Monofunctional Platinum(II) Anticancer Complexes Based on Multidentate Phenanthridine-Containing Ligand Frameworks

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Phenanthriplatin is a leading preclinical anticancer Pt complex distinguished by a phenanthridine ligand that facilitates DNA-targeted covalent binding via intercalation. We report here that Pt(II) complexes incorporating phenanthridine into a chelating, multidentate ligand scaffold exhibit a superior in vitro therapeutic index compared with phenanthriplatin and cisplatin.

Cispaltin1 and related platinum(II) drugs are key tools in modern cancer treatment.2, 3 Notwithstanding its history of transformative clinical implementation, Pt chemotherapy can be limited by severe side effects caused by off-target activity and reduced efficacy due to acquired or intrinsic resistance in certain types of cancers.4 One strategy for increasing potency and expanding the spectrum of activity of a class of compounds while mitigating side effects is to search out analogues that operate by novel mechanisms of action.5 In this respect, monofunctional platinum anticancer complexes, a class of platinum drug candidates containing only a single labile ligand first studied in earnest in the late 1980s, are attracting renewed interest.6 Compared with bifunctional anticancer complexes such as cisplatin which deform DNA strands via formation of inter- and intrastrand crosslinks,7 monofunctional Pt(II) complexes can only bind to DNA through a single coordination site opened up by the one vacating chloride. The antineoplastic activity of monofunctional complexes such as phenanthriplatin ([cis-Pt(NH3)2(phenanthridine)Cl][NO3]; Figure 1a)8 thus arises from different biochemical interactions compared to compounds like cisplatin (cis-Pt(NH3)2Cl2), with a distinct spectrum of action and potential for altered resistance/side-effect profiles.

While phenanthriplatin shows heightened activity,8

![Figure 1. (a) Structures of cispaltin, phenanthriplatin and the monofunctional phenanthridine-ligand supported Pt complexes described herein. (b) Synthesis of chelating N\(^{\text{N}}\)(H)\(^{\text{O}}\) proligands L1-L2 and their Pt(II) complexes 1-2.](image)

pyrplatin, in which phenanthridine is replaced with the parent N-heterocycle pyridine, is ten-fold less potent.9 Single-molecule DNA-stretching experiments revealed a two-step binding process for phenanthriplatin, where rapid unwinding of DNA triggered by intercalation of the phenanthridine unit is followed by slower covalent modification.10 The smaller pyridine does not associate as effectively with duplex DNA prior to covalent binding, lowering efficacy. The disposition of the N-heterocycle to the labile ligand is also important; DNA intercalation of the stereoisomer of phenanthriplatin with the heterocycle trans disposed to the chloride ([trans-Pt(NH3)2(phenanthridine)Cl][NO3])11 competes with - rather than enhancing - covalent binding, reducing the number of Pt-DNA adducts formed. Trans-phenanthriplatin is nevertheless still an effective anticancer agent, with quite different activity compared to phenanthriplatin.11 This is not true of transplatin. Covalent binding of phenanthriplatin to platinum to form a true monofunctional drug in phenanthriplatin also has superior activity compared with the simple combination of an intercalator such as ethidium bromide and cisplatin, which do not form a stable adduct in solution.12

We have recently developed synthetic mechanisms for incorporating phenanthridine into multidentate ligand architectures to explore their coordination chemistry with late
transition metals. By appending additional donors, the heterocycle can be forced cis to the labile chloride and exhibits a diminished tendency to dissociate irreversibly from the metal thanks to the chelate effect.\textsuperscript{16, 17} As the attenuation of chemical reactivity and possible side effects of bifunctional platinum drugs such as carboplatin and oxaliplatin are attributed in part to the stabilizing impact of chelating ligand structures,\textsuperscript{18} we pursued the synthesis and characterization of multidentate phenanthridine-based ligands (L1 and L2) and their platinum complexes (1 and 2, Figure 1b) to evaluate the potential of Pt(II) derivatives of chelating phenanthride-based ligands as monofunctional chemotherapeutics. We find these chelate-supported phenanthriplatin analogs show a superior therapeutic index compared to cisplatin and phenanthriplatin in vitro.

