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C_1-Symmetrical Titanium(IV) Complexes of Salan Ligands with Differently Substituted Aromatic Rings: Enhanced Cytotoxic Activity

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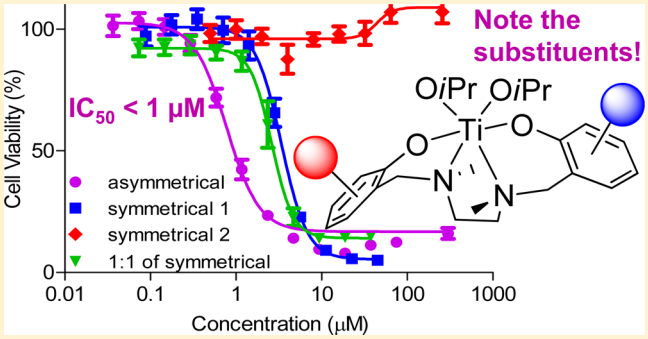
Supporting Information

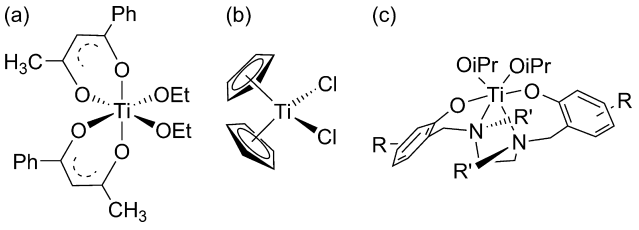
ABSTRACT: Diaminobis(phenolato) ("salan") titanium(IV) complexes of differently substituted aromatic rings were synthesized, and their hydrolytic stability and cytotoxicity were analyzed and compared to those of the C_2-symmertrical analogues and their equimolar mixtures. The hydrolytic stability of the asymmetrical complexes was in between those of the symmetrical analogues, implying an additive influence of the ligand structural parameters. Most mixed halogenated/nitrated complexes showed a marked improvement of cytotoxic activity relative to the symmetrical analogues and their mixtures, with IC_{50} values as low as <1 μM corresponding to activity exceeding that of cisplatin by up to 30-fold. In contrast, asymmetrical complexes with substitutions of similar properties revealed an added influence of both, with cytotoxicity in between those of the symmetrical analogues. With the presumption that the active species is generally a polynuclear hydrolysis product kept in mind, it is overall evident that particular ligand design and fine-tuning of the parameters of influence including hydrophilicity and hydrophobicity are essential for maximizing biological efficiency.



INTRODUCTION

Titanium(IV) complexes have been studied as antitumor compounds for several decades.[1] Budotitane ($(bzac)_2Ti(OEt_2)$) (Scheme 1a), titanocene dichloride (Cp_2TiCl_2) (Scheme 1b),

Scheme 1



and their derivatives demonstrated high anticancer activity toward a range of cancer cells with reduced toxicity relative to cisplatin; their main limitation was their relatively rapid hydrolysis in water environments.[2−13] We have introduced the "salan" antitumor Ti^{IV} complexes, which are based on tetradentate diaminobis(phenolato) ligands (Scheme 1c).[14−22] Complexes of this family showed enhanced hydrolytic stability relative to known compounds. These C_2-symmetrical complexes also demonstrated markedly higher anticancer activity than those of $(bzac)_2Ti(OiPr)_2$, Cp_2TiCl_2, and cisplatin toward various human and murine, drug-sensitive and -resistant, cancer cell lines.[14] Structural parameters of the ligands, including various aromatic substitutions, markedly affected the complex reactivity. Particularly, *ortho* halogenation increased the hydrolytic stability,[17] and large steric bulk negatively influenced the cytotoxic activity,[18,23] presumably by reducing the solubility of the complexes and potential for cellular penetration. Later studies have evinced that the active species operating in the cell is a rather bulky trinuclear hydrolysis product forming therein, following labile ligands hydrolysis.[24] Nevertheless, a correlation between stability and activity was mostly detected for active complexes.[15−18]

To expand the structure−activity relationship established for the salan-Ti^{IV} complexes, we investigated the anticancer features of C_1-symmetrical salan-Ti^{IV} complexes, bearing different substitutions on the two aromatic rings.[25,26] In our recent communication we reported on the highly cytotoxic asymmetrical salan Ti^{IV} complexes, which proved markedly more efficient than the two C_2-symmetrical analogues and their equimolar mixtures.[27] This paper reports the expanded study on this family of complexes to include various combinations of aromatic substitutions, their influence on the complex performance, and mechanistic implications.

RESULTS AND DISCUSSION

Synthesis and Characterization. Ligands with different aromatic substitutions ($H_2L^{X,Y}$, Scheme 2) were synthesized stepwise based on known procedures by reacting a substituted

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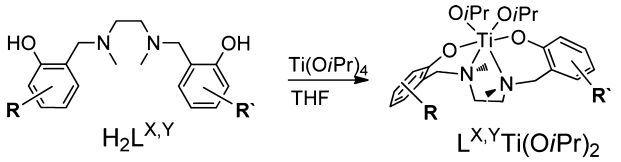
Scheme 2



$L^{1,2}$: R = *p*-NO_2; R` = *o*,*p*-Cl
$L^{1,3}$: R = *p*-NO_2; R` = *o*,*p*-Br
$L^{1,4}$: R = *p*-NO_2; R` = *o*,*p*-I
$L^{1,5}$: R = *p*-NO_2; R` = *o*-Br
$L^{1,6}$: R = *p*-NO_2; R` = H
$L^{1,7}$: R = *p*-NO_2; R` = *o*,*p*-F
$L^{2,3}$: R = *o*,*p*-Br; R` = *o*,*p*-Cl
$L^{1,1}$: R = R` = *p*-NO_2
$L^{2,2}$: R = R` = *o*,*p*-Cl
$L^{3,3}$: R = R` = *o*,*p*-Br
$L^{4,4}$: R = R` = *o*,*p*-I
$L^{5,5}$: R = R` = *o*-Br
$L^{6,6}$: R = R` = H
$L^{7,7}$: R = R` = *o*,*p*-F

salicylaldehyde with *N*,*N*′-dimethylethylenediamine, followed by reaction with the differently substituted benzylchloride.[25,26] The ^{1}H NMR features were consistent with the formation of C_S-symmetrical ligands, with several different signals for the aromatic region as follows: four in $H_2L^{2,3}$; five in $H_2L^{1,2}$, $H_2L^{1,3}$, $H_2L^{1,4}$, and $H_2L^{1,7}$; six in $H_2L^{1,5}$, and seven in $H_2L^{1,6}$. Synthesis of C_1-symmetrical Ti^{IV} complexes, $L^{X,Y}Ti(OiPr)_2$ (Scheme 2), was performed analogously to that of known compounds under an inert atmosphere by mixing the ligand with 1 equiv of $Ti(OiPr)_4$ in THF at room temperature and stirring for at least 2 h.[27] Removing the solvent under reduced pressure produced the product in quantitative yield. The complexes were analyzed by ^{1}H NMR, which verified that the desired C_1-symmetrical complexes had been obtained based on, for instance, the two different septet signals of the isopropoxo groups and AB doublets for the methylene protons. C_2-symmetrical analogues (Scheme 2) were generally synthesized as previously described.[15,17,28,29]

Single crystals of $L^{1,2}Ti(OiPr)_2$,[27] $L^{1,3}Ti(OiPr)_2$, and $L^{2,3}Ti(OiPr)_2$ that were obtained from dichloromethane were analyzed by X-ray crystallography. Selected bond lengths and angles for $L^{1,3}Ti(OiPr)_2$ and $L^{2,3}Ti(OiPr)_2$ are given in Table 1 and ORTEP drawings of the structures are presented in Figure 1. The X-ray structures exhibit similar general geometry to those of C_2-symmetrical salan Ti^{IV} complexes previously reported,[17,18,27] where the symmetry was reduced to C_1 due to the different aromatic rings. The two labile isopropoxo ligands bound to the octahedral metal center in *cis* configuration and the $L^{X,Y}$ ligand bound with a *trans* geometry of the phenolato units. The structures are all similar in bond lengths and angles, and there is no apparent clear influence of the different aromatic substitutions on the metal coordination center. All Ti−O bonds and Ti−N bonds are in the typical ranges of covalent and coordinative bonds, respectively, with typically shorter bonds to the monoanionic ligands.

