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Cytotoxic Titanium(IV) Complexes: Renaissance

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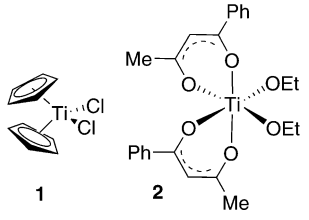
In this paper we overview our studies on amine–phenolato Ti^{IV} complexes as cytotoxic agents, in the context of the results reported thus far with titanocene dichloride, budotitane and derivatives. In particular, we emphasize the studies about the structure–activity relationship performed and important insights gained with the known compounds and our complexes, in regards to their hydrolytic behavior and cytotoxic activity, while pointing to potential mechanistic aspects. Titanocene dichloride and budotitane show cytotoxic activity towards cells that are resistant to cisplatin with reduced side effects. Their main drawback is their hydrolytic instability that has impeded mechanistic investigations and pharmaceutical use. Our new family of cytotoxic complexes was designed to include a single highly electron-donating chelating ligand to afford octahedral Ti^{IV} complexes of relatively high hydrolytic stability, with the aim to retain ligand binding throughout the biological activity for achieving controlled processes and allowing mechanistic evaluation. The effect of several parameters on hydrolysis and cytotoxicity were investigated, including those relating to the tetradentate ligand and those relating to the labile groups. Overall we observed high cytotoxic activity that is strongly dependent on the ligand, and which is strongly correlated to the complex hydrolytic behavior. Additional mechanistic studies provide insights regarding the time frame of activity and cell penetration. Some comparisons to titanocene dichloride, budotitane and analogues are highlighted.

Introduction

Despite significant progress in the treatment of cancer, new therapeutics are continuously sought for due to the intractability of certain types of tumors. Cisplatin,[1–4] discovered in 1969, was the first inorganic cancer chemotherapeutic agent, and remains a front-line treatment for testicular and ovarian cancers. Cisplatin operates by chelate binding to DNA following the hydrolysis of the two more labile ligands, the chloro groups. Following its discovery, there has been considerable exploration of the anti-cancer properties of other transition metal complexes.[5–17] Among the metals studied to date – which comprise much of the periodic table – complexes of titanium have shown particular promise due to high activity against tumors that are resistant to cisplatin combined with low toxicity.[13,18–28] In particular, the titanium complexes bis(cyclopentadienide)titanium dichloride (Cp_2TiCl_2, titanocene dichloride, **1**) and *cis*-diethoxybis(1-phenylbutane-1,3-dionato)titanium(IV) [$(bzac)_2Ti(OEt)_2$, budotitane, **2**] (Scheme 1) stand out as the first metal-based chemotherapeutics to reach Phase I clinical trials since the development of cisplatin. Although both **1** and **2** showed promise in these preliminary studies, **1** has not progressed beyond Phase II due to its low efficacy vs. toxicity ratio,[29,30] and **2** has not progressed past Phase I due to formulation problems.[31] These difficulties have spurred the development of titanium complexes that display higher potency and hydrolytic stability. The purpose of this overview is to highlight some particular developments in the field of cytotoxic Ti^{IV} complexes, while discussing recent work aimed toward uncovering the biological mechanism of action of these complexes, which remains mysterious. Consequently we will describe, in this context, some of the work that has been developed in our laboratory on a new family of complexes that does not include cyclopentadienide- or diketonato-based ligands with particular emphasis on the relation between cytotoxicity and hydrolysis.



Scheme 1.

The strong cytotoxic activity of **1** against implanted Ehrlich ascites tumors (EAT) in mice was reported in 1979 where cure rates greater than 80% were observed with little of the heavy-metal toxicity observed with cisplatin.[32] In subsequent studies the authors reported the key observation

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that while **1** and vanadocene dichloride displayed similar activities against EAT in-vivo, the cytotoxicity of **1** was ca. 100 times less than that for vanadocene dichloride in-vitro.[33] The reason for this drop in activity was hypothesized to be due to the hydrolytic instability of **1**, which rapidly forms insoluble aggregates in aqueous solution with eventual formation of TiO_2, a feature that is known to occur with many Ti^{IV} complexes due to their electron-poor and oxophilic nature. A detailed study of the hydrolytic behavior of **1** in neat water at various pH ranges revealed that at pH 5, following a rapid loss of the first chloride ion, complete hydrolysis of both chloro groups occurs with a $t_{1/2}$ of ca. 50 min, while hydrolysis of the Cp ligands is slower ($t_{1/2}$ ca. 54 h).[34] In contrast, vanadocene dichloride was stable toward hydrolysis at pH 7, which likely explains the difference between the in-vitro and in-vivo results for these two compounds. The in-vitro cytotoxicity could be restored by employing a galenic formulation,[13] and a subsequent patent reported a water-soluble formulation of **1**, MKT-4, which has allowed for its intravenous administration during clinical trials.[35]

The anticancer properties of Budotitane (**2**) were first reported in 1982.[36] It possesses activity towards animal tumors such as EAT and colon tumors.[19,21,31] When examining its hydrolysis, addition of **2** to neat water resulted in a suspension where the complex remained completely undecomposed.[21] However, if water was added to a solution of **2** in methanol or acetonitrile, rapid hydrolysis ensued. The half-life for hydrolysis of the ethoxy groups in water was calculated as $t_{1/2}$ = 20 s, while hydrolysis of the diketonato ligand in acetonitrile/water solutions of **2** is observed after 2.5 h. The use of smaller diketonato ligands was noted to lead to even more rapid ligand hydrolysis.

NMR studies indicate that **2** exists as the *cis* isomer.[21] MM3 studies suggest that the predominance of the *cis* compound is primarily due to steric factors. In an interesting demonstration of the additional structural complexities that can accompany the use of transition metals as drugs, the unsymmetrical ligands of **2** can potentially give rise to three sets of enantiomeric *cis* complexes. In $CDCl_3$ solution at 23 °C, **2** exists as a 4:1 mixture of two *cis* isomers. The relative cytotoxicity of these two compounds has not been investigated, although the crystal structure of one of them has been determined.[37]

Structure–Activity Relationships of Titanocene Dichloride (1) and Related Compounds

Considerable work has been performed in developing therapeutic analogues of **1** by varying the central metal (M), the labile ligands (Cl) and the bis-cyclopentadienyl moiety. The study of cytotoxic metallocenes other than titanium has been described in detail elsewhere.[5,12,20,38] Substitution of the chloro ligands has been explored in order to produce analogues with greater water solubility, but in general has not been shown to have a strong impact on cytotoxic activity. For example, titanocenes of the general formula Cp_2TiX_2 (X = Cl, Br, NCS, maleate, O_2CCl_3) all achieved cure rates of 100% in mice with fluid EAT, consistent with the hypothesis that the cytotoxic Ti^{IV} species is generated from displacement of the labile halide ligands in **1**.[39,40] In contrast, small changes to the Cp ligand can strongly affect the hydrolytic stability and water solubility properties of the metallocenes and thereby impact the cytotoxic activity. In addition, it has been proposed that substitution can also affect the ability of the ligand to intercalate with DNA, although further work needs to be done to establish this mechanism.

Substitution of one of the hydrogen atoms of the Cp ligand by R [R = Et (**3**), R = $Si(CH_3)_3$ (**4**)] (Scheme 2) was reported to reduce cure rates of mice with fluid EAT to 60–80%, while substitution of both Cp ligands by R reduced cure rates dramatically to 10–30%.[13] Pentamethyl-substituted titanocenes were reported to be completely devoid of cytotoxic activity. The extent to which this lowered activity represents the reduced water solubility of these compounds

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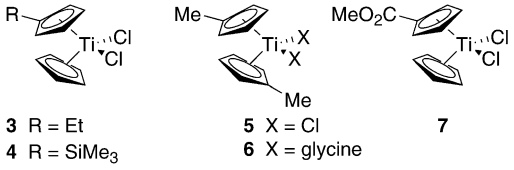
is uncertain. Studies on the hydrolytic behavior of the methylated metallocene compound $(C_5H_4Me)_2TiCl_2$ (**5**; C_5H_4Me = 1-methylcyclopentadienide) complexes (Scheme 2) revealed that the CpMe ligands were less readily hydrolyzed than **1** at physiological pH. Furthermore, when the chloride ligands were replaced with glycine in this system **6** (Scheme 2), water-soluble complexes with high stability at physiological pH were obtained. The complexes were observed to form stable complexes with nucleotides at pH 7, although further biological studies were not reported.[41]

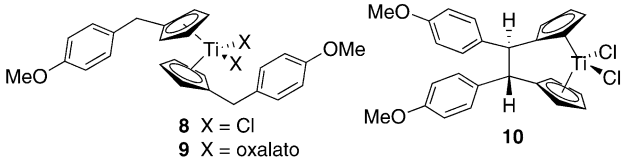


3 R = Et
4 R = $SiMe_3$
5 X = Cl
6 X = glycine
7

Scheme 2.

Substitution of the Cp ring by electron-poor substituents (R = CO_2Me **7**) (Scheme 2) was reported to lead to increased cytotoxicity in a human small-cell lung-cancer cell line but revealed little cytotoxicity towards a number of other cells studied.[42] The latter may be explained by reduced hydrolytic stability due to decreased ligand-to-metal electron donation.

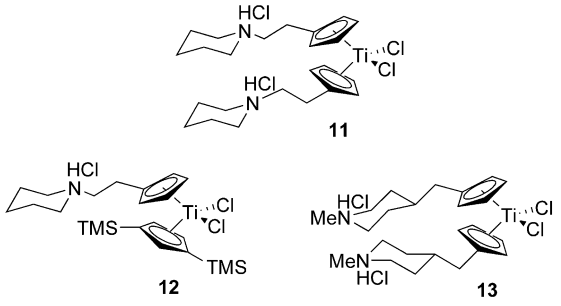
A large number of titanocene analogues containing aromatic groups appended to the Cp ligand have also been synthesized.[28] Although generalizations regarding structure–activity relationships are not yet clear, these complexes have shown good in-vitro activity against the LLC-PK cell line. When tested against a panel of 36 human tumor-cell lines, the most promising candidate, titanocene Y (**8**) (Scheme 3), was found to have good activity against renal cell cancer. Interestingly, the oxalato complex **9** (Scheme 3), possessing a substantially less labile chelating ligand in replacement of the chloro groups, was reported to have a 13-fold increase in activity relative to **8** during in-vitro studies against the LLC-PK cell line,[43] yet in-vivo studies showed almost identical activity to **8**.[44] Although the hydrolysis behavior of **8** and **9** was not studied in detail, it is possible that differences in the IC_{50} values of **8** and **9** obtained in-vitro may in fact reflect the greater hydrolytic stability of **9**. In general, lower activity was found in analogues where the two Cp units were bound by a two-carbon bridge, obtained as mixtures of stereoisomers (**10**, Scheme 3), although other *ansa*-metallocene complexes studied recently revealed promising activity.[45,46]



8 X = Cl
9 X = oxalato
10

Scheme 3.

