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Platinum(IV) Prodrugs with Haloacetato Ligands in the Axial Positions can Undergo Hydrolysis under Biologically Relevant Conditions**

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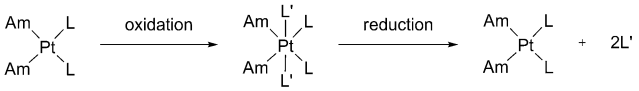
Platinum-based drugs are very effective anticancer agents that are used routinely in the clinic, and nearly 50% of all chemotherapeutic regimens administered to patients include a platinum drug.[1] Cisplatin, carboplatin, and oxaliplatin are square planar d^8 Pt^{II} complexes that trigger cancer cell death by binding to nuclear DNA, distorting its structure.[2] Owing to its reactivity and lack of selectivity most of the cisplatin administered reacts with nucleophiles in the blood, never reaching the tumor. These undesirable interactions limit the bioavailability of the drug and preclude oral administration.[3]

One approach to overcome the drawbacks of cisplatin is to use Pt^{IV} complexes as prodrugs. They are prepared by oxidatively adding two axial ligands to a Pt^{II} drug. The design of these prodrugs is predicated on the assumption that the low spin octahedral d^6 Pt^{IV} complexes are inert and will not undergo hydrolysis or ligand substitutions in the blood but will be activated in cells by reductive elimination, releasing the cytotoxic square-planar Pt^{II} drugs (Scheme 1).[4] Yet it was



Scheme 1. Octahedral Pt^{IV} prodrugs like *ctc*-[Pt(Am)$_2$L′$_2$L$_2$], where Am = neutral am(m)ine and L or L′ are anionic ligands, are assumed to remain intact until reduced in cancer cells, releasing the cytotoxic Pt^{II} moiety and the intact axial ligands (L′).

reported that in some of the biotransformation products of *ctc*-[$PtCl_2(OAc)_2(NH_3)$(c-hexylamine)] the chlorido ligands were replaced by OH while the axial OAc ligands remained intact. Negligible aquation (< 5%) was observed for *ctc*-[$PtCl_2(OAc)_2(NH_3)_2$] over 3–4 weeks.[5] Thus, the axial ligands can be used to impart favorable pharmacological properties to the drugs. Many reports describe the use of axial ligands to control the reduction potentials, lipophilicity, charge, selectivity, targeting, and cell uptake of the Pt^{IV} complexes.[6]

In most Pt^{IV} derivatives of cisplatin or oxaliplatin, the axial ligands are tethered to the metal center through carboxylates. Reduction potentials, and reduction rates depend on the electron withdrawing power of the axial carboxylato ligands. When acetates are replaced by trifluoroacetate (tfa), the reduction potentials are lower and the rates of reduction are much faster.[7]

Khokhar, Siddik and co-workers reported the synthesis and anticancer properties of [Pt(dach)(L)$_2$L′] where L = acetato or tfa and L′ = oxalato, malonato, or cbdca (Figure 1).[8]

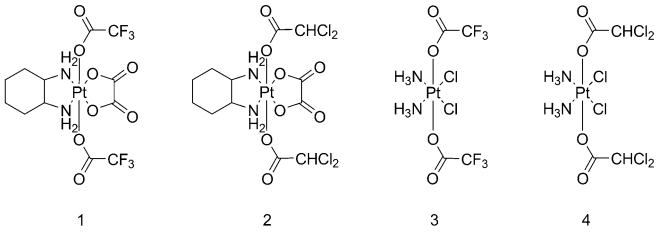


Figure 1. The Pt^{IV} prodrugs used in this study. 1) [Pt(dach)(tfa)$_2$(ox)]; 2) [Pt(dach)(dca)$_2$(ox)]; 3) *ctc*-[$Pt(NH_3)_2(tfa)_2Cl_2$], and 4) *ctc*-[$Pt(NH_3)_2$-$(dca)_2Cl_2$].

