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PAPER

Highly cytotoxic vanadium(v) complexes of salan ligands; insights on the role of hydrolysis†

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Vanadium(v) oxo complexes with tetradentate diamine bis(phenolato) "salan" ligands of the type LVO(OiPr) (L is salan) with different steric and electronic substitutions at the *ortho* and *para* positions to the binding phenolato moiety were synthesized and their hydrolytic stability and cytotoxicity were analyzed. With one exception bearing large steric groups, all complexes examined displayed marked cytotoxic activity, comparable to, and often higher than, that of cisplatin. While the hydrolytic stability changed significantly depending on the substituent at the *ortho* position relative the O-donor with little effect of *para* substitutions, the cytotoxic activity largely was not affected, and high cytotoxicity was recorded also for relatively unstable complexes. Additional measurements revealed that the cytotoxicity is largely maintained following pre-incubation of up to 18 hours of the complexes in the biological medium prior to cell addition, suggesting that hydrolysis products might serve as the active species. In addition, appreciable cytotoxic activity was measured for an isolated hydrolysis product that was analyzed crystallographically to exhibit a dimeric structure with bridging oxo ligand where both metal centers are bound to the salan ligand, supporting the aforementioned conclusions.

Introduction

Since the discovery of the anti-tumor activity of cisplatin, the research of novel metal compounds for cancer treatment has been rapidly evolving.[1–7] Due to the limited activity range resulting from inherent or acquired resistance of some cell types, and severe toxicity of platinum compounds, other transition metals are being investigated, which may introduce different coordination features leading to different mechanisms of action, where structure–activity relationship studies aim at elucidating the influences of different electronic and steric properties of the metal center on its medicinal potential.

One of the most promising metals studied is vanadium. It is a physiologically essential element and was found to activate or inhibit several enzymes and influence gene expression.[8–11] Additionally, vanadium compounds possess therapeutic potential in several diseases including diabetes[11,12] and cancer.[13–15] In particular, recent studies of *in vivo* influence of an anticancer oxo-vanadium complex of the type $L_2VO(X)$ (L is a methylated phenanthroline ligand and X is a sulfato ligand) on mice revealed favorable pharmacokinetics and toxicity profiles.[16] These features, combined with evidence of significantly higher vanadium concentration in cancerous compared to normal breast tissue,[17] contribute to the promise of vanadium compounds in cancer therapy and encourage the search for new V-based cytotoxic compounds.

The chemistry of vanadium in aqueous solutions is complicated due to rapid hydrolysis, polymerization and multiple oxidation states.[8,10] Therefore for biological applications it is of interest to develop compounds with improved hydrolytic stability and defined hydrolysis products.[18] Salans are known diamine bis(phenolato) tetradentate ligands, for which Ti(iv) complexes showed remarkable stability towards hydrolysis, where structure–activity relationship studies revealed a significant influence of the different substituents on the phenolato rings on the complex properties.[19–23] Therefore, in this study we present a new family of cytotoxic agents based on vanadium(v) complexes of salan ligands. We investigated the cytotoxic activity of complexes with different substituents on the salan ligand, while analyzing their hydrolytic behavior and its effect on cytotoxicity. Most related studies reported on salen vanadium(iv) and salicylaldehyde semicarbazone vanadium(v) complexes with fair cytotoxicity[24–26] and on practically inactive vanadium(v) species with a carboxylated salan ligand of NH donors.[27]

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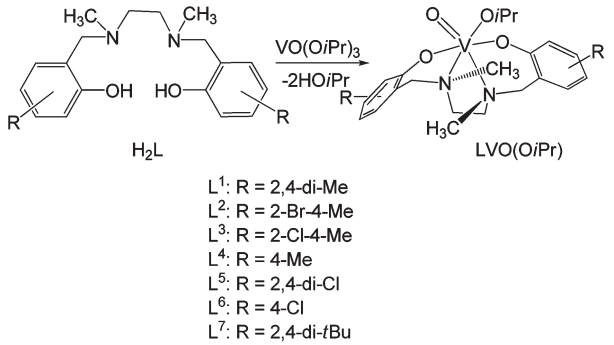
† Electronic supplementary information (ESI) available: Crystallographic data for $L^1VO(OiPr)$ and $(L^1VO)_2\mu$-O, plots of cytotoxicity measurements towards OVCAR-1 cells for $L^{1-7}VO(OiPr)$ and $(L^1VO)_2\mu$-O and for $L^1VO(OiPr)$ following different periods of pre-incubation in biological medium without cells, apoptosis *vs.* necrosis measurements plots for $L^1VO(OiPr)$ and cyclic voltammetry measurements of $L^1VO(OiPr)$ and $(L^1VO)_2\mu$-O. CCDC reference numbers 838966 and 838967. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c2dt11514j



Scheme 1 Synthesis of vanadium(v)–salan complexes.

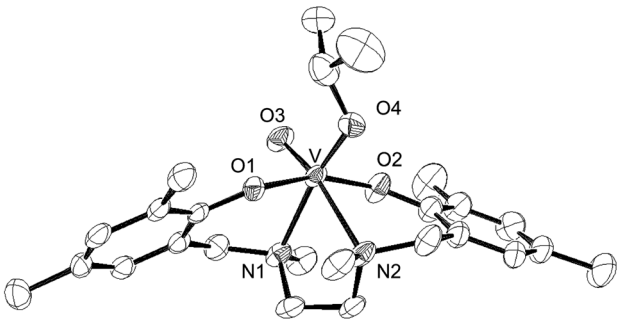


Fig. 1 ORTEP drawing of $L^1VO(OiPr)$ in 50% probability ellipsoids; H atoms and disorder in the isopropoxo group were omitted for clarity.

