Inorganic Chemistry

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Trans Titanium(IV) Complexes of Salen Ligands Exhibit High Antitumor Activity

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Supporting Information

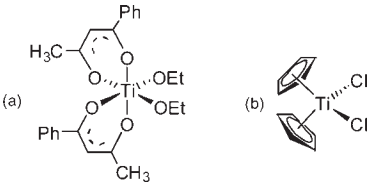
ABSTRACT: Salen−titanium(IV) complexes are introduced as a new family of highly efficient antitumor complexes, being the first cytotoxic titanium(IV) complexes of trans labile ligands, as characterized crystallographically. Four complexes with different aromatic substitutions were analyzed, reveling a meaningful effect of the ligand structure on the complex performance. All complexes exhibit high hydrolytic stability, where the labile OAr ligands hydrolyze in a 10% D_2O solution with $t_{1/2}$ ranging from 2 to 11 h. The IC_{50} values obtained for three of the salen complexes studied on HT-29 colon and OVCAR-1 ovarian cells demonstrate activity that exceeds those of the known tianium(IV) complexes Cp_2TiCl_2 and $(bzac)_2Ti(OiPr)_2$ and that of cisplatin, where the most active *para*-chlorinated complex exhibits activity enhancement relative to cisplatin by 10-fold.

Since the discovery of the cytotoxic activity of cisplatin, there is increasing interest in finding additional inorganic compounds of different metals with cytotoxic activity.[1] Two titanium(IV) complexes that reached clinical trials are titanocene dichloride (Cp_2TiCl_2) and budotitane [$(bzac)_2Ti(OEt)_2$] (Scheme 1), demonstrating cytotoxic activity toward cisplatin-resistant and -sensitive cells with reduced toxicity relative to platinum compounds, where modified complexes with various ligand substitutions exhibited improved activity.[2−11] The main general disadvantage of the titanium(IV) complexes is their low stability in aqueous environments and their tendency to undergo rapid hydrolysis, within seconds to minutes for labile ligand dissociation,[5,7,12,13] which also impeded the medicinal utility of Cp_2TiCl_2 and $(bzac)_2Ti(OEt)_2$.

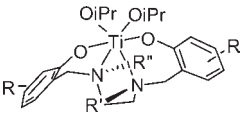
We have recently introduced a new family of cytotoxic titanium(IV) complexes based on diaminobis(phenolato) "salan" ligands (Scheme 2).[14−18] These complexes demonstrate exceptional antitumor activity and hydrolytic stability, and structure−activity relationship studies have revealed that the ligand significantly influences both parameters.

All titanium complexes that were investigated so far as antitumor agents include two labile ligands in a cis configuration, a feature deigned to enable chelate binding to a proper target as occurs for cisplatin.[19] Transplatin, the geometric isomer of cisplatin, is clinically ineffective because of its inability to perform the necessary intrastrand cross-linking to two adjacent bases in the DNA, although other *trans*-platinum complexes

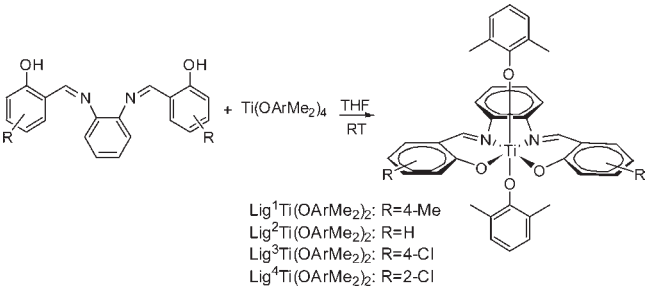
Scheme 1



Scheme 2



Scheme 3



showed cytotoxic activity by different binding mechanisms.[20] Nevertheless, the mechanism of activity of the titanium compounds is still unknown, as is the importance of this particular structural factor.

Herein we report on the investigation of titanium(IV) complexes with two labile ligands in a trans configuration, with the aim of gaining additional information on the mechanism and possible diversity of activity relating to diaminobis(phenolato) complexes. We employed ligands from the salen family that are similar in coordination features to the ligands of the salan family but are known to afford equatorial binding due to the planar imine moiety, leading to trans labile ligands.[21] We employed

