

New VIV, VIVO, VVO, and VVO₂ Systems: Exploring their Interconversion in Solution, Protein Interactions, and Cytotoxicity

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1 **New V^{IV}, V^{IV}O, V^VO and V^VO₂ Systems:**
2 **Exploring their Interconversion in Solution,**
3 **Protein Interactions and Cytotoxicity**

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1 ABSTRACT

2 The synthesis and characterization of one oxidoethoxidovanadium(V) [$V^{VO}(L^1)(OEt)$] (**1**)
3 and two non-oxidovanadium(IV) complexes, [$V^{IV}(L^{2-3})_2$] (**2** and **3**), with aroylhydrazone
4 ligands incorporating naphthalene moieties, are reported. The synthesized oxido and non-
5 oxido vanadium complexes are characterized by various physicochemical techniques and
6 their molecular structures are solved by single crystal X-ray diffraction (SC-XRD). This
7 revealed that in **1** the geometry around the vanadium atom corresponds to a distorted square
8 pyramid, with a O_4N coordination sphere, whereas that of the two non-oxido V^{IV} complexes
9 **2** and **3** corresponds to a distorted trigonal prismatic arrangement with a O_4N_2 coordination
10 sphere around each “bare” vanadium center. In aqueous solution, the V^{VO} moiety of **1**
11 undergoes a change to V^{VO}_2 species, yielding [$V^{VO}_2(L^1)$]⁻ (**1'**), while the non-oxido V^{IV} -
12 compounds **2** and **3** are partly converted into their corresponding $V^{IV}O$ complexes,
13 [$V^{IV}O(L^{2-3})(H_2O)$] (**2'** and **3'**). Interaction of these V^{VO}_2 , $V^{IV}O$ and V^{IV} systems with two
14 model proteins, ubiquitin (Ub) and lysozyme (Lyz), is investigated through docking
15 approaches, which suggest the potential binding sites: the interaction is covalent for species
16 **2'** and **3'**, with the binding to Glu16, Glu18 and Asp21 for Ub, and His15 for Lyz, and it is
17 non-covalent for species **1'**, **2** and **3**, with the surface residues of the proteins. The ligand
18 precursors and complexes are also evaluated for their *in vitro* antiproliferative activity against
19 ovarian (A2780) and prostate (PC3) human cancer cells, and in normal fibroblasts (V79) to
20 check the selectivity of the compounds for cancer cells.

21
22
23 *Keywords:* Oxidoethoxidovanadium(V)/ Non-oxidovanadium(IV)/ Solution studies / Docking
24 studies/ Protein interaction/ Antiproliferative activity.

1 INTRODUCTION

2 Due to the prospective application of vanadium compounds as therapeutic agents,¹⁻⁵
3 vanadium coordination chemistry has been an area of extensive research. For metal-based
4 compounds to show pharmacological effects, one of the relevant aspects is their ability to
5 interact with proteins, in order to allow their storage and/or enzymatic action in organisms.^{5,6}
6 In this context, attention has been devoted towards the study of vanadium binding to proteins,
7 as interactions taking place with a number of peptides and proteins can regulate their
8 transport in blood and cells, as well as cellular uptake, and is relevant for the mechanisms of
9 action. For instance, vanadium in human blood serum is expected to be bound to human
10 serum transferrin (hTf),⁷⁻⁹ and binding to hemoglobin is also relevant once vanadium ions
11 enter red blood cells.¹⁰⁻¹² In their turn, oxido vanadates(IV,V) are well known for their insulin
12 enhancing activity, an effect that appears to be related to protein tyrosine phosphatase
13 inhibition.^{2,13-15}

14 However, proper understanding of the three-dimensional interactions of vanadium
15 compounds with most relevant biomolecules, namely cellular proteins, is lacking. Single-
16 crystal X-ray diffraction (SC-XRD) analysis and spectroscopic techniques such as nuclear
17 magnetic resonance (NMR) for diamagnetic vanadium(V) and electron paramagnetic
18 resonance (EPR) for vanadium(IV) compounds, as well as circular dichroism (CD) and mass
19 spectrometry (MS) techniques have been employed to study the interactions of metal
20 complexes with biological “receptors” at molecular level. Yet, these techniques seldom
21 provide an explicit idea about the region of the protein where the metal is bound, as well as
22 about the specific amino acid residues involved in the coordination. Therefore,
23 implementation of computational methods along with the use of the above techniques is
24 required; such approach can provide relevant information on the biological and

1 pharmacological roles of metal complexes by enabling the prediction of the nature of the
2 metallodrug–protein interactions.

3 Molecular docking can produce fast and accurate predictions of the binding of synthesized
4 metal complexes with various biomolecules and both relative affinities and geometries can be
5 calculated, which are of prime importance in drug design.^{16,17} Recently, docking techniques
6 have been extended by some of us to the field of bioinorganic chemistry improving their
7 scoring functions to evaluate the metal mediated binding.^{18–22}

8 On the other hand, the nature of the ligand is important in the development of metal
9 complexes and on their particular biological and reactivity characteristics; namely, the
10 electron donor and acceptor properties, functional groups present on its backbone and their
11 relative position in the coordination sphere are relevant aspects.^{23–25} The interest in the study
12 of hydrazone-based ligands has been increasing significantly because of the varied ligation
13 properties due to the presence of several coordination sites,²⁶ as well as due to their
14 applications in the fields of analytical²⁷ and medicinal chemistry.^{28–31}

15 Over the past few years, we have been working in the field of vanadium chemistry
16 incorporating O,N donor aroylhydrazone and azine ligands, and we have successfully isolated
17 several oxidovanadium(V),^{32–37} oxidovanadium(IV),^{38–40} mixed valence
18 oxidovanadium(IV,V)^{41–43} and some rare stable non-oxidovanadium(IV) complexes,^{44,45} and
19 accessed their solution chemistry and biological activity such as protein/DNA interaction,
20 insulin enhancing and antiproliferative activity.

21 Herein, we report the syntheses and characterization by several techniques, including SC-
22 XRD, of one oxidoethoxidovanadium(V) (**1**) and two non-oxidovanadium(IV) (**2–3**)
23 complexes incorporating three tridentate ONO containing aroylhydrazone ligands (L^{1-3})²⁻
24 derived from 2-hydroxy-1-naphthaldehyde. The observed UV-Vis and EPR spectroscopic
25 properties were further interpreted by DFT simulation studies.