Their in vivo efficacy studies against L1210 cancer xenografts demonstrated that compounds with axial tfa ligands were substantially more active than the corresponding axial–acetato analogues. Specifically [Pt(dach)(tfa)$_2$(ox)] exhibited low toxicity (50 mg kg^{-1}) and impressive in vivo activity (T/C>700, where T/C = lifespan of tumor-bearing mice that were treated relative control mice (untreated)) compared with cisplatin (T/C = 300 at 5 mg kg^{-1}). The in vivo efficacy was attributed to enhanced intracellular accumulation resulting from the higher lipophilicity of the fluorinated complexes. They also reported on the preparation and cytotoxicity of [Pt^{IV}(en)(L)$_2$(L′)] where L = acetato or tfa and L′ = Mal, cbcda, or $(O_2CR)_2$ and concluded that complexes with axial tfa groups were superior to those with acetato ligands.[9]

Dichloroacetate (DCA) is an inhibitor of pyruvate dehydrogenase kinase that can enhance apoptosis by causing a metabolic change from glycolysis to glucose oxidation and sensitizing cells to chemotherapy through the mitochondrial apoptotic pathway.[10] Mitaplatin, *ctc*-[$Pt(NH_3)_2(dca)_2Cl_2$], a Pt^{IV} derivative of cisplatin with two dichloroacetates in the axial positions (Figure 1) was designed as a multi-targeted drug that upon activation will release within the cancer cell one molecule of cisplatin and two molecules of DCA, thereby simultaneously attacking nuclear DNA and the mitochondria.

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In a variety of cancer cell lines the cytotoxicity of mitaplatin was as good as—or better—than all known Pt^{IV} compounds and was comparable to that of cisplatin.[11]

HPLC studies of [Pt(dach)(tfa)$_2$(ox)] revealed that it is unstable in aqueous solution (pH 7, 37 °C). The peak of the starting material (Figure 2 A) transformed rapidly into a new peak (Figure 2 B) having a retention time of 11.5 minutes (Method 1), which slowly transformed to a peak with a retention time 6.2 minutes (Figure 2 C). The peaks shown

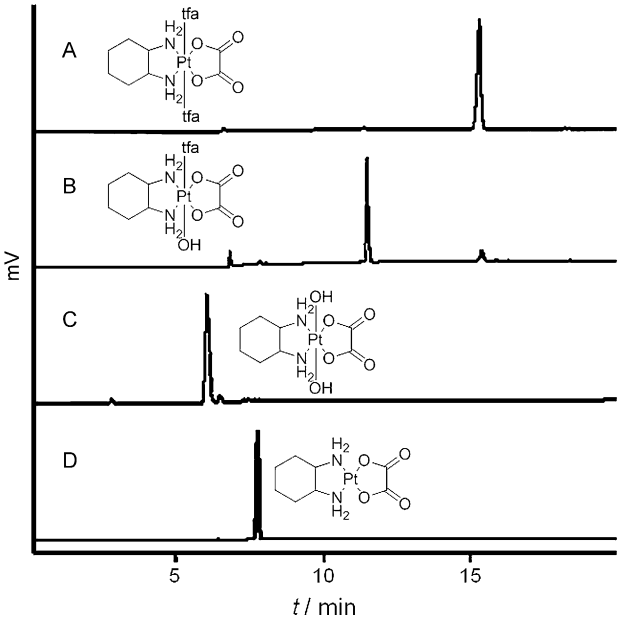


Figure 2. HPLC chromatograms of the hydrolysis of [Pt(dach)(tfa)$_2$(ox)] at A) $t = 0$ B) $t = 10$ min, and C) $t = 20$ h. D) oxaliplatin reference.

in Figure 2 B,C were collected and analyzed by ESI-MS and by ^{195}Pt NMR spectroscopy. The ^{195}Pt NMR ($\delta = +1425$ ppm) and the mass spectrum suggested that the peak in Figure 2 B corresponds to [Pt(dach)(tfa)(OH)(ox)], indicating hydrolysis of [Pt(dach)(tfa)$_2$(ox)]. Similarly the ^{195}Pt NMR ($\delta = +1310$ ppm) and the ESI-MS suggested that the peak in Figure 2 C corresponds to [Pt(dach)(OH)$_2$(ox)], which is probably obtained by subsequent hydrolysis of [Pt(dach)-(tfa)(OH)(ox)]. No reduction to oxaliplatin was observed (Figure 2 D).