Hydrolysis. The hydrolytic stability of the complexes was evaluated by ^{1}H NMR, adding 10% D_2O to a THF-d_8 solution of the complexes and monitoring changes in the signals integration, as previously described.[17,18] Although these conditions do not mimic the biological environment, this measurement provides a comparative tool to assess relative stability. The $t_{1/2}$ values for isopropoxo hydrolysis to give free isopropanol are summarized in Table 2. The C_1-symmetrical complexes exhibit marked hydrolytic stability with $t_{1/2} > 1$ h, for which *ortho* halogenation increased the stability as previously reported.[17] These values represent hydrolytic stability similar to that previously reported for related salan Ti^{IV} complexes and greater than that of Cp- and diketonato-based compounds.[11,12,17,18] The rates of isopropoxo hydrolysis obtained for the asymmetrical complexes were generally, as expected, in the same order of magnitude as, or in between, those of their C_2-symmetrical analogues. This implies that the influence of the aromatic substitutions on the hydrolytic stability is mostly additive.

Table 1. Selected Bond Lengths (Å) and Angles (°) for $L^{1,3}Ti(OiPr)_2$ and $L^{2,3}Ti(OiPr)_2$

$L^{1,3}Ti(OiPr)_2$		$L^{2,3}Ti(OiPr)_2$	
Lengths			
O(1)−Ti	1.919(2)	O(1)−Ti	1.918(5)
O(2)−Ti	1.939(2)	O(2)−Ti	1.894(5)
O(5)−Ti	1.772(2)	O(5)−Ti	1.790(5)
O(6)−Ti	1.798(2)	O(6)−Ti	1.780(5)
N(1)−Ti	2.355(2)	N(1)−Ti	2.312(6)
N(1)−Ti	2.328(2)	N(1)−Ti	2.311(6)
Angles			
O(1)−Ti−O(2)	161.49(9)	O(1)−Ti−O(2)	164.4 (2)
O(1)−Ti−O(5)	96.2(1)	O(1)−Ti−O(5)	96.4(2)
O(1)−Ti−O(6)	97.28(9)	O(1)−Ti−O(6)	91.6(2)
O(2)−Ti−O(5)	96.5(1)	O(2)−Ti−O(5)	93.2 (2)
O(2)−Ti−O(6)	92.08(9)	O(2)−Ti−O(6)	97.4(2)
O(5)−Ti−O(6)	106.1(1)	O(3)−Ti−O(4)	106.5(2)
N(1)−Ti−N(2)	76.16(8)	N(1)−Ti−N(2)	76.0(2)
O(1)−Ti−N(1)	80.39(8)	O(1)−Ti−N(1)	81.1(2)
O(2)−Ti−N(1)	83.95(9)	O(2)−Ti−N(1)	86.7(2)
O(5)−Ti−N(1)	165.4(1)	O(5)−Ti−N(1)	89.6(2)
O(6)−Ti−N(1)	88.5(1)	O(6)−Ti−N(1)	163.1(2)
O(1)−Ti−N(2)	86.19(8)	O(1)−Ti−N(2)	87.3(2)
O(2)−Ti−N(2)	80.52(8)	O(2)−Ti−N(2)	80.3(2)
O(5)−Ti−N(2)	89.5(1)	O(5)−Ti−N(2)	164.4(2)
O(6)−Ti−N(2)	163.5(1)	O(6)−Ti−N(2)	88.5(2)

Cytotoxicity. The cytotoxic activity of the complexes was studied on human colon HT-29 cancer cell line, employing the methylthiazolyldiphenyltetrazolium bromide(MTT) assay.[30] Relative IC_{50} and maximal cell growth inhibition values are summarized in Table 3. Cytotoxicity plots are given in Figures 2 and 3.

By examination of the activity of $L^{1,2}Ti(OiPr)_2$, $L^{1,3}Ti(OiPr)_2$, and $L^{1,4}Ti(OiPr)_2$ (Figure 2), the cytotoxic activity of the asymmetrical complexes is especially high, with IC_{50} values corresponding to activity exceeding that of cisplatin by up to 30-fold. Most importantly, the cytotoxicity is significantly higher than that of each of the C_2-symmetrical analogues. This may be regarded as a "synergistic" rather than additive influence of the aromatic substitutions on activity.[27] It is particularly interesting that for $L^{1,3}Ti(OiPr)_2$ and $L^{1,4}Ti(OiPr)_2$, a marked enhancement of activity relative to the nitrated symmetrical analogue $L^{1,1}Ti(OiPr)_2$ is observed, despite the complete inactivity of the symmetrical brominated and iodinated derivatives $L^{3,3}Ti(OiPr)_2$ and $L^{4,4}Ti(OiPr)_2$, respectively (Figure 2b,c). The inactivity of $L^{3,3}Ti(OiPr)_2$ and $L^{4,4}Ti(OiPr)_2$ is presumably a result of steric effects, reducing the solubility, membrane penetration ability, and thus general accessibility of the complexes to the biological target;[18,24] it thus appears that the increased activity of the asymmetrical complexes results from reducing the size below a particular threshold by employing a single halogenated ring. On the other hand,

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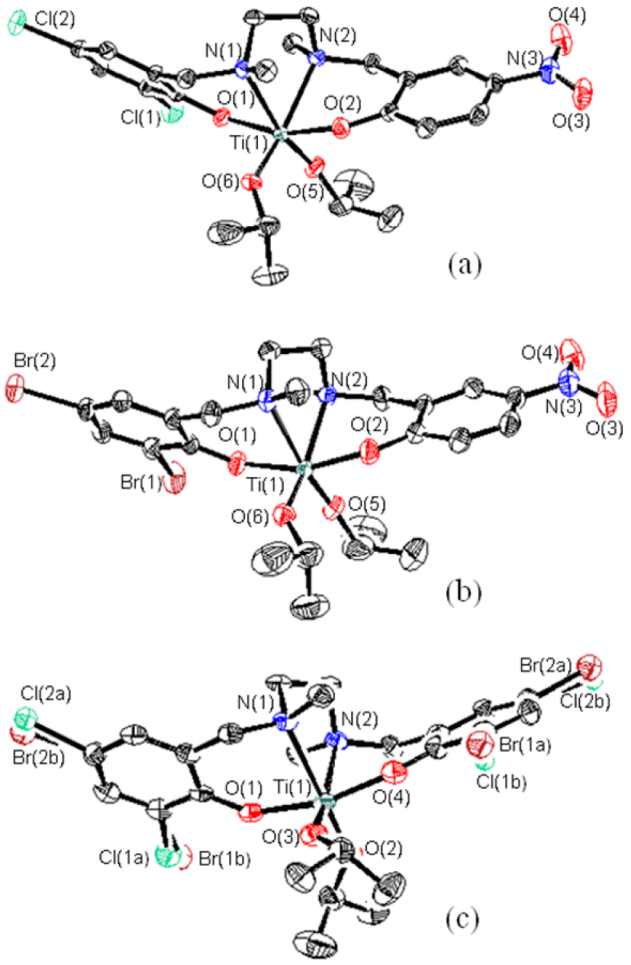

Figure 1. ORTEP drawings of $L^{1,2}Ti(OiPr)_2$ (a),[27] $L^{1,3}Ti(OiPr)_2$ (b), and $L^{2,3}Ti(OiPr)_2$ (c) in 50% probability ellipsoids; H-atoms were omitted for clarity.