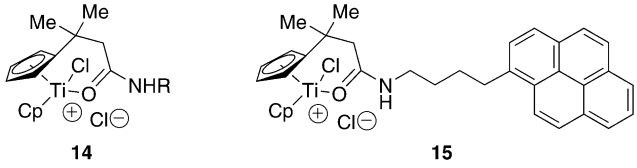
Several attempts have been made to improve the aqueous solubility of **1** through appendage of polar side chains to the Cp ligands. For example, titanocenes with alkylammonium hydrochloride moieties afforded improved aqueous solubility. Compound **11** (Scheme 4) showed good activity against both A2780 and the cisplatin-resistant A2780/CP cell lines.[47] Interestingly, an analogue of **11** with a bulkier Cp ligand (**12**, Scheme 4) possessed comparable cytotoxicity.[48] Concurrently, in a parallel study, a significant number of similar alkylammonium complexes were synthesized and their in-vitro cytotoxic properties assessed against A549, H209, and A2780 cell lines in direct comparison with the water soluble preparation of **1**, MKT-4.[49] In initial studies, an intriguing difference in cytotoxicity was found between bis(alkylammonium)cyclopentadienide complexes and those containing a single alkylammonium Cp ligand. Subsequent studies on related complexes, however, have found that this "bis(alkylammonium)" effect is not necessarily general.[50]



11
12
13

Scheme 4.

A number of cationic, water-soluble derivatives of **1** have been prepared through incorporation of an ester or a secondary amide on to the Cp ligand.[51,52] By employing an acylation reaction, a diverse array of amide and ester-containing titanocenes were synthesized. In-vitro studies of complexes with the general structure **14** (Scheme 5) showed good activity against BJAB (leukemia)cell lines.[53] Notably, complex **15** (Scheme 5) possesses a fluorescent aminopyrene unit which may be of potential application in studies on the biological activity mechanism.

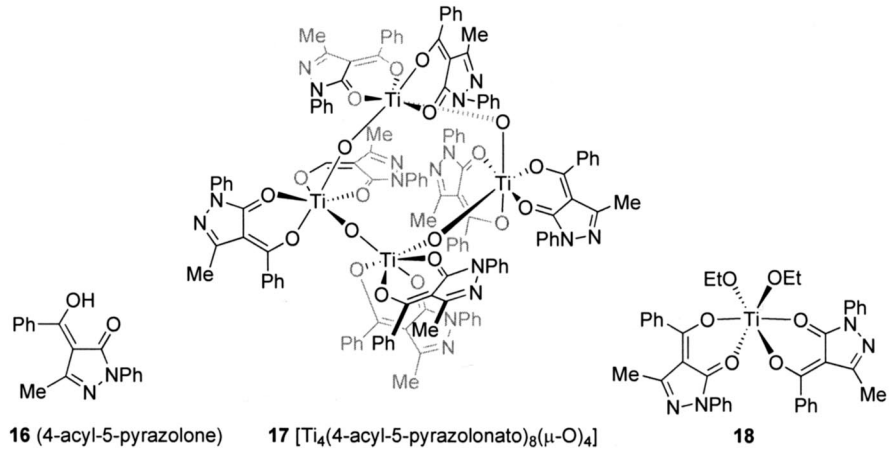


14
15

Scheme 5.

Structure–Activity Relationships of Budotitane (2) and Related Compounds

The structure–activity information gleaned from the synthesis of >200 derivatives of budotitane (**2**) with the general formula D_2MX_2 (D = 1,3 dicarbonyl; M = metal; X = labile ligand) and their activity against the sarcoma 180 ascitic tumor model were reported.[21] Among the metals investi-



Scheme 6.

gated, tumor-inhibiting effect decreased in the order Ti > Zr > Hf >Mo > Sn > Ge. No significant difference in cytotoxicity was found between compounds incorporating the labile groups X (X = F, Br, Cl, OEt), although the OEt group was eventually chosen due to its greater hydrolytic stability. Among the 1,3-dicarbonyl groups studied, acetylacetonato (acac) compounds possessed very low activity, while optimal antitumor activity was found for the benzoylacetonato (bzac) ligand. Incorporation of electron-withdrawing (Cl, NO_2) or donating (OMe) groups on the aromatic ring had a slightly deleterious effect upon the observed activity, as did the replacement of phenyl for *tert*-butyl, although considerable cytotoxicity was retained.

The cytotoxicity of structurally related mononuclear and polynuclear titanium(IV) 4-acyl-5-pyrazolonato (**16** Scheme 6) species that are structurally related to **2** were explored.[24,54] The cytotoxicity of a tetranuclear species **17** (Scheme 6) was encapsulated in a liposome due to insufficient water solubility and found to display cytotoxicity against TA-3 and HEP-2 cell lines in-vitro. Synthetic studies found that selectivity for mononuclear compounds could be obtained by rigorous avoidance of moisture during their synthesis. Notably, addition of trace water to a solution of the monomeric compound **18** (Scheme 6) in anhydrous [D_6]benzene formed a highly stable species that was hypothesized to be hydrolyzed oxo-bridged oligomers retaining the chelating ligand within 15 min, unlike the observations with budotitane.[21]

Comparison of the Biological Profiles of Titanocene Dichloride (1) and Budotitane (2)

In general, the biological activity profiles of **1** and **2** show many congruencies. When in-vivo studies are compared, **1** and **2** show good activity against slow-growing tumors of the colon, liver, and kidney, and relatively poor activity against faster growing cancers such as leukemia. Notably, emesis and other symptoms associated with the toxicity of cisplatin were absent during clinical trials of **1** and **2**. Both compounds hydrolyze easily, and require galenic preparations with water-soluble ligands in order to impart water solubility and stability. Additionally, both complexes contain a set of aromatic ligands that undergo hydrolysis under physiological conditions within several hours. The observation that **1** and **2** do not exert their maximal cytotoxic effects until >24 h after administration – in stark contrast to cisplatin – certainly leads to the question of how this relates to the half life of ligand hydrolysis.[55] In addition, the observation that **1** possess enhanced cytotoxicity following aging in certain organic co-solvents[56] raises questions regarding the particular lability required of active complexes.

While a number of detailed biological studies have been performed on **1**, analogous biological studies for **2** are largely absent. Although it is reasonable to hypothesize that **1** and **2** share a common mechanism of action, too little is known at this stage to draw definite conclusions. The following section will attempt to summarize what is currently known about the mode of action of these cytotoxic titanium complexes.

Mechanistic Aspects of Biological Activity

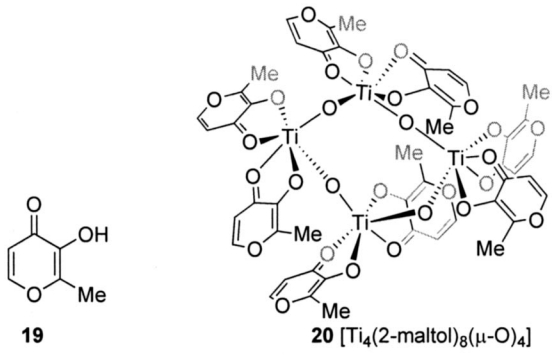
Early hypotheses that **1** share a mechanism of action with cisplatin[32] have been challenged by the efficacy of **1** against cisplatin-resistant cell lines, as well as by the disparate chemical behavior of "hard", hydrolytically unstable Cp_2TiCl_2 with "soft", water soluble $Cl_2Pt(NH_3)_2$ observed in aqueous solution under physiological conditions.[34] Nucleic acid metabolism appears to be disturbed by titanocene complexes. Analysis of the intracellular localization of titanium after treatment of **1** found titanium accumulation in cellular regions rich in nucleic acids.[57] Interestingly, while cisplatin immediately causes cessation of DNA-metabolic activity, **1** does not affect cell transit through the S phase but cells are irreversibly unable to perform mitosis.[58] It has been concluded that the three main phenomena observed in cells after treatment with **1** – specifically, the formation of giant cells, the activation of endogenous viruses, and the development of cellular necrosis – are all consistent with an interaction of **1** with intracellular DNA.[59] Adducts of **1**

(Cp_2Ti–DNA and CpTi–DNA) with calf thymus DNA has been detected spectroscopically,[60] as was also supported by model studies with particular nucleotides.[61] The specific interaction of the active titanium species with DNA remains unclear, although an interaction with the phosphate backbone has been proposed.[62] As far as budotitane (**2**) is concerned, the contribution of the aromatic diketonato ligand toward higher cytotoxicity has led to hypotheses involving DNA intercalation.[21,54] However, in light of the considerable activity retained when C_6H_5 is replaced by *tert*-butyl, the uncertain nature of the active species, and the paucity of biological studies on **2**, this proposal remains speculative.

Another work described the interaction of **1** with topoisomerase II, an enzyme catalyzing the opening and rejoining of the DNA phosphate backbone during the cell cycle. Compound **1** was reported to be a potent inhibitor of topoisomerase II, which may also lead to antiproliferative effects.[63]

Much recent work has focused on the mechanism by which anticancer titanium complexes are transported into cells. Importantly, the question of whether ligands remain attached to the titanium during transport into the cell or whether the ligands merely serve to protect the titanium center from hydrolysis in the intercellular medium remains an important unanswered question. Uncovering the mode of titanium transport would have important implications for the design of more effective and potent cytotoxic titanium complexes.

Noting the increased number of transferrin receptors on the surface of cancer cells and the implication of transferrin in transport of metal ions such as Ga^{III} and Ru^{III} to cancer cells,[64–67] it has been proposed that transferrin (Tr) is responsible for uptake of titanium from the intercellular medium.[67–69] Transferrin, the 80 kDa protein responsible for iron transport into cells, is present in the intercellular medium at a concentration of ca. 35 μM and contains two metal binding domains that are about 30% saturated with Fe^{III} in blood plasma. Additional studies have shown that Ti^{IV} binds more strongly to human serum transferrin than does Fe^{III}.[70,71] UV/Vis and ^1H NMR studies support the conclusion that **1** as [$Cp_2Ti(citrate)_2$] binds to transferrin with complete hydrolysis of the Cp ligands. After reaction with transferrin for 30 min at pH 7, ^1H NMR signals attributed to bound Cp in **1** disappeared, replaced by resonances characteristic of free cyclopentadiene. Interestingly, at low pH (<5.5) titanium was released from Ti_2Tr to bind to ATP. In this mechanism, **1**, or its analogues,[72,73] could possibly serve as a pro-drug, with the ligands protecting the titanium center from hydrolysis into titanium dioxide in the intercellular medium. Although **1** is completely hydrolyzed by transferrin in the presence of added citrate, the binding of transferrin to the potentially oxo-bridged hydrolysis products of **1** may occur with different rates and/or selectivities. It is also noteworthy, however, that no such observations were reported for **2** or its derivatives. In addition, the water-stable tetranuclear titanium complex **20** based on the diketonato analogue maltol ligand **19** (Scheme 7) does not donate Ti^{IV} to transferrin, yet is active against HT-29 cell lines in-vitro with values comparable to that of **1**, unlike other titanocene derivatives studied in comparison.[74] Thus, it would appear that further work is necessary in establishing transferrin as the exclusive agent of active transport for cytotoxic titanium complexes.



