A Marked Synergistic Effect in Antitumor Activity of Salan Titanium(IV) Complexes Bearing Two Differently Substituted Aromatic Rings

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S *Supporting Information*

ABSTRACT: Salan titanium(IV) complexes of differently substituted aromatic rings, where one ring is *para*-nitrated and another is *ortho,para*-halogenated, demonstrate exceptionally high anticancer activity, with IC$_{50}$ values of <1 μM, exceeding that of cisplatin by ∼30-fold. Whereas an additive effect in hydrolytic stability was detected for these highly stable complexes, an unexpected synergistic effect in anticancer activity makes these hybrid complexes substantially more active than both their symmetrical analogues alone and their equimolar mixture.

Scheme 1

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^{1,1}: R = R` = *p*-NO$_2$
L^{2,2}: R = R` = *o,p*-Cl
L^{3,3}: R = R` = *o,p*-Br

The discovery of the anticancer therapeutic properties of cisplatin initiated wide research on complexes of other transition metals.[1−7] In particular, the titanium(IV) complexes budotitane ((bzac)$_2$Ti(OEt)$_2$), titanocene dichloride (Cp$_2$TiCl$_2$) and their derivatives demonstrated high anticancer activity toward a range of cell lines with relatively minor toxicity, where the main disadvantage is in their relatively rapid hydrolysis.[8−19] We have recently introduced the cytotoxic C_2-symmetrical Ti^{IV} complexes that include diaminobis(phenolato) "salan" ligands (Scheme 1).[20−24] Leading compounds demonstrate high hydrolytic stability in mixed organic/water solutions with markedly higher anticancer activity than those of (bzac)$_2$Ti(O*i*Pr)$_2$, Cp$_2$TiCl$_2$, and cisplatin toward several cell lines. In addition, structural parameters of the ligands, including different substitutions on the aromatic rings, substantially influenced the complex features. For instance, *ortho*-halogenation increased both the cytotoxicity and hydrolytic stability,[20] whereas nitro substituents enhanced solubility in a biological environment. Herein we present C_1-symmetrical salan Ti^{IV} "hybrid" complexes bearing different substituents on the two aromatic rings of the ligand, demonstrating exceptionally high antitumor activity with a pronounced inherent synergistic effect.

To broaden the range of structural influence on the properties of the Ti^{IV} center in the salan complexes, we explored the cytotoxicity of asymmetric complexes, prepared similarly to known Zr^{IV} catalysts,[25,26] relative to their two symmetrical analogues alone and to the combination of these analogues, especially as synergistic effects in combination anticancer therapy are not uncommon.[27−29] In an attempt to combine high activity and stability with improved solubility, complexes halogenated on one ring and nitrated on the other were prepared (Scheme 2).

H$_2$L^{1,2} and H$_2$L^{1,3} (Scheme 2) were synthesized based on known procedures by reacting the halogenated salicylaldehyde with *N,N*-dimethylethylenediamine, followed by reaction with the nitrated benzylchloride.[25,26] ^{1}H NMR verified that the desired C_s-symmetrical ligands were obtained based, for instance, on five different signals for the aromatic region. L^{1,2}Ti(O*i*Pr)$_2$ and L^{1,3}Ti(O*i*Pr)$_2$ (Scheme 2) were synthesized quantitatively by reacting H$_2$L^{1,2} and H$_2$L^{1,3} with Ti(O*i*Pr)$_4$. The ^{1}H NMR features are consistent with the formation of C_1-symmetrical complexes, with two different septet signals of the isopropoxo groups and AB doublets of the methylene protons. All C_2-symmetrical analogues (Scheme 2) were prepared as previously described.[20,30−32]
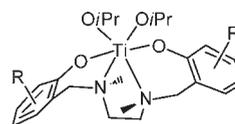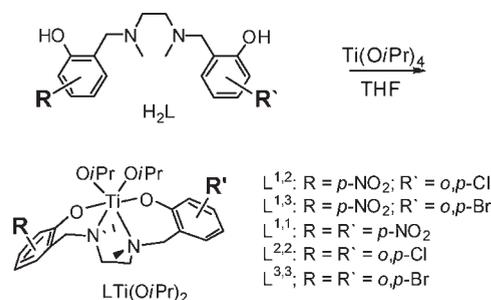
Single crystals of L^{1,2}Ti(O*i*Pr)$_2$ suitable for X-ray crystallography were obtained from dichloromethane. The X-ray structure (Figure 1) exhibits similar features both to those of related C_1-symmetrical Zr^{IV} complexes with differently substituted aromatic rings[25,26] and to those of C_2-symmetrical Ti^{IV} complexes previously reported.[20−22,30,33−37] The octahedral metal center is bound to the asymmetrical L^{1,2} ligand with a *trans* configuration of the phenolato groups, leaving the two additional isopropoxo ligands in a *cis* geometry. This indicates substantial similarity in the structure of the hybrid complex to that of its symmetrical analogues.