Hydrolysis of the first tfa ligand to give [Pt(dach)-(tfa)(OH)(ox)] was very rapid while the hydrolysis of the second tfa ligand to yield [Pt(dach)(OH)$_2$(ox)] was much slower (Figure 3). The starting material completely transformed to [Pt(dach)(tfa)(OH)(ox)] after 15 minutes, but no [Pt(dach)(OH)$_2$(ox)] was observed. The second hydrolysis had a half-life of under five hours and after 16 hours only 10 % of [Pt(dach)(tfa)(OH)(ox)] remained in solution (Figure 3). This surprising result prompted us to study the hydrolysis of three more compounds: [Pt(dach)(dca)$_2$(ox)], *ctc*-[Pt(NH$_3$)$_2$(tfa)$_2$Cl$_2$], and *ctc*-[Pt(NH$_3$)$_2$(dca)$_2$Cl$_2$] (Figure 1).

In all cases relatively rapid hydrolysis was observed. The half-life for the hydrolysis of [Pt(dach)(tfa)$_2$(ox)] was only

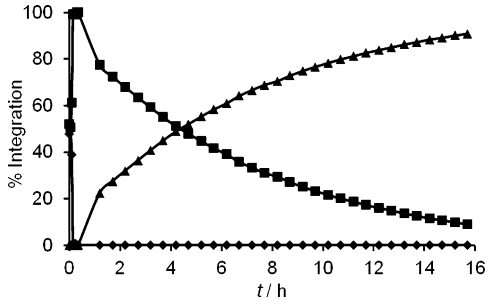


Figure 3. The reaction profile of the hydrolysis of [Pt(dach)(tfa)$_2$(ox)]. Rapid hydrolysis of [Pt(dach)(tfa)$_2$(ox)] (◆) yields [Pt(dach)-(tfa)(OH)(ox)] (■) that slowly hydrolyzes to [Pt(dach)(OH)$_2$(ox)] (▲).

about six minutes while that of [Pt(dach)(dca)$_2$(ox)] was 30-fold slower (180 minutes), suggesting that the electronegativity of the substituents strongly affects the rate of hydrolysis. Yet this was not the case for the hydrolysis of *ctc*-[Pt(NH$_3$)$_2$-(tfa)$_2$Cl$_2$] and *ctc*-[Pt(NH$_3$)$_2$(dca)$_2$Cl$_2$], whose half-lives were similar (130 and 120 minutes, respectively).

Thus, the Pt^{IV} compounds studied by Khokhar, Siddik, and co-workers with axial tfa ligands[8,9] probably hydrolyzed with half-lives of six minutes for [Pt(dach)(tfa)$_2$(ox)] or 130 minutes for *ctc*-[Pt(NH$_3$)$_2$(tfa)$_2$Cl$_2$]. Because in vitro cytotoxicity studies entail 24–96 hours incubations of the drugs in the extracellular medium, the starting materials are completely hydrolyzed during the course of the experiment. So, unless the cellular uptake was very rapid the axial ligands might be lost prior to interacting with the cells, and the observed effects may be due to the hydrolysis products rather than the starting materials.

Similarly, mitaplatin, *ctc*-[Pt(NH$_3$)$_2$(dca)$_2$Cl$_2$], was designed to simultaneously release two anti-proliferative agents. However in biologically relevant conditions, after two hours, 50 % of the mitaplatin hydrolyzed, and after nine hours, no starting material was left.

Because Pt^{IV} complexes with haloacetato axial ligands are potential anticancer drugs for either oral or intravenous administration, it is important to study their stability in plasma. [Pt(dach)(tfa)$_2$(ox)] was incubated in human plasma and its stability was monitored by ^{19}F NMR spectroscopy. Fortunately, it was easy to distinguish between the chemical shifts of [Pt(dach)(tfa)$_2$(ox)] at −74.95 ppm and that of [Pt(dach)(tfa)(OH)(ox)] at −75.4 ppm and that of free tfa at −76.0 ppm (Supporting Information, Figure S1). The decay curve for [Pt(dach)(tfa)$_2$(ox)] in plasma is similar to the one observed in aqueous phosphate buffer giving a half-life of under five minutes (Figure S2).