1 Ligand-exchange and redox chemistry of vanadium in biological conditions, leading to
2 unpredictable pharmacokinetics have hampered the introduction of V-based drugs into
3 clinical practice.^{5,15,46-49} The biological activity of several non-oxido V^{IV}-complexes has
4 recently received attention,^{15,31,44,46,50} and for example the insulin-like properties of
5 $\{[V^{IV}(\text{catecholato})_3]^{2-}\}^{15}$ and the antiproliferative activity of $[V^{IV}(\text{dtbc})_3]^{2-}$ (dtbc = 3,5-di-
6 *tert*-butylcatecholato)⁴⁶ were ascribed to the products of their hydrolysis/oxidation, i.e. V^{IV}-
7 and V^V-oxido species. In this study, we also address the hydrolysis and redox behaviour of
8 complexes **1-3**.

9 In aqueous solution, the oxidoethoxidovanadium V^VO-complex (**1**) undergoes a change to a
10 V^VO₂ species $[V^{V}O_2(L^1)]^-$ (**1'**), while the non-oxido V^{IV}-complexes (**2** and **3**) are partly
11 converted into their corresponding V^{IV}O species, yielding $[V^{IV}O(L^2)(H_2O)]$ (**2'**) and
12 $[V^{IV}O(L^3)(H_2O)]$ (**3'**). We evaluated the interaction of these V^VO₂, V^{IV}O and V^{IV} species with
13 two model proteins, ubiquitin (Ub) and lysozyme (Lyz), using docking approaches, which
14 allowed predicting the most stable binding sites. The *in vitro* antiproliferative activity of
15 complexes **1-3** and of the corresponding ligand precursors were also examined against
16 ovarian (A2780) and prostate (PC3) human cancer cells. To check the selectivity of the
17 compounds for cancer cells, the cytotoxic studies were also performed against normal
18 fibroblasts (V79).

19

20 **EXPERIMENTAL AND COMPUTATIONAL SECTION**

21 **General Methods and Materials.** The metal precursor $[V^{IV}O(\text{acac})_2]$ was prepared
22 according to standard procedures described in the literature.⁵¹ Most of the used chemicals
23 were acquired from commercial sources and utilized without further purification; reagent
24 grade solvents were dried and distilled prior to use as described in the literature.⁵² IR spectra
25 of the compounds were recorded using a Perkin Elmer Spectrum RXI spectrophotometer. A

1 Vario ELcube CHNS elemental analyzer was used for the determination of elemental
2 analyses and PerkinElmer spectrophotometers (Lambda 25 and Lambda 35) were used to
3 record the electronic spectra. NMR spectra (^1H , ^{13}C , and ^{51}V NMR) data were recorded on
4 400 MHz Bruker Ultrashield spectrometer utilizing SiMe_4 as an internal standard to measure
5 the corresponding chemical shifts (δ_{H} and δ_{C}) in ppm. V^{VOCl_3} was used as an external
6 reference sample for ^{51}V NMR to measure the chemical shifts (δ_{V}), also expressed in ppm.
7 EPR spectra were recorded in dichloromethane (DCM), in aqueous solution or in a phosphate
8 buffer, from 0 to 8000 Gauss at liquid nitrogen temperature (77 K), with an X-band Bruker
9 EMX spectrometer equipped with a HP 53150A microwave frequency counter. The
10 microwave frequency was in the range 9.40-9.41 GHz, microwave power was 20 mW, time
11 constant was 81.92 ms, modulation frequency 100 kHz, modulation amplitude 0.4 mT,
12 resolution 4096 points. To extract the experimental spin Hamiltonian parameters, the spectra
13 were generated with WinEPR SimFonia⁵³ using a linewidth for the x , y and z axes of 12, 12
14 and 7-8 Gauss and a ratio Lorentzian/Gaussian of 1.

15 The cyclic voltammograms of complexes **1–3** were obtained under N_2 from solutions of the
16 complexes in DCM using tetrabutylammonium tetrafluoroborate (Bu_4NBF_4) 0.10 M as
17 electrolyte. A three compartment cell provided with platinum wire electrodes (working and
18 secondary) and a silver wire (reference) electrode interfaced with a VoltaLab PST050
19 equipment was used, and data were acquired by a Pentium(R) Dual-Core computer. The
20 potentials were measured in Volt (± 10 mV) *versus* SCE at 200 mV/s using $[\text{Fe}(\eta^5\text{-C}_5\text{H}_5)_2]^{0/+}$
21 ($E_{1/2}^{\text{red}} = 0.475$ V, DCM) or camphorquinone ($E_{1/2}^{\text{red}} = -1.34$ V, DCM) as internal reference. A
22 LCQ FleetTM Ion Trap Mass Spectrometer from Thermo Scientific was used to measure
23 Electrospray Ionisation Mass Spectra (ESI-MS) of aqueous/methanolic solutions of
24 complexes **1** and **2**, in the negative- and positive-ion mode, for characterization of their

1 aqueous solution behavior. In the case of complex **2** a H₂O/MeOH/acetonitrile solution was
2 also used.

3
4 **Synthesis of the ligand precursors (H₂L¹⁻³).** The aroylhydrazone Schiff base compounds,
5 H₂L¹⁻³ (see **Scheme 1**), were synthesized by the condensation of 2-hydroxy-1-
6 naphthaldehyde in ethanol with their respective acid hydrazides (for H₂L¹ = 3-hydroxy-2-
7 naphthoic hydrazide, H₂L² = salicyloyl hydrazide and H₂L³ = anthranilic acid hydrazide) in
8 equimolar proportions.⁵⁴ The resultant yellowish compounds were filtered, washed with hot
9 water and dried over CaCl₂. Elemental analysis, NMR (¹H and ¹³C) and IR data for all
10 compounds verified their composition. Characterization of H₂L¹ is listed below, whereas that
11 of H₂L²⁻³ was reported earlier.⁵⁴

12 **H₂L¹.** Yield: 71%. Anal. calcd for C₂₂H₁₆N₂O₃: C, 74.15; H, 4.53; N, 7.86. Found: C, 74.03;
13 H, 4.61; N, 7.59. IR (KBr pellet, cm⁻¹): 3489 ν(O–H); 3059 ν(N–H); 1649 ν(C=O);
14 1599 ν(C=N). ¹H NMR (400 MHz, DMSO-*d*₆, ppm): δ_H 11.79 (s, 1H, naphthoyl
15 hydrazide–OH), 11.65 (s, 1H, OH), 10.91 (s, 1H, NH), 10.03 (s, 1H, HC=N), 8.67–7.22 (m,
16 12H, aromatic). ¹³C NMR (100 MHz, DMSO-*d*₆, ppm): δ_C 166.8, 155.8, 154.2, 153.1, 135.7,
17 132.8, 130.3, 129.9, 129.0, 128.1, 127.7, 127.0, 126.4, 125.1, 124.5, 124.0, 123.5, 121.6,
18 119.7, 118.7, 111.2, 110.4.

