPh_3){(κ^2-N,O)-[1(CHN(OCH$_2$CH$_2$Cl)-2(O)$C_{10}H_6$]}] (**5**)

A Shlenk tube equipped with a magnetic stirrer was charged with a 1,2-DCE solution of **4** (69.9 mg, 0.103 mmol in 10.0 ml), a 0.02 M solution of NaOH in water (10.0 mL) and a catalytic amount of tetrabutylammonium chloride (TBACl, 5 mol-%). The mixture was stirred at room temperature (25 °C) and the complete conversion of the precursor into a single product was checked spectroscopically (^{31}P NMR, 24 h). After separating the two phases, the aqueous phase was extracted with portions of 1,2-DCE (3 x 10 mL). The collected organic phases were dried over anhydrous Na_2SO_4, filtered and concentrated up to 10 mL. The addition of heptane (20 mL) caused the precipitation of an orange solid, which was filtered, washed with heptane and dried under vacuum (40.5 mg, 53 % isolated yield). $C_{31}H_{26}Cl_2NO_2PPt\cdot H_2O$. Anal. Calc.: C 49.0, H 3.7, N 1.8 %; Exp.: C 49.4, H 3.7, N 1.6 %. IR (ATR, $\tilde{\nu}$, cm^{-1}): 2964; 2906; 1619; 1602; 1541; 1507; 1481; 1429; 1407; 1335; 1259; 1084; 1015; 946; 863; 796; 753; 710. ^{1}H NMR: 9.42 (d, 1H, H_{arom}); 7.89-7.79 (m, 8H, CHN + H_{arom}); 7.63 (d, 2H, H_{arom}); 7.55-7.46 (m, 13H, H_{arom}); 7.24 (d, 1H, H_{arom}); 4.70 (t, 2H, OCH_2); 3.83 (t, 2H, CH_2Cl). ^{13}C NMR: 151.0; 135.0 (d, J=10.3 Hz); 133.6; 131.0; 128.3 (d, J=80.5 Hz); 128.2 (d, J=10.9 Hz); 123.1; 119.5; 76.4; 41.1. ^{31}P NMR: 6.01 (J_{P-Pt}=3807 Hz). ^{195}Pt NMR: -2849 (J_{P-Pt}=3807 Hz).

[PtCl(PPh_3){(κ^2-N,O)-[1(CHN(OR)-2(O)$C_{10}H_6$]}] General procedure.

A Shlenk tube equipped with a magnetic stirrer was charged with a solution of **4** in $CHCl_3$ (about 0.15 mmol in 10.0 mL), RBr (RBr/Pt = 1.3 molar ratio), a catalytic amount (5 mol-%) of TBACl and a 0.02 M solution of NaOH in water (10.0 mL). The mixture was stirred at room temperature (25 °C) and the complete conversion of the precursor was

checked spectroscopically (^{31}P NMR, 2-5 h). After separating the two phases, the aqueous phase was extracted with portions of $CHCl_3$ (3 x 10 mL). The collected organic phases were dried over anhydrous Na_2SO_4, filtered and concentrated up to 10 mL. The addition of heptane (20 mL) caused the precipitation of an orange solid, which was filtered, washed with heptane and dried under vacuum. For each O-alkylated complex the alkyl bromide used, the isolated yield and the characterization are reported:

*[PtCl(PPh$_3$){(κ^2-N,O)-[1(CHN(OBz)-2(O)C$_{10}$H$_6$]}] (**6**): BzBr, 50 %*

IR ($\tilde{\nu}$, cm^{-1}): 3055; 2963; 1619; 1599; 1533, 1434, 1332, 1300, 1189; 1097; 1016; 949; 799, 743; 692. ^{1}H NMR: 9.06 (d, 1H, J=8.4Hz, CHN); 7.94-7.85 (m, 4H, HPPh$_3$); 7.65 (m, 2H, HAr); 7.62-7.46 (m, 14H, HAr + HPPh$_3$); 7.33-7.35 (m, 2H, HAr); 7.27-7.20 (m, 1H, HAr); 6.27 (d, 1H, HAr); 5.45 ppm (s, 2H, CH_2Ph). ^{13}C NMR: 163.5, 151.4, 151.3, 135.4 135.0, (d, J=11 Hz), 134.9, 133.3, 131.0, 129.9, 128.7, 128.5, 128.3, 128.1 (d, J=10 Hz), 128.4 (d, J= 67 Hz), 127.6, 123.3, 122.9, 119.4, 105.7, 79.7. ^{31}P NMR: 6.47 (J=3783 Hz). ^{195}Pt NMR: -2843 (J=3783 Hz).

[PtCl(PPh$_3$){(κ^2-N,O)-[1(CHN(O(CH$_2$)$_4$Br)-2(O)C$_{10}$H$_6$]}] (**7**): Br(CH$_2$)$_4$Br, 71 %.

$C_{33}H_{30}BrClNO_2PPt$. Anal. Calc.: C 48.7, H 3.7, N 1.7 %; Exp.: C 48.7, H 3.4, N 2.0 %. IR ($\tilde{\nu}$, cm^{-1}): 3055, 2943, 1615, 1596, 1533, 1504, 1435, 1389, 1323, 1256, 1098, 1032, 961, 900, 868, 837, 746, 691. ^{1}H NMR: 9.27 (d, 1H, J = 8.6Hz, C<u>H</u>N); 7.88-7.83 (m, 7H, H$_{Ar}$); 7.63 (d, 1H, J = 7.7 Hz, H$_{Ar}$); 7.59-7.44 (m, 11H, H$_{Ar}$); 7.28 (m, 1H, H$_{Ar}$); 6.26 (d, 1H, J = 7.7 Hz, H$_{Ar}$); 4.45 (t, 2H, J=6.5 Hz, NOCH$_2$); 3.53 (t, 2H, J = 6.5 Hz, CH$_2$Br); 2.15 (quint, 2H, J = 6.5 Hz, NOCH$_2$C<u>H$_2$</u>); 1.97 (quint, 2H, J = 6.5 Hz, C<u>H$_2$</u>CH$_2$Br). ^{13}C NMR: 163.6, 150.6, 150.5, 135.0 (d, J = 11 Hz), 134.8, 133.2, 131.0, 128.8, 128.2 (d, J = 10 Hz), 127.6 (d, J = 64 Hz), 127.7, 127.4, 123.0, 119.4, 106.0, 76.6, 33.9, 29.5, 26.6. ^{31}P NMR: 6.45 (J=3792 Hz). ^{195}Pt NMR: -2843 (J=3792 Hz).

Inhibition Growth Assay

HeLa (human cervix adenocarcinoma cells) were grown in Nutrient Mixture F-12 [HAM] (Sigma Chemical Co.); A2780 (human ovarian carcinoma) and A2780cis (human ovarian carcinoma cisplatin-resistant) were grown in RPMI 1640 (Sigma Chemical Co.). 1.5g/L $NaHCO_3$, 10 % heat-inactivated fetal calf serum (Invitrogen), 100 U/mL penicillin, 100 µg/mL streptomycin, and 0.25 µg/mL amphotericin B (Sigma Chemical Co.) were added to the media. The cells were cultured at 37 °C in a moist atmosphere of 5 vol.-% carbon dioxide in air. HeLa cells (4 x 10^4), A2780 (2.5 x 10^4) or A2780cis (2.5 x 10^4) were seeded into each well of a 24-well cell culture plate. After incubation for 24 h, various concentrations of the test agents were added to the complete medium and incubated for a further 72 h. Stock solutions of new complexes were made in dimethylsulfoxide at 20 mM concentration and then diluted with complete medium in such a way that the final amount of solvent in each well did not exceed 0.5 vol.-%. Cisplatin was dissolved in 0.9 wt.-% NaCl. A Trypan blue assay was performed to determine cell viability. Cytotoxicity data were expressed as GI_{50} values, i.e., the concentration of the test agent inducing 50 % reduction in cell number compared with control cultures.

