An unexpected in-solution instability of diiodido analogue of picoplatin complicates its biological characterization†

Pavel Štárha, Bohuslav Drahoš and Radovan Herchel

Complex cis-[PtI₂(NH₃)(pic)] (1; pic = 2-methylpyridine), a diiodido analogue of clinically studied picoplatin (2), is unstable in solution, which is intriguingly connected with the release of its pic ligand. This observation complicates the biological testing of e.g. cytotoxicity in human cancer cells for 1.

Platinum-based drugs (e.g., cisplatin; Fig. 1) represent a clinically successful group of complexes, because they have been used worldwide for the treatment of cancer for more than 40 years. Among Pt(II) complexes, an infinite number of compounds have been studied for their anticancer activity, following multiple strategies developed over the years for the improvement of potency and reducing the numerous side-effects of cisplatin and its analogues.

One of the strategies is based on rational chemical modification in order to reduce the interactions with various intracellular biomolecules, such as reduced glutathione (GSH), known to deactivate Pt(II) drugs. To date, the cis-ammine-dichlorido-2-methylpyridineplatinum(II) complex (picoplatin, JM473; Fig. 1) is the most successful of complexes following this strategy.² Picoplatin showed high anticancer activity in vitro³ and in vivo⁴ as well as in various clinical trials in patients suffering from e.g. small-cell lung cancer.⁴ The main advantage of picoplatin is reported as lying in its efficacy toward cancer cells with acquired resistance against other Pt-based drugs.¹,³,⁵ However, the in vitro cytotoxicity of picoplatin is lower than that observed for cisplatin and oxaliplatin even in the used cisplatin- (DMS53CisR) or oxaliplatin- (HCT116oxalR) resistant cancer cells, respectively.

The mentioned low cytotoxicity of picoplatin indicates that there is room for improvement of its activity. One of the successful strategies for the improvement of potency of Pt(II) dichlorido complexes (such as picoplatin) is based on the replacement of both chlorido ligands by iodido ones.⁶ In this manner, multiple Pt(II) iodido complexes have been recently reported as having various pharmacological advantages over their direct chlorido analogues as well as over conventional Pt drugs. Complex cis-[PtI₂(NH₃)₂], an iodido analogue of cisplatin, was more effective than cisplatin in various cancer cells including the cells resistant towards Pt drugs.⁷ In contrast to cisplatin, cis-[PtI₂(NH₃)₂] kept both its iodido ligands, while one of its NH₃ ligand was replaced by the His15 imidazole of hen egg white lysozyme (HEWL), used as the model protein.⁸ Similarly, cytotoxic cis-diiodido Pt(II) complexes released N-donor aliphatic amines in the presence of various biomolecules.⁹ On the other hand, cis-[PtI₂(naza)₂] complexes, containing 7-azaindole (naza) derivatives, did not release their heterocyclic N-donor ligands and did not interact (in contrast to their chlorido analogues) with GSH and DNA models.¹⁰ Complexes trans-[PtI₂(am)(py)] (am = methanamine or propan-2-amine) released pyridine (py) only when irradiated.¹¹

With respect to the formerly reported observations for picoplatin and simple Pt(II) diiodido complexes, it was of interest for us to investigate complex cis-[PtI₂(NH₃)(pic)] (1; Fig. 1). Complex 1, an iodido analogue of picoplatin, is known in the literature as an intermediate in the synthesis of picoplatin (used in this work for comparative purposes; 2, Fig. 1).¹² Complex 1 was identified using elemental analysis, NMR, ESI-mass spectrometry (MS) and FT-IR spectroscopy. All H and C atoms of 1 were detected in the ²H and ¹³C NMR spectra and assigned using 2D experiments (ESI, Fig. S1–S4†).