19 **Synthesis of the oxidoethoxidovanadium(V) compound [V^VO(L¹)(OEt)] (**1**).** The metal
20 precursor [V^{IV}O(acac)₂] was added to a hot ethanolic (30 mL) solution of H₂L¹ in a
21 stoichiometric ratio and refluxed for 3 h. The resulting brownish mixture was filtered and the
22 filtrate was kept for crystallization by slow evaporation. After 4 days, single crystals suitable
23 for SC-XRD structure determination were isolated from the filtrate. An outline of synthesis
24 of this V^VO complex is depicted in **Scheme 1**.

1 **[V^{VO}(L¹)(OEt)]**. Yield: 63%. Anal. calcd for C₂₄H₁₉N₂O₅V: C, 61.81; H, 4.11; N, 6.01.
2 Found: C, 61.77; H, 4.08; N, 6.37. IR (KBr pellet, cm⁻¹): 3436 ν(O–H), 1597 ν(C=N), 1244
3 ν(C–O)_{enolic}, 982 ν(V=O). UV-vis (DCM) [λ_{max} , nm (ϵ , M⁻¹ cm⁻¹)]: 445 (1966), 356 (4912),
4 309 (4613). ¹H NMR (400 MHz, DMSO-*d*₆, ppm): δ_{H} 11.23 (s, 1H, naphthoyl
5 hydrazide–OH), 10.02 (s, 1H, HC=N), 8.67–7.19 (m, 12H, aromatic), 3.43 (m, 2H, CH₂
6 (OEt)), 1.06 (t, 3H, CH₃ (OEt)). ¹³C NMR (100 MHz, DMSO-*d*₆, ppm): δ_{C} 164.6, 161.4,
7 154.7, 143.8, 139.5, 136.3, 135.8, 131.8, 130.5, 129.5, 129.1, 128.7, 127.2, 126.2, 125.5,
8 124.2, 122.9, 120.5, 119.4, 115.6, 111.0, 56.5, 18.9. ⁵¹V NMR (DMSO-*d*₆, ppm): δ_{V} -552.5.

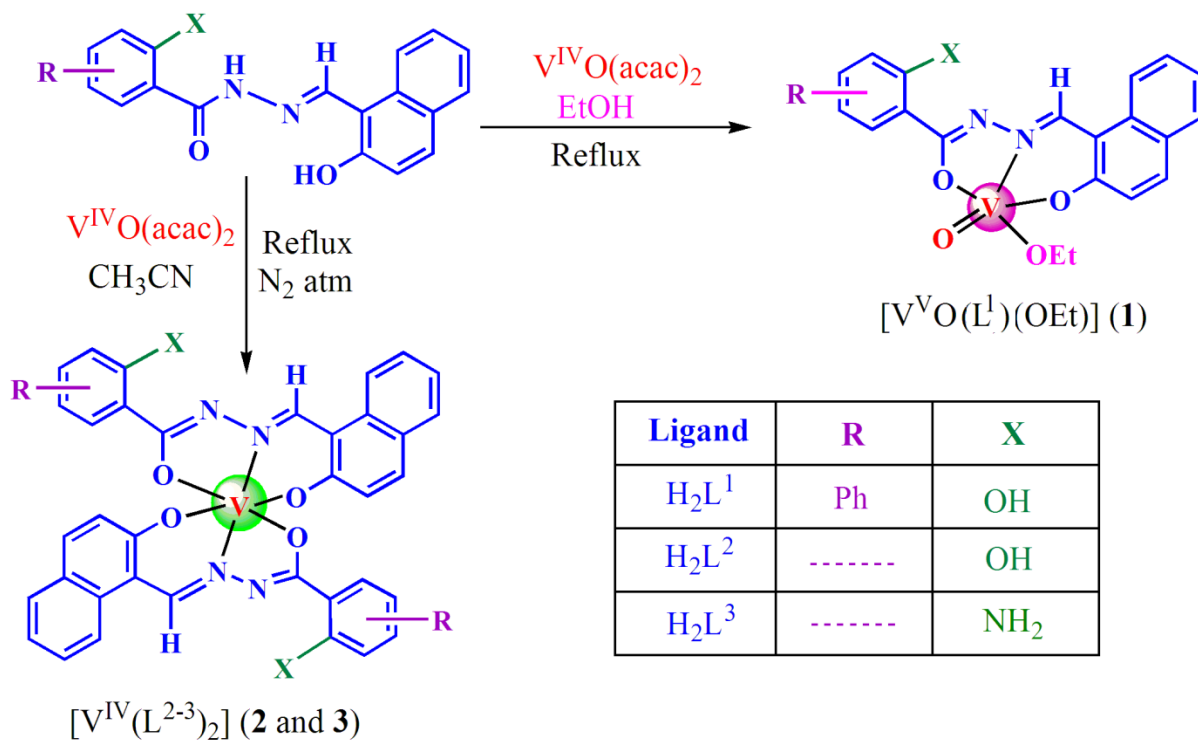
9
10 **Synthesis of non-oxidovanadium(IV) complexes [V^{IV}(L²⁻³)₂] (2 and 3)**. Ligand precursors
11 (H₂L² or H₂L³) (2 mmol) were added to a hot CH₃CN (30 mL) solution of the metal
12 precursor [V^{IV}O(acac)₂] (1 mmol) under nitrogen atmosphere.⁴⁴ The color immediately
13 changed to greenish black and the mixture was allowed to reflux for 3 h. Greenish black
14 crystals of **2** were directly obtained from the reaction mixture, while a greenish black
15 microcrystalline compound was obtained in case of **3**, which was recrystallized in CH₂Cl₂ to
16 obtain [V^{IV}(L³)₂]·2CH₂Cl₂. The crystals were then filtered off, washed thoroughly with
17 ethanol, and dried over CaCl₂. **Scheme 1** depicts an outline of the steps to obtain the non-
18 oxidovanadium(IV) complexes.

