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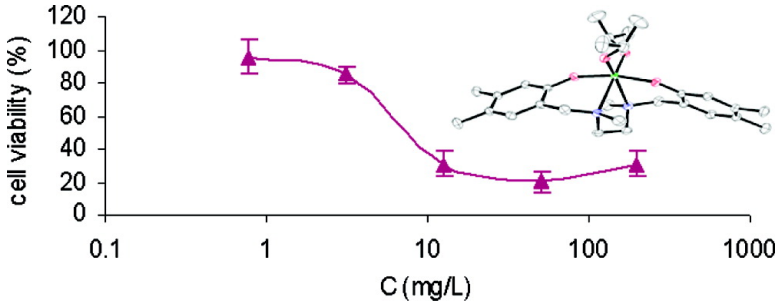

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Active Cytotoxic Reagents Based on Non-metallocene Non-diketonato Well-Defined C_2-Symmetrical Titanium Complexes of Tetradentate Bis(phenolato) Ligands

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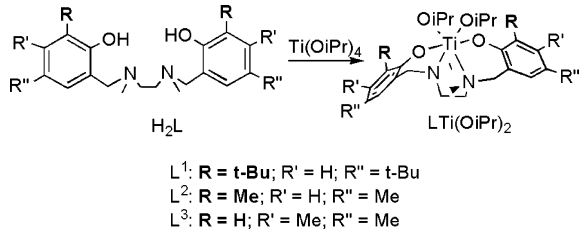
Much research has been devoted to the identification of new cytotoxic non-platinum metal complexes,[1−4] among which, Ti(IV) complexes revealed promising antitumor activity toward various cell lines.[5−12] Notably, research in this area for the last two decades has been restricted to two families of complexes, the titanocene dichloride (Cp_2TiCl_2) and Budotitane ($(bzac)_2Ti(OEt)_2$) and their derivatives. These compounds undergo rapid hydrolysis of, first, the cis labile ligands (Cl, OR), followed by the inert ones (Cp, bzac), leading to unidentified aggregates.[5,12,13] Their exact mechanism of activity is thus poorly understood, yet it is normally assumed that the ligand hydrolysis leads to formation of the active species,[14,15] although some ligand inertness is apparently required.[16,17] Additional studies indicated that the serum protein transferrin leads to complete ligand stripping from Cp_2TiCl_2 and transfers the Ti ion to the cell.[18−21] However, an early loss of the inert ligands should abolish their influence on the interaction with the cellular target and hampers their use as a target for structure−reactivity relationship studies. Herein we report a new family of *cis*-bis(isopropoxide)Ti(IV) complexes of diamine bis(phenolato) ligands, obtained as single isomers in quantitative yields, leading to appreciable in-vitro cytotoxic activity against colon and ovarian cells, where the ligand features have strong influence on reactivity which is not transferrin-dependent and apparently involves a ligand-bound active species.

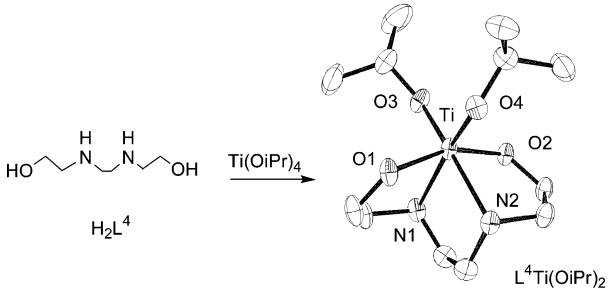
As a part of our interest to develop cytotoxic well-defined Ti(IV) complexes, we focus on chelating alkoxo ligands suitable for forming strong binding to the oxophilic Ti(IV) ion. The biologically active Budotitane which includes two monoanionic diketonato ligands exhibits several cis and trans isomers, and the symmetrical analogues of the active cis isomer feature C_2-symmetry. To minimize the number of isomers and increase thermodynamic stability, yet maintain the general symmetry, we turned to dianionic diamine−dialkoxo ligands, which were expected to lead to $LTiX_2$ type octahedral complexes. The diamine bis(phenolato) ligand family, easily synthesized in a single-step procedure,[22] conveniently leads to the desired racemic C_2 symmetrical complexes as single isomers in quantitative yields.[23−25] We thus synthesized three such complexes exhibiting ortho groups to the donor atom of varying sizes: *t*-Bu, Me, and H (Scheme 1). In comparison, we studied the aliphatic analogous ligand (Scheme 2), having no major steric demands.