Results and discussion

Synthesis and characterization

The complexes prepared and studied herein are of the type $LV^VO(OiPr)$ (Scheme 1) where L is a dianionic tetradentate salan ligand. The influence of steric and electronic effects on the cytotoxic activity and hydrolytic stability of the complex was examined by employing *N*-methylated ligands[28] with different substituents at the *ortho* and *para* positions of the aromatic rings.

The synthesis of the known salan ligands H_2L^{1-7} (Scheme 1) was conducted through one of two procedures previously described;[19–21,29–31] $H_2L^{1-3,5-7}$ were prepared from commercially available substituted phenols, dimethylethylenediamine and formaldehyde by a single step Mannich condensation and H_2L^4 was prepared by a two-step procedure from the substituted benzaldehyde and ethylene diamine with a following methylation step. The synthesis of vanadium(v)–salan complexes was conducted under an inert atmosphere similarly to known procedures,[32] by reacting the commercially available $VO(OiPr)_3$ with equimolar amounts of the ligand precursor, and treating the product with cold hexane (Scheme 1). The complexes $L^{1-7}VO(OiPr)$ (Scheme 1) were obtained in quantitative yields, as evident by ^1H NMR, which is consistent with the low symmetry of the complexes.

A representative complex $L^1VO(OiPr)$ was crystallized from cold pentane, to give single crystals suitable for X-ray crystallography. Its ORTEP drawing is presented in Fig. 1, and a list of selected bond lengths and angles is provided in Table 1. The structure displays disorder in the isopropoxo group.

Table 1 Selected bond lengths (Å) and angles (°) for $L^1VO(OiPr)$

Atoms	Value	Atoms	Value
Lengths			
O(1)–V	1.880(3)	N(1)–V	2.276(4)
O(2)–V	1.861(3)	N(2)–V	2.375(3)
O(3)–V	1.595(3)	—	—
O(4)–V	1.777(3)	—	—
Angles			
O(1)–V–O(2)	161.5(1)	O(1)–V–N(1)	82.3(1)
O(1)–V–O(3)	97.9(2)	O(2)–V–N(1)	86.7(2)
O(1)–V–O(4)	90.4(1)	O(3)–V–N(1)	59.5(2)
O(2)–V–O(3)	96.9 (2)	O(4)–V–N(1)	163.1(1)
O(2)–V–O(4)	96.0(2)	O(1)–V–N(2)	81.8(1)
O(3)–V–O(4)	106.7(2)	O(2)–V–N(2)	81.2(1)
N(1)–V–N(2)	76.7(1)	O(3)–V–N(2)	166.1(2)
—	—	O(4)–V–N(2)	87.2(1)

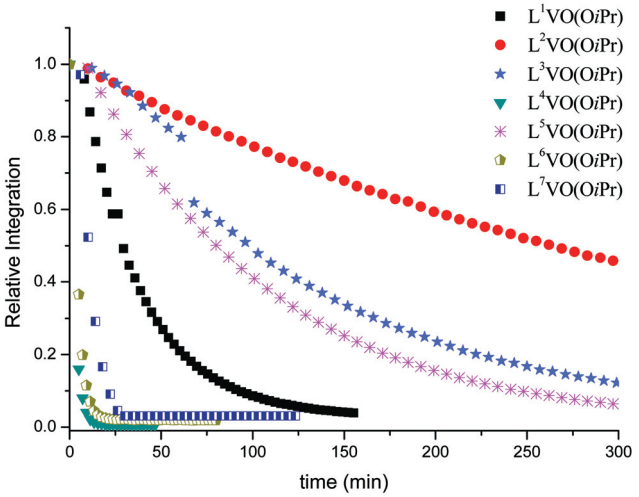


Fig. 2 Plots of the relative integration of the isopropoxo signal in the ^1H-NMR spectra as a function of the time following the addition of 10% D_2O to THF-d_8 solution of $L^{1-7}VO(OiPr)$.

The structure (Fig. 1) features an octahedral complex, with a C_1-symmetry that was reduced from C_2 due to in equivalency of the two monodentate ligands. The two phenolato oxygen donors bind in a *trans* configuration, as observed for related group (IV) metal complexes,[29,33–40] and the two monodentate ligands are *cis* to one another. Interestingly, the V–N(2) bond to the N-donor *trans* to the oxo group (2.38 Å) is substantially longer that the V–N(1) from the metal to the N-donor *trans* to the isopropoxo ligand (2.28 Å).

Hydrolysis

The relative stability of the complexes towards hydrolysis was evaluated by adding of 10% (>1000 equivalents) D_2O to a THF-d_8 solution of a complexes and measuring the rate of hydrolysis by NMR based on pseudo first order reaction, monitoring the integration of the isopropoxo group and isopropanol relative to an internal standard, as previously described.[19,20] Fig. 2 depicts the integration change with time for $L^{1-7}VO(OiPr)$, and $t_{\frac{1}{2}}$ values for isopropoxo hydrolysis are provided in Table 2. Additional

Table 2 $t_{\frac{1}{2}}$ values for the release of the isopropoxo group to give isopropanol for complexes $L^{1-7}VO(OiPr)$

Complex	Substituents	$t_{\frac{1}{2}}$ (min)
$L^1VO(OiPr)$	*o*-Me, *p*-Me	20
$L^2VO(OiPr)$	*o*-Br, *p*-Me	230
$L^3VO(OiPr)$	*o*-Cl, *p*-Me	90
$L^4VO(OiPr)$	*o*-H, *p*-Me	<5
$L^5VO(OiPr)$	*o*-Cl, *p*-Cl	70
$L^6VO(OiPr)$	*o*-H, *p*-Cl	<5
$L^7VO(OiPr)$	*o*-*t*Bu, *p*-*t*Bu	<5

changes in the spectrum included growing signals of the hydrolysis products, while no signals belonging to free salan ligands were detected.