19 **[V^{IV}(L²)₂]**. Yield: 73%. Anal. Calcd. for C₃₆H₂₄N₄O₆V: C, 65.56; H, 3.67; N, 8.49. Found: C,
20 65.59; H, 3.64; N, 8.44. IR (KBr pellet, cm⁻¹): 3321 ν(O–H), 1609 ν(C=N), 1234 ν(C–
21 O)_{enolic}. UV–Vis (DCM) [λ_{max} , nm (ϵ , M⁻¹ cm⁻¹)]: 654 (780), 544 (989), 381 (2855), 335
22 (3979).

23 **[V^{IV}(L³)₂]·2CH₂Cl₂**. Yield: 69%. Anal. Calcd. for C₃₈H₃₀Cl₄N₆O₄V: C, 55.16; H, 3.65; N,
24 10.16. Found: C, 55.19; H, 3.72; N, 10.22. IR (KBr pellet, cm⁻¹): 1652 ν(C=N), 1242 ν(C–

1 O_{enolic}. UV-Vis (DCM) [λ_{max} , nm (ϵ , M⁻¹ cm⁻¹): 642 (978), 546 (1384), 398 (3074), 324
 2 (3793).

3



4

5 **Scheme 1.** Schematic representation of the synthesis of $[V^V O(L^1)(OEt)]$ (**1**) and $[V^{IV}(L^{2-3})_2]$
 6 (**2** and **3**) compounds.

7

8 X-ray Crystallography

9 A red needle shaped prism of **1** (size: 0.60×0.02×0.01 mm³), and black prisms of **2**
 10 (0.22×0.19×0.07 mm³) and **3** (0.25×0.21×0.12 mm³) were each mounted on a loop with oil.
 11 The X-ray diffraction data of **1** and **3** were collected at 19 °C on a Bruker APEX II single
 12 crystal X-ray diffractometer and at -143 °C on Nonius Kappa CCD FR590 single crystal X-
 13 ray diffractometer, respectively, using Mo K α radiation. Diffraction data of **2** were collected
 14 at -173 °C on a SuperNova diffractometer (Rigaku) with a CCD detector and CuK α radiation.
 15 The data of **1** was integrated and scaled using SAINT, SADABS within the APEX2 software
 16 package by Bruker,⁵⁵ and the data of **3** was integrated and scaled using hkl-SCALEPACK.⁵⁶

1 This program applies a multiplicative correction factor (S) to the observed intensities (I) and
2 has the following form: $S = (e^{-2B(\sin^2 \theta) / \lambda^2}) / \text{scale}$, where S is calculated from the scale and the
3 B factor determined for each frame and is then applied to I to give the corrected intensity
4 (I_{corr}).

5 The structures of **1–3** were solved by direct methods (SHELXS, SIR97⁵⁷ SHELXT⁵⁸) to
6 produce complete heavy atom phasing model consistent with the proposed structures and then
7 completed by difference Fourier synthesis with SHELXL97⁵⁹ and SHELXL.⁶⁰ Indexing and
8 unit cell refinement indicated that complex **1** crystallizes in orthorhombic space group $Pnca$
9 2_1 , complex **2** in monoclinic space group $C2/c$, and complex **3** in triclinic space group $P\bar{1}$.

10 **Table S1** contains all the crystallographic data as well as structure solution and refinement
11 details of **1–3**. The observed data / R(int) = 5139, 0.0969 (**1**), 3152, 0.041 (**2**) and 8583,
12 0.1204 (**3**) statistics indicate that the data were appropriate. Structure refinements were
13 carried out on F^2 , applying Scattering factors from Waasmaier and Kirfel.⁶¹ All hydrogen
14 atoms were placed in geometrically idealized positions and constrained to ride on their parent
15 atoms with C···H distances in the range 0.95-1.00 Å. Isotropic thermal parameters U_{eq} were
16 fixed such that they were 1.2 U_{eq} of their parent atom U_{eq} for CH's, and 1.5 U_{eq} of their
17 parent atom U_{eq} in case of methyl groups. Hydroxyl hydrogen atoms were freely refined
18 without any constraints or restraints. All non-hydrogen atoms were refined anisotropically by
19 full-matrix least-squares. Disordered solvent within a void of 76 Å³ in **2** required to use the
20 solvent mask procedure implemented in OLEX2.⁶² Crystallographic data integrity was
21 validated with PLATON.⁶³ CCDC numbers 1976254 (**1**), 1961784 (**2**), and 1976255 (**3**)
22 contain the supplementary crystallographic data for these compounds. These data can be
23 obtained free of charge from the Cambridge Crystallographic Data Centre via
24 www.ccdc.cam.ac.uk/data_request/cif.

25

1

2 **Computational studies**

3 The geometry of V complexes was optimized and harmonic frequencies were computed with
4 Gaussian 09 software⁶⁴ at DFT theory level, combining the B3P86 functional^{65,66} with the
5 Pople basis set 6-311g. This method has been successfully applied and discussed in the
6 literature for the geometry prediction of vanadium complexes.^{67,68} DFT stability calculations
7 in solution have been carried out with the SMD continuum model⁶⁹ for water and including
8 diffuse and polarization functions (basis set 6-311++g(d,p)) for all atoms. The ⁵¹V hyperfine
9 coupling tensor A was calculated with ORCA software⁷⁰ on the optimized structures with the
10 double hybrid functional B2PLYP⁷¹ and the basis set VTZ. The ⁵¹V HFC tensor A was
11 calculated from the contributions of the isotropic Fermi contact (A^{FC}), dipolar hyperfine
12 interaction (A^D) and spin-orbit (SO) term (A^{SO}).^{72,73} The percent deviation (PD) of the
13 absolute calculated value, $|A_z|^{calcd}$, from the absolute experimental value, $|A_z|^{exptl}$, was obtained
14 as follows: $100 \times [(|A_z|^{calcd} - |A_z|^{exptl}) / |A_z|^{exptl}]$.