Scheme 7.

A recent paper proposes an alternate theory for titanium transport to cells through the action of serum albumin (HSA).[75] HSA, which is present in high concentrations in the intercellular medium (ca. 700 μM), possesses a variety of metal-binding sites and has been shown to bind intact metal complexes such as photosensitizers and contrast agents. NMR studies showed that, in contrast to albumin-free controls, solutions of **1** in the presence of HSA showed no visible signals for either free Cp_2TiCl_2 or cyclopentadiene after several days. Instead, two proton signals were observed, and proposed to represent Ti^{IV}-bound Cp protons. These results strongly suggest that HSA stabilizes the Cp_2Ti moiety at physiological pH, and that the extent to which albumin binding stabilizes complexes may be crucial to bioactivity. This was supported by studies on other metallocene analogues.[76] In contrast, binding studies with the dichloro analogue of **2** showed less than 1 equiv. of Ti bound to the protein and the presence of free ligand in the NMR spectrum. It was also reported that while transferrin outcompeted HSA for titanium citrate, exchange of Ti from a preformed HSA-Ti complex to transferrin was slow and incomplete. The important distinction of this model is that the ligands of the cytotoxic titanium complex remain bound, allowing for a biological mechanism implicating the ligands in a putative DNA-binding event within the cell. An important unanswered question in this model is whether there is a mechanism by which the HSA-Ti complex is transported into the cell, or whether HSA serves as a protecting group for the active titanium complex until it is transported by passive diffusion into the cell. The discovery that serum albumin can stabilize otherwise hydrolytically unstable titanium complexes has, therefore, added a new dimension to the study of these compounds.

It is thus clear that there is significantly more to be studied than is known about Ti^{IV} complexes as cytotoxic agents. Despite the many years since the initial findings regarding the involvement of Ti^{IV} centers in biological interactions of a therapeutic potential, very little is known about

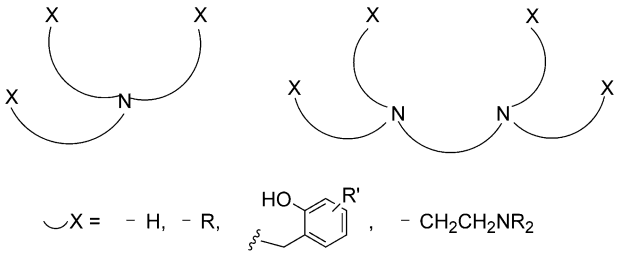
the actual fruitful biological interactions involved in the cytotoxicity mechanism, as well as about the exact requirements of a suitable complex and its attendant ligands. Thus far, only complexes of Cp, diketonato, or related ligands have been explored, although some recent derivatives demonstrate markedly improved features. Nevertheless, the enormous potential of the Ti^{IV} center due to its notably low toxicity calls for a reinvestigation of its diverse complexes. For a better exploration of the parameters affecting activity and its mechanistic aspects, the investigation of particularly designed complexes based on different strongly coordinating ligands is thus of great merit, which should primarily lead to complexes of enhanced hydrolytic stability and well-defined hydrolytic behavior. Our studies are aimed at this particular objective.

A New Family of Cytotoxic Ti^{IV} Complexes of Amine–Phenolato Ligands

Ligand and Complex Design

As the hydrolytic instability of Ti^{IV} complexes is a major obstacle that needs to be addressed, we design our ligands to enforce stable binding to Ti^{IV} and enhance hydrolytic stability. The parameters taken under consideration are the following: (a) we aim at neutral complexes for allowing cell penetration by passive diffusion and the most stable oxidation state we employ for the Ti center is (IV); (b) the preferred geometry for the Ti^{IV} center is octahedral; (c) we aim at complexes of the general structure $LTiX_2$, L being an inert ligand that will hopefully remain bound to the metal center throughout the biological interactions and allow for activity in a controlled manner, and X_2 being two more labile monoanionic ligands, preferably in a *cis* configuration, to allow potential chelate binding to the biological target of activity, as occurs for cisplatin and as may be proposed for titanocene dichloride (**1**) and budotitane (**2**) (Scheme 1), both featuring this characteristic.[21] Therefore, to fit these requirements, the inert ligands need to be tetradentate and dianionic.

We chose to work with diamine–bis(phenolato) ligands,[77–80] since the basic N-donors form strong coordinative bonds and the phenolato donors form strong Ti–O covalent bonds to the oxophilic Ti^{IV} center.[81] Such ligands have been reported for various different applications, such as in 1-olefin polymerization by Ti^{IV} catalysis.[82–86] Various constitutions of branched and sequential modes can be employed by varying the connectivity of the N atoms (Scheme 8). In addition, various different coordination numbers (such as 7 or 8) may be explored by incorporating additional dative N atoms, and different ratios of covalent/coordinative donors may also affect the number of labile ligands in the end complex. The latter enables the evaluation of the labile ligand effect and its necessity for obtaining the desired biological reactivity. Asymmetric ligands with different phenolato moieties may also be explored.



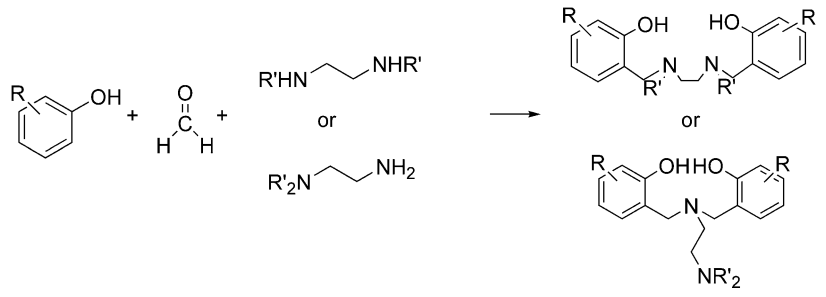
Scheme 8.

Synthesis and Characterization

General Procedures

The amine–phenolato ligands are synthesized by a highly convenient single-step procedure by a Mannich condensation from available starting materials including the amine, formaldehyde, and a substituted phenol (Scheme 9).[80,87] Variation of the phenol and amine components that are largely available allows for the preparation of a wide variety of ligands with different properties. Alternatively, the synthesis of amine–phenolato ligands may be achieved through reductive amination of substituted benzaldehydes with secondary amines.[88] This synthetic approach is undertaken where a primary amine needs to be employed, in order to avoid an additional reaction with formaldehyde to give an undesired methylene bridge.[89]

The Ti^{IV} complexes are generally synthesized by addition of the ligand to a solution of $Ti(OiPr)_4$ in an ethereal solvent at room temperature under an inert atmosphere.[77–80,90] This convenient synthesis affords the complexes as single isomers in quantitative yields, which is an advantage over the known families of compounds. Numer-

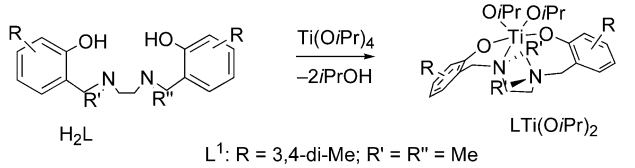


Scheme 9.

ous complexes with different steric and electronic effects may thus be prepared by employing various ligands. Diamine bis(phenolato) ligands with sequential donor connectivity (Scheme 9, top) generally give C_2-symmetrical bis(isopropoxo) Ti^{IV} complexes, where the phenolato groups are bound in a *trans* fashion, and the two isopropoxo ligands are in a *cis* configuration. Diamine bis(phenolato) ligands with branched donor connectivity (Scheme 9, bottom) give C_s-symmetrical complexes upon binding of a side donor arm to the metal center. The C_2-symmetrical complexes are the closer analogues to budotitane (**2**) in terms of not only the O-donor types, but also in the symmetry and general geometry;[21,81] however, in contrast to **2**, they are synthesized as single (racemic) isomers and should exhibit enhanced hydrolytic stability due to their chelate nature.

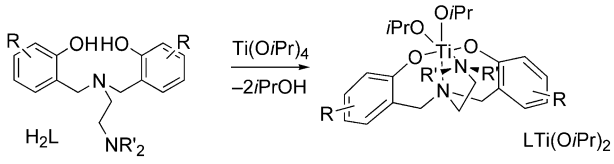
Amine–Phenolato Ligand Variations[90–92]

The bis(isopropoxo) complexes synthesized thus far are presented in Schemes 10 and 11. Steric variations on C_2-symmetrical complexes include different substituents on the aromatic rings and N-donors, which may be either proximal or distal to the metal center to assess their influence upon metal interactions or other peripheral parameters such as solubility and hydrophobicity. By incorporating electron-withdrawing or electron-donating groups on the aromatic rings, the electronic requirements of a strongly donating ligand may also be investigated. The complexes of this type that were analyzed by X-ray crystallography all reveal highly similar general coordination features, as discussed above (Figure 1). Particularly noteworthy is the similar Ti–N bond length observed for $L^{1,5}Ti(OiPr)_2$, (2.34 vs. 2.37 Å), despite the difference in the N-substituent steric demands. C_s-Symmetrical complexes were investigated, to evaluate the effect of the different symmetry and ligand arrangement (Scheme 11). The X-ray analysis of $L^{12}Ti(OiPr)_2$ (Figure 1) is especially indicative of the similar coordination features of the C_s-symmetrical complexes to the C_2-symmetrical analogues, namely, they all include similar coordination number, donor types, *trans* phenolato binding and *cis* isopropoxo groups. Nevertheless, one Ti–N bond length is somewhat longer for $L^{12}Ti(OiPr)_2$, of 2.45 Å, due to the flexibility of the side donor.



L^1: R = 3,4-di-Me; R' = R'' = Me
L^2: R = 2,4-di-Me; R' = R'' = Me
L^3: R = 2,4-di-*t*Bu; R' = R'' = Me
L^4: R = 4-*t*Bu; R' = R'' = Me
L^5: R = 3,4-di-Me; R' = R'' = Et
L^6: R = 3,4-di-Me; R' = Me, R'' = H
L^7: R = 4-Me; R' = R'' = Me
L^8: R = 4-Cl; R' = R'' = Me

Scheme 10.



L^9: R = 3,4-di-Me; R' = R'' = Me
L^{10}: R = 2,4-di-Me; R' = R'' = Me
L^{11}: R = 2,4-di-*t*Bu; R' = R'' = Me
L^{12}: R = 2,4-di-Me; R' = R'' = Et

Scheme 11.

Labile Ligand Variations[90,91]

Variations on the labile ligands were also made to evaluate their role in hydrolysis and biological activity. Thus,

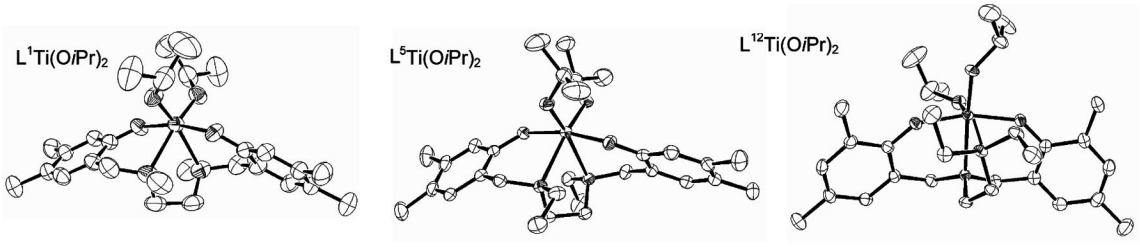


Figure 1. Representative X-ray structures of bis(isopropoxo) complexes of diamine bis(phenolato) ligands.