Two N\textsuperscript{2}N\textsuperscript{2}(H)\textsuperscript{2}/O proligands containing phenanthridinyl units were prepared by acid-catalyzed condensation of 4-aminophenanthridines\textsuperscript{19} with acetylacetone (Figure 1b). The electronic influence of the substituent in the 2-position did not significantly influence the progress of the reaction. Proligands bearing electron-releasing tBu (L1) and electron-withdrawing CF\textsubscript{3} substituents (L2) could be isolated in similar yields (~65%). Single crystals of L1 suitable for X-ray crystallography were grown from mixtures of diethylether and chloroform (Figure 2a). The structural metrics are consistent with a keto/enamine tautomer. In particular, the solid-state structure revealed a short C(21)-O(1) bond distance of 1.244(3) Å. This assignment was corroborated by comparing solution NMR and IR parameters with related compounds.\textsuperscript{20} Density functional theory (DFT; RIJCOSX-PBE0/def2-TZVP) predicted IR spectra of the optimized structures of L1 and L2 accordingly reproduce the two notable absorptions observed experimentally between 1550-1650 cm\textsuperscript{-1}. The medium-strength, narrow peaks at 1617 cm\textsuperscript{-1} (L1) and 1634 cm\textsuperscript{-1} (L2) are consistent with C=O stretching modes, while the stronger absorptions at 1570 cm\textsuperscript{-1} (L1) and 1579 cm\textsuperscript{-1} (L2) are attributed to N-H bends.

Metallation of the proligands was carried out using PtCl\textsubscript{2} in the presence of 0.5 equivalents of silver oxide in THF at elevated temperatures. Again, the electronics of the phenanthridinyl unit did not impact the progress of the reaction. Platinum complexes 1 and 2 were isolated as air-stable orange solids in similar yields (~86-87%). Ligand bonding was confirmed by disappearance of the downfield 1H NMR signal attributed to the acidic N-H proton of the proligands (L1: 13.44 ppm; L2: 13.72 ppm) and a shift in the CH resonance in the 6-position of the phenanthridinyl ring system, which shows coupling to the 195Pt nuclei in 1 and 2 (1: 10.03 ppm, 3J\textsubscript{PH} = 39 Hz; 2: 10.20 ppm, 3J\textsubscript{PH} = 39 Hz). A similar deshielding of this particular hydrogen nucleus was observed for complexes of bis(phenanthridinyl)amido ligands\textsuperscript{15b} and can be interpreted as diagnostic of phenanthridinyl bonding to a late transition metal. Preparations using oxygenous or nitrogenous Brønsted bases (e.g., NET\textsubscript{3} or NaOtBu) in place of Ag2O were similarly successful in generating the target platinum(II) complexes. Single-crystals suitable for crystallographic analysis of 1 were also obtained. The solid-state structure (Figure 2b) reveals the co-planarity of the phenanthridinyl moiety and the coordination plane of platinum, with an angle between the two planes of 3.5°. The short C(21)-O(1) of 1.278(6) Å is consistent with retention of the keto/enamide structure upon coordination to Pt. Complexes 1 and 2 are generally soluble in organic solvents, though insoluble in aqueous media.

To assess the biological potential of chelated phenanthridine-containing monofunctional Pt(II) compounds 1 and 2, in vitro cytotoxicities were evaluated using MTT assays (MTT = [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]; see Supporting Information). Table S1 reports IC\textsubscript{50} (50% growth inhibition concentrations) for two separate ovarian cancer cell lines. The results revealed promising activity for both 1 and 2 compared to cisplatin, as well as a dependence on substituent structure. For example, 2 (R = CF\textsubscript{3}) showed much higher in vitro efficacy as compared to cisplatin than 1 (R = tBu), as well as less resistance than cisplatin (IC\textsubscript{50} of A2780cis/IC50 of A2780) against both A2780 (cisplatin sensitive) and A2780cis (cisplatin resistant) ovarian cancer cell lines. The higher in vitro efficacy of 2 (R = CF\textsubscript{3}) vs 1 (R = tBu) highlights the opportunity to fine-tune biological activity via ligand backbone substitution.