The results imply that substituents at the *ortho* positions to the binding O-donor induce significant steric and electronic effects on the hydrolytic stability, while the *para* substitutions are of lesser influence. Both *o*-H complexes $L^{4,6}VO(OiPr)$ hydrolyze fairly quickly, where complete dissociation of isopropoxo ligands to give isopropanol is observed within a few minutes, despite the different electronic nature of the *para*-substituent. However, increasing the steric bulk at the *ortho* position increased the stability by ~5 fold for the methylated complex $L^1VO(OiPr)$, and by ~20 fold and ~40 fold for the halogenated complexes, the chlorinated ones $L^{3,5}VO(OiPr)$ and the brominated one $L^2VO(OiPr)$, respectively, where the bigger increased in stability is achieved for the complex of the bigger *o*-Br substituent, and little effect of the *para* position is again observed. This is similar to the observation with salan Ti(IV) complexes, where negligible effect of *para* substitutions on hydrolysis was observed, and a stabilizing effect of *ortho*-halogenation was detected, where steric hindrance at *ortho* positions was proposed to inhibit formation of polynuclear hydrolysis products.[19,20] An exception is $L^7VO(OiPr)$, for which, despite the large *o*-*t*Bu substitution, a poor stability is measured.

In order to elucidate the structure of the hydrolysis products of the vanadium(V)–salan complexes, a representative complex $L^1VO(OiPr)$ was reacted with 1000 equivalents of water for 3 days. The product was characterized by mass spectrometry and elemental analysis. A dinuclear species of the type $(L^1VO)_2\mu$-O formed as the main product of the hydrolysis of $L^1VO(OiPr)$, in which two vanadium centers are bridged by an oxo ligand, and each center is still attached to the salan ligand.[41,42] Further support for the structure was obtained from X-ray crystallography conducted on single crystals obtained from a cold 1 : 1 mixture of toluene and pentane (Fig. 3, Table 3). It is evident that the C_2-symmetrical dimeric structure includes two octahedral metal centers bound to the oxo group and the salan ligand, where the two phenolato O-donors of each ligand bind in a *trans* configuration similar to the monomeric structure but different to the related dimeric structures obtained with other metals.[28] Other structural features of each metal center are also highly similar to that of the monomeric structure of $L^1VO(OiPr)$ (Fig. 1), including the two unequivalent V–N bonds. The two metal centers are bridged by a single oxo ligand, with a V···V distance of 3.51 Å. Interestingly, the nearest distance between two *ortho*-methyl substituents is 4.33 Å, whereas the corresponding distance between two *para*-methyl groups is 3.66 Å.

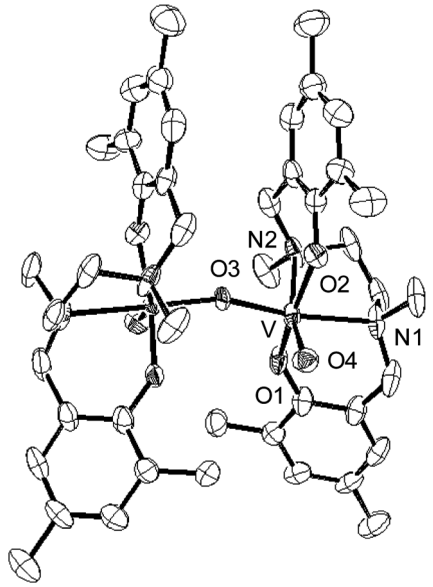


Fig. 3 ORTEP drawing of $(L^1VO)_2\mu$-O, the hydrolysis product of $L^1VO(OiPr)$, in 50% probability ellipsoids; H atoms and disordered solvent were omitted for clarity.

Table 3 Selected bond lengths (Å) and angles (°) for $(L^1VO)_2\mu$-O, the hydrolysis product of $L^1VO(OiPr)$

Atoms	Value	Atoms	Value
Lengths			
O(1)–V	1.866(3)	N(1)–V	2.164(4)
O(2)–V	1.875(3)	N(2)–V	2.387(4)
O(3)–V	1.785(1)	V···V	3.514
O(4)–V	1.603(4)	—	—
Angles			
O(1)–V–O(2)	161.6(2)	O(1)–V–N(1)	82.6(2)
O(1)–V–O(3)	91.5(2)	O(2)–V–N(1)	86.3(2)
O(1)–V–O(4)	98.5(2)	O(3)–V–N(1)	162.3(1)
O(2)–V–O(3)	94.8 (2)	O(4)–V–N(1)	89.1(2)
O(2)–V–O(4)	95.9(2)	O(1)–V–N(2)	82.2(1)
O(3)–V–O(4)	108.3(2)	O(2)–V–N(2)	81.1(2)
N(1)–V–N(2)	76.9(2)	O(3)–V–N(2)	85.8(1)
—	—	O(4)–V–N(2)	165.8(2)

This finding supports the notion that steric hindrance near and around the metal center increases kinetic stability by inhibiting formation of this dimer. It is also plausible that the large *ortho* and *para* *t*Bu groups in $L^7VO(OiPr)$ prohibit formation of the dimeric product altogether as observed for related Ti(IV) complexes,[20] thus resulting in a different, and apparently faster, hydrolysis reaction.