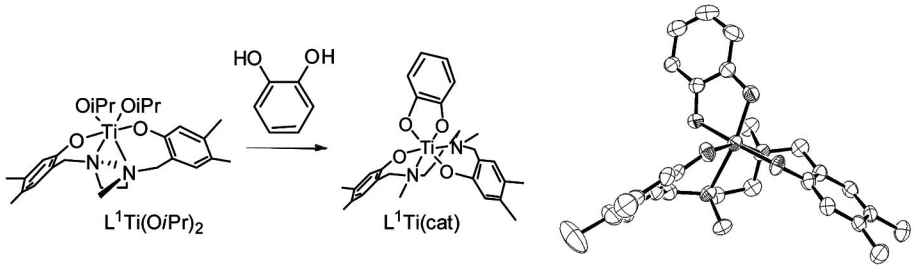


Figure 2. Variations of the labile ligands.

complexes of different ligand lability were prepared by reacting the bis(isopropoxo) compounds with a chelating dialkoxo agent. Reacting $L^1Ti(OiPr)_2$ with catechol gave the catecholato complex $L^1Ti(cat)$, which according to the crystallographic analysis, includes *cis* phenolato binding unlike the geometry of the isopropoxo precursor (Figure 2).[93] This complex should exhibit a substantially reduced lability.

Hydrolysis[90]

General Procedures

The hydrolytic behavior of the complexes was investigated in order to assess their stability under biologically relevant conditions. In analogy to observations for titanocene dichloride (**1**)[34] and budotitane (**2**)[19,21] (Scheme 1), two potentially separate processes need to be taken into account: (a) the hydrolysis of the "labile" ligands, namely the isopropoxo groups or their substitutes, which should occur first rather rapidly, and (b) the hydrolysis of the chelate amine–phenolato ligand which should occur more slowly. Three methods were employed: UV/Vis was helpful for monitoring the LMCT band of the Ti–OAr ligand at ca. 350 nm, providing indications for phenolato ligand hydrolysis, while stopped-flow instrumentation assisted in determining particularly rapid changes. ^{1}H NMR measurements allowed the identification of new species forming over relatively long periods, with the change in integration providing information regarding the reaction yield and its rate. In addition, new species that form were structurally characterized by X-ray crystallography.

Amine–Phenolato Ligand Variations

The particular amine–phenolato ligand employed has a major influence on the hydrolytic properties of Ti^{IV} complexes. In general, complexes of this type, and in particular, C_2-symmetrical bis(isopropoxo) complexes of certain steric and electronic features [$L^{1–4}Ti(OiPr)_2$] (Scheme 10), demonstrate surprisingly high hydrolytic stability, where release of the most labile isopropoxo groups is observed only within a time scale of several hours, and not several minutes as might have been expected based on observations with known compounds including titanocene dichloride (**1**) and budotitane (**2**).[19,21,34,94,95] Moreover, steric and electronic effects were found to have a major influence on both the rate of hydrolysis and also on the general hydrolytic behavior and nature of products. Where steric crowding near the metal site allows [$L^{1,2,4,6}Ti(OiPr)_2$], the isopropoxo groups indeed dissociate first to give a new polynuclear O-bridged species with bound phenolato ligand which is itself stable

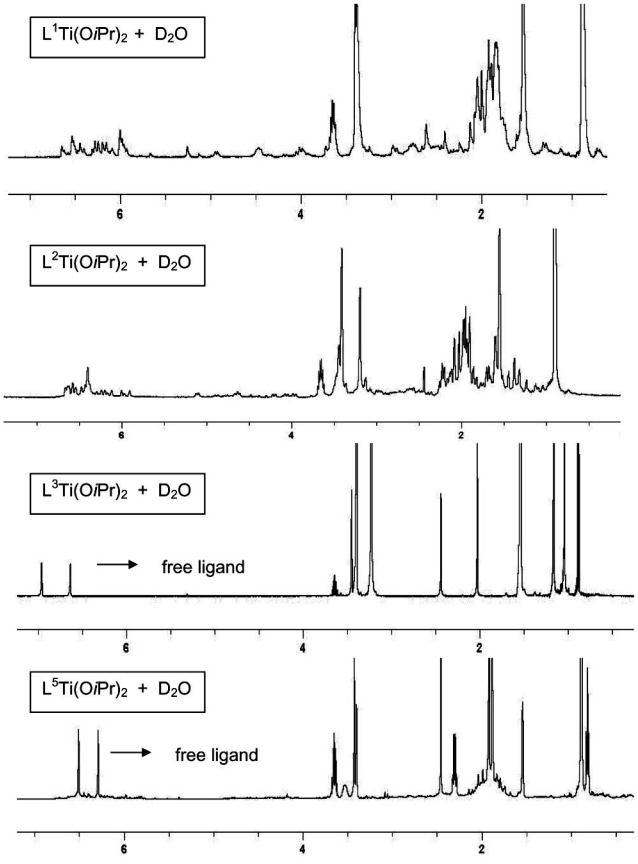


Figure 3. Representative ^{1}H NMR spectra of $L^{1–3,5}Ti(OiPr)_2$ in [D_8]THF/D_2O after completion of hydrolysis reaction.

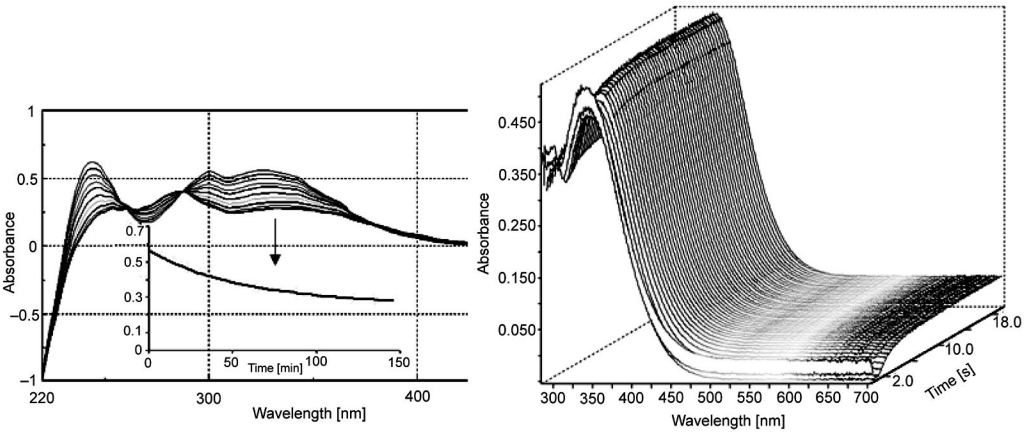


Figure 4. UV/Vis absorption over time for $L^1Ti(OiPr)_2$ upon addition of water.

for days as observed for complex **17** (Scheme 6)[24,54] and complex **20** (Scheme 7)[74] However, where steric crowding around the metal center is too large, despite strong ligand binding leading to relatively slow hydrolysis [L^3Ti-(O*i*Pr)$_2$],[96] or where the electronic features weaken ligand–metal binding leading to rapid hydrolysis [$L^{5,8}$Ti(O*i*Pr)$_2$], the cluster is not obtained and simple ligand release as bisphenol is observed instead (Figure 3). As an example, UV/Vis studies with L^1Ti(O*i*Pr)$_2$ are presented in Figure 4; formation of a new species with bound ligand following isopropoxo release is detected by the LMCT band shift as well as by the decay in absorbance to a value greater than zero, which is only observed within several hours, while no change is observed in the first seconds based on stopped-flow measurements. As these slow reactions enabled NMR characterization, the plots of integration of representative

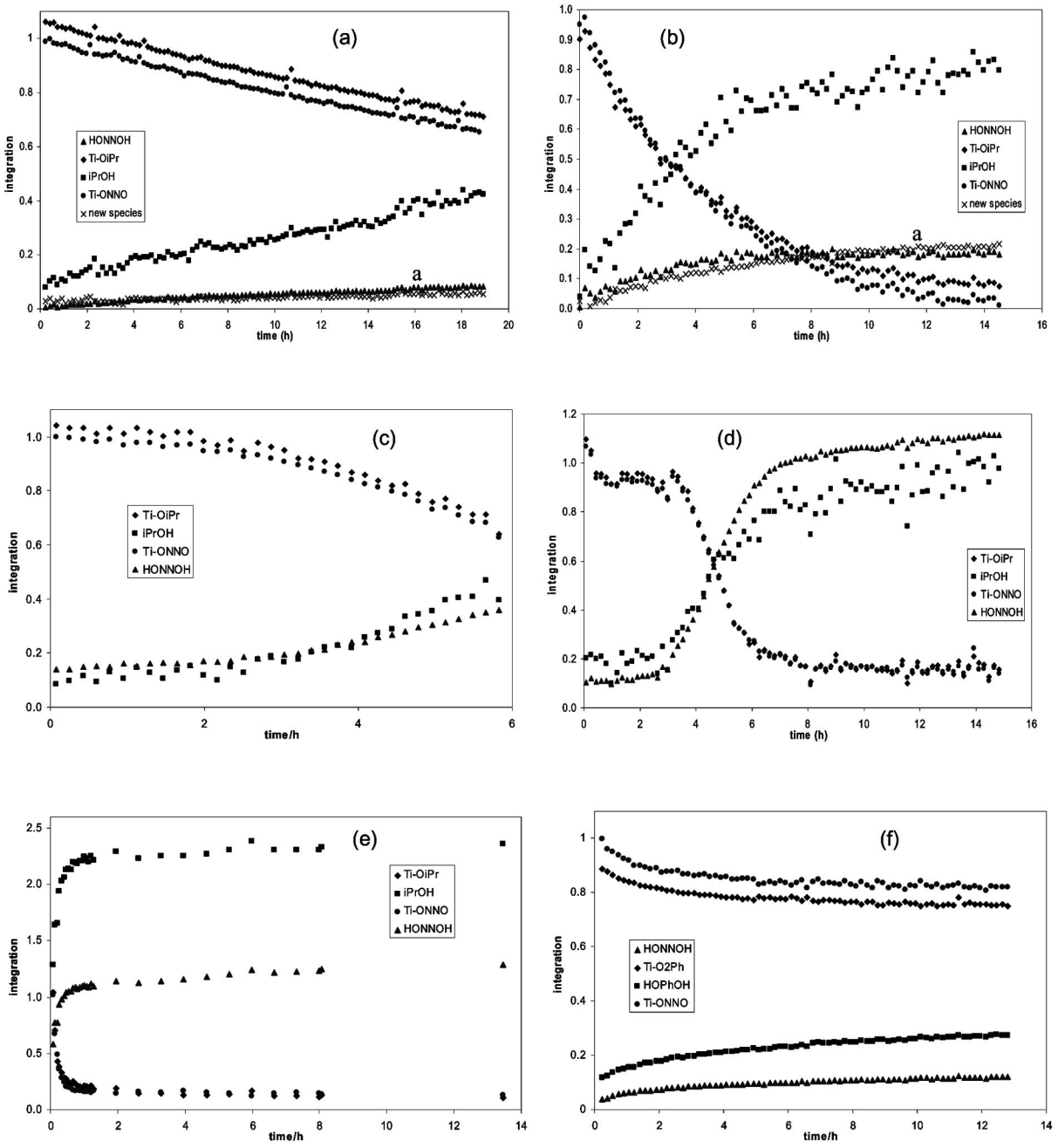


Figure 5. Plots of integration of selected signals including bound isopropoxo ('Ti-O*i*Pr'), free 2-propanol ("*i*PrOH"), bound phenolato ("Ti-ONNO"), free bis(phenol) ("HONNOH") and new phenolato-bound polynuclear species where applicable ("new species") in the ^{1}H NMR of L^2Ti(O*i*Pr)$_2$ (A,B), L^3Ti(O*i*Pr)$_2$ (C,D), L^5Ti(O*i*Pr)$_2$ (E) and L^1Ti(cat) (F) vs. time following addition of D_2O to the [D_8]THF solution at room temp. (A,C,E,F) and at 37 °C (B,D); (a) the integration values for this particular plot of the "new species" formed under the reaction conditions were not calibrated as the rest to represent the same number of protons due to the complexity of the spectrum and difficulty in assigning particular proton identity.