15 Time-dependent density functional theory (TD-DFT) calculations⁷⁴ were used to predict the
16 excited-states and electronic absorption spectrum of the non-oxido complex **3**. The
17 simulations were carried out on the geometry optimized in the gas phase using CAM-B3LYP
18 functional and 6-31+g(d) basis set, according to the method established previously.^{45,75} The
19 predicted electronic spectrum of **3** was generated using Gabedit software.⁷⁶

20 The docking calculations were performed with GOLD 5.8 software⁷⁷ according to the
21 procedures recently reported^{18,20-22,78-80} to study the possible binding to ubiquitin and
22 lysozyme of all the enantiomers (both clockwise and anticlockwise enantiomers were
23 considered) of non-oxido complexes **2** and **3** and oxidovanadium species $[V^VO_2(L^1)]^-$ (**1'**)
24 derived from **1**, and $[V^{IV}O(L^{2-3})(H_2O)]$ (**2'** and **3'**) derived from **2-3**. $[V^VO_2(L^1)]^-$ (**1'**) and
25 $[V^{IV}(L^{2-3})_2]$ (**2**, **3**) could bind to Ub and Lyz only through second coordination sphere

1 interactions (non-covalent binding), while for oxidovanadium(IV) fragments **2'** and **3'** the
2 binding may occur in an accessible equatorial position upon the replacement of monodentate
3 H₂O ligand (covalent binding). With the aim to individuate the most exposed potential
4 coordinating residues, the relative Solvent Excluded Surface (SES) of Ub and Lyz was
5 computed.⁸¹ Docking calculations were carried out building an evaluation sphere of 12 Å
6 containing the potential amino acid donors identified by SES analysis. Surface dockings in
7 the systems with non-covalent interactions between **1'**, **2** and **3** and Ub and Lyz were
8 performed considering the whole rigid protein, while for the docking simulations with
9 covalent bonds vanadium–protein (**2'** and **3'**), the side chains flexibility was taken into
10 account using the rotamers libraries implemented in GOLD.⁸² Genetic algorithm (GA)
11 parameters have been set in 50 GA runs and a minimum of 100,000 operations. The other
12 parameters of GA were set to default. The scoring (*Fitness* of GoldScore) was evaluated
13 through the recent validated versions of GoldScore accounting for coordination bonds and
14 surface interactions.²⁵ The best solutions (binding poses) were evaluated taking into account
15 the mean (F_{mean}) and the highest value (F_{max}) of the scoring and population of the cluster
16 containing the best pose.

17

18 **Determination of cytotoxic activity**

19 The cytotoxicity of the ligand precursors and their corresponding vanadium complexes was
20 evaluated in ovarian (A2780) and prostate (PC3) cancer cells and in normal fibroblasts (V79).
21 All cell lines were grown in RPMI medium supplemented with 10% FBS and maintained at
22 37 °C in an incubator (Heraeus, Germany) with 5% CO₂ and humidified atmosphere. MTT
23 assay was used to assess the cellular viability.^{37,83} For the assays, cells were seeded in 96-well
24 plates, at a density of $1-2 \times 10^4$ cells/200 μL medium, and allowed to adhere overnight. Ligand
25 and complexes were first diluted in DMSO and then in culture medium to prepare a series of

1 dilutions in the range of 10^{-7} – 10^{-4} M. The maximum concentration of DMSO in the medium
2 was 1% and, at this percentage, no cytotoxic effect was observed due to DMSO. After
3 incubation for 48 h with the compounds, the medium was discarded and 200 μ L of MTT
4 solution in PBS (0.5 mg/mL) were added to each well. After 3 h at 37 °C, the medium was
5 removed and replaced by 200 μ L DMSO to solubilise the formazan crystals. The cellular
6 viability (% control) was assessed by measuring the absorbance at 570 nm using a plate
7 spectrophotometer (Power Wave Xs, Bio-Tek). The IC₅₀ values were calculated using the
8 GraphPad Prism software (version 5.0). Results are shown as the mean \pm SD of at least two
9 independent experiments done with six replicates each.

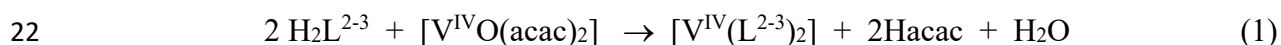
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11 **RESULTS AND DISCUSSION**

12 **Synthesis**

13 **Scheme 1** outlines the synthesis of monomeric oxidoethoxidovanadium(V) ($V^V O$) (**1**) and
14 non-oxidovanadium(IV) (V^{IV}) (**2** and **3**) complexes. Complex **1** was synthesized by refluxing
15 the metal precursor [$V^{IV} O(acac)_2$] in a hot ethanolic solution of aroylhydrazone H_2L^1 in
16 stoichiometric amounts (1:1 mol ratio). Under reflux conditions, V^{IV} was oxidized by the
17 aerial oxygen and an ethoxido coordinated vanadium(V) complex was isolated as final
18 product.

19 In contrast, the reaction of [$V^{IV} O(acac)_2$] with H_2L^{2-3} in CH_3CN medium under N_2
20 atmosphere in 1:2 mol ratio led to the formation of the non-oxidovanadium [$V^{IV}(L^{2-3})_2$]
21 (compounds **2–3**). This reaction can be represented by equation (1).



23

24

25

1 IR Spectroscopy

2 Most of the IR spectral information is summarized in the Experimental Section. The ligands
3 (H_2L^{1-3}) show a band in the range 3489–3320 cm^{-1} due to the aromatic –OH stretching
4 frequency present in the naphthalene moiety, which is absent in their corresponding
5 complexes because of deprotonation upon coordination. However, **1** and **2** possess a band in
6 the range 3436–3321 cm^{-1} , for the uncoordinated –OH group attached to the aroylhydrazide
7 moiety of their corresponding ligands.^{36,40} The free ligands also exhibit characteristic
8 stretching bands in the region 3059–3022 (ν_{N-H}) and 1649–1637 ($\nu_{C=O}$) cm^{-1} for their amide
9 moiety, which are not observed in the spectra of their respective complexes (**1–3**).
10 Additionally, the appearance of a new bands in the complex within 1244–1234 cm^{-1} , due to
11 the stretching vibration of the conjugated $C=N-N=C-O^-$ moiety, clearly indicate the
12 coordination of the ligands in their deprotonated enolic form.^{45,84–87} Moreover, the presence
13 of one sharp band at 982 cm^{-1} for **1**, due to the $\nu(V=O)$ stretching, indicates the mono-oxido
14 nature of the complex,^{33,34,43,88} while its absence in the spectra of **2** and **3** agrees with the
15 presence of “bare” vanadium centers.^{44,45,75}