$L^{1-3}Ti(OiPr)_2$ (Scheme 1) were synthesized according to known procedures by reacting H_2L^{1-3} with one equiv of $Ti(OiPr)_4$ to give the Ti(IV) complexes quantitatively.[23−25] ^{1}H NMR analysis has verified that the desired isomers were formed solely. Single crystals of $L^3Ti(OiPr)_2$ were obtained from diethylether at room temperature and the crystal structure (Figure 1) features a C_2 symmetrical octahedral complex with two cis-isopropoxide groups. $L^4Ti(OiPr)_2$ (Scheme 2) was synthesized by reacting H_2L^4 with 1 equiv of $Ti(OiPr)_4$. The resulting bis(isopropoxide) complex crystallized from toluene at −5 °C to give yellow single crystals. The crystal structure

Scheme 1



Scheme 2. Preparation and ORTEP Drawing of $L^4Ti(OiPr)_2$ at 50% Probability Ellipsoids



(Scheme 2) reveals a C_2 symmetrical isomer with similar structural features to those of $L^{1-3}Ti(OiPr)_2$ in terms of the coordination sphere and general geometry.

The cytotoxicity was studied on ovarian OVCAR-1 and colon HT-29 cell lines, employing the MTT assay for establishing cell viability. The IC_{50} values are summarized in Table 1.

The bulkiest complex $L^1Ti(OiPr)_2$ is inactive against both cell types. However, decreasing the steric bulk around the metal to Me or H groups leads to significant reactivity, which is not observed for any of the free ligands (Figure 2, S1). The IC_{50} values obtained for both $L^2Ti(OiPr)_2$ and $L^3Ti(OiPr)_2$ are significantly lower than those measured for Cp_2TiCl_2 and $(bzac)_2Ti(OiPr)_2$, and are even lower than those measured for Cisplatin[16] (Table 1). Interestingly, the aliphatic analogue $L^4Ti(OiPr)_2$ is inactive against both cell types, despite having similar symmetry, donor atoms, and general geometry as well as no significant steric demands. To further evaluate the role of the complex symmetry, we synthesized the C_s-symmetrical analogue $L^5Ti(OiPr)_2$ as previously described (Supporting Information, Scheme S1). This complex is inactive toward both cell types, despite having similar coordination number, donor atoms, and substitutions.

We also explored the influence of the protein transferrin on reactivity and cell insertion.[18−21] As expected, addition of the protein to the biological medium increases the reactivity of Cp_2TiCl_2 to some extent (Table 1). However, different behavior is observed for the complexes described herein, which resembles more the one observed with $(bzac)_2Ti(OiPr)_2$. No change in reactivity was observed for the unreactive $L^1Ti(OiPr)_2$, suggesting some resistance

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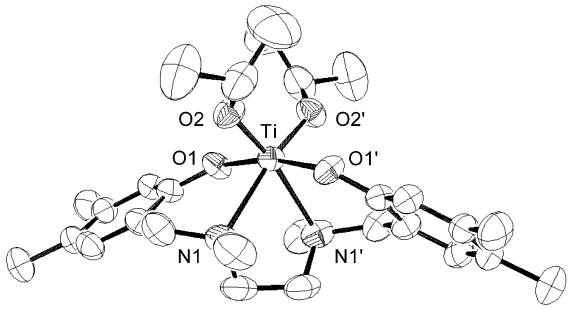

Figure 1. ORTEP drawing of $L^3Ti(OiPr)_2$ at 50% probability ellipsoids.

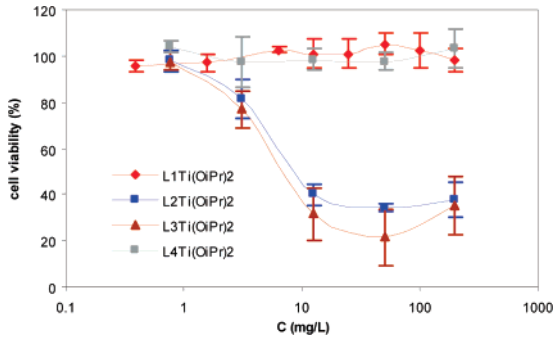

Figure 2. Dependence of HT-29 cell viability on added concentration

Table 1. IC_{50} (μM) Values[a] for $L^{1-4}Ti(OiPr)_2$ on HT-29 and OVCAR-1 Cells and Comparison to Known Compounds