Table 2. $T_{1/2}$ (h) Values for Isopropoxo Hydrolysis from C_1-Symmetrical Complexes in 1:9 D_2O/THF-d_8 Solution at Room Temperature Based on Pseudo-First-Order Fit and Comparison to Analogous C_2-Symmetrical Complexes

complex	R	R′	$t_{1/2}$ (h)
$L^{1,2}Ti(OiPr)_2$	*p*-NO_2	*o*,*p*-Cl	10
$L^{1,1}Ti(OiPr)_2$	*p*-NO_2	*p*-NO_2	1[17]
$L^{2,2}Ti(OiPr)_2$	*o*,*p*-Cl	*o*,*p*-Cl	100[17]
$L^{1,3}Ti(OiPr)_2$	*p*-NO_2	*o*,*p*-Br	20
$L^{3,3}Ti(OiPr)_2$	*o*,*p*-Br	*o*,*p*-Br	100
$L^{1,4}Ti(OiPr)_2$	*p*-NO_2	*o*,*p*-I	10
$L^{4,4}Ti(OiPr)_2$	*o*,*p*-I	*o*,*p*-I	150
$L^{1,5}Ti(OiPr)_2$	*p*-NO_2	*o*-Br	30
$L^{5,5}Ti(OiPr)_2$	*o*-Br	*o*-Br	150[15]
$L^{1,6}Ti(OiPr)_2$	*p*-NO_2	H	3
$L^{6,6}Ti(OiPr)_2$	H	H	2[15]
$L^{1,7}Ti(OiPr)_2$	*p*-NO_2	*o*,*p*-F	4
$L^{7,7}Ti(OiPr)_2$	*o*,*p*-F	*o*,*p*-F	10
$L^{2,3}Ti(OiPr)_2$	*o*,*p*-Cl	*o*,*p*-Br	70

greater hydrolytic stability relative to the nonhalogenated derivative $L^{1,1}Ti(OiPr)_2$ is achieved (Table 2),[17] while solubility is increased with the nitro substitution on the second ring. Of further interest is the reduced activity of the 1:1 mixtures of the C_2-symmetrical analogues to give an identical final Ti^{IV} concentration; in this equimolar mixture, participation of both compounds in activity is apparent, with little to no synergistic effect. The difference in activity may be attributed to different active species forming in the cell;[17,18] the asymmetrical complexes should yield well-identified trinuclear hydrolysis product/s,[31] whereas a larger mixture of clusters may be envisioned for the 1:1 combination, and particularly, the homoligated clusters should be less active as evident from the reduced activity of the symmetrical monomeric precursors. Notably, the $t_{1/2}$ for formation of the clusters from the two symmetrical precursors is markedly different (Table 2; see for example, $L^{1,1}Ti(OiPr)_2$ vs $L^{2,2}Ti(OiPr)_2$). Thus, it is logical that for the 1:1 mixture, the homoligated clusters would form with preference. This explains the additive behavior observed for the mixture, emphasizing the advantage of the asymmetrical derivatives that apparently yield new species with higher activity. Nevertheless, as the stability of symmetrical *ortho*-halogenated complexes is fairly high, their participation as active species prior to hydrolysis is certainly reasonable. These observations overall emphasize the importance of fine-tuning the structural parameters of the ligands for maximizing the cytotoxic activity.

Table 3. Relative IC_{50} (μM) and Maximal Cell Growth Inhibition (%)[a] Values for HT-29 Cells of C_1-Symmetrical Complexes in Comparison to Analogous C_2-Symmetrical Complexes, Their Combinations, and Cisplatin[b]

complex	R	R′	IC_{50} (μM)
$L^{1,2}Ti(OiPr)_2$	*p*-NO_2	*o*,*p*-Cl	0.7 ± 0.4 (95%)[27]
$L^{1,1}Ti(OiPr)_2$	*p*-NO_2	*p*-NO_2	3.3 ± 0.3 (95%)[27]
$L^{2,2}Ti(OiPr)_2$	*o*,*p*-Cl	*o*,*p*-Cl	2.8 ± 0.7 (87%)[27]
$L^{1,1}/L^{2,2}Ti(OiPr)_2$[c]			2.4 ± 0.9 (71%)[27]
$L^{1,3}Ti(OiPr)_2$	*p*-NO_2	*o*,*p*-Br	0.8 ± 0.3 (84%)[27]
$L^{3,3}Ti(OiPr)_2$	*o*,*p*-Br	*o*,*p*-Br	inactive[27]
$L^{1,1}/L^{3,3}Ti(OiPr)_2$[c]			3.1 ± 0.6 (85%)[27]
$L^{1,4}Ti(OiPr)_2$	p-NO_2	*o*,*p*-I	1.3 ± 0.3 (84%)
$L^{4,4}Ti(OiPr)_2$	*o*,*p*-I	*o*,*p*-I	inactive
$L^{1,1}T/L^{4,4}Ti(OiPr)_2$[c]			6 ± 3 (86%)
$L^{1,5}Ti(OiPr)_2$	*p*-NO_2	*o*-Br	1.8 ± 0.4 (95%)
$L^{5,5}Ti(OiPr)_2$	*o*-Br	*o*-Br	2.4 ± 0.9 (87%)
$L^{1,1}/L^{5,5}Ti(OiPr)_2$[c]			3.4 ± 0.7 (91%)
$L^{1,6}Ti(OiPr)_2$	*p*-NO_2	H	4.4 ± 0.7 (95%)
$L^{6,6}Ti(OiPr)_2$	H	H	16 ± 8 (92%)
$L^{1,1}/L^{6,6}Ti(OiPr)_2$[c]			6 ± 3 (92%)
$L^{1,7}Ti(OiPr)_2$	*p*-NO_2	*o*,*p*-F	3 ± 1 (93%)
$L^{7,7}Ti(OiPr)_2$	*o*,*p*-F	*o*,*p*-F	2.13 ± 0.07 (90%)
$L^{1,1}/L^{7,7}Ti(OiPr)_2$[c]			3.1 ± 0.8 (93%)
$L^{2,3}Ti(OiPr)_2$	*o*,*p*-Cl	*o*,*p*-Br	[d]
$L^{2,2}/L^{3,3}Ti(OiPr)_2$[c]			[d]
cisplatin[32]			20 ± 2

[a]Maximal cell growth inhibition refers to the % inhibition recorded at the highest compound concentration tested, relative to untreated control. [b]Error values are based on standard deviations. [c]1:1 mixture of the two; concentration determined per Ti^{IV} center. [d]Negligible activity (does not reach 50% inhibition).

$L^{1,5}Ti(OiPr)_2$ does not exhibit significantly higher cytotoxicity than the C_2-symmetrical analogue $L^{5,5}Ti(OiPr)_2$ (Figure 3a); this probably results from the decreased size of only a single halide, enabling the enhanced accessibility and cytotoxicity also of the symmetrical derivative $L^{5,5}Ti(OiPr)_2$.[15] $L^{1,6}Ti(OiPr)_2$ and $L^{1,7}Ti(OiPr)_2$ also show an additive effect of the two ring substitutions with IC_{50} values similar to those of

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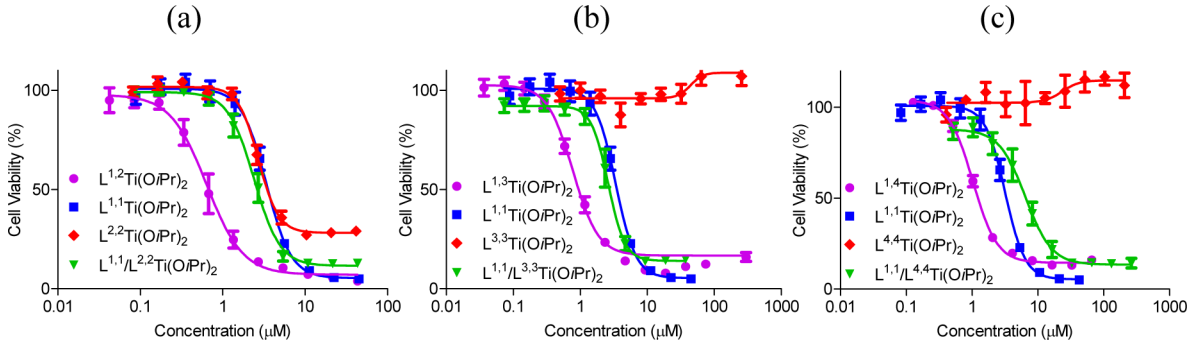

Figure 2. Cytotoxicity against human colon cancer HT-29 cells, of $L^{1,2}Ti(OiPr)_2$,[27] $L^{1,3}Ti(OiPr)_2$,[27] and $L^{1,4}Ti(OiPr)_2$. The analysis was conducted following a 3 day incubation period, based on the MTT assay. The C_1-symmetrical complexes are compared to the C_2-symmetrical analogues and their combination (1:1 mixture; concentration determined per Ti^{IV} center).