16

17 Description of the molecular structures of 1–3

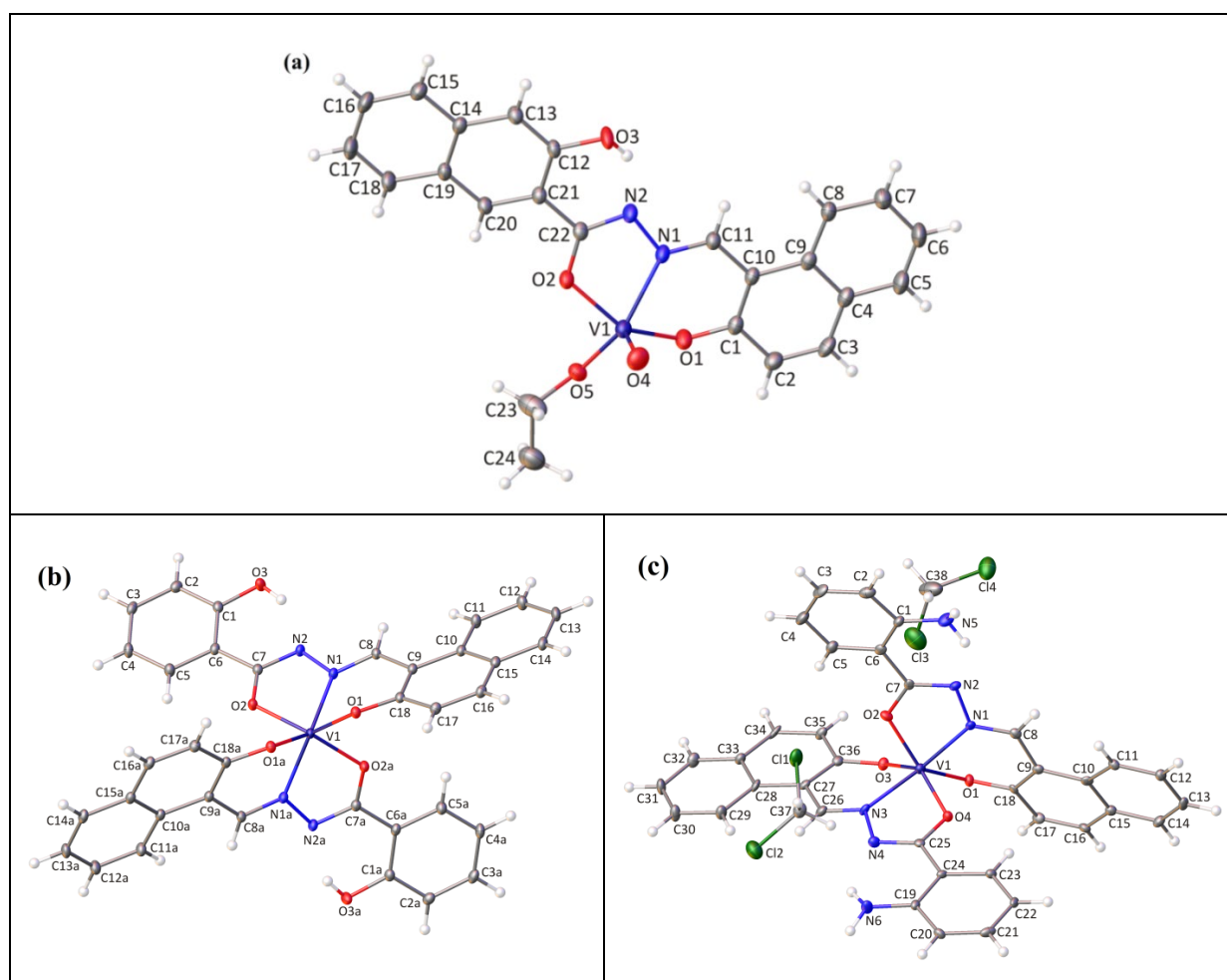
18 Detailed information on the stereochemistry of the complexes, in particular the coordination
19 mode of the ligands, and confirmation of the non-oxido nature of compounds **2** and **3** was
20 gathered through single-crystal X-ray diffraction experiments, which allowed to obtain the
21 molecular structures of $[V^V O(L^1)(OEt)]$ (**1**), $[V^{IV}(L^2)_2]$ (**2**) and $[V^{IV}(L^3)_2] \cdot 2CH_2Cl_2$ (**3**).
22 **Figure 1** shows the molecular structures of one of the molecules of each asymmetric unit of
23 complexes **1–3**, and selected bond distances and angles are collected in **Table S2A**.

24 Complex **1** (**Figure 1a**) corresponds to discrete units of monomeric $V^V O(OEt)$ -species
25 having a $V^V O^{3+}$ moiety, where the V^V -center is in a five-coordinated distorted square

1 pyramidal $V^V O_4 N$ geometry. The basal plane is made up of the naphtholate oxygen (O1),
2 imine nitrogen (N1), enolic oxygen (O2) of the tridentate binegative ONO-donor
3 aroylhydrazone ligand (L^1), and the oxygen (O5) from an alkoxide, which came from the
4 ethanol used as solvent. The remaining apical position of the square pyramid is occupied by
5 the oxido ligand (O4). This structure also revealed different V–O bond lengths in the
6 following order: V1–O4 of the oxido moiety (1.570(2) Å) < V–O5 of EtO^- (1.748(2) Å) <
7 V–O1 of naphtholate (1.843(2) Å) < V–O2 of enolate (1.930(19) Å), as usually observed in
8 such oxidoalkoxidovanadium complexes.^{36,43,45,89} There is a small disorder in the EtO^-
9 ligand; the crystal packing diagrams show that the pending hydroxyl O3 atom, attached with
10 the naphthalene moiety of hydrazide from a neighboring molecule, interacts weakly with the
11 vanadium, as depicted in **Figure S1**.

12 The molecular structures of $[V^{IV}(L^2)_2]$ (**2**) and $[V^{IV}(L^3)_2] \cdot 2CH_2Cl_2$ (**3**) are similar, (**Figure**
13 **1b-c**), and some selected geometric parameters are given in **Table S2A**. The molecular
14 structures contain a V^{IV} -center coordinated by a pair of doubly deprotonated tridentate ONO-
15 donor aroylhydrazones. Each of the ligands bind the V-atom *via* the naphtholate-O, imine-N
16 and enolic-O atoms of the tridentate binegative ONO-donor aroylhydrazones. In summary,
17 the vanadium atoms comprise trigonal prismatic $V-N_2O_4$ coordination geometries where each
18 of the hydrazone ligands is forming a five-membered CN_2OV and a six-membered C_3NOV
19 chelate ring. Within the crystal lattice of molecule **2** (**Figure 1b**), asymmetric unit contains
20 half of the mononuclear non-oxidovanadium(IV) complex. The vanadium cation is located on
21 a special position, namely crystallographic two-fold axis which generates the complete
22 mononuclear complex molecule. However, such type of symmetry is not observed in case of
23 **3**. Additionally, there are two dichloromethane solvent molecules of crystallization present in
24 the crystal lattice of **3**, as shown in **Figure 1c**.