Cytotoxicity

The cytotoxic activity of the vanadium(V) complexes was tested on colon HT-29 and ovarian OVCAR-1 cells and analyzed by the methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay.[19] Generally, similar results were obtained towards both cell lines. A plot of the cell viability dependence on the complex concentration (Fig. 4) enables calculating the IC_{50} and maximal cell growth inhibition values, as summarized in Table 4.

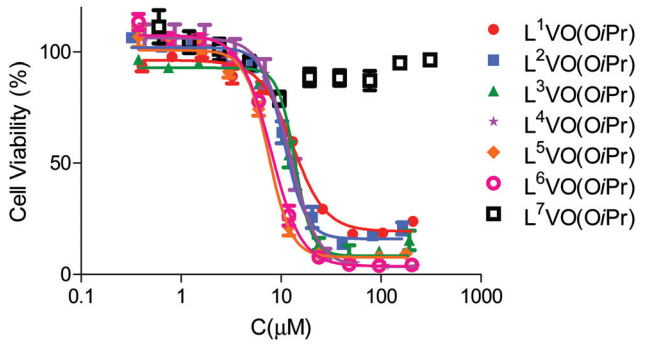


Fig. 4 Dependence of HT-29 cell viability on administered concentration of $L^{1-7}VO(OiPr)$ following 3 day incubation obtained by the MTT assay.

Table 4 Relative IC_{50} values (μM) and maximum cell growth inhibition (%) values for $L^{1-7}TiO(OiPr)$, the vanadium precursor $VO(OiPr)_3$ and cisplatin towards colon HT-29 and ovarian OVCAR-1 cell lines

Complex	OVCAR-1	HT-29
$L^1VO(OiPr)$	12.0 ± 2.4 (81%)	13 ± 2 (81%)
$L^2VO(OiPr)$	7.5 ± 1.3 (89%)	10.9 ± 1.3 (84%)
$L^3VO(OiPr)$	8.3 ± 2.2 (92%)	10.5 ± 2.8 (92%)
$L^4VO(OiPr)$	9.5 ± 1.9 (96%)	10.3 ± 1.6 (97%)
$L^5VO(OiPr)$	5.9 ± 1.1 (93%)	6.8 ± 0.9 (92%)
$L^6VO(OiPr)$	8.0 ± 0.7 (97%)	8.4 ± 0.8 (96%)
$L^7VO(OiPr)$	Inactive	Inactive
$VO(OiPr)_3$	21.0 ± 6.9 (89%)	25 ± 9 (87%)
Cisplatin	8.8 ± 2.1 (91%)	12.2 ± 2.3 (90%)

The vanadium(v)–salan complexes $L^{1-6}VO(OiPr)$ exhibit marked cytotoxic activity with IC_{50} values that represent activity that is comparable to, and often lower than, that of cisplatin for the examined cells, with clear activity enhancement relative to the vanadium(v) precursor $VO(OiPr)_3$. Moreover, additional measurements have revealed that apoptosis is probably the main pathway of cell death, rather than necrosis (see ESI†). When comparing the cytotoxicity of the different vanadium(v)–salan complexes $L^{1-6}VO(OiPr)$, no significant difference is detected. An exception, again, is the *tert*-butylated complex $L^7VO(OiPr)$ which did not display significant cytotoxic activity. The lack of activity may be attributed to the large steric bulk inhibiting cell penetration and/or interaction with cellular target, or to indirect parameters such as reduced solubility.

It is thus obvious that for this family of cytotoxic agents, hydrolytic stability is not a pre-requisite for activity. Thus, to assess the period through which the complexes may be exposed to biological environment and still retain cytotoxic activity, $L^1VO(OiPr)$, a complex of relatively minor stability (Table 2) was pre-incubated in an aqueous biological medium for different periods without cells, followed by administration to the cancer cells for additional incubation of 3 days and measuring cell viability (Fig. 5). A summary of the IC_{50} values obtained is displayed in Table 5. A slight improvement in the cytotoxic activity was observed after short periods of pre-incubation (0.5 and 1 hours), probably as a result of enhanced solubility of the active species. Surprisingly, despite the relatively rapid hydrolysis of the complex, even following pre-incubation of 18 hours, the

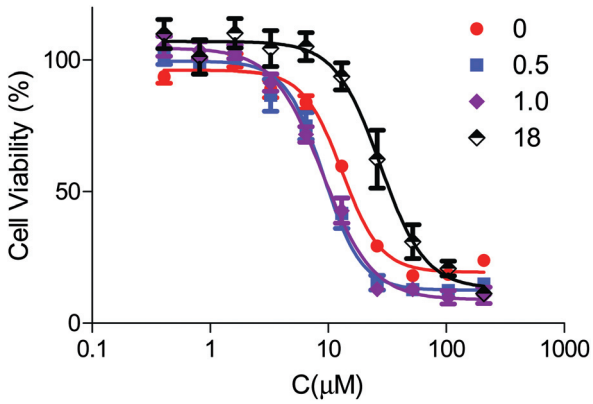


Fig. 5 Dependence of HT-29 cell viability on administered concentration of $L^1VO(OiPr)$ following pre-incubation in aqueous medium for varying periods (given in hours) prior to cell addition and 3 day incubation with cells, obtained by MTT assay.