signals vs. time for representative bis(isopropoxo) complexes is given in Figure 5, with the summary of selected $t_{1/2}$ hydrolysis rates extrapolated from the plots given in Table 1. These plots clearly show that complete chelate ligand hydrolysis occurs simultaneously to isopropoxo release for complexes that do not yield a cluster [$L^{3,5}Ti(OiPr)_2$], which is surprising considering their expected different lability, while for the others [$L^2Ti(OiPr)_2$], a new polynuclear species with bound phenolato ligands forms within the time scale of isopropoxo release. We may thus conclude and say that steric effects near the metal center have the most crucial effect on hydrolysis as expected, because they affect the ability of two metal centers to approach each other to form a polynuclear cluster. In addition, complexes bearing electron-withdrawing groups, large groups on the N-donor, and C_s-symmetry due to a pending donor arm, demonstrate a more rapid hydrolysis rate, which is attributed to weaker ligand binding. In general, when comparing to **1** and **2**, some of our complexes demonstrate substantially higher hydrolytic stability, where modifications on the "inert" ligands may affect hydrolysis rate as observed with derivatives of **1** (**5**, Scheme 2).[41]

Table 1. $t_{1/2}$ Values for hydrolysis of O*i*Pr of selected complexes.

Complex	$t_{1/2}$
$L^1Ti(OiPr)_2$	5 h
$L^2Ti(OiPr)_2$	31 h
$L^3Ti(OiPr)_2$	10 h
$L^4Ti(OiPr)_2$	3 h
$L^5Ti(OiPr)_2$	5 min

Analysis of the structure of the polynuclear complex formed from $L^1Ti(OiPr)_2$ (Scheme 10) was enabled by X-ray crystallography (Figure 6). This complex was only obtained when >50 water equivalents were added and the reaction was stirred for 3 d, which again emphasizes the unique hydrolytic stability of these complexes relative to known compounds, as for example, traces of water are sufficient to form polynuclear complexes of acyl-pyrazolonato ligands within 15 minutes (**17**, Scheme 6).[24,54] Interestingly, binding of phenolato ligands in a *cis* fashion is observed for two out of the three metal centers in the trinuclear cluster,[93] as was also obtained with the catecholato complex

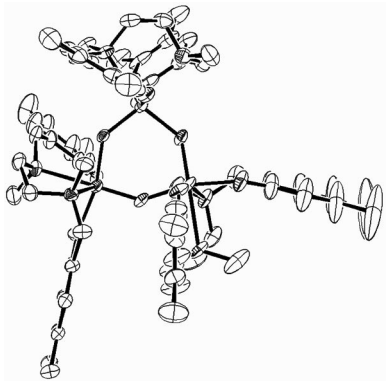


Figure 6. X-ray structure of the hydrolysis product of $L^1Ti(OiPr)_2$.

$L^1Ti(cat)$ (Figure 7), suggesting a similar ligand-replacement mechanism. The bulk that is induced by the phenolato ligands around the metal centers in this cluster explains the dependence of cluster formation on suitable steric properties. It is also noteworthy that preliminary recent results obtained for $L^6Ti(OiPr)_2$ suggest that a dinuclear complex is formed rather than a trinuclear complex, due to the small difference in steric effects between L^1 and L^6. The two Ti^{IV} centers in the dinuclear complex feature *cis* phenolato units as well.

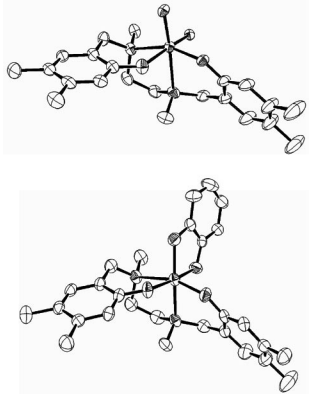


Figure 7. ORTEP drawing of a single Ti center of the hydrolysis product of $L^1Ti(OiPr)_2$ (top) and $L^1Ti(cat)$ (bottom).

Labile Ligand Variations

The catecholato complex $L^1Ti(cat)$ (Figure 2) exhibits an enhanced hydrolytic stability as expected. As the L^1 ligand LMCT band in the UV/Vis overlaps with the LMCT of the catecholato ligand, its analysis was performed by NMR only. High stability is observed for days, where only minor (ca.10–20%) release of free ligands is observed, and the formation of a new species could not be detected (Figure 5). Thus, it is concluded that the lability of the L and X_2 ligands in the $LTiX_2$ complex impact each other to a large extent. Where the L ligand is relatively labile [$L^5Ti(OiPr)_2$], the hydrolysis of both L and X_2 occurs rapidly, while where the L ligand is rather inert [$L^{1-3}Ti(OiPr)_2$], the hydrolysis of the X_2 is also delayed. This phenomenon that was not observed with titanocene dichloride (**1**) and budotitane (**2**), where generally different lability was observed for the two ligand types.[19,21,34] Nevertheless, where hydrolysis of the X_2 allows cluster formation, the L groups remain attached in the highly stable cluster formed. In addition, where the X_2 groups are also particularly inert [$L^1Ti(cat)$], the hydrolysis of the L groups is also delayed and a highly stable complex is observed.

Cytotoxicity[90–92]

General Procedures and Reference Measurements[97]

To date, the cytotoxicity of the phenolato complexes has been measured on two types of cancer-cell lines, colon HT-29 and ovarian OVCAR-1 cells. The MTT (methylthiazolyl-

diphenyl-tetrazolium bromide) assay was employed to evaluate cell viability based on cellular metabolic activity.[98] In general, similar activity patterns were observed for both cell types. In this overview we will demonstrate the activity towards ovarian cells only, while activity towards colon cells can be found elsewhere.[90–92] In some cases, measurements were conducted in the presence of a supplement whose effect we were interested to explore, such as apo-transferrin. In addition, variations of incubation times enabled us to estimate the general stability and the time scale of activity and cell penetration.

The cytotoxicity of particularly labile Ti^{IV} complexes such as $Ti(OiPr)_4$, $TiCl_4$, and $TiCl_4(THF)_2$ was investigated to confirm that a stabilizing ligand is required and not every source of Ti^{IV} ion can be active following rapid ligand hydrolysis. No cytotoxicity was observed for any of these complexes towards both cell types, including when apo-transferrin was added to the biological medium.[97] This suggests that the O-bridged aggregates that form upon exposure to water are inactive, and their formation is too rapid to allow any interaction with transferrin that may deliver the stripped ion to its biological target. These results are also in agreement with the observation that the maltol tetranuclear complex (**20**, Scheme 7) does not deliver Ti^{IV} to transferrin.[74] It is thus obvious that a carefully designed inert ligand is required to stabilize an active species and enable cytotoxic activity, and support such role for the Cp and diketonato ligands in **1** and **2**.[56,74]

Amine–Phenolato Ligand Variations

Complexes of the diamine bis(phenolato) ligands of C_2-symmetry possess appreciable cytotoxic activity towards the colon and ovarian cells measured, which is higher than those of Cp_2TiCl_2, $(bzac)_2Ti(OiPr)_2$ and cisplatin (Table 2). Moreover, the activity is strongly dependent on the ligand type, as different structural variations have a dramatic effect on the cell viability measured. We thus see that this ligand family enables stabilization of the active species, and appears to be involved in the interaction with the biological target.[74]

Steric effects on the N-donors and on the aromatic rings, both at positions near the metal site as well as distant from it, were explored. In general, steric bulk has a negative effect on cytotoxicity as reported for titanocene derivatives (**3**, **4**, Scheme 2),[13] with no particularly larger influence of the near-metal site bulk (Figure 8). The complexes with the best activity identified thus far are $L^1Ti(OiPr)_2$ and $L^2Ti(OiPr)_2$ (Scheme 10), which include two methyl groups on each aromatic ring yet at different positions, and two *N*-methyl substituents. Preliminary results indicate that a similarly high activity is also observed for $L^7Ti(OiPr)_2$ featuring a single methyl group distant from the metal center. These results point to a small influence of the second methyl group. In contrast, no activity whatsoever is observed for complex $L^3Ti(OiPr)_2$ featuring two *tert*-butyl groups on each ring including at positions *ortho* to the metal site, while mediocre activity is obtained for $L^4Ti(OiPr)_2$, which includes a single bulky group on each ring distant from the metal site. In addition, complex $L^5Ti(OiPr)_2$ possessing larger ethyl groups on the N-donors relative to the highly active $L^1Ti(OiPr)_2$ is also completely inactive, despite its highly similar X-ray structure (Figure 1). Interestingly, a strong correlation is observed between the hydrolytic behavior of the complexes and the cytotoxicity, although somewhat different steric-dependence is observed for the two. Only complexes of steric bulk enabling formation of a polynuclear O-bridged complex show any cytotoxic activity, which leads us to suspect that formation of this cluster or the parameters involving it such as isopropoxo hydrolysis, are essential for a fruitful biological interaction, as may be proposed for known complexes that lose their labile ligands quite rapidly.[19,21,34] In addition, the particular rate of hydrolysis is also significant yet to a different extent. Therefore, complexes that hydrolyze particularly rapidly such as $L^5Ti(OiPr)_2$, are completely inactive, yet complexes that reveal slower hydrolysis are not necessar-

Table 2. IC_{50} (μM) values for selected diamine bis(phenolato) bis(isopropoxo) complexes on HT-29 and OVCAR-1 cells and comparison to known compounds with or without a supplement of apo-transferrin (Tr).

