Synthesis, characterization and antiproliferative activity on mesothelioma cell lines of bis(carboxylato) platinum(IV) complexes based on picoplatin†

Mauro Ravera,[a] Elisabetta Gabano,[a] Ilaria Zanellato,[a] Ilaria Bonarrigo,[a] Esther Escribano,[b] Virtudes Moreno,[b] Mercè Font-Bardia,[c,d] Teresa Calvet[c] and Domenico Osella*[a]

[a] Dipartimento di Scienze dell'Ambiente e della Vita, Università del Piemonte Orientale "Amedeo Avogadro", Viale Michel 11, 15121 Alessandria, Italy. E-mail: domenico.osella@mfn.unipmn.it; Fax: +39-0131-360250; Tel: +39-0131-360266

[b] Departament de Química Inorgànica, Universitat de Barcelona, Martí i Franquès 1-11, 08028 Barcelona, Spain

[c] Departament de Cristal·lografia, Mineralogia i Dipòsits Minerals, Universitat de Barcelona, Martí i Franquès s/n, 08028 Barcelona, Spain

[d] Unitat de Difracció de RX, Centre Científic i Tecnològic de la Universitat de Barcelona (CCITUB), Solé i Sabarís 1-3, 08028 Barcelona, Spain

Abstract

The synthesis and characterization of a series of picoplatin-based (picoplatin = $[PtCl_2(mpy)(NH_3)]$, mpy = 2-methylpyridine), Pt(IV) complexes with axial carboxylato ligands of increasing length are reported. The synthesis is based on the oxidation with hydrogen peroxide of picoplatin to give the *cis,cis,trans*-$[PtCl_2(mpy)(NH_3)(OH)_2]$ intermediate and then its transformation into the dicarboxylato complexes cis, *cis,trans*-$[PtCl_2(mpy)(NH_3)(RCOO)_2]$ (R = $CH3(CH2)_n$, n = 0–4) with the corresponding anhydride. $Pt_{(IV)}$ complexes with n = 0–2 were selected to be tested on four malignant pleural mesothelioma (MPM) cell lines, on human mesothelial cells (HMC), and on the cisplatin-sensitive ovarian A2780 cell line along with cisplatin as a metallo-drug reference. In general, the longer the axial chain, the more cytotoxic and selective the $Pt_{(IV)}$ complex is. $Pt_{(IV)}$ analogs show good activity on the MPM cell lines, approaching or in some case bypassing that of cisplatin and represent quite promising drug candidates for the treatment of tumors whose chemoresistance is mainly based on glutathione overexpression, such as MPM.

1. INTRODUCTION

The basic US patent for the antitumor drug cisplatin (US 4,177,263) was issued in December 1979.[1] This is probably a milestone in the history of antitumoral chemotherapy considering that today cisplatin still plays a pivotal role in the systemic treatment of a variety of solid tumors.[2]

The two major problems associated with the use of cisplatin are the severe toxic side-effects (namely nephrotoxicity,[3] neurotoxicity[4] and ototoxicity[5]), as well as the intrinsic or acquired resistance manifested in various types of cancers.[6] As such, a large number of $Pt_{(II)}$-based compounds structurally similar to cisplatin have been developed and tested, to increase the selectivity towards cancer cells and to limit the chemoresistance.

Chemoresistance is a multifactorial phenomenon, in particular the reaction of cisplatin with nucleophiles other than DNA, especially S-donor biomolecules, can cause inactivation of the drug.[7] For instance, cisplatin is inactivated in cells by reduced glutathione (GSH, present in millimolar concentrations in cells) and by metallothioneins (MTs, small proteins having 20 Cys groups per molecule), the main cellular detoxification agents.[8] It is important to recall, however, that GSH may not be the primary target of cisplatin, as reported by Gibson and coworkers. [9] A bulky carrier ligand coordinated to the $platinum_{(II)}$ core reduces the level of these undesired substitution reactions in the square-planar complexes.[10] This rationale prompted the design, synthesis, preclinical tests and clinical trials of cis-amminedichlorido(2-methylpyridine)$platinum_{(II)}$ (picoplatin, ZD0437, AMD-437), an active $Pt_{(II)}$ antitumor drug able to circumvent the acquired Pt-chemotherapy resistance.[11] The presence of the sterically demanding 2-methylpyridine (mpy) hinders the axial approach of nucleophiles to the platinum center without detriment of the level of DNA platination.[12,13] Picoplatin was granted orphan drug designation in 2007 for the treatment of small cell lung cancer (EU/3/07/502).

$Platinum_{(IV)}$ complexes, which undergo ligand substitution reactions much more slowly than their $platinum_{(II)}$ counterparts, are tested as orally administrable pro-drugs. It is

generally accepted that the reduction of $Pt_{(IV)}$ compounds can be carried out by intracellular reductants such as ascorbic acid, GSH or MTs. $Pt_{(IV)}$ complex is believed to be reduced to its $Pt_{(II)}$ active metabolite, which, in turn, will be activated by hydrolysis and bind DNA (Fig. 1).[14] The rational choice of axial ligands L is thus the key for modulating the lipophilicity (the ability to enter the tumor cells by passive diffusion) and the redox potential (the ability to be reduced under the hypoxic conditions typical of tumor tissues) of the $Pt_{(IV)}$ complexes.[15]

In this context, the choice of carboxylic anions with a carbon chain of increasing length as the axial ligands was particularly useful since a strict correlation between antiproliferative activity and length of the carbon chain was observed for other $Pt_{(IV)}$ complexes in vitro (at least as far as these complexes were soluble enough in water) and in vivo in mouse tumor models.[16,17]

Finally, the characteristics of the remaining A and X/L ligands determine the potency of the cytotoxic $Pt_{(II)}$-metabolite that is eventually generated by reduction of the $Pt_{(IV)}$ parent complex (Fig. 1).[18]

It is interesting to consider the dual role of GSH, which on the one hand promotes the reduction of $Pt_{(IV)}$ to $Pt_{(II)}$, activating the pro-drug in cells, and on the other hand hinders the action of the resulting $Pt_{(II)}$ metabolite. Thus, the insertion of the picoplatin square-planar structure in a $Pt_{(IV)}$ octahedral scaffold should afford an ideal pro-drug candidate, easily reduced but hardly deactivated by GSH, often over-expressed by resistant tumor cells.

Malignant pleural mesothelioma (MPM) is a rare and aggressive asbestos-related cancer associated with poor prognosis. Currently, all the polychemotherapeutic protocols for MPM include $Pt_{(II)}$ complexes, namely cisplatin or carboplatin.[19] MPM cells of both phenotypes (namely epithelioid and sarcomatoid) are characterized by strong chemoresistance,[20] which has been ascribed mainly to mechanisms associated with GSH[21] and, then, represent an ideal challenge for picoplatin-based $Pt_{(IV)}$ compounds. It is important

104 to recall that picoplatin has been tested in a phase II trial as a second-line therapy in
105 mesothelioma, [22] demonstrating a manageable tolerability profile.

106 In the present paper, the synthesis, characterization and antiproliferative activity on
107 mesothelioma cell lines of a series of picoplatin-based $Pt_{(IV)}$ complexes having carboxylic
108 acids with carbon chains of increasing length as axial ligands is reported (Fig. 2). This
109 synthesis is based on the oxidation with hydrogen peroxide of picoplatin to give the
110 *cis,cis,trans*-$[PtCl_2(mpy)(NH_3)\ (OH)_2]$ intermediate and then its transformation into
111 dicarboxylato with the corresponding anhydride. Hambley and co-workers followed a
112 similar strategy, but the specific reaction of the above said the $Pt_{(IV)}$ intermediate in neat
113 acetic anhydride (at r.t. instead of 0 °C) resulted in extensive decomposition only.[23]

114

115

116

2. RESULTS AND DISCUSSION

Synthesis of the Pt(IV) complexes

Platinum(IV) complexes containing amine ligands are generally prepared by oxidation of the corresponding platinum(II) precursors using hydrogen peroxide or chlorine. Picoplatin was prepared in high yields according to a published method[23] involving the formation of the platinum dimeric species $[Pt_2I_4(mpy)_2]$ directly from $[PtI_4]^{2-}$ and mpy in water. The reaction of the dimer with ammonia produces a mixed diiodido species, *cis*-$[PtI_2(mpy)(NH_3)]$, which represents the synthon in Dhara's procedure24 to obtain the desired picoplatin. Further treatment with excess hydrogen peroxide in water gave the dihydroxidoplatinum(IV) derivative.[33] The carboxylation reaction was carried out in neat anhydride, at low or room temperature (**1** and **2**), or in refluxing acetonitrile with the corresponding anhydride (**3**–**5**) affording the *bis*(carboxylato)platinum(IV) complexes in high (**1** and **2**) or moderate (**3**–**5**) yield (Scheme 1).