X-Ray determinations

Crystals of **4** were selected at room temperature (296 K), glued to glass fibers and analyzed with a Bruker Smart Breeze CCD diffractometer. Table **3** summarizes the lattice parameters and the space group. Intensity data were collected in the range of 2θ angles reported in Table **3**. After correction for Lorentz and polarization effects and for absorption, the structure solution was obtained using the direct methods contained in SHELXS program.[13] The hydrogen of oxime hydroxyl residue was found in the difference Fourier map, while the other hydrogen atoms were introduced in calculated positions. The final reliability factors of the refinement procedure, done using SHELXL program, [14] are listed in Table **3**. Other control calculations were performed with the programs contained in the WINGX suite.[15]

CCDC 1590522 for **4** contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre.

Table 3. Crystal data and structure refinement for complex **4.**

Compound	**4**	
Empirical formula	$C_{29}H_{23}ClNO_2PPt$	
Formula weight	678.99	
Temperature	296(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	C 2/c	
Unit cell dimensions	a = 17.0248(7) Å	α= 90°.
	b = 9.7147(4) Å	β= 101.871(2)°.
	c = 30.9507(17) Å	γ = 90°.
Volume	5009.5(4) Å^3	
Z	8	
Density (calculated)	1.801 Mg/m^3	
Absorption coefficient	5.800 mm^{-1}	
F(000)	2640	
Crystal size	0.250 x 0.204 x 0.030 mm^3	
Theta range for data collection	2.894 to 33.732°.	
Index ranges	-26<=h<=26, -15<=k<=12, -46<=l<=37	
Reflections collected	33996	
Independent reflections	9433 [R(int) = 0.0382]	
Completeness to theta = 25.242°	99.8 %	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	9433 / 0 / 316	
Goodness-of-fit on F^2	0.961	
Final R indices [I>2sigma(I)]	R1 = 0.0383, wR2 = 0.0818	
R indices (all data)	R1 = 0.0678, wR2 = 0.0915	
Extinction coefficient	n/a	
Largest diff. peak and hole	1.628 and -0.879 e.Å^{-3}	

Acknowledgements

The authors thank the Università di Pisa (Fondi di Ateneo 2016) and MIUR (PRIN 2015, "Design of Innovative Metal-Ligand Systems for Catalysis and Energy Applications") for financial support and Interuniversity Consortium CHEMICAL REACTIVITY and CATALYSIS (CIRCC). S. S. is grateful to the financial support provided by Università di Pisa—Progetti di Ricerca di Ateneo 2017—'Composti di metalli di transizione come possibili agenti antitumorali' (PRA_2017_25). L. D. V. is grateful to the financial support provided by Dipartimento di Scienze del Farmaco-Università di Padova-Progetti di Ricerca di Dipartimento PRID 2017- "An in depth investigation on novel Pt-based agents to shed light on cancer resistance mechanims" and thanks the Consorzio Interuniversitario di Ricerca in Chimica dei Metalli nei Sistemi Biologici (CIRCMSB).Thanks are due to Dr. D. Scittarelli for preliminary experiments.

Keywords: platinum • antitumor agents • oxime ligands • functionalization

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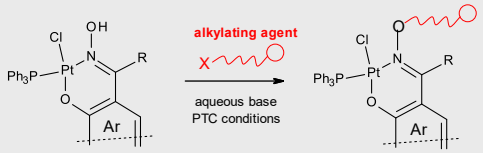

cytotoxic complex

Anticancer complexes

*Daniela Belli Dell' Amico, Marialuigia Colalillo, Lisa Dalla Via, Martina Dell' Acqua, Aída N. García-Argáez, Mariafrancesca Hyeraci, Luca Labella, Fabio Marchetti and Simona Samaritani**

Page No. – Page No.

Synthesis and reactivity of cytotoxic platinum(II) complexes of bidentate oximes: a step towards the functionalization of bioactive complexes