1 The observed V–O and V–N bond lengths around the central vanadium atom and angles for **2**
2 and **3** are within the expected range as reported earlier in the literature.^{44,45}
3 The crystal structure of non-oxidovanadium(IV) complexes (**2** and **3**) revealed a near perfect
4 trigonal prismatic coordination geometry of the vanadium. The two opposing triangular faces
5 (consisting of O1, O2 and N1 and their symmetry related ones) are rotated off each other by
6 about 2.17(4)° (for complex **2**) and 6.70(7)° (for complex **3**), but this is within the margins by
7 which the triangular angles deviate from 60° [(63.04°, 57.95°, and 59.01°) (**2**) and
8 (61.07/62.38°, 58.39/58.02° and 60.54/59.60° (**3**)]. The corner lengths of the trigonal prism
9 deviate from each other by ca. 0.13 Å for both the complexes (**2** and **3**). The corresponding
10 geometrical parameters are also listed in **Table S2B**.

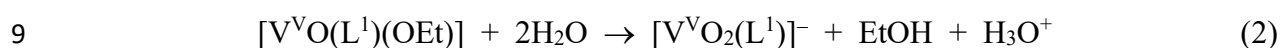


11 **Figure 1.** Molecular structures of (a) $[V^VO(L^1)(OEt)]$ (**1**), (b) $[V^{IV}(L^2)_2]$ (**2**) and (c)
12 $[V^{IV}(L^3)_2] \cdot 2CH_2Cl_2$ (**3**), drawn with thermal ellipsoids at the 50% probability level.

1 ESI Mass Spectrometry

2 Electrospray Ionisation Mass Spectra (ESI-MS) of water:methanol (ca. 90:10) solutions of
3 complex **1** were recorded, in the negative- and positive-ion mode, for characterization of their
4 aqueous solutions; the corresponding ESI-MS spectra are depicted in **Figures S2-S3** and
5 **Tables S3-S4**.

6 From the ESI-MS spectra recorded in the negative-ion mode it is clear that upon dissolving **1**,
7 the complex is converted to a $V^V O_2(L)$ -type complex (peak at $m/z = 437.34$, 100%). Thus the
8 process occurring is:



10 In principle, the $[V^V O_2(L^1)]^-$ species formed can be 5- or 6-coordinated with an additional
11 water molecule bound to vanadium; in fact, a monodentate solvent molecule could have been
12 removed from the first metal coordination sphere during the ionization process.^{78,90,91} To
13 consider this possibility, the relative stability of $[V^V O_2(L^1)]^-$ and $[V^V O_2(L^1)(H_2O)]^-$ was
14 calculated by DFT methods modeling the solvent effects with SMD.⁶⁹ The results indicated
15 that for $[V^V O_2(L^1)(H_2O)]^-$ an energy minimum could not be found; the H_2O ligand leaves
16 spontaneously the axial site, suggesting that the 6-coordinated species is not stable at all in
17 aqueous solution. Globally, these experiments indicate that when measuring the cytotoxicity
18 of complex **1**, once this compound is added to the incubation media, it will be converted to a
19 dioxidovanadium(V) compound $[V^V O_2(L^1)]^-$.

20 Similarly, the ESI-MS spectra of the non-oxidovanadium compound **2** were recorded upon
21 dissolving it in water:methanol (ca. 90:10) solutions (**Figures S4-S7** and **Tables S5-S6**). In
22 the ESI-MS (+) experiments in water:methanol (ca. 90:10) solutions, complex **2** hardly
23 dissolved and only gave a peak at $m/z = 157.0$ (100%) probably due to the fragmented ligand.
24 Upon addition of acetonitrile to this solution, a clear peak at $m/z = 660.22$ (100%) showed
25 up, corresponding to $[V^{IV}(L^2)_2]+H^+$, as well as others: at $m/z = 700.71$ (~5%,

1 $[\text{V}^{\text{IV}}(\text{L}^2)_2]\cdot\text{CH}_3\text{CN}+\text{H}^+$), at $m/z = 403.10$ (~12%, $[\text{V}^{\text{VO}}(\text{L}^2)(\text{MeOH})]^+$ or
2 $[\text{V}^{\text{IV}}\text{O}(\text{HL}^2)(\text{MeOH})]^+$, see SI section) and $m/z = 412.81$ (~20%, $[\text{V}^{\text{IV}}\text{O}(\text{L}^2)(\text{H}_2\text{O})]+\text{Na}^+$).
3 When doing the “washing” procedures of the MS instrument with MeOH, a clear peak at m/z
4 $= 659.40$ (100%, ESI-MS(-)) showed up, apparently indicating that complex **2** is stable
5 enough in water:MeOH solvent for several hours, so that this non-oxido V^{IV} complex can be
6 detected by ESI-MS. Thus, from the ESI-MS data it is clear that $[\text{V}^{\text{IV}}(\text{L}^2)_2]$ is fairly stable in
7 contact with aerated $\text{H}_2\text{O}/\text{MeOH}$ solvents, but that significant amounts of $\text{V}^{\text{IV}}\text{O}(\text{L}^2)$ -type
8 complexes also form in these solutions. We did not manage to detect $\text{V}^{\text{V}}\text{-L}^2$ species by ^{51}V
9 NMR, which might be due to the presence of high amounts of paramagnetic V^{IV} -complexes,
10 but a minor peak was detected by ESI-MS(+) (see above and Figure S6 and Table S5), that
11 might be due to a V^{V} -species ($m/z = 403.10$, ~12%, $[\text{V}^{\text{VO}}(\text{L}^2)(\text{MeOH})]^+$); therefore it is
12 plausible that some amount of V^{V} -species are also formed in the conditions prevailing in cell
13 media.