Single-crystal X-ray quality crystals were obtained for **2**, as described in the experimental section. The ORTEP representation with thermal ellipsoids of the structure of 2 is reported in Fig. 3, while the relevant information concerning data collection and details of structure refinement are summarized in Table 1. The molecule shows disorder in one of the two propanoato ligands: with the terminal methyl group being equally disordered over two sites (C3 and C3′) and the H atoms of the adjacent carbon C2 being equally disordered over two pairs of sites.

The coordination geometry around platinum is octahedral, as expected for oxidation state IV. The equatorial plane is occupied by two chloride atoms [Pt–Cl1 2.311(1) and Pt–Cl2 2.319(1) Å] and two ammine groups [Pt–N1 2.050(5) and Pt–N2 2.065(5) Å] whereas the axial positions are occupied by propanoato ligands [Pt–O1 1.993(4) and Pt–O5 2.008(4) Å]. The coordinated oxygens are slightly misaligned with respect to an ideal octahedron [O1–Pt–O3 173.2(1)]. The bond lengths and angles can be regarded as normal compared with those described in the literature. The non-coordinated oxygen of two of the carboxylate ligands are hydrogen-bonded to the protons of one ammine and also there are hydrogen-bonds with other molecules. This hydrogen-bonding scheme was also found in previously

146 reported crystal structures of $Pt_{(IV)}$ complexes.[25,26] Distortion of the octahedron, which is
147 probably due to the presence of the methyl group of the picoline, is proved by the values of
148 the angles N–Pt–N 96.1°, Cl–Pt–Cl 89.1° and O–Pt–O 173.2°.

149

150 **Electrochemical behavior**

151 In cyclic voltammetry (CV), complexes 1–5 show a $2e^-$ reduction process
152 complicated by a chemically irreversible following reaction (*i.e.*, the detachment of the two
153 axial ligands upon reduction of octahedral $Pt_{(IV)}$ to square-planar $Pt_{(II)}$, E_2C mechanism).
154 The peak potentials are reported in Table 2.

155 It should be noted that all picoplatin $Pt_{(IV)}$-complexes but 1 showed low solubility in
156 pure water and addition of 10% of ethanol was useful to observe a better shaped peak. The
157 measured E_p values are identical within the experimental error for experiments with ethanol
158 contents lower than 20%. As the percentage of the co-solvent becomes higher than 20%, the
159 E_p values decrease almost linearly (Fig. 4).

160 As previously reported for the cisplatin-, nedaplatin-, and $[PtCl_2(dach)]$- (dach = 1,2-
161 diaminocyclohexane) based series of $Pt_{(IV)}$ complexes,[26] the E_p values increase (becomes
162 less negative) as the axial chain length increases. Fig. 5 shows the trends of E_p *vs*. the total
163 number of secondary carbons (nCs) for the picoplatin-based $Pt_{(IV)}$ complexes in comparison
164 with the abovementioned series of compounds. Linear relations are observed in each case (r
165 > 0.98) and the lines are almost parallel (slope = 23–27 mV).

166 In pure organic solvent $Pt_{(IV)}$ complexes with different axial chains show very
167 similar E_p values, pointing out that the chain length of the carboxylato ligand does not
168 influence *per se* the reduction potential, indeed it does not substantially alter the electronic
169 characteristics of the Pt center, where the reduction is centered. [27] However, in water
170 different solvation effects on the species involved in the E_2C reduction mechanism do
171 influence the final E_p value. The hydrophilicity/hydrophobicity of the Pt(IV) complexes and

the produced fragments, in particular of the carboxylates, account for the trends shown in Fig. 4 and 5.[26] Thus, along with the increase in lipophilicity, the chain length of the carboxylato ligand offers a further beneficial effect on antiproliferative activity of these complexes, lowering their reduction potential.

Biological tests

Complexes **1**–**3** were selected to be tested on the cisplatinsensitive ovarian A2780 cell line, on four mesothelioma cell lines, and on human mesothelial cells (HMC), along with cisplatin as reference standard (Table 3). The heavier congeners (**4** and **5**) were barely soluble in water and required consequently a too high amount of co-solvent to perform meaningful biological tests.

On A2780 **1** is less potent whereas **3** has higher activity than cisplatin. A similar behavior was seen for the cisplatin-based Pt(IV) analogs.[17] On MPM cells complexes **1**–**3** show an increased activity as the carbon chain length increases, approaching or in one case bypassing that of cisplatin itself. Moreover, derivatives **1**–**3** always exhibit a lower resistance factor RF (RF = IC_{50} (MM98R)/IC_{50} (MM98)) than cisplatin.

Noteworthy, it has been demonstrated that the cisplatinresistant cell line MM98R shows a higher increase in intracellular GSH (when treated with Pt derivatives at sub-lethal concentrations) with respect to the wild counterpart MM98. This could partially explain the acquired resistance.[28] For this reason, the observed low RF for compounds **1**–**3** may be related to a generally low inactivation of $Pt_{(IV)}$ complexes under study, or their metabolites, by GSH (see following section).

In general, as previously observed for other series of homologous $Pt_{(IV)}$ complexes,[17] the longer the axial chain is, the more cytotoxic the $Pt_{(IV)}$ complex is. On the basis of complexes **1**–**3** only, the relationship between activity and length of the axial chain is confirmed.

Table 3 shows also the IC_{50} measured on human mesothelial cells (HMC; i.e., cells isolated from patients with no history of malignant disease, see experimental for details) and the selectivity index (SI) is defined as the ratio between IC_{50} (HMC) and the mean of IC_{50}

on BR95, MG06, and MM98. The ability to discriminate between normal and malignant cells is of paramount importance for developing clinically applicable chemotherapeutics. Cisplatin, as expected, exhibits low selectivity (SI = 1.5), while SI increases for **1**–**3** as the axial chain length.

A longer chain means both higher lipophilicity (and in turn likely higher uptake) and higher (less negative, i.e., easier reduction) E_p values (Fig. 5). Therefore, these data are consistent with the previous finding that the cytotoxicity of $Pt_{(IV)}$ complexes depends on both lipophilic and electronic features.[17,26]

It is noteworthy to recall, however, that a parabolic relationship between IC_{50} and the length of the carbon chain has been reported for [Pt(carboxylato)$_2$Cl$_2$(1R,2R-diaminocyclohexane)] complexes.[16] This behavior may be due to the very low solubility in water of complexes with too long chains; the solubility is a parameter not included in the above-mentioned relationships.[17]

Reduction with glutathione (GSH)

The occurrence of reduction of complexes **1**–**3** by GSH, the prevailing intracellular non-protein thiol with concentrations of up to 8 mM,[12] was verified. Complexes were dissolved in DMSO and these solutions were quickly diluted with ultrapure water to a final 1 mM compound concentration (the final content of DMSO was 1%). GSH (5 eq) was added and the behavior of the resulting solutions was monitored over 4 h at 37 °C by means of RP-HPLC and ESI-MS.

Both chromatograms and mass spectra indicated the disappearance of the signals relating to the $Pt_{(IV)}$ compounds, which was almost complete after 4 h.

