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trans-Platinum Planar Amine Compounds with $[N_2O_2]$ Ligand Donor Sets: Effects of Carboxylate Leaving Groups and Steric Hindrance on Chemical and Biological Properties

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Replacement of NH_3 by a planar amine L to give *trans*-$[PtCl_2(L)(L')]$ (L = NH_3, L′= pyridine or substituted pyridine, quinoline, isoquinoline, thiazole; L = L′= pyridine, thiazole), greatly enhances the cytotoxicity of the transplatinum geometry. The "parent" compound *trans*-$[PtCl_2(NH_3)_2]$ is therapeutically inactive. Modification of the ligands to an $[N_2O_2]$ donor set, where O represents an acetate leaving group, enhances the aqueous solubility while retaining the cytotoxicity of the parent chloride compounds. The effect of two mutual trans leaving groups with weak trans influence is to impart remarkable chemical stability on the structure. This strategy is analogous to the use of the inert dicarboxylate leaving groups in the clinical compounds carboplatin and oxaliplatin. In this paper, systematic modification of the steric effects of carrier pyridine groups and, especially, carboxylate leaving groups in *trans*-$[Pt(O_2CR)_2(NH_3)(pyr)]$ is shown to modulate aqueous solubility and hydrolysis to the activated aqua species. The results presented here demonstrate the utility of the "carboxylate strategy" in "fine-tuning" the chemical and pharmacokinetic properties in the design of clinically relevant transplatinum complexes.

Platinum complexes in the trans geometry are of interest for their biological properties. Substitution of NH_3 in *trans*-$[PtCl_2(L)(L')]$ gives complexes with cytotoxicity in the micromolar range. Since the first publication of this phenomenon using planar amines[1−3] (pyridine, thiazole, quinoline, isoquinoline, etc.) a range of amine ligands have been employed, including iminoethers, alicyclic amines, and heterocyclic aliphatic amines.[4−8] In general, these complexes exhibit enhanced cytotoxicity with respect to the parent transplatin and are usually non-cross-resistant with cisplatin. Complexes containing trans-planar amines (TPA compounds) exhibit a unique cytotoxicity profile in the NCI tumor panel and induce topoisomerase I-DNA complexes in human tumor cells.[9,10] To address the poor aqueous solubility and relatively high chemical reactivity of the *trans*-$[PtCl_2(L)(L')]$ structure, we have introduced the use of carboxylates as leaving groups in the first examples of cytotoxic transplatinum complexes containing $[N_2O_2]$ ligand donor sets.[11] Complexes such as *trans*-$[PtOAc)_2(pyr)_2]$ are very water-soluble and surprisingly stable toward hydrolysis, resembling carboplatin in their reactivity. As a series, the complexes are cytotoxic in both cisplatin and oxaliplatin-resistant cells and show remarkably high cellular uptake.[11,12]

In our initial reports we suggested that the "carboxylate strategy" could be extended to all classes of transplatinum complexes. A report on some simple examples, using compounds containing the alicyclic amine motif,[13] analogues of compounds first developed by Navarro-Raninger et al.,[7] has since confirmed this suggestion.

The pharmacological properties of *trans*-$[Pt(O_2CR)_2(L)(L')]$ can in principle be modified by steric and electronic effects of the donor groups, as well as in the leaving carboxylate ligands. In this paper, we report on the synthesis and characterization of a structurally similar set, *trans*-$[Pt(O_2CR)_2(NH_3)(L)]$, and show that systematic variation of the

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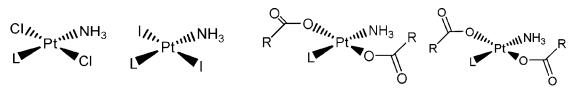
Chart 1. TPA Carboxylate Compounds.