Hydrolytic stability was assessed by ^{1}H NMR, adding 10% D$_2$O to a THF-d_8 solution of the complex, as reported for
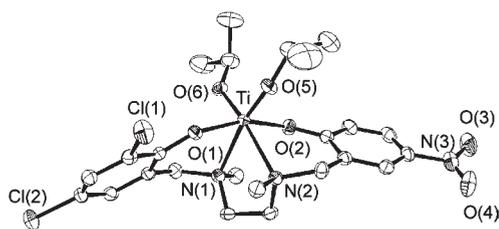
Figure 1. ORTEP drawing of $L^{1,2}Ti(OiPr)_2$ in 50% probability ellipsoids; H-atoms omitted for clarity. Selected bond lengths (Å) and angles (deg): Ti(1)−N(1) 2.362(2), Ti(1)−O(1) 1.920(2), Ti(1)−O(5) 1.772(2). O(1)−Ti(1)−O(2) 161.93(8), N(1)−Ti(1)−N(2) 76.18(8), O(5)−Ti(1)−O(6) 106.4(1).
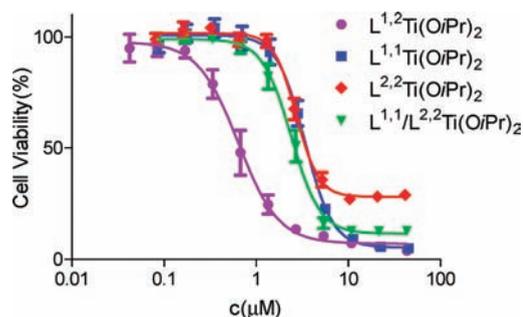
Figure 2. Dependence of HT-29 cell viability on administered concentration of $L^{1,2}Ti(OiPr)_2$ and comparison to its C_2-symmetrical analogues and their combination (1:1 mixture; concentration determined per Ti^{IV} center), following 72 h of incubation based on 3×3 repetitions.

Figure 3. Dependence of HT-29 cell viability on administered concentration of $L^{1,3}Ti(OiPr)_2$ and comparison to its C_2-symmetrical analogues and their combination (1:1 mixture; concentration determined per Ti^{IV} center), following 72 h of incubation based on 3×3 repetitions.

Table 1. Relative IC$_{50}$ (μM) Values^a for $L^{1,2}Ti(OiPr)_2$ and $L^{1,3}Ti(OiPr)_2$ on HT-29 and OVCAR-1 Cells and Comparison to Analogous C_2-Symmetrical Complexes, Their Combinations, and Cisplatin

Complex	HT-29 (μM)	OVCAR-1 (μM)
$L^{1,2}Ti(OiPr)_2$	0.7 ± 0.4	0.7 ± 0.4
$L^{1,1}Ti(OiPr)_2$	3.3 ± 0.3	2.5 ± 0.9
$L^{2,2}Ti(OiPr)_2$	2.8 ± 0.7	3.0 ± 0.7
$L^{1,1}/L^{2,2}Ti(OiPr)_2$[b]	2.4 ± 0.9	1.8 ± 0.7
$L^{1,3}Ti(OiPr)_2$	0.9 ± 0.3	0.9 ± 0.4
$L^{3,3}Ti(OiPr)_2$	inactive	inactive
$L^{1,1}/L^{3,3}Ti(OiPr)_2$[b]	3.1 ± 0.6	2.4 ± 0.6
Cisplatin[39]	20 ± 2	13 ± 1

^{a} Error values are based on standard deviations. ^{b} 1:1 mixture of the two; concentration determined per Ti^{IV} center.