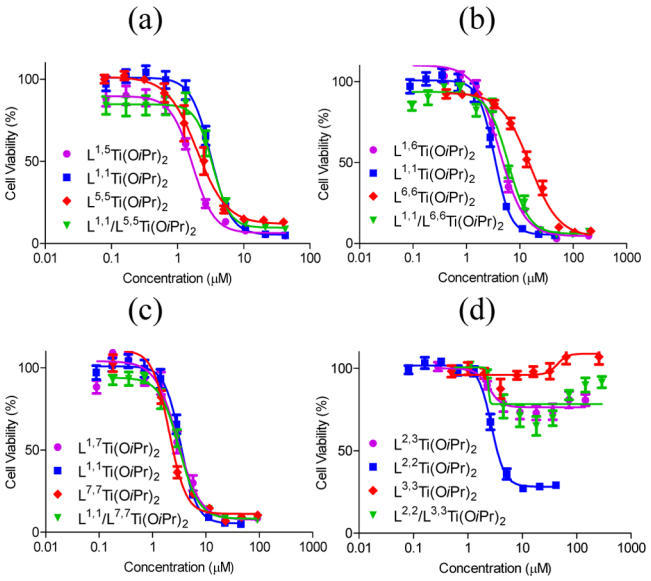

Figure 3. Cytotoxicity against human colon cancer HT-29 cells, of $L^{1,5}Ti(OiPr)_2$, $L^{1,6}Ti(OiPr)_2$, $L^{1,7}Ti(OiPr)_2$, and $L^{2,3}Ti(OiPr)_2$. The analysis was conducted following a 3 day incubation period, based on the MTT assay. The C_1-symmetrical complexes are compared to the C_2-symmetrical analogues and their combination (1:1 mixture; concentration determined per Ti^{IV} center).

the C_2-symmetrical analogues (Figure 3b,c, Table 3). This implies that accessibility is not a significant limitation in the symmetrical derivatives, which overall feature similar solubility, stability, and cytotoxicity properties (Tables 2−3). This is also supported by the similar cytotoxicity observed for the 1:1 mixtures of the symmetrical analogues. For $L^{2,3}Ti(OiPr)_2$ (Figure 3d, Table 3), another additive influence of the ligand structural parameters is observed despite the markedly different cytotoxicity of the symmetrical complexes, one being inactive; the activity is between those of the two symmetrical analogues, similarly to that of their 1:1 mixture. This may reflect the similar properties of the halide substituents, both decreasing the complex solubility and accessibility, and thus an intermediate cytotoxicity is recorded. Interestingly, the $t_{1/2}$ for cluster formation is similar for both symmetrical derivatives $L^{2,2}Ti(OiPr)_2$ and $L^{3,3}Ti(OiPr)_2$; thus, whatever the ratio of clusters that form (if any) from their 1:1 mixture is, an additive behavior results because there is apparently no clear advantage in the heteroligated cluster.

CONCLUSION

This paper presents an extended structure−activity study on C_1-symmetrical Ti^{IV} complexes of salan ligands with differently substituted aromatic rings, as anticancer compounds. The cytotoxicity and hydrolytic stability of the complexes was measured and compared to those of the symmetrically substituted analogues. Very high cytotoxic activity toward human colon cancer cells was recorded, with IC_{50} values as low as <1 μM, corresponding to activity greater than that of cisplatin by an order of magnitude. In fact, the highest activity recorded is for asymmetrical compounds, for which fine-tuning of the ligand structural parameters has proven crucial in determining the biological activity.

When comparing the properties of the asymmetrical complexes to those of the symmetrical analogues, it appears that the influence of ligand substitution on the hydrolytic stability is additive, whereas that on the cytotoxicity ranges from additive to synergistic. Structural parameters that increase hydrolytic stability, such as *ortho*-halogenation,[17] may often decrease cytotoxicity due to limited solubility of the symmetrical complex and potentially hampered cellular penetration due to increased size.[24] Thus, the replacement of one ring substitution with a hydrophilic moiety such as NO_2, although it somewhat decreases the stability, enables biological accessibility. Consequently, better activity is obtained for the asymmetrical derivative relative to both symmetrical analogues and their equimolar mixture. In contrast, for asymmetrical complexes with substituents of similar characters, an additive influence is normally obtained, even in cases where a markedly different activity was recorded for both symmetrical derivatives. It is thus obvious that any combination of substitutions should be examined separately and particular ligand design is essential for obtaining maximal biological efficiency.

Previous studies have revealed that the hydrolysis products of salan Ti^{IV} complexes are oxo-bridged trinuclear salan-bound clusters,[17,18] and suggested that such clusters participate as active species following their formation in the cell.[24] It is thus obvious that a single asymmetrical molecule is better than a mixture of C_2-symmetrical derivatives for obtaining combined properties, which minimizes the number of different active species forming in the biological environment, as supported by the cytotoxicity results described herein. The clusters are normally inactive when administered directly in a non-formulated manner,[33] presumably due to their reduced solubility and hampered cellular penetration;[24,34] this further emphasizes the importance of not only the direct biological

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activity of a potential drug in terms of its interaction with the cellular target, but also its biological accessibility,[35] as is also evident from the exceptionally high cytotoxicity of the C_1-symmetrical complexes. A recent report on the cellular biodistribution of related compounds and their accumulation in the nuclei further emphasizes the need for compact structures.[36] Future studies should therefore take into consideration, among other aspects, the balance between hydrophobicity (for cellular permeability) and hydrophilicity (for water solubility), in the fine design of future hydrolytically stable and particularly active antitumor titanium(IV) complexes. Nevertheless, it should be noted that other structural parameters of the different derivatives may also be of influence on the biological reactivity, the exact mechanism of which is yet to be determined.

EXPERIMENTAL SECTION

All symmetrically substituted ligands $H_2L^{1,1}$–$H_2L^{7,7}$ and the symmetrical bis(isopropoxo) Ti^{IV} complexes $L^{1,1}Ti(OiPr)_2$–$L^{3,3}Ti(OiPr)_2$ and $L^{5,5}Ti(OiPr)_2$–$L^{7,7}Ti(OiPr)_2$ were synthesized according to published procedures.[15,17,25,28,29,37] $H_2L^{1,2}$, $H_2L^{1,3}$, $L^{1,2}Ti(OiPr)_2$, and $L^{1,3}Ti(OiPr)_2$ were prepared as described in our previous communication.[27] The syntheses of differently substituted ligands and complexes were based on those previously reported for related compounds (see details below).[25,26] All complexes were obtained in quantitative yields with >95% purity. All reagents were purchased from Aldrich Chemical Co. Inc., Acros Organics, Fluka Riedel-deHaën, or TCI. All solvents were purchased dry from Aldrich Chemical Co. Inc., purified on alumina column on M. Braun Drying Solvent System SPS-800, or distilled from potassium or potassium/benzophenone under nitrogen. All experiments requiring dry atmosphere were performed in a M. Braun drybox under nitrogen atmosphere. NMR data were recorded using AMX-400 MHz or AMX-500 MHz Bruker spectrometer. X-ray diffraction data were obtained with Bruker Smart Apex diffractometer, running the SMART software package. After collection, the raw data frames were integrated by the SAINT software package. The structures were solved and refined using SHELXTL software package. Crystal data for $L^{1,3}Ti(OiPr)_2$ and $L^{2,3}Ti(OiPr)_2$ are summarized in Table 4. HRMS analyses were preformed in the microanalytical laboratory in our institute on 6520 Accurate-Mass Q-TOF LC/MS.