Department of Inorganic Chemistry, Faculty of Science, Palacký University in Olomouc, 17. listopadu 1192/12, 771 46 Olomouc, Czech Republic.
E-mail: pavel.starha@upol.cz

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Being aware of a contradiction between the results reported for Pt(n) diiodido complexes with NH₃ or aliphatic amines (release of N-donor ligand(s) in the presence of biomolecules)⁸,⁹ as compared with complexes containing heterocyclic ligands (no release of N-donor ligands),¹⁰ and with an intention to investigate the cytotoxicity of 1, it was imperative for us to pay special attention to its solution behaviour studies (¹H NMR, HPLC, UV-Vis), since speciation under gate more deeply, whether the herein reported instability of 1 means just its undesirable extracellular deactivation¹⁴,¹⁶ or there is a possibility of intracellular activation, depending on the cellular accumulation rate of 1. A similar effect has recently been investigated for the unstable, highly cytotoxic PtV complex, which is supposed to be beneficial for a decrease of toxicity in the treated patients.¹⁷

Also of interest, the formerly reported Pt(n) diiodido complexes were more stable in solution than their chlorido analogues,⁷,¹⁰,¹¹ which was not the case of the herein studied picoplatin (2) and its markedly less stable diiodido analogue 1. It should be noted that the N-donor ligand release, as observed for some Pt diiodido complexes in the presence of various biomolecules,⁸,⁹ or when irradiated¹¹ (see above), was also reported for some Pt dichlorido complexes studied in the cell culture medium¹⁰ or blood serum supplemented with methionine or N-acetylcyesteine,²⁰ but, to the best of our knowledge, it has never been observed directly in solution (i.e., without any biomolecules), as described in this work for 1.

The HPLC chromatogram of the freshly prepared solution of picoplatin (2) in 50% MeCN/50% H₂O (v/v) contained two dominant peaks (tᵣ = 2.25 and 3.90 min; Fig. 3). Two new peaks were observed in the HPLC chromatogram of 2 at tᵣ = 5.23 and 6.12 min during its incubation up to 96 h of standing at 37 °C (Fig. 3 and ESI, Fig. S7†). For two reasons, these new signals are connected with the hydrolysis of 2. At first, their coupled ESI+ MS showed the [Pt(NH₃)₂(pic) – H]⁺ (304.1 m/z; calc. 304.0 m/z), [Pt(pic) + MeCN – H]⁺ (328.0 m/z; calc. 328.0

Regrettably, the release of the NH₃ ligand can be ruled out because the total integral intensity of the broad multiplet centred at 4.40 ppm in the ¹H NMR spectrum of 1 in DMF-d₇ correlated well with the total integral intensity of three detected C7–H₄ resonances belonging to 1, 1' and free pic (ESI, Fig. S7†).

In total for the ¹H NMR studies, although the extent of hydrolysis of 1 (ca. 25% at t = 96 h) is comparable with that of picoplatin (2), only ca. 10% of the initial complex 1 was detected in the ¹H NMR spectrum after 96 h of standing at 37 °C, which is caused by the pic release from 1 (ca. 13% at t = 96 h) and by the trans-isomerization of 1 (ca. 50% at t = 96 h).

After 6 h of standing, ca. 95% of 1 was still present in 50% DMF-d₇/50% D₂O, while only ca. 53% of 1 remained intact after 24 h (Fig. 2). This is important for the upcoming studies of the cellular accumulation by human cancer cells, which should shed light on whether 1 could be subjected to the in vitro cytotoxicity testing. In other words, we aim to investigate more deeply, whether the herein reported instability of 1 means just its undesirable extracellular deactivation¹⁴,¹⁶ or there is a possibility of intracellular activation, depending on the cellular accumulation rate of 1. A similar effect has recently been investigated for the unstable, highly cytotoxic PtV complex, which is supposed to be beneficial for a decrease of toxicity in the treated patients.¹⁷

The ¹H NMR studies indicated that a hydrolysis, which is usually involved in the mechanism of action of cytotoxic Pt(n) complexes (including picoplatin, 2),¹⁵ occurred also for the iodido complex 1 (Fig. 2), because the mentioned doublet appeared at 7.54 ppm also for 1. Remarkably for 1 and in sharp contrast to 2, two other sets of pic signals were detected in the ¹H NMR spectra of 1 along with the mentioned signals of 1 and its hydrolysate. This implied that two other processes are in progress for 1 under the used conditions. In particular, a metabolism of 1 is unambiguously connected with the release of the pic ligand, as proved by the presence of the C6–H and C7–H₄ resonances of free pic detected in the ¹H NMR spectrum of 1 at 8.49 and 2.58 ppm, respectively (Fig. 2 and ESI, S7†).