Received: June 16, 2011
Published: July 28, 2011

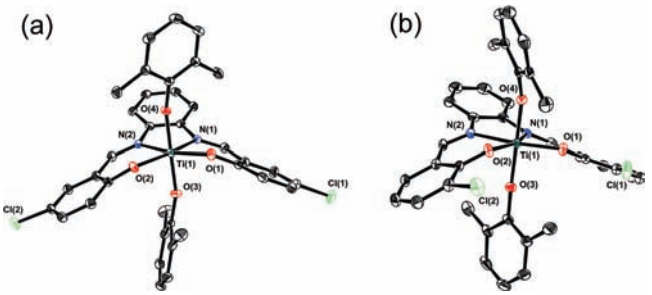


Figure 1. ORTEP drawings of $Lig^3Ti(OArMe_2)_2$ (a) and $Lig^4Ti(OArMe_2)_2$ (b). Selected bond lengths (Å) and angles (deg) for (a) Ti(1)−N(1) 2.145(2), Ti(1)−O(1) 1.909(2), Ti(1)−O(3) 1.857(2), O(1)−Ti(1)−O(2) 117.18(7), N(2)−Ti(1)−N(1) 73.71(7), and O(3)−Ti(1)−O(4) 178.18(7) and for (b) Ti(1)−N(1) 2.164(2), Ti(1)−O(1) 1.912(2), Ti(1)−O(3) 1.843(2), O(1)−Ti(1)−O(2) 118.32(8), N(2)−Ti(1)−N(1) 73.77(9), and O(3)−Ti(1)−O(4) 177.00(9).

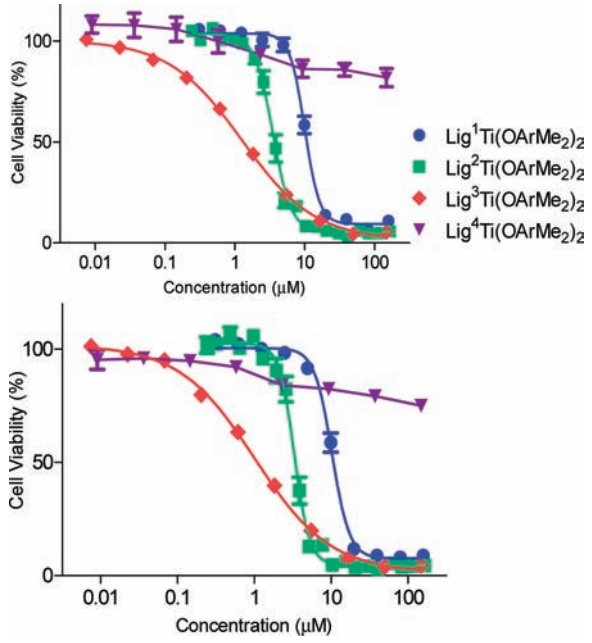


Figure 2. Dependence of OVCAR-1 (top) and HT-29 (bottom) cell viability based on the MTT assay following a 3 day incubation period upon added concentration of $Lig^{1-4}Ti(OArMe_2)_2$ presented on a logarithmic scale.

"salophen" ligands that include a planar phenylenediamine bridge (Scheme 3),[22] which were more efficient than ethylenediamine in producing pure trans products. High cytotoxicity is obtained, identifying salen−titanium(IV) complexes as a new family of antitumor agents and as the first *trans*-titanium(IV) complexes with cytotoxic activity.

Ligands H_2Lig^{1-4} were prepared based on a published procedure from differently substituted salicylaldehyde compounds and phenylenediamine.[23] Because of the difficulty in isolating clean isopropoxo complexes of these ligands starting from $Ti(OiPr)_4$ and previous observations suggesting that labile ligands are of lesser influence on cytotoxicity,[7] $Ti(OArMe_2)_4$ was employed instead.[24] Thus, $Lig^{1-4}Ti(OArMe_2)_2$ were synthesized by reacting H_2Lig^{1-4} with 1 equiv of $Ti(OArMe_2)_4$ to give

Table 1. IC_{50} (μM) Values of $Lig^{1-4}Ti(OArMe_2)_2$ and Known Reference Compounds toward HT-29 and OVCAR-1 Cancer Cell Lines and Additional Reference Values

complex	aromatic substitution	HT-29 (μM)	OVCAR-1 (μM)
$Lig^1Ti(OArMe_2)_2$	*p*-Me	10 ± 2	9 ± 1
$Lig^2Ti(OArMe_2)_2$	none	3.5 ± 0.6	3.3 ± 0.5
$Lig^3Ti(OArMe_2)_2$	*p*-Cl	1.2 ± 0.3	1.0 ± 0.3
$Lig^4Ti(OArMe_2)_2$	*o*-Cl	inactive	inactive
reference		HT-29 (μM)	OVCAR-1 (μM)
cisplatin		20 ± 2	13 ± 1
Cp_2TiCl_2		520 ± 90	550 ± 120
$(bzac)_2Ti(OiPr)_2$		11.6 ± 0.8	11.5 ± 0.2
reference[25]		LLC-PK (μM)	
titanocene Y		21 ± 1	
Cp_2TiCl_2		2000 ± 1000	
cisplatin		3.3 ± 0.5	

the titanium(IV) complexes in high yields (Scheme 3). ^1H and ^{13}C NMR analyses of the products are consistent with the formation of an isomer with high symmetry, with a single type of aromatic system and a single set of labile ligand signals. Single red crystals suitable for X-ray crystallography of $Lig^{3,4}Ti(OArMe_2)_2$ were obtained from diethyl ether at room temperature. ORTEP drawings of the structures along with selected bond lengths and angles are provided in Figure 1.