Comparative hydrolysis studies by ^{1}H NMR to monitor dissociation of isopropoxo groups were performed as previously described,[17,18] using 1–5 mM of the complex solution in THF-d_8 and adding D_2O to give a final solution of 1:9 D_2O/THF-d_8. The $t_{1/2}$ values were determined based on a pseudo first order fit for each compound. The results were verified by including *p*-dinitrobenzene as an internal standard. Structural information for hydrolysis products is provided in SI.

Cytotoxicity was measured on HT-29 colon cells obtained from ATCC Inc. using the methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay as previously described.[30] Cells (6×10^5) in medium (contains: 1% penicillin/streptomycin antibiotics; 1% L-glutamine; 10% fetal bovine serum (FBS), all purchased from Biological Industries Inc., and 88% medium RPMI-1640, purchased from Sigma Inc.) were seeded into 66 wells in a 96-well plate and allowed to attach for 24 h. The cells were consequently treated with the reagent tested at different concentrations. Solution of reagent was prepared by dissolving the reagent in THF to give final concentrations of up to 200 mg/L. From the resulting solution, 10 μL was added to each well already containing 200 μL of the aforementioned suspension of cells in the medium. After a standard of 72 h of incubation at 37 °C in 5% CO_2 atmosphere, MTT (0.1 mg in 20 μL) was added and the cells were incubated for additional 3 h. The MTT solution was then removed, and the cells were dissolved in 200 μL isopropanol. The absorbance at 550 nm was measured for 200 μL of the aforementioned solution by a Bio-Tek EL-800 microplate reader spectrophotometer. The control representing 100% viable cells was based on a reference measurement with THF alone at identical concentration. Each analysis was repeated at least 3 × 3 times, meaning three independent measurements were conducted for three wells each. Relative IC_{50} values were determined by a nonlinear regression of a variable slope (four parameters) model by Graph Pad Prism 5.0 program, where error values are based on standard deviations.

Control measurements of free ligands confirmed that the ligands are either inactive or of activities markedly lower than those of their complex, with generally 20-fold activity decrease (see SI). Previous studies have also confirmed that any activity of the salan titanium(IV) complexes does not result from those of free ligands.[18]

2-Bromo-6-((methyl(2-(methylamino)ethyl)amino)methyl)-phenol. To a stirred solution of 3-bromo-2-hydroxybenzaldehyde (0.88 gr, 4.4 mmol) in methanol (40 mL) was added a solution of *N*,*N*′-dimethylethylenediamine (0.47 mL, 4.4 mmol) in methanol (10 mL). The solution was stirred for 2 h and $NaBH_4$ (0.33gr, 8.8 mmol) was added in small portions. The solution was stirred overnight and the white precipitate was collected by vacuum filtration (0.75 gr, 62%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 (dd, J = 7.9, 1.1 Hz, 1H, Ar–H), 6.89 (dd, J = 6.9, 1.5 Hz, 1H, Ar–H), 6.53 (t, J = 7.8 Hz, 1H, Ar–H), 3.38 (s, 2H, CH_2), 2.86 (t, J = 6.4 Hz, 2H, CH_2), 2.62 (t, J = 6.4 Hz, 2H, CH_2), 2.44 (s, 3H, CH_3), 2.24 (s, 3H, CH_3). ^{13}C NMR (500 MHz, $CDCl_3$) δ 156.61, 132.11, 128.46, 124.47, 118.20, 111.32, 59.38, 54.85, 47.90, 41.93, 35.14. HRMS ($C_{11}H_{16}Br_2N_2O$ +H) Calc: 273.0597. Found: 273.0621.

2,4-Difluoro-6-((methyl(2-(methylamino)ethyl)amino)-methyl)phenol. This was synthesized similarly by reacting 3,5-fluoro-2-hydroxybenzaldehyde (1.15 gr, 7.3 mmol) with *N*,*N*′-dimethylethylenediamine (0.78 mL, 7.3 mmol) to give the product (0.79 gr, 47%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.70 (m, 1H, Ar–H), 6.50 (m, 1H, Ar–H), 7.85 (d, J = 2.0 Hz, 1H, Ar–H), 3.51 (s, 2H, CH_2), 2.88 (t, J = 6.4 Hz, 2H, CH_2), 2.63 (t, J = 6.4 Hz, 2H, CH_2), 2.45 (s, 3H, CH_3), 2.25 (s, 3H, CH_3). ^{13}C NMR (400 MHz, $CDCl_3$) δ 152.18 (dd, J = 68, 36 Hz), 125.93 (dd, J = 32, 16 Hz), 110.85 (dd, J = 88, 13 Hz), 110.16 (dd, J = 90, 12 Hz), 103.95 (dd, J = 194, 88 Hz), 103.41 (dd, J = 104, 91 Hz), 54.59, 54.22, 47.63, 42.33, 34.74. HRMS ($C_{11}H_{16}F_2N_2O$ +H) Calc: 231.1318. Found: 231.1312.

$H_2L^{1,4}$. 2,4-Diiodo-6-((methyl(2-(methylamino)ethyl)amino)-methyl)phenol[25] (1.00 gr, 2.2 mmol) in THF(50 mL) was added to a stirred solution of 2-chloromethyl-4-nitrophenol (0.42 gr, 2.2 mmol) in THF (30 mL). Triethylamine (0.4 mL) was then added and the solution was stirred for 2 h. The solution was filtrated and the volatiles were removed under vacuum. The solid was dissolved in dichloromethane and washed twice with water. The solvent was removed under vacuum and the red crude was purified on silica column and

Table 4. Crystal Data for $L^{1,3}Ti(OiPr)_2$ and $L^{2,3}Ti(OiPr)_2$

parameter/compound	$L^{1,3}Ti(OiPr)_2$	$L^{2,3}Ti(OiPr)_2$
formula	$C_{24}H_{33}Br_2N_3O_6Ti$	$C_{24}H_{32}Br_2Cl_2N_2O_4Ti$
M_W	667.25	691.14
space group	$P2_1/c$	$P2_1/c$
a (Å)	8.673(1)	8.395(2)
b (Å)	24.148(3)	12.710(3)
c (Å)	13.457(2)	23.572(6)
α (°)	90	90
β (°)	100.741(2)	95.155(4)
γ (°)	90	90
V (Å^3)	2769.1(6)	2803(1)
T (K)	173(1)	173(1)
Z	4	4
μ (Mo–Kα) (mm^{-1})	3.237	3.379
reflns measd	31787	29165
reflns unique	6607	6097
R_{int}	0.0298	0.1104
$R(f_0^2)$ [$I > 2\sigma(I)$]	0.0415	0.0919
R_w [$I > 2\sigma(I)$]	0.1145	0.2301

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washed with methanol to give the yellow product $H_2L^{1,4}$ (0.97 gr,73%). ^{1}H NMR (400 MHz, C_6D_6) δ 8.11 (d, J = 3.2 Hz, 1H, Ar−H), 8.02 (dd, J = 9.0, 3.2 Hz, 1H, Ar−H), 7.85 (d, J = 2.0 Hz, 1H, Ar−H), 7.39 (d, J = 2.0 Hz, 1H, Ar−H), 6.87 (d, J = 9.2 Hz, 1H, Ar−H), 3.73 (s, 2H, CH_2), 3.69 (s, 2H, CH_2), 2.70 (m, 4H, CH_2CH_2), 2.23 (s, 3H, CH_3), 2.22 (s, 3H, CH_3). ^{13}C NMR (500 MHz, C_6D_6) δ 164.78, 158.15, 146.26, 146.00, 141.05, 137.58, 126.19, 126.01, 125.18, 124.47, 121.96, 116.97, 60.90, 60.78, 53.57, 53.44, 41.16, 41.14. HRMS ($C_{18}H_{21}I_2N_3O_4Ti$ +H) Calc: 597.9686. Found: 597.9694.