	Reagent	Tr	HT-29	OVCAR-1
1	Cp_2TiCl_2	–	710 ± 120	780 ± 90
2	$(bzac)_2Ti(OiPr)_2$	–	53 ± 1	53 ± 1
3	cisplatin	–	33 ± 3	17 ± 4
4	$L^1Ti(OiPr)_2$	–	12 ± 1	14 ± 1
5	$L^2Ti(OiPr)_2$	–	12 ± 1	12 ± 1
6	$L^3Ti(OiPr)_2$	–	inactive	inactive
7	$L^4Ti(OiPr)_2$	–	_[a]	_[a]
8	$L^5Ti(OiPr)_2$	–	inactive	inactive
9	$L^1Ti(cat)$	–	20 ± 2	40 ± 4
10	Cp_2TiCl_2	+	460 ± 40	520 ± 30
11	$(bzac)_2Ti(OiPr)_2$	+	56.9 ± 0.6	65.0 ± 0.6
12	$L^1Ti(OiPr)_2$	+	16 ± 3	15 ± 3
13	$L^2Ti(OiPr)_2$	+	20 ± 3	39 ± 4
14	$L^3Ti(OiPr)_2$	+	inactive	inactive

[a] Cell-growth inhibition does not exceed 50%.

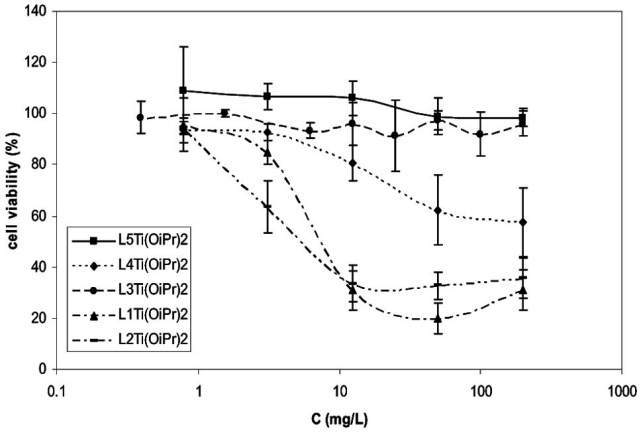


Figure 8. Dependence of OVCAR-1 cell viability after 3 d incubation period on administered concentration of $L^{1–5}Ti(OiPr)_2$ presented in a logarithmic scale.

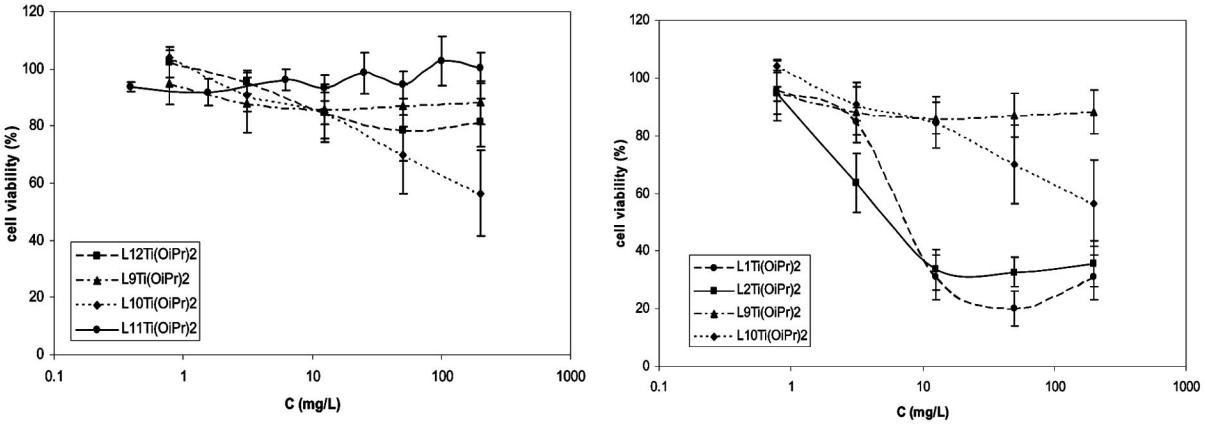


Figure 9. Dependence of OVCAR-1 cell viability after 3 d incubation period on administered concentration of $L^{9-12}Ti(OiPr)_2$ (left) and of $L^{9,10}Ti(OiPr)_2$ relative to $L^{1,2}Ti(OiPr)_2$ (right) presented in a logarithmic scale.

ily active such as $L^3Ti(OiPr)_2$, whose near-metal-site steric bulk does not allow cluster formation. Moreover, beyond a certain level of inertness, no major effect is observed for complexes of substantially different hydrolysis rate which directly results from the near-metal-site steric bulk, as demonstrated by the similar activity of $L^1Ti(OiPr)_2$ and $L^2Ti(OiPr)_2$. It is also noteworthy that other parameters that are not related to the hydrolysis rate and hydrolytic behavior are involved in the steric dependence cytotoxicity pattern, as $L^4Ti(OiPr)_2$, featuring similar near-metal-site steric bulk and similar hydrolysis to $L^1Ti(OiPr)_2$, possess a reduced cytotoxicity. This parameter may suggest the requirement of planarity to enable DNA intercalation as was proposed for **1** and **2** derivatives,[13,21] or may reflect the effect of other indirect factors, as the general hydrophobicity and solubility of the two complexes is different.

Elaborated studies on electronic effects are currently being performed, and preliminary results obtained thus far do not reveal a clear effect. At high doses, the effect of chloro substituents seems to be mostly negative, which is in agreement with their electron-withdrawing nature and more rapid hydrolysis, while a different pattern is observed at low doses. It is also noteworthy that no clear effect was observed for derivatives of titanocene dichloride (**7**, Scheme 2)[42] and of budotitane.[21] This phenomenon is currently under investigation.

The family of C_s-symmetrical complexes demonstrates significantly diminished activity relative to their C_2-symmetrical analogues, despite the high similarity in coordination features (Figure 9). This feature is attributed to the more rapid hydrolysis observed for this family of complexes. Steric bulk has a negative effect on cytotoxicity here as well, where $L^{11}Ti(OiPr)_2$ (Scheme 11), the analogue of $L^3Ti(OiPr)_2$ (Scheme 10), also possess no cytotoxic activity. Nevertheless, higher activity is observed for $L^{10}Ti(OiPr)_2$ relative to $L^9Ti(OiPr)_2$, perhaps due to its larger near-metal site bulk which directly affects hydrolysis, and thus for these compounds that are of lesser hydrolytic stability to begin with, this effect becomes clearly pronounced.

Labile Ligand Variations

The relatively inert catecholato complex $L^1Ti(cat)$ (Figure 2) is of reduced cytotoxicity relative to its bis(isopropoxo) counterpart $L^1Ti(OiPr)_2$ (Figure 10). This result is different than that obtained for titanocene analogues, where substituting the chloro groups in titanocene Y (**8**) with a more inert oxalato ligand (**9**) (Scheme 3) enhanced the activity.[43] This implies that while the inertness of the tetradentate bis(phenolato) ligand is essential to stabilize an active Ti-based species and delay the hydrolysis of the X groups, the inertness of the additional X ligands should be more finely tuned. This observation is reminiscent of the one relating to titanocene dichloride (**1**), which exhibits enhanced cytotoxicity when is aged in certain organic co-solvents assumingly due to partial decomposition.[56] It is thus plausible that ultimate hydrolysis of the X groups in our complexes occurs throughout the biological mechanism that brings about fruitful interactions, as occurs for cisplatin and as may be proposed for titanocene dichloride (**1**) and budotitane (**2**).[19,21,34] This result is also in agreement with our observation that only complexes that yield a stable polynuclear complex upon water addition are cytotoxic, as this process involves the hydrolysis of the isopropoxo groups. Nevertheless, the correlation between polynuclear complex formation and cytotoxic activity raises the possibility of the cluster itself being the active species, consistently with the results observed with budotitane derivatives such as **17** (Scheme 6),[22,99] and **20** (Scheme 7).[74] Therefore, to further establish the importance of somewhat labile X groups and to inquire about the activity of the highly stable clusters forming upon hydrolysis, we measured the cytotoxicity of the trinuclear complex $L^1{}_3Ti_3O_3$ obtained from $L^1Ti(OiPr)_2$ (Figure 6). No cytotoxicity was observed for the cluster (Figure 10), unlike the observations with **17** and **20**. This is, however, in agreement with the lack of activity observed for labile complexes forming stable O-bridged aggregates rapidly,[97] and thus supports the requirement of somewhat labile groups for obtaining cyto-

toxic metal centers. This result also suggests that some intermediate in the cluster formation rather than the cluster itself plays a part in the biological activity. Nevertheless, it is also possible that the cluster's lack of cytotoxic activity represents its inability to enter cells rather than the lack of its involvement in valuable cellular interactions, which may still occur once the cluster formation takes place within the cellular environment following cell membrane transport of the original complex or an intermediate.

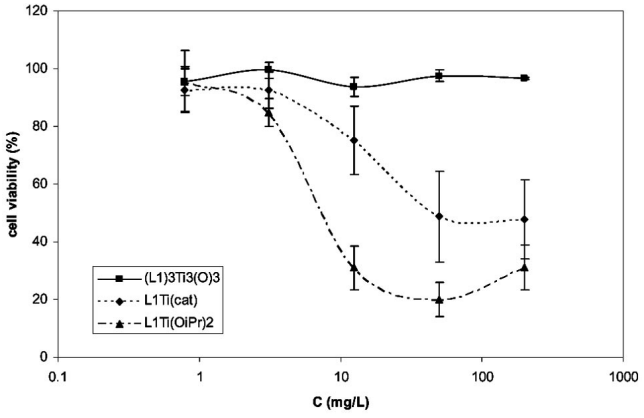


Figure 10. Dependence of OVCAR-1 cell viability after 3 d incubation period on administered concentration of $L^1Ti(OiPr)_2$, $L^1Ti(cat)$ and $L^1{}_3Ti_3O_3$ presented in a logarithmic scale.