The HPLC chromatogram of the freshly prepared solution of picoplatin (2) by ¹H NMR (50% DMF-d₇/50% D₂O), clearly detecting one new set of signals in the ¹H NMR spectra of 2 (e.g., doublet at 7.54 ppm; Fig. 2). An equilibrium was reached after 72 h with ca. 22% of 2 hydrolysed to cis-[Pt(H₂O)(NH₃)(OH)(pic)]⁺,¹¹ (according to the previously determined pK_a values¹⁵); the sample was incubated at 37 °C between the measurements.

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Fig. 2. The results of the ¹H NMR solution behaviour studies of 1 (black), given together with picoplatin (2; red) and free 2-methylpyridine (pic; green); the samples were prepared in 50% DMF-d₇/50% D₂O and incubated at 37 °C between the measurements.

[Image 48x108 to 284x250]
Concerning the diiodido complex 1, its freshly prepared solution (50% MeCN/50% H2O, v/v) analysed by HPLC/ESI+ MS showed the dominant peak at \( t_R = 6.55 \) min (Fig. 3), belonging to the initial complex \([\text{Pt}(\text{NH}_3)(\text{pic})]^+; 431.8 \text{ m/z}; \text{calc.} 431.1 \text{ m/z})\) species, which were not found in the ESI+ mass spectrum of the fresh solution. Secondly, both peaks \( (t_R = 5.23 \text{ and } 6.12 \text{ min})\) were also detected in the HPLC chromatograms of the synthetically dechlorinated picoplatin. The peak centred at \( t_R = 5.28 \text{ min} \) is consistent with the hydrolysis of 1, as observed also for picoplatin (2; see above). Another HPLC peak \( (t_R = 2.23 \text{ min})\), although at the same position as detected for 2, had a different ESI+ mass spectrum \( e.g., \text{the dominant peak at } 346.1 \text{ m/z}; \text{calc.} 346.1 \text{ m/z for } [\text{Pt}\{\text{pic}\}^+ + \text{OH} + \text{MeCN}]^+ \) than 2 with no chlorine-containing peaks detected. Most importantly, the peak at \( t_R = 8.89 \text{ min} \) was clearly detected in the HPLC chromatograms of 1 after 6 h of incubation at 37 °C (Fig. 3 and ESI, Fig. S9†) and this peak unambiguously belongs to the released pic ligand \( (94.2 \text{ m/z}; \text{calc.} 94.1 \text{ m/z})\), which is very well consistent with the above-discussed \(^1\text{H} \text{NMR results.}

The results of the time-dependent UV-Vis studies of 1 (up to 96 h of standing at 37 °C; 50% DMF/50% H2O, v/v) showed that there is a gradual change of its composition (Fig. 4). In particular, a decrease of intensity of the maximum detected at \( \lambda = 341 \text{ nm} \) \((t = 0 \text{ h})\) continued up to 72 h of standing, when an equilibrium was reached with no further changes observed. This is consistent with the observations reported for complexes \( \text{cis-[PtI}_2(\text{NH}_3)_2]^+ \) and \( \text{Pt}_2(\text{dach})_2^{16c} \) both showing a gradual decrease of the band detected at ca. 350 nm, assigned to the hydrolytic replacement of the iodo ligands. Thus, the hydrolysis of the Pt-I bonds of 1 was also proved by UV-Vis spectroscopy. Importantly, a difference between the spectra of 1 and the spectrum of the co-studied \( \text{cis-[Pt(H}_2\text{O})(\text{NH}_3)(\text{OH})(\text{pic})]^+ \) showed a gradual decrease of the band detected at \( \lambda = 350 \text{ nm} \), assigned to the solvolysis of cis-[Pt(H2O)(NH3)(OH)(pic)]+ hydrolysate (red) is given for comparative purposes.