$H_2L^{1,5}$. This was synthesized similarly by reacting 2-bromo-6-((methyl (2-(methylamino)ethyl)amino)methyl)phenol (0.71 gr, 2.02 mmol) with 2 chloromethyl-4-nitrophenol (0.38 gr, 2.02 mmol) to give yellow product (0.56 gr, 65%). ^{1}H NMR (500 MHz, THF-d_8) δ 8.05 (dd, J = 8.9, 2.7 Hz, 1H, Ar−H), 8.00 (d, J = 2.7 Hz, 1H, Ar−H), 7.36 (dd, J = 7.9, 2.9 Hz, 1H, Ar−H), 6.95 (d, J = 6.6 Hz, 1H, Ar−H), 6.83 (d, J = 8.9 Hz, 1H, Ar−H), 6.62 (t, J = 7.7 Hz, 1H, Ar−H), 3.84 (s, 2H, CH_2), 3.75 (s, 2H, CH_2), 2.74 (m, 4H, CH_2CH_2), 2.32 (s, 3H, CH_3), 2.29 (s, 3H, CH_3). ^{13}C NMR (500 MHz, THF-d_8) δ 156.08, 141.23, 133.10, 133.10, 128.87, 125.83, 125.80, 124.70, 123.87, 120.59, 117.05, 111.14, 61.96, 61.79, 54.77, 54.68, 41.81, 41.72. HRMS ($C_{18}H_{22}BrN_3O_4$ +H) Calc: 426.0844. Found: 426.0848.

$H_2L^{1,6}$. This was synthesized similarly by reacting 2-((methyl(2-(methylamino)ethyl)amino)methyl)phenol[38] (1.00 gr, 5.15 mmol) with 2 chloromethyl-4-nitrophenol (0.97 gr, 5.15 mmol) to give yellow product (0.72 gr, 40%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02 (d, J = 3.2 Hz, 1H, Ar−H), 7.98 (dd, J = 9.2, 2.8 Hz, 1H, Ar−H), 7.18 (dd, J = 7.2, 1.6 Hz, 1H, Ar−H), 7.12 (dt, J = 8.0, 1.6 Hz, 1H, Ar−H), 7.10 (m, 1H, Ar−H), 6.74 (m, 1H, Ar−H), 6.67 (d, J = 8.8 Hz, 1H, Ar−H), 3.75 (s, 2H, CH_2), 3.63 (s, 2H, CH_2), 2.78 (m, 2H, CH_2CH_2), 2.72 (m, 2H, CH_2CH_2), 2.27 (s, 3H, CH_3), 2.23 (s, 3H, CH_3). ^{13}C NMR (500 MHz, DMSO-d_6) δ 162.42, 154.82, 152.50, 136.07, 137.34, 126.32, 125.80, 124.15, 123.90, 117.49, 111.19, 110.45, 59.69, 59.48, 52.47, 52.40, 42.39, 42.36. HRMS ($C_{18}H_{23}N_3O_4$ +H) Calc: 346.1770. Found: 346.1761.

$H_2L^{1,7}$. This was synthesized similarly by reacting 2,4-difluoro-6-((methyl(2-(methylamino)ethyl)amino)methyl)phenol (0.54 gr, 2.35 mmol) with 2 chloromethyl-4-nitrophenol (0.44 gr, 2.35 mmol) to give yellow product (0.29 gr, 32%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (dd, J = 9.2, 2.8 Hz, 1H, Ar−H), 7.91 (d, J = 2.8 Hz, 1H, Ar−H), 6.85 (d, J = 8.8 Hz, 1H, Ar−H), 7.76 (m, 1H, Ar−H), 6.51 (m, 1H, Ar−H), 3.79 (s, 2H, CH_2), 3.71 (s, 2H, CH_2), 2.71 (m, 4H, CH_2CH_2), 2.34 (s, 3H, CH_3), 2.31 (s, 3H, CH_3). ^{13}C NMR (500 MHz, $CDCl_3$) δ 166.52, 153.76 (dd, J = 940, 46 Hz), 150.53 (dd, J = 966, 50 Hz), 141.07 (dd, J = 51, 12 Hz), 137.34, 126.57 (dd, J = 33, 15 Hz), 125.80, 125.15, 123.68, 116.29, 111.30 (dd, J = 90, 12 Hz), 103.15 (dd, J = 107, 91 Hz), 56.59, 56.48, 52.87, 52.80, 40.89, 40.76. HRMS ($C_{18}H_{21}F_2N_3O_4$ +H) Calc: 382.1573. Found: 382.1579.

$H_2L^{2,3}$. This was synthesized similarly by reacting 2,4-dichloro-6-((methyl(2-(methylamino)ethyl)amino)methyl)phenol[25] (2.28 gr, 9.89 mmol) with 2,4-dibromo-6- (bromomethyl)phenol (3.41 gr, 9.89 mmol) to give white product (3.91 gr, 75%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.60 (d, J = 2.0 Hz, 1H, Ar−H), 7.38 (d, J = 2.8 Hz, 1H, Ar−H), 7.31 (m, 1H, Ar−H), 7.18 (m, 1H, Ar−H), 3.76 (s, 2H, CH_2), 3.75 (s, 2H, CH_2), 2.69 (s, 4H, CH_2CH_2), 2.22 (s, 3H, CH_3), 2.18 (s, 3H, CH_3). ^{13}C NMR (500 MHz, $CDCl_3$) δ 155.08, 153.61, 135.59, 131.41, 130.14, 127.89, 125.05, 124.85, 124.64, 122.87, 112.27, 112.00, 62.28, 62.24, 55.20, 55.15, 42.97, 42.92. HRMS ($C_{18}H_{20}Br_2Cl_2N_3O_4$ +H) Calc: 526.9312. Found: 526.9319.

$L^{1,4}$Ti(O*i*Pr)$_2$. Ti(O*i*Pr)$_4$ (48 mg, 0.17 mmol) in dry THF was added to $H_2L^{1,4}$ (100 mg, 0.17 mmol) in dry THF under an inert atmosphere. The two solutions were allowed to stir at room temperature for 24 h to give the product following solvent removal (>95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (dd, J = 8.8, 3.2 Hz, 1H, Ar−H), 7.96 (m, 2H, Ar−H), 7.23 (d, J = 1.6 Hz, 1H, Ar−H), 6.67 (d, J = 9.2 Hz, 1H, Ar−H), 5.16 (sept, J = 6.0 Hz, 1H, $CHCH_3$), 4.91 (sept, J = 6.4 Hz, 1H, $CHCH_3$), 4.58 (m, 2H, CH_2), 3.29 (d, J = 14.0 Hz, 1H, CH_2), 3.12 (d, J = 14.0 Hz, 1H, CH_2), 2.91 (m, 2H, CH_2), 2.51 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 1.92 (m, 2H, CH_2), 1.28 (d, J = 6.4 Hz, 3H, $CHCH_3$), 1.24 (d, J = 6.0 Hz, 6H, $CHCH_3$), 1.24 (d, J = 6.0 Hz, 3H, $CHCH_3$), 1.23 (d, J = 5.6 Hz, 6H, $CHCH_3$). ^{13}C NMR (500 MHz, $CDCl_3$) δ 169.46, 161.75, 146.60, 139.44, 127.66, 126.92, 126.44, 125.33, 118.49, 90.77, 80.17, 79.85, 79.78, 79.08, 64.42, 64.39, 52.89, 52.73, 48.45, 47.72, 30.91, 26.84, 26.58, 26.55. HRMS ($C_{24}H_{33}I_2N_3O_6Ti$ +Na) Calc: 783.9842. Found: 783.9833.