The structures of $Lig^{3,4}Ti(OArMe_2)_2$ exhibit similar features. Octahedral C_{2v}-symmetrical complexes are obtained, with trans binding of the labile dimethylphenoxo ligands, as anticipated, characterized by an O−Ti−O angle to labile ligands of 177−178° and an O−Ti−O angle to the salen ligands of 117−118°. The bond lengths and angles around the metal center are otherwise similar to those obtained for related salan complexes, with somewhat shorter Ti−N bonds to the sp^2-type donor.[14,18]

The cytotoxicity was studied on ovarian OVCAR-1 and colon HT-29 cell lines as previously described,[18] employing the MTT assay for establishing cell viability. The results are depicted in Figure 2. A summary of the IC_{50} values is provided in Table 1.

$Lig^{1-3}Ti(OArMe_2)_2$ demonstrate high antitumor activity toward HT-29 and OVCAR-1 cells, higher than that of cisplatin. When compared to the activity of titanocene Y on other cells,[25] similar and more significant improvements relative to Cp_2TiCl_2 and cisplatin, respectively, are obtained for the salen complexes. Additionally, the *para*-chlorinated complex exhibits the highest activity, which exceeds that of cisplatin by an order of magnitude. It is also obvious that the structural parameters of the ligand play an important role in the activity because the *ortho*-chlorinated complex is completely inactive. Because the nonsubstituted complex is more active than the *para*-methylated one and the *para*-chlorinated complex is more active than all in this series, we may generally suggest that steric effects that are significantly more pronounced at the ortho positions reduce cytotoxicity and the effect of the *p*-chloro substituent is apparently electronic. Compared to the family of *cis*-salan complexes,[14,18] it is apparent that the IC_{50} values obtained for the salens are comparable to those obtained for the salans, despite the different geometries, different diamino bridges, and different labile ligands. It is

dx.doi.org/10.1021/ic201296h |*Inorg. Chem.* 2011, 50, 7946–7948

interesting, however, that the *ortho*-chlorinated substitution that yielded an especially active salan complex gave no activity for the corresponding salen compound, rendering the ortho steric effect as more meaningful for the salen family of complexes.

Because the main limitation of previously reported titanium(IV) complexes as cytotoxic agents is their rapid dissociation in water solutions, the hydrolytic stability of the salen complexes was measured under conditions similar to those previously applied for the analogous salan complexes, as previously described.[14,18] The active complexes $Lig^{1-3}Ti(OArMe_2)_2$ all gave similar $t_{1/2}$ values for hydrolysis of the labile groups of 2−4 h, which are comparable to the values obtained for the corresponding salan complexes despite the reduced availability of the lone-pair electrons of nitrogen donors that are occupied by resonance, and represent substantially higher stability than those of titanocene dichloride and budotitane.[14,18] Similar to observations with the salan complexes, no substantial effect of the para substituents on the hydrolytic stability is observed. In contrast, the *ortho*-chlorinated complex $Lig^4Ti(OArMe_2)_2$ demonstrated some enhancement of the hydrolytic stability with a $t_{1/2}$ value of 11 h, which is an effect similar to that observed for the salan analogue, although substantially less pronounced.[18] Nevertheless, because $Lig^4Ti(OArMe_2)_2$ is inactive biologically, we cannot attribute cytotoxicity variations solely to the hydrolytic stability, and parameters relating to the size, solubility, and general hydrophobicity and membrane penetration ability are surely also of significance.

The results explicitly show for the first time that titanium(IV) complexes with a trans configuration of the labile groups may exhibit high cytotoxic activity, which is similar to that of the corresponding cis complexes. We still do not know, however, whether the two labile positions in the salen complexes operate separately or whether reorganization on the metal center occurs upon the first interaction to form chelate binding, due to higher flexibility of the octahedral titanium(IV) center relative to that of the planar platinum compounds. This adds to the diversity of the possible titanium(IV) complexes to be synthesized and analyzed as antitumor compounds. Additionally, the presence of the phenylenediamine bridge in the salen complexes, which does not diminish the hydrolytic stability, adds possibilities for the use of various substitutions to fine-tune the complex performance and may be advantageous if planarity is of importance, for instance, for DNA intercalation. Another advantage of the salen complexes over the salans is in their enhanced solubility in biologically relevant solutions, and in their achiral nature, removing the requirement of chiral separation that involves the medicinal application of the C_2-symmetrical salans.[26]

In summary, the high activity observed combined with the high hydrolytic stability makes this new family of salen-based titanium(IV) complexes highly promising for antitumor applications. We are currently exploring additional members of this family of complexes and their mechanism of operation.

■ ASSOCIATED CONTENT

Supporting Information. Crystallographic data for $Lig^{3,4}Ti(OArMe_2)_2$ and experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

We thank Dr. Shmuel Cohen for crystallography. This research received funding from the European Research Council under the European Community's Seventh Framework Programme (FP7/2007-2013)/ERC Grant 239603.

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