ESI-MS analyses of the freshly prepared solutions of each compound with GSH showed the presence of unreacted $Pt_{(IV)}$ and solvated picoplatin $[PtCl(DMSO)(mpy)(NH_3)]^+$. During the reduction of the $Pt_{(IV)}$ complexes other $Pt_{(II)}$ metabolites can be observed corresponding to the retention of the original axial ligands. In particular, in case of complex **3** it has been possible to identify low amounts of the

[Pt(butanoato)Cl(mpy)(NH_3)] species and relatively higher quantities of the corresponding solvated species [Pt(butanoato)(DMSO)(mpy)(NH_3)]$^+$. Therefore, the reduction is associated with some rearrangements according to Gibson and co-workers[18] who demonstrated that the reduction of complexes like *cis,trans,cis*-[$Pt^{IV}Cl_2(CH_3CO_2)_2(NH_3)$(amine)] yielded the expected *cis*-[PtII$Cl_2(NH_3)$(amine)] metabolite, along with other $Pt_{(II)}$ complexes containing one or two carboxylate ligands. Likely, the reduction proceeds by several pathways where only am(m)ines are constantly maintained in the coordination sphere. The reduction was found to follow a pseudo-first-order kinetic, as previously reported for several $Pt_{(IV)}$ complexes (see for instance *bis*-(acetato) (1-adamantylamine) amminedichloroplatinum$_{(IV)}$, coded as LA-12, structurally very similar to **1**).[29] The linearization of the data points over a 2 h time interval gave the following half-time of reduction: 32 min for **3**, 30 min for 2 and 25 min for **1**. It is important recall that an "easy" reduction (from a thermodynamic point of view) it is not always related with a short reduction time.[14a,30,31] In fact, Gibson *et al*. found that reduction of the Pt(IV) analogs of oxaliplatin with two axial carboxylate ligands is extremely slow while reduction of their dihydroxido analogs is much faster. However, the reduction potential of the former is higher than the latter.[32] For this behavior it was suggested that the reductive elimination reaction occurs *via* an attack by the reductant *via* halido- or oxygen-bridge electron transfer.[14a,31] The interaction between the reducing agent and the axial ligand, and hence the reduction time, follow the order Cl, OH > COOH.

In the actual case, as the *t*½ are very similar, the reduction potential appears to be the paramount factor influencing IC_{50}. Picoplatin-based $Pt_{(IV)}$ complexes can then be reduced by GSH to produce active $Pt_{(II)}$ metabolites, supporting the general view of "activation by reduction" mechanism. Moreover, the resulting $Pt_{(II)}$ metabolites always contain the mpy ligand, which guarantees a low inactivation of the complex by GSH. This is the reason of the good antiproliferative activity of **1–3** observed on the cisplatin resistant MM98R cell line.

3. EXPERIMENTAL

All chemicals (analytical grade) were obtained from Aldrich, except K_2PtCl_4 from Alfa Aesar, and used as received. Picoplatin[23] and *cis,cis,trans*-$[PtCl_2(mpy)(NH_3)(OH)_2]$[33] were prepared according to literature procedures. Elemental analyses were carried out with a EA3000 CHN Elemental Analyzer (EuroVector, Milano, Italy). Platinum was quantified by means of a Spectro Genesis ICP-OES spectrometer (Spectro Analytical Instruments, Kleve, Germany) equipped with a crossflow nebulizer. In order to quantify the platinum concentration the Pt 299.797 nm line was selected. A platinum standard stock solution of 1000 mg L^{-1} was diluted in 1.0% v/v nitric acid to prepare calibration standards. The elemental analyses and Pt content were within ±0.3% absolute of the theoretical value.

The multinuclear NMR spectra were measured on a JEOL Eclipse Plus operating at 400 MHz (^1H), 100.5 MHz (^{13}C), and 85.9 MHz (^{195}Pt with a spectral window of 1200 ppm), respectively (Fig. 6). ^1H and ^{13}C NMR chemical shifts were reported in parts per million referenced to solvent resonances. ^{195}Pt NMR spectra were recorded using a solution of K_2PtCl_4 in saturated aqueous KCl as external reference. The shift for $K_2[PtCl_4]$ was adjusted to −1628 ppm relative to $Na_2[PtCl_6]$ (δ = 0 ppm).

Electrospray ionization mass spectra (ESI-MS) were obtained using a Micromass ZMD mass spectrometer. Typically, a dilute solution of compound in acetone–methanol 1 : 99 was delivered directly to the spectrometer source at 0.01 mL min^{-1}, using a Hamilton microsyringe controlled by a single-syringe infusion pump. The nebulizer tip operated at 3000–3500 V and 150 °C, with nitrogen used both as a drying and as a nebulizing gas. The cone voltage was 30 V. Quasi-molecular ion peaks $[M+H]^+$ or sodiated $[M+Na]^+$ peaks were assigned on the basis of the *m/z* values and of the simulated isotope distribution patterns.

Synthesis of *trans,cis,cis*-[Pt(acetato)$_2$Cl$_2$(mpy)(NH$_3$)], 1

cis,cis,trans-[PtCl$_2$(mpy)(NH$_3$)(OH)$_2$] (107 mg, 0.261 mmol) was suspended in neat acetic anhydride (243 μL, 2.57 mmol) at 0 °C. Then the mixture was stirred at room temperature in the dark for 4 d. The final pale yellow precipitate was separated by centrifugation and washed with diethyl ether and dried *in vacuo* (116 mg, 90%). ^{1}H NMR (400 MHz; D$_2$O): δ 8.65 (H6, dd, ^3J = 6.22 Hz, ^4J = 1.28 Hz, ^{1}H), 8.06 (H4, td, ^3J = 7.69 Hz, ^4J = 1.28 Hz, 1H), 7.57 (H3, dd, ^3J = 7.69 Hz, 4J = 1.64 Hz, 1H), 7.49 (H5, td, ^3J = 6.96 Hz, ^4J = 1.64 Hz, 1H), 2.77 (H2, s, 3H), 2.14 (H8, s, 3H) ppm; ^{13}C NMR (100.5 MHz; D$_2$O): δ 181.45 (C7, Cquat), 161.50 (C2, Cquat), 151.37 (C6, CH), 142.26 (C4, CH), 129.75 and 124.91 (C3 and C5, 2 × CH), 23.26 and 22.61 (C1 and C8, 2 × CH3) ppm; ^{195}Pt NMR (85.9 MHz; D2O): δ 1365 ppm. ESI-MS (methanol : acetone 99 : 1) 493.15 (65.42%), 494.00 (74.32%), 495.12 (100.00%), 495.98 (54.50%), 497.20 (58.95%), 498.23 (13.97%), 499.30 (15.71%) m/z, calcd for C$_{10}$H$_{17}$Cl$_2$N$_2$O$_4$Pt m/z: 493.02 (65.46%), 494.02 (74.20%), 495.02 (100.00%), 496.02 (54.55%), 497.02 (59.01%), 498.02 (13.94%), 499.02 (15.76%) [M+H]$^+$.

Synthesis of *cis,cis,trans*-[PtCl$_2$(mpy)(NH$_3$)(propanoato)$_2$], 2

cis,cis,trans-[PtCl$_2$(mpy)(NH$_3$)(OH)$_2$] (121 mg, 0.295 mmol) was added to neat propanoic anhydride (583 μL, 4.55 mmol) and allowed to react for 5 d at room temperature in the dark. The mixture was then centrifuged and the pale yellow precipitate was washed with diethyl ether and dried *in vacuo* (134 mg, 87%). ^{1}H NMR (400 MHz; acetone-d6) δ: 8.88 (H6, dd, ^3J = 6.22 Hz; ^4J = 1.46 Hz, 1H), 8.10 (H4, td, ^3J = 7.69 Hz, ^4J = 1.46 Hz, 1H), 7.62 (H3 and H5, m, 2H), 3.27 (NH3, m, 3H), 2.86 (H1, s, 3H), 2.29 (H8, quart, ^3J = 7.69 Hz, 2H), 0.98 (H9, t, ^3J = 7.69 Hz, 3H) ppm; ^{13}C NMR (100.5 MHz; acetone-d6) δ: 182.82 (C7, Cquat), 161.69 (C2, Cquat), 151.89 (C6, CH), 138.67 (C4, CH), 129.10 and 123.44 (C3 and C5, 2 × CH), 30.01 (C8, CH$_2$), 23.16 (C1, CH$_3$), 9.59 (C9, CH$_3$) ppm; ^{195}Pt NMR (85.9 MHz; acetone-d6) δ: 1399 ppm; ESI-MS (methanol : acetone 99 : 1) 521.13 (64.34%),

522.01 (74.48%), 523.10 (100.00%), 524.15 (55.83%), 525.31 (59.21%), 526.14 (15.00%), 527.07 (15.78%) m/z, calcd for $C_{12}H_{21}Cl_2N_2O_4Pt$ m/z: 521.05 (64.36%), 522.05 (74.44%), 523.05 (100.00%), 524.05 (55.88%), 525.05 (59.28%), 526.05 (15.04%), 527.05 (15.81%) $[M+H]^+$.