1a : L = py **1b** : L = py R = CH_3 L = 4-pic
2a : L = 2-pic **2b** : L = 2-pic **1c** : L = py **4d** : R = CH_2OH
3a : L = 3-pic **3b** : L = 3-pic **2c** : L = 2-pic **4e** : R = H
4a : L = 4-pic **4b** : L = 4-pic **3c** : L = 3-pic **4f** : R = CH_2Cl
4c : L = 4-pic **4g** : R = CF_3
4h : R = Bz

Table 1. Solubility of TPA Carboxylates and pK_a of the Corresponding TPA Diaqua Complexes at Ambient Temperature

TPA acetates	pK_{a1}	pK_{a2}	solubility (mg/mL)	TPA-substituted carboxylates	solubility (mg/mL)
1c[11]	3.73	6.80	11.6	**4d**	22.0
2c	4.03	7.10	10	**4e**	35.0
3c[11,14]	3.97	6.78	11.4	**4f**	0.29
4c[11,14]	3.94	6.88	13.2	**4g**	insoluble
				4h	insoluble

donor ligand (L = pyridine or substituted pyridine, 2-pic, 3-pic, or 4-pic) and R allows for a range of aquation rates and also cytotoxicity. Thus the general $[PtN_2O_2]$ structure is able to be "fine-tuned" to enhance biological activity.

The platinum complexes from this study are presented in Chart 1. The synthesis and characterization of the intermediates and complexes **1a**−**c**,[11] **2a**, **3a**, **4a**,[14] **3b**−**c**, and **4b**−**c**[12] have been previously reported. The syntheses of complexes **2b**, **2c**,[16] **4d**,[17] **4e**,[18] **4f**,[19] **4g**,[20] and **4h**[21] were adapted from literature procedures,[11] and purity was confirmed by HPLC.[22] The ^{1}H NMR chemical shifts and elemental analysis of these compounds is reported in the Supporting Information. The silver salts of the carboxylates were prepared as described below.[15] The pathway to the final carboxylate complex takes advantage of the increased trans influence of the halides over the N-donor ligands. In all examples, synthesis from the chloride complex to the iodide intermediate was quantitative in yield, and the yields of the final carboxylate complexes were much higher (50−70%) than those of recently published methods.[13]

The solubility of the complex series was measured at 37 °C and is presented in Table 1. Replacement of the halogens with acetate ligands significantly enhanced the aqueous solubility of TPAs, as previously reported.[11] The aqueous solubility of the acetate series is influenced by the steric hindrance of the heterocycle (pyridine, quinoline, etc.),[11] but there is little difference seen for the various substitution patterns around the pyridine ring. In contrast, significant differences are noted when the carboxylate group was changed. For the series of 4-pic complexes, an increase in hydrogen bonding capability leads to enhanced solubility as seen for the formate, **4e**, and hydroxyacetate, **4d,** derivatives, while the chloroacetate, **4f**, trifluoroacetate, **4g**, and benzoate, **4h,** derivatives show reduced solubility.

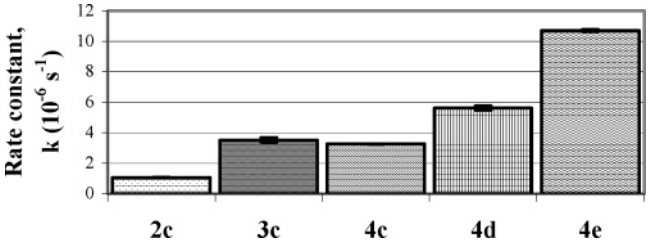


Figure 1. Initial hydrolysis rate constants determined from HPLC experiments in H_2O at 37 °C.

The pK_a of **1c** was determined from the potentiometric titration of solutions of *trans*-$[Pt(H_2O)_2(L)(L')]^{2+}$ as per Ma.[11] This is presented Table 1 in comparison to the pK_a values of the other compounds of the series **2**−**4c**. The result is consistent with previous data[11,14] and confirms the effect on pK_{a1} of substitution of an amine NH_3 or diamine (ethylenediamine, 1,2-diaminocyclohexane) by a π-acceptor ligand.[14,24]