In addition, neither the proligand L2 or precursors 4-amino-(2-tert-butyl)phenanthridine or 4-amino-(2-trifluoromethyl)-phenanthridine (which may be generated upon hydrolysis of L1 or L2) were found to be effective in the absence of Pt(II). The differing profile compared to cisplatin (i.e., higher in vitro efficacy and lower cross-resistance) implies a different mechanism of operation from cisplatin, which, considering the planar structure of 1 and 2 compared to phenanthriplatin\textsuperscript{5} may involve a more prominent role for intercalation. As noted above, intercalation enhances covalent binding and ultimately boosts the number of complex-DNA adducts observed for phenanthriplatin, but only when these two processes are concurrent.\textsuperscript{10} The lack of activity in the absence of Pt(II) highlights a key role for the metal centre in the cytotoxicity of 1 and 2. Phenanthridines in general are anticipated to interact with DNA via an intercalation mechanism, similar to the...
This journal is compared with cisplatin of each of the three different platinum compounds. The SKOV3 cells were incubated for 24 h with 2 μM concentrations of each of the three different platinum compounds. The treated cells were then harvested and digested for GFAAS analysis. Complex 2 exhibits higher cellular uptake (4.09 ± 0.138 pmol Pt per million cells) compared with cisplatin (2.12 ± 0.129 pmol Pt per million cells) or phenanthrplatin (2.88 ± 0.023 pmol Pt per million cells; Figure 4a). Similar to cisplatin,22 phenanthrplatin uptake has been shown to be mediated by organic cation transporters (OCT). phenanthrplatin is considered a high affinity substrate for OCT2, while showing a lower apparent affinity for the multi-drug and toxin extrusion proteins (MATE) responsible for excretion of platinum into the urine.23 Though not a cation itself, a similar affinity for transport and extrusion proteins might be plausibly expected for the chemically related 2, as also has been observed for initially neutral platinas such as cisplatin and oxalipatin.24 The enhanced uptake of 2 compared with phenanthrplatin does not clearly correlate with decreased cell viability for SKOV3 cells. This effect plausibly also features in the lower toxicity observed in vitro towards non-cancer cell lines.

With respect to cellular responses, a dual staining Annexin V/PI flow cytometry assay was used to probe the occurrence of apoptosis. In particular, SKOV3 ovarian cancer cells were treated with and without 2. The results in Figure 4b clearly indicate that 2 induced apoptosis, stimulating SKOV3 cells to undergo early (0.87%) and late (11.82%) stage apoptosis after 72 h of incubation, the populations of which were much higher than those of control. The evidence compiled from the cell-based studies suggest that planar phenanthrde-ligated Pt(II) complexes such as 2 can readily enter cancer cells and trigger apoptosis.

Monofunctional phenanthrplatin-type complexes based on chelating tridentate N-heterocycle-containing ligands thus show promising in vitro anticancer activity, highlighting the potential of this new class of anticancer agents. The high activity towards cisplatin-resistant cancer cells is a critical finding, as tumors resistant to cisplatin often show cross-resistance to a diverse range of unrelated antitumour drugs.25 The activation of independent pathways by the molecular structure of phenanthrde-based Pt(II) complexes similar to what is observed with phenanthrplatin26 is likely responsible for the increased sensitivity of resistant cells to 1 and 2.26

In addition, a distinguishing feature of both cis-phenanthrplatin and trans-phenanthrplatin is the orientation of the phenanthrde ligand with respect to the coordination plane of platinum. The heterocycle is nearly orthogonal in the cis isomer (dihedral angle ~ 89°)24 and slightly less so in trans-phenanthrplatin (~ 67°).11 Coupled with the asymmetry of phenanthrde with respect to the position of benzannulation relative to the nitrogen atom, phenanthrplatin is chiral.27 While racemization upon rotation about the Pt-N(phenanthrde) bond is rapid enough to preclude requiring...
administration of a single enantiomer, there is a preference for
diastereomer formation upon binding to DNA.\textsuperscript{27} Forcing the
phenanthridinyl unit coplanar with the metal coordination
plane obviates this chirality and raises the interesting question
of why 1 and 2 show enhanced anticancer efficacy compared
with phenanthriplatin \textit{in vitro}. Identification of the molecular
targets of 1 and 2 and investigation of potential intercalation-
based mechanisms\textsuperscript{28} therefore represent the next steps in this
line of inquiry.

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Conflicts of interest
There are no conflicts to declare.

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