Mechanistic Insights[90–92]

Transferrin Effect

To estimate whether the protein transferrin plays a part in the cytotoxic activity of our Ti^{IV} complexes as was proposed for titanocene dichloride (**1**) and other metal-based cytotoxic complexes,[64–67,69–72,74] we measured the cytotoxicity with a supplement of this apo-protein. Although enhanced activity was observed for **1** in the presence of the protein, no positive impact was observed for $(bzac)_2Ti(OiPr)_2$ and for all bis(phenolato) complexes studied to date, including active and inactive ones (Figure 11). Therefore, an alternative mode of delivery exists for our complexes, which seems to be in general more similar to that of **2** than to that of **1**, as may also have been anticipated due to the higher structural resemblance. These results support previous findings with budotitane analogues suggesting that when a strongly bound ligand exists to stabilize the Ti^{IV} center, transferrin is no longer required (**20**, Scheme 7),[74] and thus the ligand should remain bound for long enough time to allow the complex to reach its biological target. Therefore, the cytotoxic activity may be controlled and tuned by ligand structural modifications. It is also noteworthy that binding of an intact ligand-bound complex to a delivery protein such as albumin is still possible.[75]

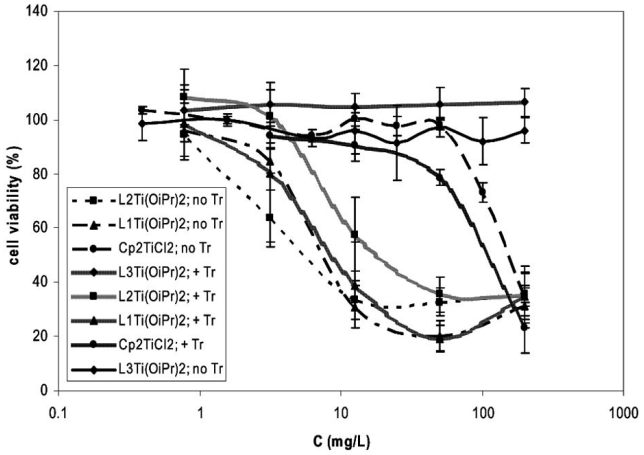


Figure 11. Dependence of OVCAR-cell viability after 3 d incubation period on administered concentration of $L^{1-3}Ti(OiPr)_2$ and Cp_2TiCl_2 presented in a logarithmic scale with or without added apo-transferrin (10 μg/mL).

Cytotoxicity Dependence on Incubation Times

To establish the time frame of activity of our complexes, we varied the incubation times of the complexes with the cells. We generally observed that the cytotoxicity increases with longer incubation times, as higher activity is observed after three days of incubation than is after single incubation day (Figure 12). This is contrary to the observations with cisplatin, for which meaningful activity is observed within the first 24 h of administration.[55] Interestingly, exposing the complexes to the biological medium for two days in the absence of cells, and consequently incorporating the cells for an additional incubation day, leads to complete loss of cytotoxic activity. It is thus clear that dissociation of the complexes under conditions of the medium leads to activity loss, and thus a form of the complex prior to dissociation is responsible for relatively rapid cell insertion and demonstration of activity, while once in the cell, the activity is retained for longer periods. These results are also in agreement with our observations that the stable cluster obtained from $L^1Ti(OiPr)_2$ (Figure 6) upon hydrolysis is inactive, and support the involvement of the original complex or an in-

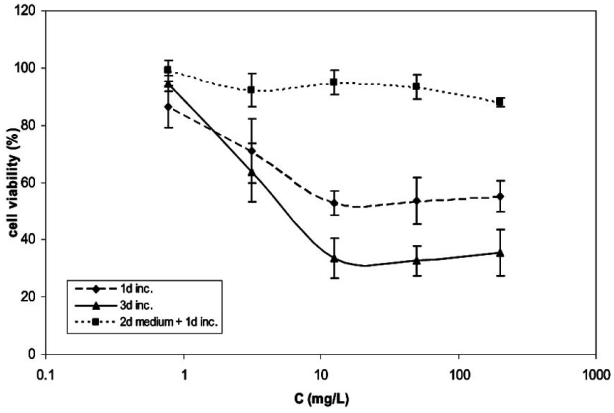


Figure 12. Dependence of OVCAR-cell viability on administered concentration of $L^2Ti(OiPr)_2$ presented in a logarithmic scale following different incubation periods.

termediate formed prior to the cluster in fruitful biological interactions. Moreover, we have recently observed that addition of the biological medium in a volume that corresponds to 100 equiv. of water to a THF solution of $L^1Ti(OiPr)_2$ also yields crystals of the cluster $L^1{}_3Ti_3O_3$ (Figure 6), which supports similar hydrolytic behavior in the biological medium to that occurring in neat water, pointing to the cluster formation as the potential dissociation process responsible for cytotoxic activity loss.

Summary and Perspective

In this paper we overviewed our recent studies with Ti^{IV} complexes of amine–phenolato complexes as cytotoxic agents. This family of compounds is highly promising due to two main features: (a) appreciable cytotoxic activity is observed towards colon and ovarian cells, which is higher than that of Cp_2TiCl_2, $(bzac)_2Ti(OiPr)_2$ and cisplatin, and is strongly dependent on the ligand structural features; and (b) particularly favorable features regarding their hydrolytic behavior are observed, with another strong dependence on ligand structure and strong correlation to the cytotoxic activity. A high hydrolytic stability is observed for the most active complexes, where the labile ligands hydrolyze with a time scale of several hours rather than several minutes or less,[19,21,34] and the phenolato ligands remain attached in the ultimate formation of a highly stable O-bridged polynuclear complex upon the labile ligand dissociation, which is itself inactive. As complexes that hydrolyze rapidly are inactive, we conclude that a relatively inert tetradentate ligand is required to decelerate the hydrolysis of the labile groups upon formation of the inactive cluster for long enough periods to enable cell penetration and demonstration of cytotoxic activity. Nevertheless, some lability of the OR groups is required for allowing the fruitful biological interaction to occur, as replacing the isopropoxo groups with an inert catecholato ligand diminished the cytotoxic activity, contrary to the results obtained with oxalato titanocene Y (**9**, Scheme 3),[43] although other parameters may be engaged in the activity decrease that relate to the general different structure of the catecholato complex.

Additional mechanistic studies have led us to propose the following: (a) a relatively rapid cell insertion of the active species occurs, which is unstable in the absence of cells but is active in the cellular environment; and (b) the complexes insert the cells without the aid of the protein transferrin. We may thus conclude that the tetradentate bis(phenolato) ligand indeed remains bound to the Ti^{IV} center and is resistant to transferrin stripping, although protein binding, such as transferrin or albumin, of the ligand-bound complex cannot be ruled out, and may actually be advantageous for enhancing selectivity to tumor tissue.[74,75]

The high versatility of this family of complexes enables us to perform various studies in regards to additional structural parameters of interest, including the complex coordination number, the number of anionic labile ligands, the complex symmetry, and more, which are currently underway. We are currently also performing additional in-depth mechanistic studies to evaluate the cell penetration mode of these complexes, the nature of the active species and the important biological interactions, while designing better-suited compounds based on the insights gained in regards to the steric and electronic demands of the ligands, and in particular, to their water solubility. In-vivo studies are also underway, and the relation between the hydrolytic stability and in-vitro activity will be established. As Ti^{IV} complexes generally demonstrate substantially lower toxicity and reduced side effects relative to platinum and other metal compounds, which is the main limitation in their therapeutic use, we believe that based on the promising results obtained thus far for our compounds as well as on other recent achievements in this field, proving that it is possible to create protective ligands for Ti^{IV}, exciting times involving research of Ti^{IV} agents are ahead, and the true potential of Ti^{IV} complexes as cytotoxic drugs is yet to be realized.

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[1] A. S. Abu-Surrah, M. Kettunen, *Curr. Med. Chem.* **2006**, *13*, 1337–1357.
[2] K. R. Barnes, S. J. Lippard, *Met. Ions Biol. Systems* **2004**, *42*, 143–177.
[3] V. Cepeda, M. A. Fuertes, J. Castilla, C. Alonso, C. Quevedo, J. M. Perez, *Anti-Cancer Agents Med. Chem.* **2007**, *7*, 3–18.
[4] P. Yang, M. Guo, *Coord. Chem. Rev.* **1999**, *185–186*, 189–211.
[5] M. J. Clarke, F. Zhu, D. R. Frasca, *Chem. Rev.* **1999**, *99*, 2511–2543.
[6] B. Desoize, *Anticancer Res.* **2004**, *24*, 1529–1544.
[7] P. C. A. Bruijnincx, P. J. Sadler, *Curr. Opin. Chem. Biol.* **2008**, *12*, 197–206.
[8] A. M. Evangelou, *Crit. Rev. Oncol. Hemat.* **2002**, *42*, 249–265.
[9] M. Galanski, V. B. Arion, M. A. Jakupec, B. K. Keppler, *Curr. Pharm. Des.* **2003**, *9*, 2078–2089.
[10] M. A. Jakupec, M. Galanski, V. B. Arion, C. G. Hartinger, B. K. Keppler, *Dalton Trans.* **2008**, 183–194.
[11] N. Katsaros, A. Anagnostopoulou, *Crit. Rev. Oncol. Hemat.* **2002**, *42*, 297–308.
[12] P. Köpf-Maier, *Eur. J. Clin. Pharmacol.* **1994**, *47*, 1–16.
[13] P. Köpf-Maier, H. Köpf, *Chem. Rev.* **1987**, *87*, 1137–1152.
[14] I. Kostova, *Curr. Med. Chem.* **2006**, *13*, 1085–1107.
[15] F. Kratz, M. T. Schutte, *Cancer J.* **1998**, *11*, 176–182.
[16] I. Ott, R. Gust, *Arch. Pharm. Chem. Life Sci.* **2007**, *340*, 117–126.
[17] G. Xu, Y. B. Cui, K. Cui, S. H. Gou, *Prog. Chem.* **2006**, *18*, 107–113.
[18] F. Caruso, M. Rossi, C. Pettinari, *Expert Opin. Ther. Patents* **2001**, *11*, 969–979.
[19] E. Mel'ndez, *Crit. Rev. Oncol. Hemat.* **2002**, *42*, 309–315.
[20] P. M. Abeysinghe, M. M. Harding, *Dalton Trans.* **2007**, 3474–3482.
[21] B. K. Keppler, C. Friesen, H. G. Moritz, H. Vongerichten, E. Vogel, *Struct. Bonding (Berlin)* **1991**, *78*, 97–127.
[22] F. Caruso, M. Rossi, *Mini-Rev. Med. Chem.* **2004**, *4*, 49–60.