reagent	HT-29	OVCAR-1	HT-29 + Tr[b]	OVCAR-1 + Tr[b]
Cp_2TiCl_2	710 ± 120	780 ± 90	460 ± 40	520 ± 30
$(bzac)_2Ti(OiPr)_2$	53 ± 1	53 ± 1	57 ± 1	65 ± 1
$L^1Ti(OiPr)_2$	unreactive	unreactive	unreactive	unreactive
$L^2Ti(OiPr)_2$	12 ± 1	12 ± 1	20 ± 3	40 ± 4
$L^3Ti(OiPr)_2$	12 ± 1	14 ± 1	16 ± 3	15 ± 3
$L^4Ti(OiPr)_2$	unreactive	unreactive	unreactive	unreactive
Cisplatin[c]	33 ± 3	17 ± 4		

[a] Obtained after 3 d incubation. [b] Tr: transferrin. [c] See reference 16.

to the protein promoted ligand striping (Table 1). Additionally, $L^{2-3}Ti(OiPr)_2$ did not reveal any improvement in reactivity in the presence of the protein, supporting involvement of an alternative active species and transport mechanism. No reactivity was observed for $L^4Ti(OiPr)_2$ and $Ti(OiPr)_4$[16] with added protein as well, presumably due to more rapid formation of unreactive inert aggregates.

Clearly, the chelating ligand in $L^{1-3}Ti(OiPr)_2$ and its steric demands play a significant role in the reactivity observed. This supports the notion that the active species either consists of a bound chelating ligand that allows transferrin-independent cell penetration or its formation strongly depends on the ligand steric demands. In addition, given that other labile complexes such as $Ti(OiPr)_4$ are inactive toward the cell types analyzed,[16] some inertness of the chelating ligand is obviously important.[16,17] Initial hydrolysis studies of $L^{2-3}Ti(OiPr)_2$ we performed by ^1H NMR revealed $t_{1/2}$ values for ligand hydrolysis of several hours, which is in the same order of magnitude as observed for Cp_2TiCl_2 and $(bzac)_2Ti(OEt)_2$.[5,13,26] To shed some light on the role of ligand hydrolysis, we studied the activity of our complexes after 2d interaction with the biological medium at 37 °C prior to administration to the cells. $L^{2-3}Ti(OiPr)_2$ lost their activity, suggesting that the active species possesses a rather rapid cell penetration mechanism, and once in the cell, the reactivity increases with longer incubation times (Figure 3, S2). In addition, the lack of activity observed for $L^4Ti(OiPr)_2$ may result

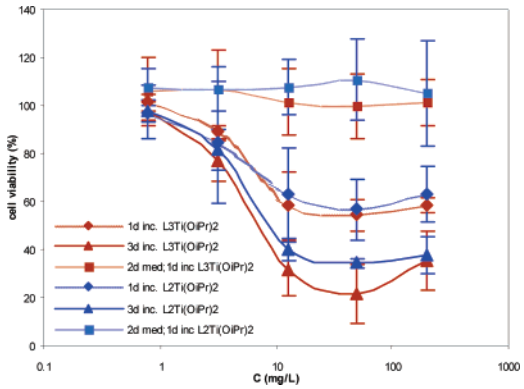

Figure 3. Dependence of HT-29 cell viability at different incubation and/or medium interaction times on added concentration

from faster hydrolysis rate or may suggest a role of the planar aromatic moieties in $L^{2-3}Ti(OiPr)_2$ in DNA interchelation as suggested for Budotitane,[10] although this activity clearly depends on the Ti center as the free ligands are unreactive. We are currently studying additional mechanistic aspects of the hydrolysis and reactivity of this new family of easily accessible well-defined highly cytotoxic Ti(IV) complexes.

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Supporting Information Available: Crystallographic data for $L^{3-4}Ti(OiPr)_2$ and experimental procedures including Scheme S1 of the preparation of the C_s-symmetrical analogue and Figures S1−S2 representing measurements on OVCAR-1 cells. This material is available free of charge via the Internet at http://pubs.acs.org.

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