Table 5 Relative IC_{50} values (μM) and maximum cell growth inhibition (%) values for $L^1VO(OiPr)$ following pre-incubation in aqueous medium for varying periods prior to cell addition

Pre-incubation time (hours)	OVCAR-1	HT-29
0	12.0 ± 2.4 (81%)	13 ± 2 (81%)
0.5	6.8 ± 1.4 (89%)	9.6 ± 2.9 (87%)
1	7 ± 2 (88%)	9.8 ± 3.8 (91%)
18	27 ± 13 (82%)	30.4 ± 18.4 (87%)

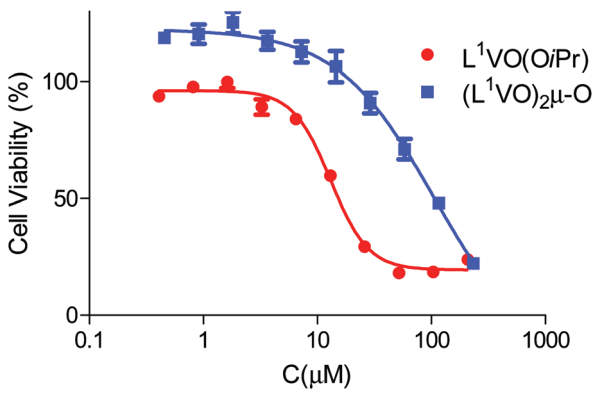


Fig. 6 Dependence of HT-29 cell viability on administered concentration of $L^1VO(OiPr)$ and its hydrolysis product $(L^1VO)_2\mu$-O following 3 day incubation obtained by the MTT assay.

cytotoxic activity of the complex was not completely abolished, but only slightly diminished, seemingly accompanied by partial precipitation of the dimeric product of hydrolysis. This implies that the dimeric hydrolysis product, or related polynuclear complexes formed in the biological environment, might play a role in the cytotoxicity mechanism, as also suggested for Ti(IV) complexes of diketonato ligands.[43–46]

Consequent to these results, and following the hypothesis that the product of hydrolysis may penetrate into the cell, the cytotoxic activity of $(L^1VO)_2\mu$-O, the isolated hydrolysis product of $L^1VO(OiPr)$ (Fig. 3), was studied (Fig. 6). Indeed, $(L^1VO)_2\mu$-O

exhibits cytotoxic activity, although lower than that of the precursor $L^1VO(OiPr)$, which may be attributed to decreased solubility in aqueous medium. This supports the notion that this dimer or related clusters may indeed manifest activity in the cellular environment.

Conclusions

In this paper we presented a new family of cytotoxic vanadium(V)–salan complexes and evaluated the effect of different electronic and steric properties on the cytotoxic activity and hydrolytic stability of the complexes. We observed significant electronic and steric effects of groups located at the *ortho* positions on the hydrolytic stability of the complexes. However, when these groups are situated at the *para* position, no significant effect on the hydrolytic stability is observed, presumably as they are more distant from the metal center. Hence it can be deduced that the hydrolytic stability of the complexes can be controlled by structural changes at the *ortho* position. In contrast to these observations, the cytotoxic activity did not display variation between the studied complexes, with one exception with particularly large steric bulk. In previous studies a correlation was found between hydrolytic stability of related Ti(IV)-salan complexes and their cytotoxic activity, where cytotoxicity generally increased with hydrolytic stability.[19,20] This behavior was considered to arise from formation of polynuclear hydrolysis products that were either inactive or unable to penetrate through the cellular membrane. In the current study we showed that a dimeric hydrolysis products of a precursor vanadium(V) complex demonstrates cytotoxic activity, and therefore it is presumably able to enter into the cell and interact with the cellular target. This makes the dimer a good candidate for the active species formed in the biological environment when starting from the precursor complex, although other related polynuclear products may also be participating in the fruitful biological interactions. Consequently, the hydrolytic stability does not play a significant role in the cytotoxic ability of these precursor complexes, which is of significance for their potential medicinal utility allowing for longer shelf lives. Additionally, the indication that different structural changes at both the *ortho* and the *para* positions do not affect much the cytotoxic activity of the complexes enables the design of complexes with other desired qualities, such as improved water solubility, without diminishing the cytotoxic activity. This may enable isolating dimeric hydrolysis products that would be of sufficient solubility to serve themselves as potential drugs. The only property found in this study to demolish the cytotoxicity of this family of complexes is particularly large steric bulk at the *ortho* and the *para* positions, which is also in accordance with the argument that the active species is the dimeric hydrolysis product, formation of which may be inhibited where large steric hindrance occurs near and around the metal center. Nevertheless, the effect of other parameters relating to solubility, cell penetration ability and more cannot be ruled out.

The vanadium(V)–salan complexes presented herein exhibit high promise as cytotoxic agents, as their activity towards the examined HT-29 and OVCAR-1 cell lines may even reach levels higher than those achieved by cisplatin, while involving an apoptotic cell death pathway. A marked improvement in cytotoxicity is observed relative to the vanadium(V) precursor, pointing to an important effect of the salan ligand both in enhancing cytotoxicity and in enabling formation of more defined hydrolysis products that are themselves stable for days in biological environment, which may be the active species as discussed above. These results encourage further development of this family of complexes, and full analysis of their mechanism of action.