[23] F. Caruso, M. Rossi, C. Opazo, C. Pettinari, *Bioinorg. Chem. Appl.* **2005**, *3*, 317–329.
[24] F. Caruso, M. Rossi, J. Tanski, R. Sartori, R. Sariego, S. Moya, S. Diez, E. Navarrete, A. Cingolani, F. Marchetti, C. Pettinari, *J. Med. Chem.* **2000**, *43*, 3665–3670.
[25] A. M. Christoforou, P. A. Marzilli, L. G. Marzilli, *Inorg. Chem.* **2006**, *45*, 6771–6781.
[26] G. Kelter, N. J. Sweeney, K. Strohfeldt, H.-H. Fiebig, M. Tacke, *Anti-Cancer Drugs* **2005**, *16*, 1091–1098.
[27] P. Köpf-Maier, H. Köpf, *Struct. Bonding (Berlin)* **1988**, *70*, 103–185.
[28] K. Strohfeldt, M. Tacke, *Chem. Soc. Rev.* **2008**, *37*, 1174–1187.
[29] N. Kruger, U. R. Kleeberg, K. Mross, L. Edler, G. Saβ, D. K. Hossfeld, *Onkologie* **2000**, *23*, 60–62.
[30] G. Lummen, H. Sperling, H. Luboldt, T. Otto, H. Rubben, *Cancer Chemother. Pharmacol.* **1998**, *42*, 415–417.
[31] T. Schilling, K. B. Keppler, M. E. Heim, G. Niebch, H. Dietzfelbinger, J. Rastetter, A. R. Hanauske, *Invest. New Drugs* **1995**, *13*, 327–332.
[32] H. Köpf, P. Köpf-Maier, *Angew. Chem. Int. Ed. Engl.* **1979**, *18*, 477–478.
[33] P. Köpf-Maier, W. Wagner, H. Köpf, *Cancer Chemother. Pharmacol.* **1981**, *5*, 237–241.
[34] J. H. Toney, T. J. Marks, *J. Am. Chem. Soc.* **1985**, *107*, 947–953.
[35] B. W. Müller, R. Müller, S. Lucks, W. Hohr, *US patent* **1994**, 5,296,237.
[36] J. J. Keller, B. K. Keppler, D. Schmahl, *Arzneim.-Forsch./Drug Res.* **1982**, *32*, 806–807.
[37] E. Dubler, R. Buschmann, H. W. Schmalle, *J. Inorg. Biochem.* **2003**, *95*, 97–104.
[38] M. M. Harding, G. Mokdsi, *Curr. Med. Chem.* **2000**, *7*, 1289–1303.
[39] P. Köpf-Maier, B. Hesse, R. Voigtlander, H. Köpf, *J. Cancer Res. Clinical Oncol.* **1980**, *97*, 31–39.
[40] M. C. Valadares, M. L. S. Queiroz, *Eur. J. Pharmacol.* **2002**, *439*, 35–42.
[41] G. Mokdsi, M. M. Harding, *J. Organomet. Chem.* **1998**, *565*, 29–35.
[42] J. R. Boyles, M. C. Baird, B. G. Campling, N. Jain, *J. Inorg. Biochem.* **2001**, *84*, 159–162.
[43] J. Claffey, M. Hogan, H. Müller-Bunz, C. Pampillon, M. Tacke, *ChemMedChem* **2008**, *3*, 729–731.
[44] I. Fichtner, C. Pampillon, N. J. Sweeney, K. Strohfeldt, M. Tacke, *Anti-Cancer Drugs* **2006**, *17*, 333–336.
[45] S. Gomez-Ruiz, G. N. Kaluderovic, D. Polo-Ceron, S. Prashar, M. Fajardo, Z. Zizak, Z. D. Juranic, T. J. Sabo, *Inorg. Chem. Commun.* **2007**, *10*, 748–752.
[46] S. Gomez-Ruiz, G. N. Kaluderovic, S. Prashar, D. Polo-Ceron, M. Fajardo, Z. Zizak, T. J. Sabo, Z. D. Juranic, *J. Inorg. Biochem.* **2008**, *102*, 1558–1570.
[47] O. R. Allen, L. Croll, A. L. Gott, R. J. Knox, P. C. McGowan, *Organometallics* **2004**, *23*, 288–292.
[48] O. R. Allen, A. L. Gott, J. A. Hartley, J. M. Hartley, R. J. Knox, P. C. McGowan, *Dalton Trans.* **2007**, 5082–5090.
[49] P. W. Causey, M. C. Baird, S. P. C. Cole, *Organometallics* **2004**, *23*, 4486–4494.
[50] G. D. Potter, M. C. Baird, S. P. C. Cole, *J. Organomet. Chem.* **2007**, *692*, 3508–3518.
[51] A. Gansauer, D. Franke, T. Lauterbach, M. Nieger, *J. Am. Chem. Soc.* **2005**, *127*, 11622–11623.
[52] A. Gansauer, I. Winkler, D. Worgull, D. Franke, T. Lauterbach, A. Okkel, M. Nieger, *Organometallics* **2008**, *27*, 5699–5707.
[53] A. Gansauer, I. Winkler, D. Worgull, T. Lauterbach, D. Franke, A. Selig, L. Wagner, A. Prokop, *Chem. Eur. J.* **2008**, *14*, 4160–4163.
[54] F. Caruso, C. Pettinari, F. Marchetti, P. Natanti, C. Phillips, J. Tanski, M. Rossi, *Inorg. Chem.* **2007**, *46*, 7553–7560.
[55] R. J. Knox, F. Friedlos, D. A. Lydall, J. J. Roberts, *Cancer Res.* **1986**, *46*, 1972–1979.
[56] M. Ravera, C. Cassino, E. Monti, M. Gariboldi, D. Osella, *J. Inorg. Biochem.* **2005**, *99*, 2264–2269.
[57] P. Köpf-Maier, D. Krahl, *Naturwissenschaften* **1981**, *68*, 273–274.
[58] P. Köpf-Maier, W. Wagner, E. Liss, *J. Cancer Res. Clinical Oncol.* **1981**, *102*, 21–30.
[59] P. Köpf-Maier, *J. Cancer Res. Clinical Oncol.* **1982**, *103*, 145–164.
[60] M. L. McLaughlin, J. M. Cronan, T. R. Schaller, R. D. Snelling, *J. Am. Chem. Soc.* **1990**, *112*, 8949–8952.
[61] Z. Zhang, P. Yang, M. Guo, H. Wang, *J. Inorg. Biochem.* **1996**, *63*, 183–190.
[62] M. Tacke, *Lett. Drug Des. Discov.* **2008**, *5*, *332*–335.
[63] G. Mokdsi, M. M. Harding, *J. Inorg. Biochem.* **2001**, *83*, 205–209.
[64] P. T. Gomme, K. B. McCann, *Drug Discov. Today* **2005**, *10*, 267–273.
[65] H. Li, Z. M. Qian, *Med. Res. Rev.* **2002**, *22*, 225–250.
[66] H. Sun, H. Li, P. J. Sadler, *Chem. Rev.* **1999**, *99*, 2817–2842.
[67] M. Guo, H. Sun, H. J. McArdle, L. Gambling, P. J. Sadler, *Biochemistry* **2000**, *39*, 10023–10033.
[68] M. Guo, P. J. Sadler, *J. Chem. Soc., Dalton Trans.* **2000**, 7–9.
[69] H. Sun, H. Li, R. A. Weir, P. J. Sadler, *Angew. Chem. Int. Ed.* **1998**, *37*, 1577–1579.
[70] A. D. Tinoco, A. M. Valentine, *J. Am. Chem. Soc.* **2005**, *127*, 11218–11219.
[71] A. D. Tinoco, C. D. Incarvito, A. M. Valentine, *J. Am. Chem. Soc.* **2007**, *129*, 3444–3454.
[72] L. M. Gau, R. Hernández, J. Matta, E. Meléndez, *J. Biol. Inorg. Chem.* **2007**, *12*, 959–967.
[73] Y. Perez, V. López, L. Rivera-Rivera, A. Cardona, E. Mel'ndez, *J. Biol. Inorg. Chem.* **2005**, *10*, 94–104.
[74] R. Hernández, J. Lamboy, L. M. Gau, J. Matta, F. R. Roman, E. Meléndez, *J. Biol. Inorg. Chem.* **2008**, *13*, 685–692.
[75] A. D. Tinoco, E. V. Eames, A. M. Valentine, *J. Am. Chem. Soc.* **2008**, *130*, 2262–2270.
[76] A. Vessières, M.-A. Plamont, C. Cabestaing, J. Claffey, S. Dieckmann, M. Hogan, H. Müller-Bunz, K. Strohfeldt, M. Tacke, *J. Organomet. Chem.* **2009**, DOI:10.1016/j.jorganchem.2008.11.071.
[77] J. Balsells, P. J. Carroll, P. J. Walsh, *Inorg. Chem.* **2001**, *40*, 5568–5574.
[78] A. J. Chmura, M. G. Davidson, M. D. Jones, M. D. Lunn, M. F. Mahon, A. F. Johnson, P. Khunkamchoo, S. L. Roberts, S. S. F. Wong, *Macromolecules* **2006**, *39*, 7250–7257.
[79] S. Gendler, S. Segal, I. Goldberg, Z. Goldschmidt, M. Kol, *Inorg. Chem.* **2006**, *45*, 4783–4790.
[80] E. Y. Tshuva, I. Goldberg, M. Kol, Z. Goldschmidt, *Inorg. Chem.* **2001**, *40*, 4263–4270.
[81] J. Lamboy, A. Pasquale, A. L. Rheingold, E. Meléndez, *Inorg. Chim. Acta* **2007**, *360*, 2115–2120.
[82] S. Gendler, A. L. Zelikoff, J. Kopilov, I. Goldberg, M. Kol, *J. Am. Chem. Soc.* **2008**, *130*, 2144–2145.
[83] S. Groysman, I. Goldberg, M. Kol, E. Genizi, Z. Goldschmidt, *Inorg. Chim. Acta* **2003**, 137–144.
[84] S. Groysman, E. Y. Tshuva, I. Goldberg, M. Kol, Z. Goldschmidt, M. Shuster, *Organometallics* **2004**, *23*, 5291–5299.
[85] S. Segal, I. Goldberg, M. Kol, *Organometallics* **2005**, *24*, 200–202.
[86] E. Y. Tshuva, I. Goldberg, M. Kol, Z. Goldschmidt, *Chem. Commun.* **2001**, 2120–2121.
[87] E. Y. Tshuva, N. Gendeziuk, M. Kol, *Tetrahedron Lett.* **2001**, *42*, 6405–6407.
[88] A. Yeori, S. Groysman, I. Goldberg, M. Kol, *Inorg. Chem.* **2005**, *44*, 4466–4468.
[89] C. M. Manna, M. Shavit, E. Y. Tshuva, *J. Organomet. Chem.* **2008**, *693*, 3947–3950.
[90] D. Peri, S. Meker, M. Shavit, E. Y. Tshuva, *Chem. Eur. J.* **2009**, *15*, 2403–2415.

[91] Full experimental details for unpublished work described herein will be reported elsewhere.
[92] M. Shavit, D. Peri, C. M. Manna, J. S. Alexander, E. Y. Tshuva, *J. Am. Chem. Soc.* **2007**, *129*, 12098–12099.
[93] S. Groysman, E. Sergeeva, I. Goldberg, M. Kol, *Eur. J. Inorg. Chem.* **2005**, 2480–2485.
[94] C. F. Campana, Y. Chen, V. W. Day, W. G. Klemperer, R. A. Sparks, *J. Chem. Soc., Dalton Trans.* **1996**, 691–702.
[95] T. Kemmitt, N. I. Al-Salim, G. J. Gainsford, *Inorg. Chem.* **2000**, *39*, 6067–6071.
[96] V. Ugrinova, G. A. Ellis, S. N. Brown, *Chem. Commun.* **2004**, 468–469.
[97] M. Shavit, D. Peri, A. Melman, E. Y. Tshuva, *J. Biol. Inorg. Chem.* **2007**, *12*, 825–830.
[98] M. C. Alley, D. A. Scudiero, A. Monks, M. L. Hursey, M. J. Czerwinski, D. L. Fine, B. J. Abbott, J. G. Mayo, R. H. Shoemaker, M. R. Boyd, *Cancer Res.* **1988**, *48*, 589–601.
[99] F. Caruso, L. Massa, A. Gindulyte, C. Pettinari, F. Marchetti, R. Pettinari, M. Ricciutelli, J. Costamagna, J. C. Canales, J. Tanski, M. Rossi, *Eur. J. Inorg. Chem.* **2003**, 3221–3232.

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