The chemical reactivity toward the solvolysis of complex 1 was also studied by theoretical methods employing the ORCA 4.2 computational package and using density functional theory (DFT) with the PBE0 hybrid functional. Generally, such solvolysis proceeds by the associative mechanisms in square planar Pt(II) complexes through the five-coordinated bipyramidal transition state, in which incoming and leaving ligands are weakly bound to platinum.22 Firstly, we have studied the first step in the solvolysis of both 1 and picoplatin (2) and considered not only solvolysis by water, but also by DMF, which was used during the experimental studies. As expected, the halogenido ligand in the trans-position to pic is the most prone to solvolysis by both solvents, having the lowest energy of the transition state (TS), albeit the second halogenido ligand reached only slightly higher values of \( \Delta G \text{‡} \). The Gibbs free energies and selected structural parameters are listed in ESI, Table S1.† Interestingly, the energies of TS related to the solvolysis of N-donor ligands (pic and NH3) are lowered for 1 in comparison with 2.

Next, both steps of the solvolysis of halogenido ligands were computed and relative Gibbs energies are depicted in Fig. 5 and selected structural parameters are listed in ESI, Table S1.† Obviously, replacing chlorido ligands by iodo ones led to lower energies of TS states in both steps of hydrolysis thus speeding up the kinetics, which is in accordance with our experimental observations. Furthermore, we may notice that these reactions are less endergonic (\( \Delta G \text{ is lower} \)) than for the co-studied picoplatin.
Similar results were found for solvolysis by DMF (Fig. 5), but in this case, the energies of TS are rather higher than for the hydrolysis, which means that DMF-solvolysis would be slower. This nicely agrees with the NMR studies, in which the hydrolysis was not observed in DMF unless it was mixed with water. In all geometries of TS we observed the elongation of Pt–X (X = I and Cl) and Pt–O bonds, e.g., the Pt–I bond varied between 2.998 and 3.091 Å and Pt–O was close to 2.4 Å (ESI, Table S1†). The <(X–Pt–O) angles, where X is the leaving halogenido ligand and O is the donor atom of incoming aqua or the DMF ligand, are ca. 70–80°, and this angle is increasing in the order Cl < I and H2O < DMF, which can be ascribed to steric effects (ESI, Table S1†).

Finally, thermodynamic data were computed for the cis–trans isomerization reactions of 1 and picoplatin (2) (ESI, Table S2†). The Gibbs free energy of this reaction is almost three-times higher for 1 showing deep impact of iodido ligands on the chemical properties of these complexes. Faster isomerization of 1 is also supported by the herein performed experimental study.

In particular, besides the expected hydrolysis of the Pt–I bonds of 1, we observed for the first time the release of the N-donor ligand (heterocyclic pic in the case of this work). To the best of our knowledge, the N-donor ligand release was observed for similar Pt(n) diiodido complexes only in the presence of relevant biomolecules5,9 or when irradiated.11 Since the trans-effect of ligands of 1 follows an order I > py > NH3, the NH3 release should predominate over the pic release from the structure of 1. However, since the NH3 release was disproved by the obtained results and direct evidence was obtained for the pic release, a contribution of the steric effects has to be taken into account as well for pic. Importantly, the instability of 1 under the aqueous conditions indicated that further in-solution studies (e.g., interaction with intracellular biomolecules, stability in the cell culture medium) are required to determine whether 1 could be submitted for the testing of in vitro cytotoxicity in human cancer cells. Despite DMSO being reported as an unsuitable solvent for anticancer Pt(n) complexes (as discussed above),14b we plan to investigate the solution behaviour of 1 and 2 in this solvent as well as in its mixture with water in the following study. In conclusion, the results of the performed solution behaviour studies clearly proved that the Pt(n) diiodido complex 1 undergoes several in-solution processes (Fig. 6), which is in contrast to its chlorido analogue, i.e., clinically studied Pt(n) dichlorido complex picoplatin (2).

Conflicts of interest

There are no conflicts to declare.

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**Notes and references**