Synthesis of *trans,cis,cis*-[Pt(butanoato)$_2$Cl$_2$(mpy)(NH$_3$)], 3

cis,cis,trans-[$PtCl_2$(mpy)(NH_3)$(OH)_2$] (157 mg, 0.383 mmol) was dissolved in acetonitrile (20 mL). Butanoic anhydride (644 µL, 3.82 mmol) was then added. The mixture was refluxed for 24 h. The resulting yellow solution was dried under reduced pressure. The residual oil was dissolved in few mL of acetone and precipitated with diethyl ether to get a pale yellow powder (158 mg, 75%). ^{1}H NMR (400 MHz; acetone-d6) δ: 8.90 (H6, dd, ^3J = 6.40 Hz, ^4J = 1.46 Hz, 1H), 8.11 (H4, td, ^3J = 7.69 Hz, ^4J = 1.46 Hz, 1H), 7.60 (H3 and H5, m, 2H), 2.86 (H1, s, 3H), 2.24 (H8, m, 2H), 1.53 (H9, m, 2H), 0.88 (H10, t, ^3J = 7.50 Hz, 3H) ppm; ^{13}C NMR (100.5 MHz; acetone-d_6) δ: 182.07 (C7, C_{quat}), 161.73 (C2, C_{quat}), 152.12 (C6, CH), 141.46 (C4, CH), 129.00 and 123.98 (C3 and C5, 2 × CH), 38.86 (C8, CH_2), 23.25 (C1, CH_3), 19.13 (C9, CH_2), 13.24 (C10, CH_3) ppm; ^{195}Pt NMR (85.9 MHz; acetone-d_6) δ: 1407 ppm; ESI-MS (methanol : acetone 99 : 1) 549.12 (63.23%), 550.15 (74.58%), 551.06 (100.00%), 552.03 (57.23%), 553.14 (59.55%), 554.11 (16.16%), 555.05 (15.91%) m/z, calcd for $C_{14}H_{25}Cl_2N_2O_4Pt$ m/z: 549.08 (63.26%), 550.08 (74.62%), 551.08 (100.00%), 552.08 (57.21%), 553.08 (59.53%), 554.08 (16.12%), 555.08 (15.89%) $[M+H]^+$.

Synthesis of *cis,cis,trans*-[PtCl$_2$(NH$_3$)(mpy)(pentanoato)$_2$], 4

cis,cis,trans-[$PtCl_2$(mpy)(NH_3)$(OH)_2$] (140 mg, 0.341 mmol) was dissolved in acetonitrile (20 mL). Pentanoic anhydride (700 µL, 3.44 mmol) was then added and the mixture was refluxed for 24 h. The resulting yellow solution was dried under reduced pressure. The residual oil was re-crystallized from methanol–diethyl ether and washed with

diethyl ether to get a pale yellow powder (98 mg, 50%). ^{1}H NMR (400 MHz; acetone-d_6) δ: 8.90 (H6, dd, ^{3}J = 6.59 Hz, ^{4}J = 1.10 Hz, 1H), 8.11–7.50 (H3, H4 and H5, m, 3H), 3.29 (NH_3, m, 3H), 2.86 (H1, s, 3H), 2.26 (H8, m, 2H), 1.49 (H9, m, 2H), 1.31 (H10, m, 2H), 0.84 (H11, m, 3H) ppm; ^{13}C NMR (100.5 MHz; acetoned$_6$) δ: 182.21 (C7, C_{quat}), 161.75 (C2, C_{quat}), 152.08 (C6, CH), 141.50 (C4, CH), 129.06 and 124.00 (C3 and C5, 2 × CH), 36.71 (C8, CH_2), 27.91 (C9, CH_2), 23.26 and 22.07 (C1 and C10, CH_3 and CH_2), 13.33 (C11, CH_3) ppm; ^{195}Pt NMR (85.9 MHz; acetone-d_6) δ: 1408 ppm; ESI-MS (methanol : acetone 99 : 1) 577.03 (62.23%), 578.16 (74.75%), 579.14 (100.00%), 580.08 (58.52%), 581.16 (59.80%), 582.05 (17.24%), 583.07 (16.02%) m/z, calcd for $C_{16}H_{29}Cl_2N_2O_4Pt$ m/z: 577.11 (62.21%), 578.12 (74.78%), 579.11 (100.00%), 580.11 (58.50%), 581.11 (59.84%), 582.11 (17.20%), 583.11 (16.00%) $[M+H]^+$.

Synthesis of *cis,trans,cis*-[$PtCl_2$(hexanoato)$_2$(mpy)(NH_3)], 5

cis,cis,trans-[$PtCl_2$(mpy)(NH_3)$(OH)_2$] (148 mg, 0.361 mmol) was dissolved in acetonitrile (20 mL). Hexanoic anhydride (428 μL, 1.80 mmol) was then added and the mixture was refluxed for 24 h. The resulting yellow solution was dried under reduced pressure. The residual oil was re-crystallized from methanol–diethyl ether and washed with diethyl ether to get a pale yellow powder (77 mg, 35%). ^{1}H NMR (400 MHz; acetone-d6) δ: 8.90 (H6, dd, ^{3}J = 6.59 Hz, ^{4}J = 1.10 Hz, 1H), 8.10–7.40 (H3, H4 and H5, m, 3H), 3.29 (NH_3, m, 3H), 2.86 (H1, s, 3H), 2.26 (H8, m, 2H), 1.51 (H9, m, 2H), 1.29 (H10 and H11, m, 4H), 0.86 (H12, t, 3H) ppm; ^{13}C NMR (100.5 MHz; acetone-d_6) δ: 182.23 (C7, C_{quat}), 161.76 (C2, C_{quat}), 152.10 (C6, CH), 141.52 (C4, CH), 129.10 and 124.08 (C3 and C5, 2 × CH), 36.93 (C8, CH_2), 30.91 (C10, CH_2), 24.42 (C9, CH_2), 23.26 and 22.4 (C1 and C11, CH_3 and CH_2), 13.47 (C12, CH_3) ppm; ^{195}Pt NMR (85.9 MHz; acetone-d_6) δ: 1408 ppm; ESI-MS (methanol : acetone 99 : 1) 605.16 (61.14%), 606.11 (74.95%), 607.13 (100.00%), 608.18 (59.80%), 609.18 (60.10%), 610.09 (18.24%), 611.10 (16.14%) m/z, calcd for C18H33Cl2N2O4Pt m/z: 605.14 (61.17%), 606.15 (74.92%), 607.15 (100.00%), 608.15 (59.78%), 609.15 (60.14%), 610.15 (18.26%), 611.15 (16.12%) $[M+H]^+$.