$L^{1,5}$Ti(O*i*Pr)$_2$. This was synthesized similarly from Ti(O*i*Pr)$_4$ (134 mg, 0.47 mmol) and $H_2L^{1,5}$ (200 mg, 0.47 mmol) to give the product in a quantitative yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (dd, J = 8.8, 3.2 Hz, 1H, Ar−H), 7.96 (d, J = 3.2 Hz, 1H, Ar−H), 7.48 (m, 1H, Ar−H), 6.92 (m, 1H, Ar−H), 6.67 (d, J = 8.8 Hz, 1H, Ar−H), 6.58 (t, J = 8.0 Hz, 1H, Ar−H), 5.21 (sept, J = 6.0 Hz, 1H, $CHCH_3$), 4.96 (sept, J = 6.0 Hz, 1H, $CHCH_3$), 4.66 (d, J = 13.2 Hz, 1H, CH_2), 4.61 (d, J = 14.0 Hz, 1H, CH_2), 3.28 (d, J = 14.0 Hz, 1H, CH_2), 3.21 (d, J = 14.0 Hz, 1H, CH_2), 2.93 (m, 2H, CH_2), 2.50 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 1.91 (m, 2H, CH_2), 1.30 (d, J = 6.0 Hz, 3H, $CHCH_3$), 1.25 (d, J = 6.0 Hz, 6H, $CHCH_3$), 1.24 (d, J = 6.0 Hz, 3H, $CHCH_3$). ^{13}C NMR (500 MHz, THF-d_8) δ 169.90, 159.31, 139.36, 133.49, 129.88, 126.90, 126.45, 125.46, 119.38, 118.88, 118.51, 112.96, 79.93, 79.55, 65.09, 64.35, 52.91, 52.56, 47.88, 47.78, 26.44, 26.36, 26.06, 25.98. HRMS ($C_{24}H_{34}BrN_3O_6Ti$ +Na) Calc: 610.1014. Found: 610.1007.

$L^{1,6}$Ti (O*i*Pr)$_2$. This was synthesized similarly from Ti(O*i*Pr)$_4$ (113 mg, 0.40 mmol) and $H_2L^{1,6}$ (137 mg, 0.40 mmol) to give the product in a quantitative yield. ^{1}H NMR (500 MHz, THF-d_8) δ 8.04 (ddd, J = 8.9, 2.9, 0.5 Hz, 1H, Ar−H), 7.97 (d, J = 2.8 Hz, 1H, Ar−H), 7.11 (m, 1H, Ar−H), 6.98 (dd, J = 7.4, 0.4 Hz, 1H, Ar−H), 6.66 (dt, J = 7.3, 1.2 Hz, 1H, Ar−H), 6.63 (d, J = 9.0 Hz, 1H, Ar−H), 6.57 (dd, J = 8.1, 1.2 Hz, 1H, Ar−H), 4.97 (sept, J = 6.1 Hz, 1H, $CHCH_3$), 4.95 (sept, J = 6.1 Hz, 1H, $CHCH_3$), 4.61 (d, J = 13.4 Hz, 1H, CH_2), 4.57 (d, J = 13.6 Hz, 1H, CH_2), 3.38 (d, J = 13.8 Hz, 1H, CH_2), 3.26 (d, J = 13.5 Hz, 1H, CH_2), 2.93 (m, 2H, CH_2), 2.47 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 1.95 (m, 2H, CH_2), 1.28 (d, J = 6.0 Hz, 3H, $CHCH_3$), 1.25 (d, J = 6.4 Hz, 6H, $CHCH_3$), 1.24 (d, J = 6.0 Hz, 3H, $CHCH_3$). ^{13}C NMR (500 MHz, THF-d_8) δ 166.80, 159.96, 127.43, 126.99, 126.81, 123.91, 123.48, 122.69, 122.41, 116.10, 115.65, 114.68, 76.35, 76.02, 75.29, 62.28, 62.12, 61.42, 49.88, 49.57, 49.51, 44.77, 44.73, 44.68. HRMS ($C_{24}H_{35}N_3O_6Ti$ +Na) Calc: 532.1893. Found: 532.1900.

$L^{1,7}$Ti (O*i*Pr)$_2$. This was synthesized similarly from Ti(O*i*Pr)$_4$ (153 mg, 0.54 mmol) and $H_2L^{1,7}$ (201 mg, 0.54 mmol) to give the product in a quantitative yield. ^{1}H NMR (500 MHz, THF-d_8) δ 8.06 (dd, J = 8.9, 2.5 Hz, 1H, Ar−H), 8.00 (d, J = 2.9 Hz, 1H, Ar−H), 6.85 (m, 1H, Ar−H), 6.64 (d, J = 8.9 Hz, 1H, Ar−H), 6.62 (m, 1H, Ar−H), 5.00 (sept, J = 6.0 Hz, 1H, $CHCH_3$), 4.96 (sept, J = 6.4 Hz, 1H, $CHCH_3$), 4.56 (t, J = 13.8 Hz, 2H, CH_2), 3.44 (d, J = 13.5 Hz, 1H, CH_2), 3.32 (d, J = 14 Hz, 1H, CH_2), 2.95 (m, 2H, CH_2), 2.50 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 2.01 (m, 2H, CH_2), 1.25 (d, J = 3.0 Hz, 3H, $CHCH_3$), 1.24 (d, J = 5.8 Hz, 3H, $CHCH_3$), 1.24 (d, J = 3.1 Hz, 3H, $CHCH_3$), 1.23 (d, J = 5.9 Hz, 3H, $CHCH_3$). ^{13}C NMR (500 MHz, THF-d_8) δ 169.53, 154.65 (dd, J = 940, 45 Hz), 151.57 (dd, J = 1027, 49 Hz), 147.69 (m), 139.36, 127.69 (dd, J = 35, 16 Hz), 126.96, 126.52, 125.50, 118.57, 111.44 (dd, J = 90, 13 Hz), 104.31 (dd, J = 105, 92 Hz), 80.01, 79.43, 64.32, 64.28, 52.79, 52.77, 47.66, 47.65, 26.46, 26.40, 26.01, 25.98. HRMS ($C_{24}H_{33}F_2N_3O_6Ti$ +Na) Calc: 568.1723. Found: 568.1712.

$L^{2,3}$Ti (O*i*Pr)$_2$. This was synthesized similarly from Ti(O*i*Pr)$_4$ (124 mg, 0.44 mmol) and $H_2L^{2,3}$ (231 mg, 0.44 mmol) to give the product in a quantitative yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, J = 2.4 Hz, 1H, Ar−H), 7.28 (d, J = 2.4 Hz, 1H, Ar−H), 7.03 (d, J = 2.4 Hz, 1H, Ar−H), 6.86 (d, J = 2.4 Hz, 1H, Ar−H), 5.22 (sept, J = 6.0 Hz, 1H, $CHCH_3$), 5.21 (sept, J = 6.4 Hz, 1H, $CHCH_3$), 4.63 (m, 2H, CH_2), 3.15 (d, J = 4.8 Hz, 1H, CH_2), 3.12 (d, J = 4.4 Hz, 1H, CH_2), 2.91 (m, 2H, CH_2), 2.46 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 1.88 (m, 2H, CH_2), 1.29 (d, J = 6.0 Hz, 3H, $CHCH_3$), 1.29 (d, J = 6.0 Hz, 6H, $CHCH_3$), 1.22 (d, J = 6.0 Hz, 3H, $CHCH_3$). ^{13}C NMR (500 MHz, THF-d_8) δ 158.90, 135.17, 132.46, 129.60, 128.83, 128.34, 128.30, 127.92, 123.37, 121.76, 113.85, 108.79, 79.75, 79.70, 64.51, 64.47, 52.78, 52.02, 47.87, 47.66, 26.74, 26.66, 26.29, 26.17. HRMS ($C_{24}H_{32}Br_2Cl_2N_2O_4Ti$ +K) Calc: 728.9207. Found: 728.9200.