symmetrical derivatives.[20,21] The rates of isopropoxo hydrolysis obtained for the hybrid complexes were, as expected, between those of the corresponding C_2-symmetrical complexes, with a $t_{1/2}$ value of 10 h for $L^{1,2}Ti(OiPr)_2$ (relative to 1.3 and 110 h for $L^{1,1}Ti(OiPr)_2$ and $L^{2,2}Ti(OiPr)_2$, respectively)[20] and 17 h for $L^{1,3}Ti(OiPr)_2$ (relative to 1.3 and 110 h). Presuming a salan-bound polynuclear complex forming under these conditions as observed for the symmetrical analogues,[20,21,36] this behavior is apparently a consequence of combined steric and electronic influences on the metal center.

The cytotoxicity of the complexes was studied on colon HT-29 (Figures 2, 3) and ovarian OVCAR-1[38] cancer cell lines, employing the methylthiazolyldiphenyltetrazolium bromide (MTT) assay.[20] The IC$_{50}$ values are summarized in Table 1.

It is evident that the cytotoxic activity of both C_1-symmetrical compounds on the cells analyzed 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 (Figures 2, 3), marking a synergistic rather than additive effect of the two parts of the molecule. It is particularly interesting that, for $L^{1,3}Ti(OiPr)_2$, a marked enhancement of activity relative to the nitrated symmetric analogue $L^{1,1}Ti(OiPr)_2$ is observed, despite the complete inactivity of the brominated analogue $L^{3,3}Ti(OiPr)_2$ on the cells tested (Figure 3), which is probably a result of increased steric effects.[20,21] Of further interest is the comparison to the performance of the 1:1 mixtures of C_2-symmetrical analogues to give an identical final Ti^{IV}

concentration, where participation of both compounds in activity is apparent, with a markedly less pronounced synergistic effect.

The higher efficiency of the hybrid Ti^{IV} salan complexes as anticancer agents may be attributed to several possible parameters: (a) the particular symmetry is of importance to the interaction with the biological target, where the hybrid complex exhibits an overall different structure with different electronic and steric features; (b) formation of a different polynuclear salan-bound complex is expected upon hydrolysis, which as we previously reported might be involved in the cytotoxicity mechanism;[20,21,36] this may also explain the observation with the combinations of symmetrical analogues where heteroligated clusters may form; (c) indirect parameters affecting delivery to the target, such as cell penetration and solubility, might be of influence, although, at the low concentrations applied to establish the high cytotoxicity, no solubility problems were detected. In fact, an additional related advantage of the hybrid complexes is their enhanced solubility in DMSO, which is of particular interest to therapeutic applications, adding to their promise as new anticancer agents. Altogether, these observations further emphasize the importance of fine-tuning structural parameters of the ligand to achieve high anticancer activity, and the ligand involvement in the valuable cellular interactions, all of which should encourage continuous development of improved systems.

■ ASSOCIATED CONTENT

ⓈSupporting Information. Crystallographic data for $L^{1,2}Ti(OiPr)_2$ (CCDC 838323), experimental details, and cytotoxicity plots for OVCAR-1 cells. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Bruijnincx, P. C. A.; Sadler, P. J. *Curr. Opin. Chem. Biol.* **2008**, *12*, 197.

(2) Desoize, B. *Anticancer Res.* **2004**, *24*, 1529.

(3) Galanski, M.; Arion, V. B.; Jakupec, M. A.; Keppler, B. K. *Curr. Pharm. Des.* **2003**, *9*, 2078.

(4) Jakupec, M. A.; Galanski, M.; Arion, V. B.; Hartinger, C. G.; Keppler, B. K. *Dalton Trans.* **2008**, 183.

(5) Ott, I.; Gust, R. *Arch. Pharm. Chem. Life Sci.* **2007**, *340*, 117.

(6) van Rijt, S. H.; Sadler, P. J. *Drug Discov. Today* **2009**, *14*, 1089.

(7) Xu, G.; Cui, Y. B.; Cui, K.; Gou, S. H. *Prog. Chem.* **2006**, *18*, 107.

(8) Abeysinghe, P. M.; Harding, M. M. *Dalton Trans.* **2007**, 3474.

(9) Caruso, F.; Rossi, M. *Mini-Rev. Med. Chem.* **2004**, *4*, 49.