Hydrolysis reactions can be facilitated by acidic or basic conditions. We measured the rates of hydrolysis at different pH values. The results for the hydrolysis of [Pt(dach)-(dca)$_2$(ox)] are depicted in Figure 4. At pH values of 5–6, there was no hydrolysis but as the pH increased the hydrolysis became much more rapid, suggesting that the hydrolysis follows the sn1CB mechanism.[12]

Hydrolysis can proceed by a dissociative substitution of the DCA ligand catalyzed by reversible deprotonation of a coordinating amine,[13] or by hydrolysis of the ester. The

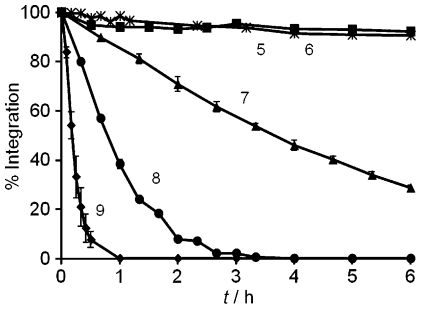
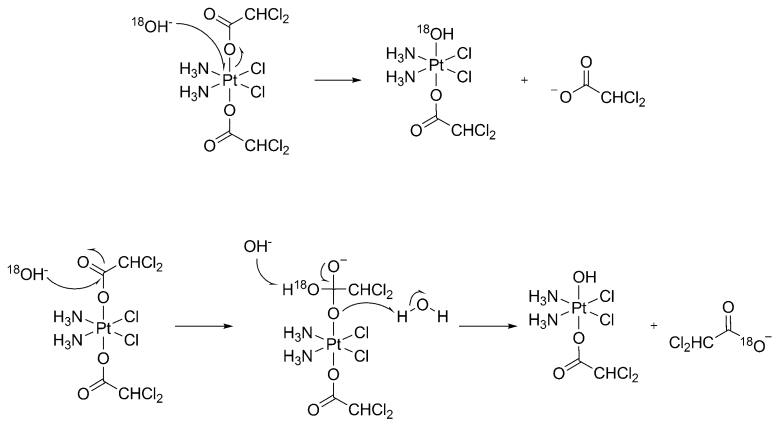


Figure 4. Variation of the rates of hydrolysis of [Pt(dach)(dca)$_2$(ox)] as a function of the pH (pH values are indicated as integers on the graph).

former involves the breaking of the Pt^{IV}–O(dca) bond and formation of Pt–OH bond, while the latter proceeds by an attack of the hydroxide on the carbonyl carbon to form a tetrahedral intermediate, followed by elimination (Scheme 2).



Scheme 2. A description of two possible pathways for the hydrolysis of *ctc*-[Pt(NH$_3$)$_2$(dca)$_2$Cl$_2$] in $H_2{}^{18}O$. Direct substitution of the DCA by $^{18}OH^-$ will result in incorporation of ^{18}O into the axial position of the complex and release of ^{16}O DCA (top). Attack of OH^- on the carbonyl carbon follows the classical path of base-catalyzed ester hydrolysis resulting in at least partial incorporation of ^{18}O into DCA.

To see if the oxygen bound to the Pt originated from the carboxylate, implying that the O–C(O)CHCl$_2$ bond was broken, or whether it came from the hydroxide, the hydrolysis of mitaplatin was carried out in $H_2{}^{18}O$ and the products were characterized by ESI-MS (Figure 5). The simulated spectrum of *ctc*-[Pt(NH$_3$)$_2$(dca)(OH)Cl$_2$] is depicted in Figure 5 A2 and that of *ctc*-[Pt(NH$_3$)$_2$(dca)(^{18}OH)Cl$_2$] in Figure 5 A3 and the experimental spectrum in Figure 5 A1.

The only hydrolysis product we detected is *ctc*-[Pt(NH$_3$)$_2$(dca)(^{18}OH)Cl$_2$]. To further confirm this conclusion we also measured by negative-ion ESI-MS the free DCA that was released into solution. The simulated spectra for OC(O)CHCl$_2$ and ^{18}OC(O)CHCl$_2$ are depicted in Figures 5B2 and 5B3, respectively and the experimental spectrum in Figure 5 B1. Only OC(O)CHCl$_2$ was observed, indicating that the O–C(O)CHCl$_2$ bond was not broken and that there is no exchange between the $H_2{}^{18}O$ and OC(O)CHCl$_2$.