X-Ray structure

Yellow crystals of **2** were obtained from slow diffusion of ether into an acetone solution at room temperature. Suitably sized crystals were selected for X-ray single-crystal diffraction measurements. A prismatic crystal (0.1 × 0.1 × 0.2 mm) was selected and mounted on a MAR345 (Marresearch GmbH, Norderstedt, Germany) diffractometer with an image plate detector. A summary of the crystal data, structure solution and refinement parameters are given in Table 1. Bond lengths (Å) and angles (°) are reported in the ESI,† along with atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($Å^2 \times 10^3$), anisotropic displacement parameters ($Å^2 \times 10^3$), hydrogen coordinates ($\times 10^4$) and isotropic displacement parameters ($Å^2 \times 10^3$), and hydrogen bonds (Å and °).†

Unit-cell parameters were determined from 7652 reflections ($3 < \theta < 31°$) and refined by least-squares method. Intensities were collected with graphite monochromatized molybdenum Kα radiation. 15 764 reflections were measured in the range $32.38 \leqslant \theta \leqslant 5022$, 4676 of which were non-equivalent by symmetry (R_{int} (on I) = 0.049). 4676 reflections were assumed as observed applying the condition $I > 2\sigma(I)$. Lorentz-polarization and absorption corrections were made. The structure was solved by direct methods, using the SHELXS computer program and refined by full-matrix least-squares method with the SHELX97 computer program,[34] using 15 764 reflections (very negative intensities were not assumed). The function minimized was $\Sigma w||Fo|^2 - |Fc|^2|^2$, where $w = [\sigma^2(I) + (0.0470P)^2 + 1.2647P]^{-1}$, and $P = (|Fo|^2 + 2|Fc|^2)/3$. Values of f, f' and f'' were taken from International Tables of X-ray crystallography.[35] All H atoms were computed and refined, using a riding model, with an isotropic temperature factor equal to 1.2 times the equivalent temperature factor of the atom which are linked. The final R(on F) factor was 0.031, $wR(\text{on } |F|^2) = 0.083$ and goodness-of-fit = 1.100 for all observed reflections. Number of refined parameters was 199. Maximum and mean shift/esd = 0.00. Maximum and minimum peaks in final difference synthesis were 1.302 and −2.089 $eÅ^{-3}$, respectively.

Electrochemical measurements

An Autolab PGSTAT12 electrochemical analyzer (Eco Chemie, Utrecht, The Netherlands) interfaced to a personal computer running GPES 4.9 electrochemical software was used for the electrochemical measurements. A standard three-electrode cell was designed to allow the tip of the reference electrode (Ag/AgCl, 3M KCl) to closely approach the working electrode (a glassy carbon, GC, disk, diameter 0.1 cm, sealed in epoxy resin). The GC working electrode was polished with alumina followed by diamond paste, then rinsed with distilled water and dried. This process yielded an almost completely reproducible surface for all experiments. All measurements were carried out under nitrogen in 0.05 M phosphate buffer (PB, pH 7.4) containing NaCl 0.15 M and different percentages of ethanol as co-solvent. Metal complex solutions were 5.0×10^{-4} M. The temperature of the solution was kept constant (25 ± 1 °C) by circulation of a thermostated water–ethanol mixture through a jacketed cell. Positive-feedback iR compensation was applied routinely. All potentials are measured at 0.2 V s^{-1} scan rate and reported vs. normal hydrogen electrode (NHE).

Reduction with glutathione

RP-HPLC for the reduction study were performed using a Waters HPLC-MS instrument equipped with Alliance 2695 separations module, 2487 Dual lambda absorbance detector, 3100 mass Detector. The chromatographic conditions were:[36] silica-based C18 gel as the stationary phase (5-μm Gemini® C^{18} column 25 × 3 mm ID); mobile phase containing 15 Mm HCOOH : MeOH 50 : 50 for **3**, 60 : 40 for **2** and 70 : 30 for **1** (flow rate = 0.5 mL min^{-1}; isocratic elution, UV-vis detector set at 210 nm). ESI-MS measurements (positive ion mode) were performed with instrumental settings reported above.

Biological tests

All compounds were tested on three primary cell lines, derived from pleural effusion of previously untreated patients suffering from MPM, called BR95 and MG06 (epithelioid) and MM98 (sarcomatoid), and on a cisplatin-resistant cell line derived from wild type MM98

by exposure to sub-lethal concentrations of cisplatin for several months, called MM98R.[37] HMC were obtained from the biobank of the Hospital of Alessandria (Pathology Unit). HMCs were isolated from patients with no history of malignant disease.[38] HMCs and epithelioid MPM cells were grown in F10 Ham medium, while Dulbecco Modified Eagle's Medium (DMEM) was used for sarcomatoid MPM cells. Human ovarian carcinoma cells A2780 were purchased from ECACC (European Collection of Cell Cultures, UK) and grown in RPMI-1640 medium. Media were obtained from Gibco (Invitrogen Life Science, San Giuliano Milanese, Italy) supplemented with L-glutamine 2 mM, penicillin 100 IU mL^{-1}, streptomycin (100 mg L^{-1}) and 10% fetal bovine serum (FBS) at 37 °C in a 5% CO_2 humidified chamber. A quantity of 2–5 × 10^3 cells per well were seeded onto 96-well flat-bottom plates and allowed to attach 24 h before drug treatment. Compounds were dissolved in DMSO to final concentration 5 mM for 1; and 2.5 mM for **2** and **3**. The mother solutions were serially diluted in complete medium, never exceeding 0.5% total DMSO, this concentration was found to be non-toxic to the cell tested. Cisplatin (Sigma) was dissolved in 0.9% NaCl and sterile filtered. Aqueous HCl was added to the cisplatin stock solution (1 mM) up to pH 3, in order to avoid hydrolysis during storage at −80 °C. Challenge with the compounds was performed for 72 h continuous treatment.

At the end of the experiment, the [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] inner salt (MTS) assay was performed using a commercial kit (CellTiter Aqueous Solution, Promega, Milan, Italy), according to the manufacturer's instructions; absorbance values were recorded at 490/620 nm by a spectrophotometric plate reader (Sirio S, SEAC, Florence, Italy) and corrected by subtraction of the absorbance of MTS alone.

Residual cell viability was also evaluated by means of the resazurin reduction assay.[39] Briefly, cells were seeded in black sterile tissue-culture treated 96-well plates. At the end of the treatment, the viability was assayed with 10 μg mL^{-1} resazurin (Acros Chemicals, France) in fresh medium for 1 h at 37 °C, and the amount of the reduced product, resorufin, was measured by means of fluorescence using an excitation wavelength of 550 nm and an emission wavelength of 585 nm with a Tecan Infinite F200 plate reader (Tecan Austria).

In each experiment, the cells were challenged with the drug candidates at different concentrations and the final data were calculated from at least three replicates of the same experiment carried out in triplicate. The absorbance or fluorescence of 8 wells containing medium without cells were used as blank. The absorbance or fluorescence data were normalized to 100% cell viability for non-treated cells, half inhibiting concentration (IC_{50}), defined as the concentration of the drug reducing cell viability by 50%, was obtained from the dose–response sigmoid using Origin Pro (version 8, Microcal Software, Inc., Northampton, MA, USA).

4. CONCLUSIONS

The synthesis and characterization of a series of picoplatin-based Pt(IV) complexes with axial carboxylato ligands of increasing length are reported. The oxidation of picoplatin with hydrogen peroxide to give the *cis,cis,trans*-[$PtCl_2$(mpy)(NH_3)$(OH)_2$] intermediate is followed by the transformation into dicarboxylato complexes *cis,cis,trans*-[$PtCl_2$(mpy)(NH_3)$(RCOO)_2$] **1–5** by reaction with the corresponding anhydride. Complexes **1–3** were selected to be tested, along with cisplatin as the prototypal metallo-drug, on four MPM cell lines, on HMC, and on the cisplatin-sensitive ovarian A2780 cell line. The longer the axial chain, the more cytotoxic and selective the corresponding $Pt_{(IV)}$ complex is, approaching or overcoming the performance of cisplatin.

When complexes **1–3** reacted with GSH, the formation of the corresponding $Pt_{(II)}$ species (with quite similar $t_{½}$ ranging between 25 and 32 min) is observed, supporting the general view of "activation by reduction" mechanism. As the cisplatin-resistant cell line MM98R exhibits high levels of intracellular GSH, especially during platinum-drug treatment, the observed low RF for compounds **1–3** may be related to a generally low inactivation of reduced $Pt_{(II)}$ metabolites.

In conclusion, provided that tests on monolayer cell culture are only indicative, especially for $Pt_{(IV)}$ pro-drug candidates, and required to be corroborated by experiments on spheroids and *in vivo*, the insertion of the picoplatin moiety in the octahedral structure of $Pt_{(IV)}$ with additional axial carboxylato ligands affords complexes quite promising for treatment of tumors whose chemoresistance is mainly based on GSH overexpression, such as mesothelioma.