Experimental

Data on H_2L^{1-7} can be found elsewhere.[19,29–31] $VO(OiPr)_3$ (98%) was purchased from Strem and used without further purification. Solvents used in the complex synthesis were distilled from K–benzophenone under nitrogen or dried over alumina columns on M. Braun SPS-800 solvent purification system. All experiments requiring a dry atmosphere were performed in a M. Braun glove box under nitrogen atmosphere. NMR data were recorded using an AMX-500 MHz Bruker spectrometer. X-ray diffraction data were obtained with a Bruker Smart Apex diffractometer, running the SMART software package. Hydrolysis studies by NMR were performed as previously described,[19,20] using a solution of the complex in THF-d_8 and adding >1000 equiv of D_2O to give a final solution of 1 : 9 D_2O–THF-d_8. The $t_{\frac{1}{2}}$ value is based on a pseudo first order fit for each compound. The results were verified by including *p*-dinitrobenzene as an internal standard.

Cytotoxicity was measured on a HT-29 colon and OVCAR-1 ovarian cells obtained from ATCC Inc. using the methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay.[19] Cells (0.6×10^6) in medium (contains: 1% penicillin/streptomycin antibiotics; 1% L-glutamine; 10% fetal bovine serum (FBS) and 88% medium RPMI-1640, all purchased from Biological Industries Inc.) were seeded into a 96-well plate and allowed to attach for a day. The cells were consequently treated with the reagent tested at 10 different concentrations. A solution of reagent was prepared by dissolving 8 mg of the reagent in 100 μL of THF, with further diluting with THF to obtain the different concentrations, and diluting 20 μL of resulting solution with 180 μL of medium. From the resulting solution, 10 μL was added to each well already containing 200 μL of the above solution of cells in the medium to give a final concentration of up to 100 mg L^{-1}. Control wells were treated with similar amounts of THF only. After a standard of 3 days 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 hours. The MTT solution was then removed, and the cells were dissolved in 200 μL isopropanol. The absorbance at 550 nm was measured by a Bio-Tek EL-800 microplate reader spectrophotometer. Each measurement was repeated at least 3 × 3 times, namely, three repeats per plate, all repeated at least 3 times on different days (9 repeats altogether). Relative IC_{50} values were determined by a nonlinear regression of a variable slope (four parameters) model.

Synthesis of $L^{1-7}VO(OiPr)$, general procedure: A solution of H_2L^{1-7} (0.42 mmol) in THF (1 ml) was added to a stirred solution of $VO(OiPr)_3$ (0.42 mmol) in THF (3 ml). The reaction mixture was stirred at room temperature for 15 minutes, and the

volatiles were removed under vacuum. A dark purple powder was obtained after adding cold hexane and removing it under vacuum. The products were obtained in quantitative yields (> 95%).

$L^1VO(OiPr)$

^1H NMR ($CDCl_3$): δ = 6.92 (s, 1H; Ar), 6.91 (s, 1H; Ar), 6.68 (s, 1H; Ar), 6.63 (s, 1H; Ar), 5.68 (sept, J = 6.1 Hz, 1H; C*H*(CH_3)$_2$), 4.89 (d, J = 14.4 Hz, 1H; CH_2), 4.61 (d, J = 13.3 Hz, 1H; CH_2), 3.55 (d, J = 14.6 Hz, 1H; CH_2), 3.11 (d, J = 13.7 Hz, 1H; CH_2), 3.05 (dt, J = 13.1, 3.4 Hz, 1H; CH_2), 2.93 (dt, J = 13.4, 3.3 Hz, 1H; CH_2), 2.59 (s, 3H; CH_3), 2.41 (s, 3H; CH_3), 2.29 (s, 3H; CH_3), 2.25 (s, 6H; CH_3), 2.22 (s, 3H; CH_3), 1.89 (m, 2H; CH_2), 1.40 (d, J = 6.1 Hz, 3H; CH(C*H*$_3$)$_2$), 1.36 (d, J = 6.1 Hz, 3H; CH(C*H*$_3$)$_2$). ^{13}C NMR ($CDCl_3$): δ 130.5, 130.3, 128.5, 128.1, 127.1, 126.9, 124.4, 120.3, 84.9, 66.8, 63.5, 53.7, 50.9, 48.5, 47.1, 25.3, 24.5, 20.7, 20.6, 17.2, 17.0 ppm. Anal. calcd for $C_{25}H_{37}N_2O_4V$: C, 62.49; H, 7.76; N, 5.83. Found: C, 62.43; H, 7.68; N, 5.74.

Crystal data for $L^1VO(OiPr)$

Crystallized from cold pentane (dark purple crystals); $C_{25}H_{37}N_2O_4V$, M = 480.51, trigonal, *a, b* = 19.8537(8), c = 67.448(5) Å, V = 23024(2) Å^3, T = 173(1) K, space group $R\bar{3}c$, Z = 36, μ(Mo Kα) = 0.419 mm^{-1}, 82 154 reflections measured, 5590 unique (R_{int} = 0.1254). $R(F_o^2)$ for [$I > 2\sigma(I)$] = 0.1157, R_w for [$I > 2\sigma(I)$] = 0.1853.

$L^2VO(OiPr)$

^1H NMR ($CDCl_3$): δ = 7.31 (s, 2H; Ar), 6.78 (s, 1H; Ar), 6.74 (s, 1H; Ar), 5.97 (sept, J = 6.1 Hz, 1H; C*H*(CH_3)$_2$), 4.90 (d, J = 14.6 Hz, 1H; CH_2), 4.60 (d, J = 13.7 Hz, 1H; CH_2), 3.58 (d, J = 14.7 Hz, 1H; CH_2), 3.16 (d, J = 13.9 Hz, 1H; CH_2), 2.96 (dt, J = 13.0, 3.3 Hz, 1H; CH_2), 2.86 (dt, J = 13.3, 3.3 Hz, 1H; CH_2), 2.67 (s, 3H; CH_3), 2.44 (s, 3H; CH_3), 2.25 (s, 6H; CH_3), 1.96 (m, 2H; CH_2), 1.45 (d, J = 6.1 Hz, 3H; CH(C*H*$_3$)$_2$), 1.43 (d, J = 6.1 Hz, 3H; CH(C*H*$_3$)$_2$). ^{13}C NMR ($CDCl_3$): δ 132.6, 132.4, 129.8, 129.6, 128.7, 128.5, 125.6, 122.2, 87.5, 66.5, 63.4, 53.7, 51.0, 48.9, 47.4, 25.3, 24.5, 20.3, 20.3 ppm. Anal. calcd for $C_{23}H_{31}Br_2N_2O_4V$: C, 45.27; H, 5.12; N, 4.59. Found: C, 45.38; H, 4.70; N, 4.62%.