Together, these data suggest that for mitaplatin the hydrolysis is carried out by an attack of the hydroxide on the Pt^{IV}, resulting in the breaking of the Pt–OC(O)CHCl$_2$ bond and formation of the Pt–^{18}OH bond. This is essentially a substitution of the axial DCA ligand with a hydroxide. We are currently investigating the reaction mechanisms.

Because there is a lot of interest in the design of novel platinum anticancer agents based on Pt^{IV} prodrugs, it is important to continue exploring the aqueous chemistry of Pt^{IV} complexes. Although it might seem that the Pt^{IV} chemistry is well understood, there have been several reports that seem to contradict some of the basic assumptions in the field. Natile and co-workers have shown that the

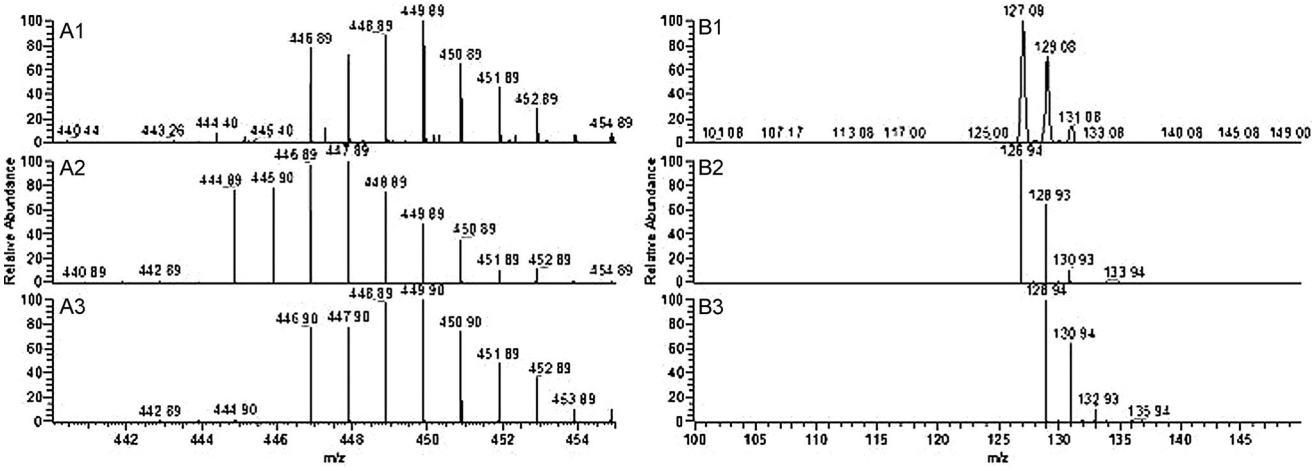


Figure 5. A1) ESI-MS of the solution resulting from hydrolysis of *ctc*-[Pt(NH$_3$)$_2$(dca)$_2$Cl$_2$] in $^{18}H_2O$, showing the sole product detected was *ctc*-[Pt(NH$_3$)$_2$(dca)(^{18}OH)Cl$_2$], A2) the simulated ESI-MS of *ctc*-[Pt(NH$_3$)$_2$(dca)(^{16}OH)Cl$_2$], A3) the simulated ESI-MS of *ctc* -[Pt(NH$_3$)$_2$(dca)-(^{18}OH)Cl$_2$]. B1) ESI-MS of the solution resulting from hydrolysis of *ctc*-[Pt(NH$_3$)$_2$(dca)$_2$Cl$_2$] in $^{18}H_2O$, showing that only ^{16}OC(O)CHCl$_2$ was in solution, B2) the simulated ESI-MS of ^{16}OC(O)CHCl$_2$, B3) the simulated ESI-MS of ^{18}OC(O)CHCl$_2$.

reduction products of a Pt^{IV} complex are dependent on the reducing agent,[14] and we and others have shown that more than one reduction product are possible.[15] Also, we have shown that for some platinum complexes there is no correlation between reduction potentials and reduction rates,[16] and this work demonstrates that Pt^{IV} complexes with axial haloacetato ligands can undergo hydrolysis under biologically relevant conditions and in timescales that are relevant to cytotoxicity studies.

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