5. ACKNOWLEDGEMENTS

We are indebted to the CRT Foundation (Turin) and Provincia di Alessandria for financial support, and to Professor P.-G. Betta (Mesothelioma Biobank Reference Center, Alessandria National Hospital) for providing cell lines. We acknowledge the EU-COST Action D39 (Metallo-Drug Design and Action), and the Inter-University Consortium for Research on the Chemistry of Metal Ions in Biological Systems (CIRCMSB, Bari, Italy) within this research was carried out. EE thanks the Spanish Ministry of Science and Innovation for a PhD grant associated to the Project CTQ2008-02064. Finally, we thank the three Referees for their stimulating criticisms and suggestions.

6. NOTES AND REFERENCES

1 R. I. Eisenstein and D. S. Resnick, Nat. Biotechnol., 2001, 19, 881–882.

2 (a) Non-Small Cell Lung Cancer Collaborative Group, Br. Med. J., 1995, 311, 899–909; (b) R. S. Go and A. A. Adjei, J. Clin. Oncol., 1999, 17, 409–422; (c) M. Q. Baggstrom, T. E. Stinchcombe, D. B. Fried, C. Poole, T. A. Hensing and M. A. Socinski, J. Thorac. Oncol., 2007, 2, 845–853; (d) K. Hotta, K. Matsuo, H. Ueoka, K. Kiura, M. Tabata and M. Tanimoto, J. Clin. Oncol., 2004, 22, 3852–3859.

3 N. Pabla and Z. Dong, Kidney Int., 2008, 73, 994–1007.

4 S. R. McWhinney, R. M. Goldberg and H. L. McLeod, Mol. Cancer Ther., 2009, 8, 10–16.

5 J. H. van den Berg, J. H. Beijnen, A. J. M. Balm and J. H. M. Schellens, Cancer Treat. Rev., 2006, 32, 390–397.

6 B. Koberle, M. T. Tomicic, S. Usanova and B. Kaina, Biochim Biophys. Acta, Rev. Cancer, 2010, 1806, 172–182.

7 J. Reedijk, Chem. Rev., 1999, 99, 2499–2510.

8 (a) M. A. Fuertes, C. Alonso and J. M. Pèrez, Chem. Rev., 2003, 103, 645–662; (b) J. Reedijk and J. M. Teuben, in Cisplatin. Chemistry and Biochemistry of a Leading Anticancer Drug, ed. B. Lippert, Wiley-VCH, Weinheim, 1999, pp. 339–362; (c) M. G. Cherian, S. B. Howell, N. Imura, C. D. Klaassen, J. Koropatnick, J. S. Lazo and M. P. Waalkes, Toxicol. Appl. Pharmacol., 1994, 126, 1–5.

9 Y. Kasherman, S. Sturup and D. Gibson, J. Med. Chem., 2009, 52, 4319–4328.

10 F. Basolo, F. J. Chatt, H. B. Gray, R. G. Pearson and B. L. J. Shaw, J. Chem. Soc., 1961, 2207–2215.

11 L. Kelland, Nat. Rev. Cancer, 2007, 7, 573–584.

12 Y. Chen, Z. Guo, S. Parsons and P. J. Sadler, Chem.–Eur. J., 1998, 4, 672–676.

13 (a) J. F. Holford, F. I. Raynaud, B. A. Murrer, K. Grimaldi, J. A. Hartley, M. J. Abrams and L. R. Kelland, Anti–Cancer Drug Des., 1998, 13, 1–18; (b) F. I. Raynaud, F. E. Boxall, P. M. Goddard, P. Valenti, M. Jones, M. A. Murrer, M. J. Abrams and L. R.

529 Kelland, Clin. Cancer Res., 1997, 3, 2063–2074.

530 14 (a) M. D. Hall and T. W. Hambley, Coord. Chem. Rev., 2002, 32, 49–67; (b) S. Choi,
531 S. Delaney, L. Orbai, E. J. Padgett and A. S. Hakemian, Inorg. Chem., 2001, 40, 5481–
532 5482; (c) S. Choi, R. B. Cooley, A. S. Hakemian, C. Y. Larrabee, R. C. Bunt, S. D.
533 Maupas, J. G. Muller and C. J. Burrows, J. Am. Chem. Soc., 2004, 126, 591–598; (d)
534 S. Choi, R. B. Cooley, A. Voutchkova, C. H. Leung, L. Vastag and D. E. Knowles, J.
535 Am. Chem. Soc., 2005, 127, 1773–1781.

536 15 (a) M. D. Hall, R. A. Alderden, M. Zhang, P. J. Beale, Z. Cai, B. Lai, A. P. J. Stamp
537 and T. W. Hambley, J. Struct. Biol., 2006, 155, 38–44; (b) M. D. Hall, H. R. Mellor,
538 R. Callaghan and T. W. Hambley, J. Med. Chem., 2007, 50, 3403–3411; (c) A. M.
539 Montaña and C. Batalla, Curr. Med. Chem., 2009, 16, 2235–2260.

540 16 (a) L. R. Kelland, B. A. Murrer, G. Abel, C. M. Giandomenico, P. Mistry and K. R.
541 Harrap, Cancer Res., 1992, 52, 822–828; (b) S. R. Ali Khan, S. Huang, S. Shamsuddin,
542 S. Inutsuka, K. H. Whitmire, Z. H. Siddik and A. R. Khokhar, Bioorg. Med. Chem.,
543 2000, 8, 515–521.

544 17 P. Gramatica, E. Papa, M. Luini, E. Monti, M. B. Gariboldi, M. Ravera, E. Gabano, L.
545 Gaviglio and D. Osella, J. Biol. Inorg. Chem., 2010, 15, 1157–1169.

546 18 A. Nemirovski, I. Vinograd, K. Takrouri, A. Mijovilovich, A. Rompel and D. Gibson,
547 Chem. Commun., 2010, 46, 1842–1844.

548 19 M. Ray and H. L. Kindler, Chest, 2009, 136, 888–896.

549 20 A. A. Mujoomdar, T. R. Tilleman, W. G. Richards, R. Bueno and D. J. Sugarbaker, J.
550 Thorac. Cardiovasc. Surg., 2010, 140, 352–355.

551 21 K. Järvinen, Y. Soini, K. Kahlos and V. L. Kinnula, Hum. Pathol., 2002, 33, 748–755.

552 22 G. Giaccone, M. E. O'Brien, M. J. Byrne, M. Bard, E. Kaukel and B. Smit, Eur. J.
553 Cancer, 2002, 38, S19–S24.

554 23 A. R. Battle, R. Choi, D. E. Hibbs and T. W. Hambley, Inorg. Chem., 2006, 45, 6317–
555 6322.

556 24 S. C. Dhara, Indian J. Chem., 1970, 8, 193–194.

557 25 (a) M. R. Reithofer, S. M. Valiahdi, M. A. Jakupec, V. B. Arion, A. Egger, M. Galanski

and B. K. Keppler, J. Med. Chem., 2007, 50, 6692–6699; (b) W. H. Ang, S. Pilet, R. Scopelliti, F. Bussy, L. Juillerat-Jeanneret and P. J. Dyson, J. Med. Chem., 2005, 48, 8060–8069.

26 J. A. Platts, G. Ermondi, G. Caron, M. Ravera, E. Gabano, L. Gaviglio, G. Pelosi and D. Osella, J. Biol. Inorg. Chem., 2011, 16, 361–372.

27 M. R. Reithofer, A. K. Bytzek, S. M. Valiahdi, C. R. Kowol, M. Groessl, C. G. Hartinger, M. A. Jakupec, M. Galanski and B. K. Keppler, J. Inorg. Biochem., 2011, 105, 46–51.

28 I. Zanellato, I. Bonarrigo, M. Sardi, M. Alessio, E. Gabano, M. Ravera and D. Osella, ChemMedChem, 2011, 6, 2287–2293.

29 F. Žák, J. Turánek, A. Kroutil, P. Sova, A. Mistr, A. Poulová, P. Mikolin, Z. Žák, A. Kašná, D. Záluská, J. Neča, L. Šindlerová and A. Kozubík, J. Med. Chem., 2004, 47, 761–763.