$L^{4,4}$Ti (O*i*Pr)$_2$. This was synthesized similarly from Ti(O*i*Pr)$_4$ (34 mg, 0.12 mmol) and $H_2L^{4,4}$ (91 mg, 0.11 mmol) to give the product in a quantitative yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, J = 2.4 Hz, 2H, Ar−H), 7.21 (d, J = 2.0 Hz, 2H, Ar−H), 5.18 (sept, J = 6.0

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Hz, 2H, $CHCH_3$), 4.59 (d, J = 13.6 Hz, 2H, CH_2), 3.10 (d, J = 14.0 Hz, 2H, CH_2), 2.88 (d, J = 10.0 Hz, 2H, CH_2), 2.47 (s, 6H, CH_3), 1.39 (d, J = 6.0 Hz, 2H, CH_2), 1.27 (d, J = 6.0 Hz, 6H, $CHCH_3$), 1.21 (d, J = 6.0 Hz, 6H, $CHCH_3$). ^{13}C NMR (500 MHz, $CDCl_3$) δ 160.66, 145.65, 138.10, 125.75, 90.45, 79.33, 77.89, 63.87, 51.77, 47.86, 26.27, 26.02. HRMS ($C_{24}H_{32}I_4N_2O_4Ti$ +Na) Calc: 990.7919. Found: 990.7915.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Integration decay plots of ^1H NMR-based hydrolysis measurements, cytotoxicity plots of representative free ligands vs their titanium(IV) complexes, structural information on representative trinuclear hydrolysis products, and X-ray structures of $L^{1,3}Ti(OiPr)_2$ and $L^{2,3}Ti(OiPr)_2$. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Buettner, K. M.; Valentine, A. M. *Chem. Rev.* **2012**, *112*, 1863−1881.
(2) Abeysinghe, P. M.; Harding, M. M. *Dalton Trans.* **2007**, 3474−3482.
(3) Caruso, F.; Rossi, M. *Mini-Rev. Med. Chem.* **2004**, *4*, 49−60.
(4) Caruso, F.; Rossi, M.; Pettinari, C. *Expert Opin. Ther. Pat.* **2001**, *11*, 969−979.
(5) Christodoulou, C. V.; Eliopoulos, A. G.; Young, L. S.; Hodgkins, L.; Ferry, D. R.; Kerr, D. J. *Br. J. Cancer* **1998**, *77*, 2088−2097.
(6) Kelter, G.; Sweeney, N. J.; Strohfeldt, K.; Fiebig, H. H.; Tacke, M. *Anti-Cancer Drugs* **2005**, *16*, 1091−1098.
(7) Keppler, B. K.; Friesen, C.; Moritz, H. G.; Vongerichten, H.; Vogel, E. *Struct. Bonding (Berlin)* **1991**, *78*, 97−127.
(8) Köpf-Maier, P.; Köpf, H. *Chem. Rev.* **1987**, *87*, 1137−1152.
(9) Meléndez, E. *Crit. Rev. Oncol. Hematol.* **2002**, *42*, 309−315.
(10) Strohfeldt, K.; Tacke, M. *Chem. Soc. Rev.* **2008**, *37*, 1174−1187.
(11) Caruso, F.; Massa, L.; Gindulyte, A.; Pettinari, C.; Marchetti, F.; Pettinari, R.; Ricciutelli, M.; Costamagna, J.; Canales, J. C.; Tanski, J.; Rossi, M. *Eur. J. Inorg. Chem.* **2003**, 3221−3232.
(12) Toney, J. H.; Marks, T. J. *J. Am. Chem. Soc.* **1985**, *107*, 947−953.
(13) Ott, I.; Gust, R. *Arch. Pharm. Chem. Life. Sci.* **2007**, *340*, 117−126.
(14) Manna, C. M.; Braitbard, O.; Weiss, E.; Hochman, J.; Tshuva, E. Y. *Chem. Med. Chem* **2012**, *7*, 703−708.
(15) Meker, S.; Manna, C. M.; Peri, D.; Tshuva, E. Y. *Dalton Trans.* **2011**, *40*, 9802−9809.
(16) Shavit, M.; Peri, D.; Manna, C. M.; Alexander, J. S.; Tshuva, E. Y. *J. Am. Chem. Soc.* **2007**, *129*, 12098−12099.
(17) Peri, D.; Meker, S.; Manna, C. M.; Tshuva, E. Y. *Inorg. Chem.* **2011**, *50*, 1030−1038.
(18) Peri, D.; Meker, S.; Shavit, M.; Tshuva, E. Y. *Chem.—Eur. J.* **2009**, *15*, 2403−2415.
(19) Tshuva, E. Y.; Peri, D. *Coord. Chem. Rev.* **2009**, *253*, 2098−2115.
(20) Tshuva, E. Y.; Ashenhurst, J. A. *Eur. J. Inorg. Chem.* **2009**, 2203−2218.
(21) Manna, C. M.; Armony, G.; Tshuva, E. Y. *Inorg. Chem.* **2011**, *50*, 10284−10291.
(22) Manna, C. M.; Armony, G.; Tshuva, E. Y. *Chem.—Eur. J.* **2011**, *17*, 14094−14103.
(23) Immel, T. A.; Groth, U.; Huhn, T. *Chem.—Eur. J.* **2010**, *16*, 2775−2789.
(24) Meker, S.; Margulis-Goshen, K.; Weiss, E.; Magdassi, S.; Tshuva, E. Y. *Angew. Chem., Int. Ed.* **2012**, *51*, 10515−10517.
(25) Cohen, A.; Kopilov, J.; Goldberg, I.; Kol, M. *Organometallics* **2009**, *28*, 1391−1405.
(26) Cohen, A.; Yeori, A.; Kopilov, J.; Goldberg, I.; Kol, M. *Chem. Commun.* **2008**, 2149−2151.
(27) Glasner, H.; Tshuva, E. Y. *J. Am. Chem. Soc.* **2011**, *133*, 16812−16814.
(28) Chmura, A. J.; Davidson, M. G.; Jones, M. D.; Lunn, M. D.; Mahon, M. F.; Johnson, A. F.; Khunkamchoo, P.; Roberts, S. L.; Wong, S. S. F. *Macromolecules* **2006**, *39*, 7250−7257.
(29) Immel, T. A.; Debiak, M.; Groth, U.; Buerkle, A.; Huhn, T. *ChemMedChem* **2009**, *4*, 738−741.
(30) Ganot, N.; Meker, S.; Reytman, L.; Tzubery, A.; Tshuva, E. Y. *J. Vis. Exp.* **2013**, DOI: 10.3791/50767.
(31) Single crystals of poor quality were obtaine for the hydrolysis product of $L^{1,2}Ti(OiPr)_2$. The X-ray structure, although unpublishable due to major disorder, pointed to the formation of a salan-bound oxo-bridged trinuclear complex as expected.[17,18] HRMS also supported formation of trinuclear hydrolysis prtoducts for additional representative complexes. See more information in SI.
(32) Tzubery, A.; Tshuva, E. Y. *Inorg. Chem.* **2011**, *50*, 7946−7948.
(33) Preliminary cytotoxicity studies of the hydrolysis products of $L^{1,2}Ti(OiPr)_2$ and $L^{1,3}Ti(OiPr)_2$ showed no activity, as did clusters previously studied.[18]
(34) Previous studies have suggested that even compounds exhibiting $t_{1/2}$ values of only several hours for labile ligand hydrolysis in 10% D_2O solutions undergo rapid cellular penetration, prior to formation of the bulky polynuclear hydrolysis products.[17,18]
(35) Shavit, M.; Peri, D.; Melman, A.; Tshuva, E. Y. *J. Biol. Inorg. Chem.* **2007**, *12*, 825−830.
(36) Schur, J.; Manna, C. M.; Deally, A.; Köster, R. W.; Tacke, M.; Tshuva, E. Y.; Ott, I. *Chem. Commun.* **2013**, *49*, 4785−4787.
(37) Gendler, S.; Segal, S.; Goldberg, I.; Goldschmidt, Z.; Kol, M. *Inorg. Chem.* **2006**, *45*, 4783−4790.
(38) Sumrit, P.; Hormnirum, P. *Macromol. Chem. Phys.* **2013**, *214*, 1845−1851.