The initial hydrolysis of selected TPA carboxylates (**2**−**4c**, **4d**, and **4e**) was monitored by HPLC over a period of 12 h at 37 °C, and the initial hydrolysis rate constants were calculated using the program SCIENTIST (version 2.0, MicroMath, Inc.); they are presented in Figure 1. The slowest initial hydrolysis rate was found for complex **2c**, *t*-$[Pt(OAc)_2(NH_3)(2\text{-pic})]$, with just 4% hydrolysis occurring after 9 h. The hydrolysis of the formate complex **4f** was the fastest, with 24% hydrolysis occurring after 9 h. The hydrolysis rates of the acetate complexes followed the pattern **2c** < **4c** < **3c**. These results may be contrasted with those for the analogous dichloride complexes where the first hydrolysis step for formation of the monoaqua complex is relatively fast with k_1 = 2.6, 12.7, and 5.2 × 10^{-5} s^{-1} (I = 0.1 M) for 2-pic, 3-pic, and 4-pic, respectively.[14] In contrast, the rates observed for the analogous acetate derivatives were k_1 = 1.07, 3.52, and 3.26 × 10^{-6} s^{-1} (I = 0.02 M) for **2c**, **3c**, and **4c**, respectively. Thus, hydrolysis of the acetate complexes is slowed by an approximate order of magnitude compared to their direct chloro analogues, but there is little difference in the present case between 3- and 4-picoline. Steric hindrance therefore predominates over the electronic effects of the methyl group. In the case of the chloride complexes, the 3-pic directs less electron density to the platinum, rendering it less nucleophilic relative to the 2-pic and 4-pic.[14] We observe the same trend in the acetate complexes, but

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(15) AgOAcOH (AgO_2CCH_2OH, OAcOH = hydroxyacetate) was prepared via the addition of hydroxyacetic acid (0.0526 mol) to a suspension of Ag_2O (4.32 mmol) in 150 mL of H_2O. The mixture was stirred for 2 h in the dark, filtered through Celite, and reduced until a white crystalline solid formed. Yield: 1.40 g (89%). AgOAcCl (AgO_2CCH_2-Cl, OAcCl = chloroacetate), AgOFm (AgO_2CH, OFm = formate), AgTfa (AgO_2CCF_3, Tfa = trifluoroacetate), and AgOBz ($AgO_2CC_6H_5$, OBz = benzoate) were prepared similarly.
(16) *trans*-$[Pt(OAc)_2(NH_3)(2\text{-pic})]$ **2c**. ^{195}Pt NMR (acetone-d_6): δ −1420. IR (cm^{-1}): 1633 m (C═O).
(17) *trans*-$[Pt(OAcOH)_2(NH_3)(4\text{-pic})]$ **4d**. ^{195}Pt NMR (D_2O): δ −1454. IR (cm^{-1}): 1645 s (C═O).
(18) *trans*-$[Pt(OFm)_2(NH_3)(4\text{-pic})]$ **4e**. ^{195}Pt NMR (D_2O): δ −1450. IR (cm^{-1}): 1614 s (C═O).
(19) *trans*-$[Pt(OAcCl)_2(NH_3)(4\text{-pic})]$ **4f**. ^{195}Pt NMR (CD_3OD): δ −1403. IR (cm^{-1}): 1619 s, 1645 m, 1668 m (C═O).
(20) *trans*-$[Pt(Tfa)_2(NH_3)(4\text{-pic})]$ **4g**. ^{195}Pt NMR (CD_3OD): δ −1402. IR (cm^{-1}): 1680, 1705 d, s(C═O).
(21) *trans*-$[Pt(OBz)_2(NH_3)(4\text{-pic})]$ **4h**. ^{195}Pt NMR (CD_3OD): δ −1420. IR (cm^{-1}): 1633 m, 1620 m (C═O).
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the effect is decreased because of the carboxylate group being a weak ligand, with a low trans influence and trans effect.[11]

The hydrolysis rates of the carboxylate complexes followed the same pattern as the solubility, with **4c** < **4d** < **4e** and values of $k_1 = 3.26$, 5.61, and 10.7×10^{-6} s^{-1} ($I = 0.02$ M), respectively. The measured rate of 10.7×10^{-6} s^{-1} for the formate complex **4e** is still slower than that of the chloro analogue and even that of cisplatin (51.8×10^{-6} s^{-1} at 20 °C).[25]