30 S. Choi, C. Filotto, M. Bisanzo, S. Delaney, D. Lagasee, J. L. Whitworth, A. Jusko, C. R. Li, N. A. Wood, J. Willingham, A. Schwenker and K. Spaulding, Inorg. Chem., 1998, 37, 2500–2504.

31 L. Chen, P. F. Lee, J. D. Ranford, J. J. Vittal and S. Y. Wong, J. Chem. Soc., Dalton Trans., 1999, 1209–1212.

32 J. Zhang, E. Wexselblatt, T. Hambley and D. Gibson, Chem. Commun., 2012, 48, 847–849.

33 C. M. Giandomenico and E. S. Y. Wong, US Pat, 6,413,953 B1, 2002

34 G. M. Sheldrick, Acta Crystallogr., Sect. A: Found. Crystallogr., 2008, 64, 112–122.

35 International Tables of X-Ray Crystallography, ed. J. A. Ibers and W. C. Hamilton, Kynoch Press, Birmingham, 1976, vol. IV, pp. 99–100 and 149.

36 J. A. Platts, S. P. Oldfield, M. M. Reif, A. Palmucci, E. Gabano and D. Osella, J. Inorg. Biochem., 2006, 100, 1199–1207.

37 I. Zanellato, C. D. Boidi, G. Lingua, P. Betta, S. Orecchia, E. Monti and D. Osella, Cancer Chemother. Pharmacol., 2011, 67, 265–273.

38 (a) S. Orecchia, F. Schillaci, M. Salvio, R. Libener and P.-G. Betta, Lung Cancer,

2004, 45, S37–S43; (b) E. Aldieri, C. Riganti, F. Silvagno, S. Orecchia, P. G. Betta, S. Doublier, E. Gazzano, M. Polimeni, A. Bosia and D. Ghigo, Am. J. Respir. Cell Mol. Biol., 2011, 45, 625–631.

39 E. Magnani and E. Bettini, Brain Res. Protoc., 2000, 5, 266–272.

600 **Table 1** Crystal data and structure refinement for complex **2**

601

	2
Formula	$C_{12}H_{20}Cl_2N_2O_4Pt$
M_r	522.29
T/K	123(2)
Crystal system	Monoclinic
Space group	$P2_1/c$
a/Å	11.576(5)
b/Å	7.980(3)
c/Å	21.461(5)
α (°)	90
β (°)	119.29(2)
γ (°)	90
V/Å^3	1729.0(11)
Z	4
D_{calc}/g cm^{-3}	2.006
Wavelength/Å	0.71073
Absorption coefficient/mm^{-1}	8.438
F(000)	1000
Crystal size/mm	0.2 × 0.1 × 0.1
θ range for data collection (°)	2.02–32.38
Limiting indices	$-16 \le h \le 16$, $-11 \le k \le 10$, $-25 \le l \le 25$
Reflections collected/unique	15 764/5022 [R_{int} = 0.0494]
Completeness to θ = 25.00	98.0%
Absorption correction	Empirical
Maximum and minimum transmission	0.43 and 0.39
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	5022/4/201
Goodness-of-fit on F^2	1.097
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.0308, wR_2 = 0.0826
R indices (all data)	R_1 = 0.0325, wR_2 = 0.0853
Largest difference peak and hole/e Å^{-3}	1.357 and −2.169

602

603

604

605 **Table 2.** Reduction peak potentials E_p for the 1–5 series of compounds ([Pt] = 0.5 mM, in
606 0.05 M PB/0.15 M NaCl + 10% ethanol, glassy carbon working electrode)

607

Compound	E_p (V *vs*. NHE at 0.2 V s^{-1})
1	–0.090
2	+0.048
3	+0.114
4	+0.150
5	+0.160

608

609

610

611

612 **Table 3** IC_{50} values for 72 h of continuous treatment

613

Complex	IC_{50} values (μM)							
	A2780	BR95	MG06	MM98	MM98R	HMC	RF[b]	SI[c]
Cisplatin	1.2 ± 0.4	6.2 ± 0.9[a]	4.1 ± 1.5[a]	3.2 ± 1.0[a]	19.4 ± 2.8[a]	6.7 ± 1.2	6.1	1.5
1	7.4 ± 1.9	17.5 ± 4.9	13.2 ± 4	12.7 ± 3	12.1 ± 1.9	22.8 ± 2.0	1.0	1.6
2	2.4 ± 1.3	15.2 ± 2.1	11.1 ± 0.6	10.1 ± 2.6	13.2 ± 2.3	21.3 ± 1.9	1.3	1.8
3	0.40 ± 0.04	5.6 ± 0.2	7.2 ± 0.8	5.6 ± 0.5	9.4 ± 0.9	18.9 ± 3.1	1.7	3.1

[a] Data from ref. 28. [b] Resistance factor RF = IC_{50} (MM98R)/IC_{50} (MM98). [c] Selectivity index SI = IC_{50} (HMC)/mean IC_{50} (BR95, MG06, MM98).

614

615

616

617

Figures Captions

Figure 1. A general scheme of the $2e^-$ reduction of a generic $Pt_{(IV)}$ complex (A = am(m)ines, X and L = chlorides or carboxylates) according to ref. 18.

Figure 2. Sketch of picoplatin and its $Pt_{(IV)}$-based derivatives.

Scheme 1. (i) **1**: neat acetic anhydride at 0 °C, then r.t. in the dark for 4 days; **2**: neat propanoic anhydride for 5 days at r.t. in the dark; butanoic (**3**), pentanoic (**4**), and hexanoic (**5**) anhydride in acetonitrile, then mixture refluxed for 24 h.

Figure 3. ORTEP representation of 2, ellipsoids at 50% probability

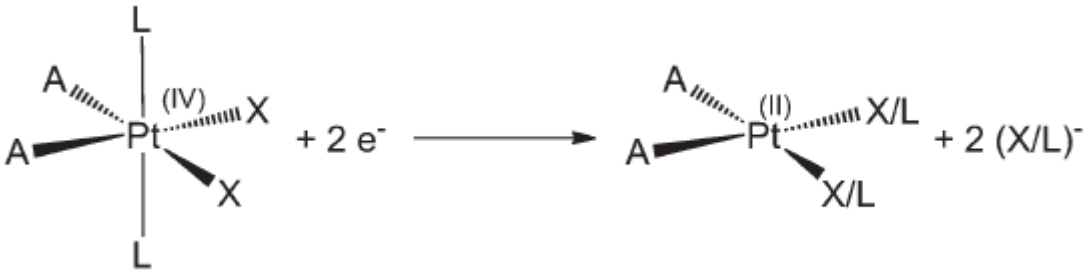
Figure 4. Plot of the Ep values of **1** (empty squares) and **3** (black squares) vs. % of co-solvent ethanol.

Figure 5. Ep vs. the total number of axial secondary carbons (nCs) for the picoplatin-based $Pt_{(IV)}$ complexes (stars) in comparison with cisplatin-(squares), nedaplatin- (triangles), and $[PtCl_2(dach)]$- (circles) based series of $Pt_{(IV)}$ complexes (see ref. 26).

Figure 6. Numbering scheme used to assign the NMR signals in complexes **1**–**5**.