$L^3VO(OiPr)$

^1H NMR ($CDCl_3$): δ = 7.12 (s, 2H; Ar), 6.73 (s, 1H; Ar), 6.70 (s, 1H; Ar), 5.94 (sept, J = 6.1 Hz, 1H; C*H*(CH_3)$_2$), 4.87 (d, J = 14.7 Hz, 1H; CH_2), 4.61 (d, J = 13.6 Hz, 1H; CH_2), 3.59 (d, J = 14.7 Hz, 1H; CH_2), 3.16 (d, J = 13.8 Hz, 1H; CH_2), 2.97 (dt, J = 13.1, 3.4 Hz, 1H; CH_2), 2.86 (dt, J = 13.3, 3.4 Hz, 1H; CH_2), 2.66 (s, 3H; CH_3), 2.44 (s, 3H; CH_3), 2.25 (s, 3H; CH_3), 2.24 (s, 3H; CH_3), 1.97 (m, 2H; CH_2), 1.45 (d, J = 6.1 Hz, 3H; CH(C*H*$_3$)$_2$), 1.43 (d, J = 6.1 Hz, 3H; CH(C*H*$_3$)$_2$). ^{13}C NMR ($CDCl_3$): δ 129.7, 129.4, 129.2, 129.1, 128.0, 127.8, 126.0, 122.4, 87.4, 66.4, 63.2, 53.8, 51.0, 48.6, 47.3, 25.0, 24.4, 20.5, 20.4 ppm. Anal. calcd for $C_{23}H_{31}Cl_2N_2O_4V$: C, 52.99; H, 5.99; N, 5.37. Found: C, 52.77; H, 5.70; N, 5.42%.

$L^4VO(OiPr)$

^1H NMR ($CDCl_3$): δ = 6.99 (d, J = 8.2 Hz 2H; Ar), 6.83 (s, 1H; Ar), 6.80 (s, 1H; Ar), 6.71 (d, J = 8.2 Hz 1H; Ar), 6.64 (d, J = 8.2 Hz 1H; Ar), 5.68 (sept, J = 6.1 Hz, 1H; C*H*(CH_3)$_2$), 4.82 (d, J = 14.6 Hz, 1H; CH_2), 4.63 (d, J = 13.6 Hz, 1H; CH_2), 3.59 (d, J = 14.7 Hz, 1H; CH_2), 3.12 (d, J = 13.6 Hz, 1H; CH_2), 3.03 (dt, J = 12.9, 3.1 Hz, 1H; CH_2), 2.90 (dt, J = 13.0, 2.9 Hz, 1H; CH_2), 2.61 (s, 3H; CH_3), 2.44 (s, 3H; CH_3), 2.28 (s, 3H; CH_3), 2.27 (s, 3H; CH_3), 1.95 (m, 2H; CH_2), 1.43 (d, J = 6.1 Hz, 3H; CH(C*H*$_3$)$_2$), 1.41 (d, J = 6.1 Hz, 3H; CH(C*H*$_3$)$_2$). ^{13}C NMR ($CDCl_3$): δ 130.6, 129.3, 129.2, 129.1, 129.0, 127.9, 125.0, 121.1, 85.7, 66.5, 63.5, 54.1, 51.0, 48.8, 47.5, 24.6, 24.5, 20.7, 20.6 ppm. Anal. calcd for $C_{23}H_{33}N_2O_4V$: C, 61.05; H, 7.35; N, 6.19. Found: C, 61.52; H, 7.41; N, 6.15%.

$L^5VO(OiPr)$

^1H NMR ($CDCl_3$): δ = 7.30 (m, 2H; Ar), 6.93 (d, J = 2.5 Hz, 1H; Ar), 6.89 (d, J = 2.6 Hz, 1H; Ar), 6.02 (sept, J = 6.1 Hz, 1H; C*H*(CH_3)$_2$), 4.84 (d, J = 14.7 Hz, 1H; CH_2), 4.55 (d, J = 13.9 Hz, 1H; CH_2), 3.61 (d, J = 14.9 Hz, 1H; CH_2), 3.18 (d, J = 14.1 Hz, 1H; CH_2), 2.94 (dt, J = 13.1, 3.4 Hz, 1H; CH_2), 2.84 (dt, J = 13.5, 3.4 Hz, 1H; CH_2), 2.67 (s, 3H; CH_3), 2.43 (s, 3H; CH_3), 2.05 (m, 2H; CH_2), 1.44 (d, J = 6.1 Hz, 3H; CH(C*H*$_3$)$_2$), 1.43 (d, J = 6.1 Hz, 3H; CH(C*H*$_3$)$_2$). ^{13}C NMR ($CDCl_3$): δ 129.0, 128.7, 128.1, 127.1, 126.7, 123.6, 123.2, 122.5, 89.0, 65.8, 62.8, 53.8, 51.2, 48.8, 47.3, 24.9, 24.5 ppm. Anal. calcd for $C_{21}H_{25}Cl_4N_2O_4V$: C, 44.86; H, 4.48; N, 4.98. Found: C, 44.95; H, 4.38; N, 5.07.