The slow hydrolysis is a unique feature of two carboxylate ligands in a trans-axial position and can be modulated by choice of carboxylate. To examine how this might affect possible reactions with biomolecules, a preliminary study of the reactions of **4e** with guanosine-5′-monophosphate (5′-GMP, model for DNA) and *N*-acetyl-l-methionine (N-AcMet, model for sulfur ligand metabolism) was made by ^{195}Pt NMR spectroscopy. For 5′-GMP, the peak of the starting material at −1450 ppm is only gradually replaced by a new peak at −2365 ppm. This shift is consistent with the formation of a species that has a PtN_4 coordination sphere[26] because of the replacement of both formate groups. Approximately 50% reaction (as judged by the intensity of the ^{1}H H8 signal and ^{195}Pt NMR signals) occurred within 24 h. For N-AcMet the starting material was approximately 50% of total intensity even after 8 h. A new species was observed with $\delta(^{195}\text{Pt})$ at −1775 ppm, which formed after 1 h, and did not exceed 15% of the total reaction product over 10 h. The chemical shift is consistent with a PtN_2O_2 coordination sphere, this could result from displacement of formate by H_2O/OH or an O-bound methionine.[27] This species preceded the formation of the N-AcMet product at −3320 ppm consistent with a PtN_2S_2 coordination sphere, *trans*-[Pt(NH$_3$)(4-pic)(N-AcMet)$_2$],[23,28] which was 40% of the total platinum species after 10 h. This reaction can be contrasted to that of *trans*-[PtCl$_2$(NH$_3$)$_2$], whose reaction with N-AcMet is complete within 5 h.[28] These results emphasize the previously made analogy between the transplatinum [N_2O_2] ligand donor set and carboplatin and show that substitution reactions with potentially deactivating biomolecules may be significantly retarded in comparison with chloro analogues. Whether the possible formation of an O-bound methionine species is a reflection of the low pK_{a1} and the electronic effects of a π-acceptor ligand is worthy of further investigation.

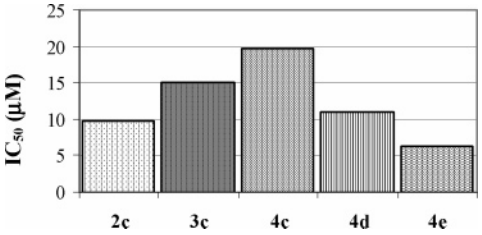

Figure 2. Cytotoxicity in A2780 human ovarian cells of the TPA carboxylate compounds.

The MTT assay was used to determine growth inhibition of the platinum drugs in the human ovarian cancer cell line A2780.[29] All TPA carboxylates tested exhibit cytotoxic behavior in the micromolar range. The most cytotoxic compound, **4e**, *trans*-[Pt(OFm)$_2$(NH$_3$)(4-pic)], was also that which displayed the fastest hydrolysis, as well as being most soluble, Figure 2. The cytotoxicity of the carboxylate compounds increases in the order **4c** < **4d** < **4e** (OAc < OAcOH < OFm), suggesting that the nature of the carboxylate leaving group is related to cytotoxicity. The cytotoxicity of the acetate compounds increases in the order **2c** < **3c** < **4c** (2-pic < 3-pic < 4-pic), suggesting that the steric hindrance of the methyl group can influence cytotoxicity, with the more sterically hindered 2-pic complex being the most cytotoxic of the acetate compounds.

It is remarkable that the complexes with carboxylate leaving groups display, in general, cytotoxicity equivalent to the parent chlorides, despite their differences in reactivity.[1,2,12,14] In contrast, carboplatin is significantly less cytotoxic than cisplatin on a molar basis. Previous studies have shown significantly enhanced cellular uptake of the acetate derivatives,[11,12] and it is reasonable to suggest that these results may reflect the overall modulation of the pharmacological properties responsible for cellular toxicity: uptake, nature and frequency of DNA adducts and the extent of metabolizing "deactivating" reactions. In summary, the results presented here demonstrate again the utility of the carboxylate strategy in the design of clinically relevant transplatinum complexes.

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Supporting Information Available: ^{1}H NMR chemical shifts and elemental analysis of reported compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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