637 **Figure 1**

638



639

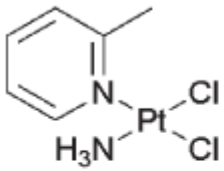
640

641

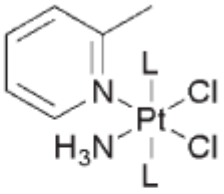
642

643 **Figure 2**

644



picoplatin
ZD0437
AMD-437



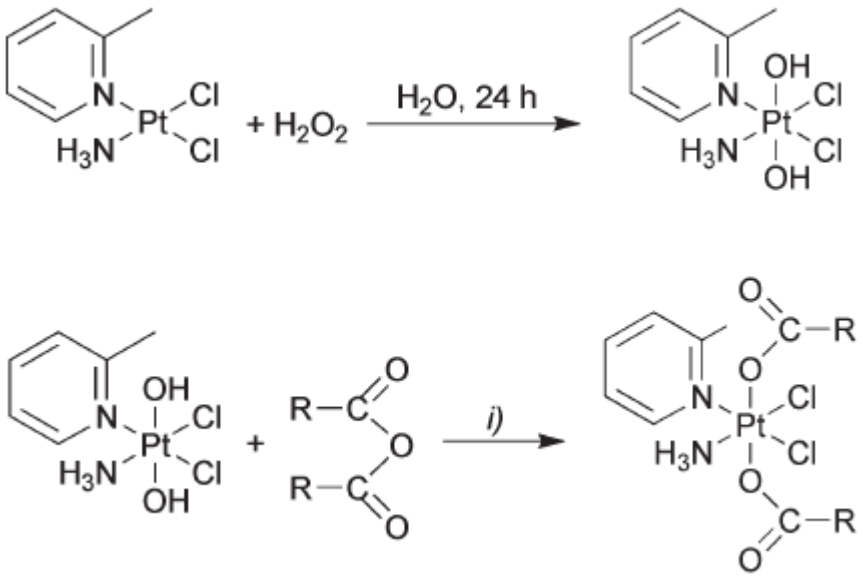
1 L = CH_3COO^-
2 L = $CH_3CH_2COO^-$
3 L = $CH_3(CH_2)_2COO^-$
4 L = $CH_3(CH_2)_3COO^-$
5 L = $CH_3(CH_2)_4COO^-$

645

646

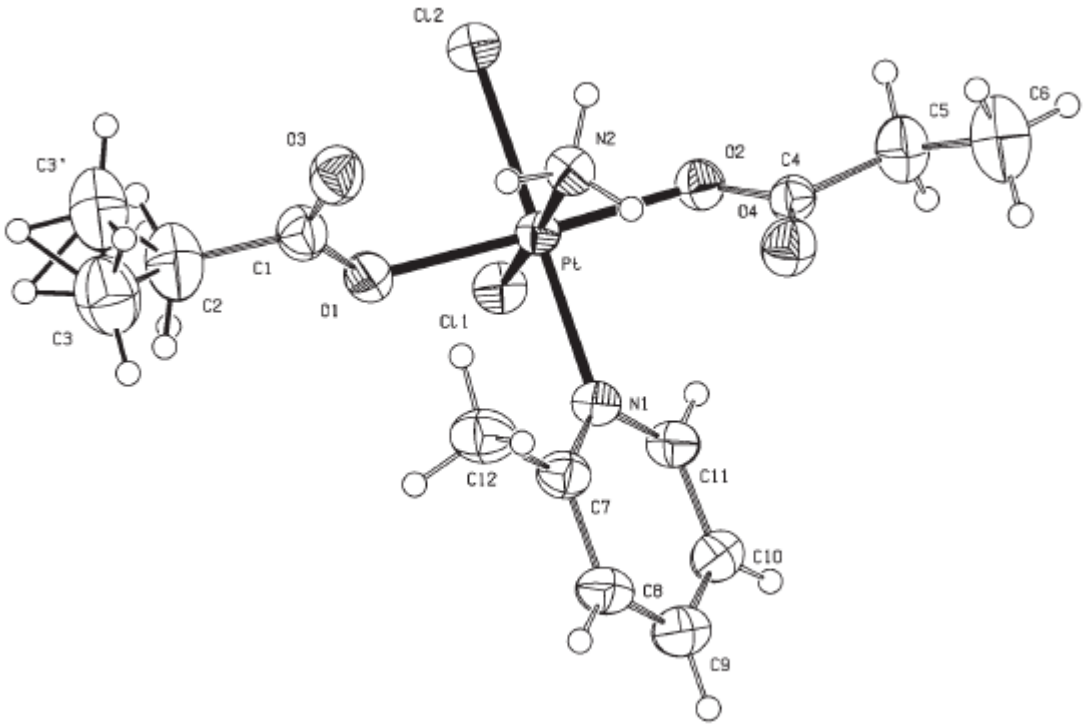
647

Scheme 1.



652 **Figure 3**

653



654

655

656

657

Figure 4

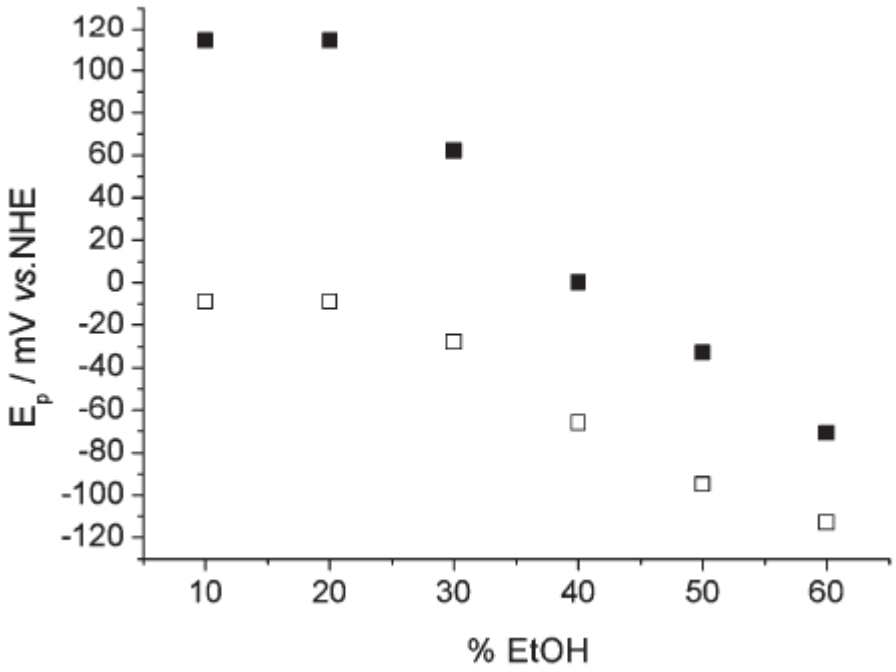

120
100
80
60
40
20
0
-20
-40
-60
-80
-100
-120
E_p / mV vs.NHE
10
20
30
40
50
60
% EtOH

Figure 5

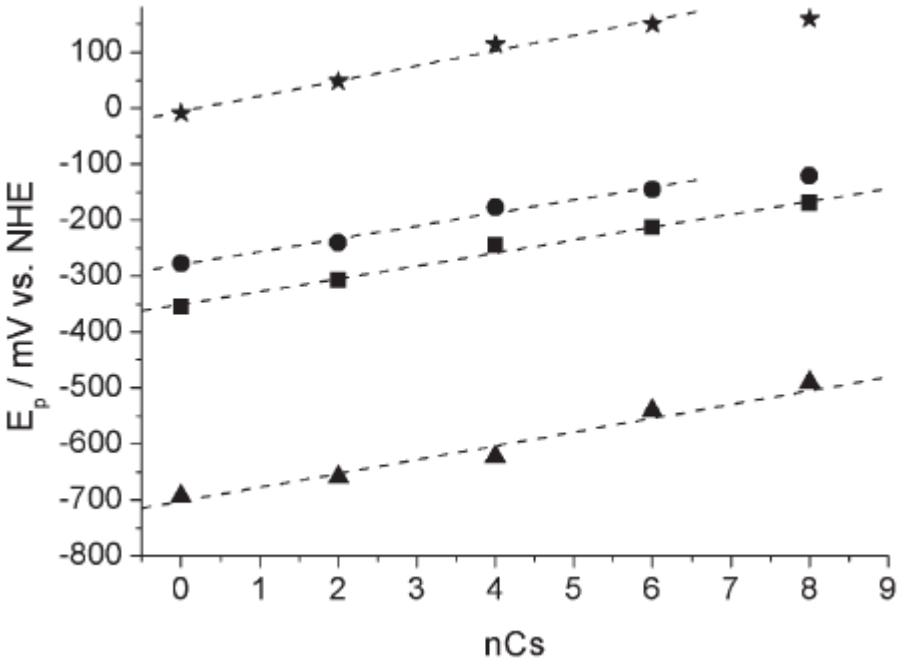

100
0
-100
-200
-300
-400
-500
-600
-700
-800
E_p / mV vs. NHE
0
1
2
3
4
5
6
7
8
9
nCs

Figure 6