(10) Caruso, F.; Rossi, M.; Pettinari, C. *Expert Opin. Ther. Pat.* **2001**, *11*, 969.

(11) Christodoulou, C. V.; Eliopoulos, A. G.; Young, L. S.; Hodgkins, L.; Ferry, D. R.; Kerr, D. J. *Br. J. Cancer* **1998**, *77*, 2088.

(12) Kelter, G.; Sweeney, N. J.; Strohfeldt, K.; Fiebig, H.-H.; Tacke, M. *Anti-Cancer Drugs* **2005**, *16*, 1091.

(13) Keppler, B. K.; Friesen, C.; Moritz, H. G.; Vongerichten, H.; Vogel, E. *Struct. Bonding (Berlin)* **1991**, *78*, 97.

(14) Köpf-Maier, P.; Köpf, H. *Chem. Rev.* **1987**, *87*, 1137.

(15) Köpf-Maier, P.; Köpf, H. *Struct. Bonding (Berlin)* **1988**, *70*, 103.

(16) Meléndez, E. *Crit. Rev. Oncol. Hemat.* **2002**, *42*, 309.

(17) Strohfeldt, K.; Tacke, M. *Chem. Soc. Rev.* **2008**, *37*, 1174.

(18) 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.

(19) Toney, J. H.; Marks, T. J. *J. Am. Chem. Soc.* **1985**, *107*, 947.

(20) Peri, D.; Meker, S.; Manna, C. M.; Tshuva, E. Y. *Inorg. Chem.* **2011**, *50*, 1030.

(21) Peri, D.; Meker, S.; Shavit, M.; Tshuva, E. Y. *Chem.—Eur. J.* **2009**, *15*, 2403.

(22) Shavit, M.; Peri, D.; Manna, C. M.; Alexander, J. S.; Tshuva, E. Y. *J. Am. Chem. Soc.* **2007**, *129*, 12098.

(23) Tshuva, E. Y.; Ashenhurst, J. A. *Eur. J. Inorg. Chem.* **2009**, 2203.

(24) Tshuva, E. Y.; Peri, D. *Coord. Chem. Rev.* **2009**, *253*, 2098.

(25) Cohen, A.; Kopilov, J.; Goldberg, I.; Kol, M. *Organometallics* **2009**, *28*, 1391.

(26) Cohen, A.; Yeori, A.; Kopilov, J.; Goldberg, I.; Kol, M. *Chem. Commun.* **2008**, 2149.

(27) Chan, A. *Annals Oncol.* **2007**, *18*, 1152.

(28) Gao, Y.; Jia, Z.; Kong, X.; Li, Q.; Chang, D. Z.; Wei, D.; Le, X.; Huang, S.; Huang, S.; Wang, L.; Xie, K. *Cancer Res.* **2011**, *71*, 5182.

(29) Jia, Z.; Gao, Y.; Wang, L.; Li, Q.; Zhang, J.; Le, X.; Wei, D.; Yao, J. C.; Chang, D. Z.; Huang, S.; Xie, K. *Cancer Res.* **2010**, *70*, 1111.

(30) 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.

(31) Gendler, S.; Segal, S.; Goldberg, I.; Goldschmidt, Z.; Kol, M. *Inorg. Chem.* **2006**, *45*, 4783.

(32) Immel, T. A.; Debiak, M.; Groth, U.; Burkle, A.; Huhn, T. *ChemMedChem* **2009**, *4*, 738.

(33) Balsells, J.; Carroll, P. J.; Walsh, P. J. *Inorg. Chem.* **2001**, *40*, 5568.

(34) Groysman, S.; Sergeeva, E.; Goldberg, I.; Kol, M. *Eur. J. Inorg. Chem.* **2005**, *2005*, 2480.

(35) Manna, C. M.; Tshuva, E. Y. *Dalton Trans.* **2010**, *39*, 1182.

(36) Meker, S.; Manna, C. M.; Peri, D.; Tshuva, E. Y. *Dalton Trans.* **2011**, *40*, 9802.

(37) Yeori, A.; Groysman, S.; Goldberg, I.; Kol, M. *Inorg. Chem.* **2005**, *44*, 4466.

(38) See Supporting Information.

(39) Tzubery, A.; Tshuva, E. Y. *Inorg. Chem.* **2011**, *50*, 7946.