$L^6VO(OiPr)$

^1H NMR ($CDCl_3$): δ = 7.13 (m, 2H; Ar), 7.00 (d, J = 2.7 Hz, 1H; Ar), 6.97 (d, J = 2.6 Hz, 1H; Ar), 6.73 (d, J = 8.6 Hz, 1H; Ar), 6.66 (d, J = 8.7 Hz, 1H; Ar), 5.79 (sept, J = 6.1 Hz, 1H; C*H*(CH_3)$_2$), 4.78 (d, J = 14.7 Hz, 1H; CH_2), 4.57 (d, J = 13.7 Hz, 1H; CH_2), 3.60 (d, J = 14.8 Hz, 1H; CH_2), 3.13 (d, J = 13.8 Hz, 1H; CH_2), 2.98 (dt, J = 13.6, 3.4 Hz, 1H; CH_2), 2.84 (dt, J = 13.9, 3.5 Hz, 1H; CH_2), 2.61 (s, 3H; CH_3), 2.42 (s, 3H; CH_3), 2.02 (m, 2H; CH_2), 1.43 (d, J = 6.1 Hz, 3H; CH(C*H*$_3$)$_2$), 1.40 (d, J = 6.1 Hz, 3H; CH(C*H*$_3$)$_2$). ^{13}C NMR ($CDCl_3$): δ 129.7, 128.8, 128.5, 128.4, 126.2, 124.0, 123.2, 122.8, 87.3, 65.8, 63.0, 54.1, 51.2, 48.9, 47.5, 24.7, 24.5 ppm. Anal. calcd for $C_{21}H_{27}Cl_2N_2O_4V$: C, 51.13; H, 5.52; N, 5.68. Found: C, 51.06; H, 5.29; N, 5.48.

$L^7VO(OiPr)$

^1H NMR ($CDCl_3$): δ = 7.29 (d, J = 2.6 Hz, 1H; Ar), 7.26 (d, J = 2.4 Hz, 1H; Ar), 6.89 (d, J = 2.5 Hz, 1H; Ar), 6.79 (d, J = 2.6 Hz, 1H; Ar), 5.49 (sept, J = 6.1 Hz, 1H; C*H*(CH_3)$_2$), 4.84 (d, J = 14.4 Hz, 1H; CH_2), 4.44 (d, J = 13.4 Hz, 1H; CH_2), 3.68 (d, J = 14.6 Hz, 1H; CH_2), 3.22 (dt, J = 13.0, 3.5 Hz, 1H; CH_2), 3.14 (d, J = 13.5 Hz, 1H; CH_2), 3.05 (dt, J = 13.3, 3.5 Hz, 1H; CH_2),

2.58 (s, 3H; N CH_3), 2.53 (s, 3H; CH_3), 2.04 (m, 2H; CH_2), 1.54 (s, 9H; *t*Bu), 1.48 (d, J = 6.1 Hz, 3H; $CH(CH_3)_2$), 1.47 (s, 9H; *t*Bu), 1.31 (s, 9H; *t*Bu), 1.30 (s, 9H; *t*Bu), 1.19 (d, J = 6.1 Hz, 3H; $CH(CH_3)_2$). ^{13}C NMR ($CDCl_3$): δ 141.0, 140.4, 124.8, 124.1, 123.4, 123.4, 122.9, 120.7, 83.7, 67.4, 64.8, 55.3, 51.8, 50.0, 48.8, 35.5, 35.3, 34.4, 34.3, 31.9, 31.8, 31.1, 30.8, 25.9, 24.4 ppm. Anal. calcd for $C_{37}H_{61}N_2O_4V$: C, 68.49; H, 9.48; N, 4.32. Found: C, 68.48; H, 9.53; N, 4.45.

$(L^1VO)_2\mu$-O, product of the hydrolysis of $L^1VO(OiPr)$

To a stirred solution of $L^1VO(OiPr)$ (0.52 mmol, 0.25 gr) in THF (4 ml) were added 1000 equivalents (0.52 mol) of distilled water. More THF was added as required. The reaction was stirred at room temperature for 3 days; afterwards the volatiles were removed by evaporation. The product can be recrystallized from a cold pentane–toluene mixture (60%). HRMS (ESI): *m*/*z* calcd for $C_{44}H_{60}N_4O_7V_2+H^+$: 859.3414 [M + H^+]; found: 859.3424. Anal. calcd for $C_{44}H_{60}N_4O_7V_2$: C, 61.46; H, 7.15; N, 6.52. Found: C, 61.69; H, 6.83; N, 6.50%.

Crystal data for $(L^1VO)_2\mu$-O

Crystallized from cold pentane–toluene mixture (dark blue crystals); $C_{44}H_{60}N_4O_7V_2{\cdot}2(C_7H_8)$, M = 1035.04, monoclinic, a = 11.704(1), b = 24.115(3), c = 19.876(2) Å, V = 5496(1) Å^3, T = 173(1) K, space group C_2/c, Z = 4, μ(Mo Kα) = 0.394 mm^{-1}, 29 432 reflections measured, 6544 unique (R_{int} = 0.0576). $R(F_o^2)$ for $[I > 2\sigma(I)]$ = 0.1199, R_w for $[I > 2\sigma(I)]$ = 0.2448.

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