14

15 UV–Vis Spectroscopy

16 The electronic spectra of **1–3** were recorded in DCM by employing concentrations in the
17 range $1\text{-}2\times 10^{-4}$ M. The $\text{V}^{\text{VO}}(\text{OEt})$ -complex (**1**) exhibits two absorptions in the range of 445-
18 356 nm, that are attributed to ligand-to-metal charge transfer (LMCT) transitions.^{33–35} The
19 other strong absorption bands observed at higher energies, below 350 nm, may be considered
20 as ligand centered transitions (**Figure S8a**).^{34,35,45} The two non-oxidovanadium (V^{IV})
21 complexes (**2** and **3**) possess similar spectral features and the spectrum of **2** is depicted in
22 **Figure S8b**. For **2** and **3**, the main bands in the visible region are LMCT, as supported by
23 TD-DFT (see **Table 1**), while the strong absorptions at higher energy (380–320 nm) are
24 composite bands due to LMCT and ligand centered transitions.

1 In order to get additional information on the nature and intensity of the transitions of the non-
 2 oxidovanadium complexes, the electronic absorption spectrum of **3** was simulated by TD-
 3 DFT methods following the protocol recently used for other 'bare' V^{IV} complexes (**Figure**
 4 **S9**).^{45,75} The absorption wavelength and oscillator strength for the most important transitions
 5 are listed in **Table 1**. The dominant character of the transitions involves excitations from
 6 ligand centered MOs to V-*d* based MOs, mainly V-*d*_{yz}, V-*d*_{x²-y²}, and V-*d*_{z²} (LMCT); however,
 7 a not negligible percent amount of ligand centered absorptions is expected. The involvement
 8 of the V-*d* orbitals in non-oxidovanadium(IV) complexes was ascertained in other studies.^{92,93}
 9 Interestingly, metal-to-ligand charge transfers (MLCT) – predicted in other systems^{45,92} – are
 10 not important for **3** and do not contribute significantly to the experimental absorptions. These
 11 data confirm that, for non-oxido V^{IV} complexes, most of the observable electronic transitions
 12 derive from LMCT transitions.⁹⁴

13

14 **Table 1.** Calculated and experimental electronic transitions for complex **3** up to 250 nm.^a

Transition (MO character) ^b	% V in MO	% transition	λ ^c	$f \times 10^5$ ^d	λ^{exptl} (ϵ^{exptl}) ^{c,e}
H ^{α} -8 \rightarrow L ^{α} +2 (<i>d</i> _{yz})	L (0% V) \rightarrow M (42% V)	61.6	887.9	20	885 (127)
H ^{α} \rightarrow L ^{α} (<i>d</i> _{x²-y²})	L (0% V) \rightarrow M (32% V)	23.3	593.5	430	642 (978)
H ^{α} -1 \rightarrow L ^{α} (<i>d</i> _{x²-y²})	L (4% V) \rightarrow M (32% V)	17.9	507.9	370	546 (1384)
H ^{α} \rightarrow L ^{α} (<i>d</i> _{x²-y²})	L (0% V) \rightarrow M (32% V)	22.3	502.6	7860	
H ^{β} \rightarrow L ^{β} (<i>d</i> _{z²})	L (0% V) \rightarrow M (12% V)	14.1	441.1	6320	435 (2312) ^f
H ^{β} \rightarrow L ^{β} (<i>d</i> _{z²})	L (0% V) \rightarrow M (12% V)	16.9	393.0	28310	398 (3074)
H ^{β} -1 \rightarrow L ^{β} (<i>d</i> _{z²})	L (1% V) \rightarrow M (12% V)	19.6	388.7	12140	
H ^{α} -1 \rightarrow L ^{α} (<i>d</i> _{x²-y²})	L (4% V) \rightarrow M (32% V)	26.4	349.8	13290	324 (3793)
H ^{α} -1 \rightarrow L ^{α} +2 (<i>d</i> _{yz})	L (4% V) \rightarrow M (42% V)	12.5	295.7	18060	

15 ^a Calculations carried out at the level of theory CAM-B3LYP/6-31+g(d). ^b H stands for
 16 HOMO and L for LUMO, and α and β indicate the electron spin. ^c λ values measured in nm.

17 ^d Oscillator strength. ^e ϵ values measured in M⁻¹ cm⁻¹. ^f Shoulder of a more intense
 18 absorption.

19

20

1 NMR Spectroscopy

2 ^1H and ^{13}C NMR of all ligand precursors H_2L^{1-3} and complex **1** were recorded in $\text{DMSO-}d_6$.

3 The spectra of H_2L^{1-3} exhibit two prominent characteristic singlet resonances in the range $\delta =$
4 11.65–10.91 ppm due to the presence of $-\text{OH}$ and $-\text{NH}$ protons, respectively. The absence of
5 these peaks in the spectrum of complex **1** indicates the deprotonation of the $-\text{OH}$ and $-\text{NH}$
6 moieties upon complexation; its ^1H NMR spectrum is depicted in **Figure S10**. All the
7 aromatic protons of **1** are observed in the expected region, $\delta = 8.67$ – 7.19 ppm. Furthermore,
8 the appearance of two peaks at $\delta = 3.43$ ppm and 1.06 ppm are attributed to the $-\text{CH}_2$ and
9 $-\text{CH}_3$ protons, respectively, of the coordinated ethoxido ligand.⁴³

10 The ^{13}C NMR spectrum of complex **1**, shown in **Figure S11**, contains two signals for the
11 $(\text{CO}=\text{N})$ and $(\text{N}=\text{CH})$ carbons in the extreme downfield region at $\delta = 164.6$ and 161.4 ppm,
12 respectively, whereas the aromatic carbons appear in the expected region between
13 154.7 – 111.0 ppm. Two resonances for the aliphatic carbons of the EtO^- ligand are found in
14 the upfield region at $\delta = 56.5$ ($-\text{CH}_2$) and 18.9 ($-\text{CH}_3$) ppm.^{36,43}

15 The ^{51}V NMR spectra of **1** displays a singlet at $\delta_{\text{V}} = -552.5$ ppm in DMSO (**Figure S12**), as
16 well in DCM (at $\delta_{\text{V}} = -565$ ppm). These chemical shifts are in the range of those obtained for
17 other mononuclear oxidoethoxidovanadium(V) complexes,^{33,35,43,45,95,96} with $(\text{L}^1)^{2-}$ depicting
18 significantly higher shielding effect.

19 Upon dissolving complex **2** in DMSO and adding water up to ca. 90% v/v, setting the pH in
20 the range 4.5–7.5 and leaving the solutions standing for several hours, no ^{51}V NMR
21 resonance was detected, except at pH 7.5, where a weak peak at $\delta_{\text{V}} = -559$ ppm was
22 observed, probably due to free vanadate.

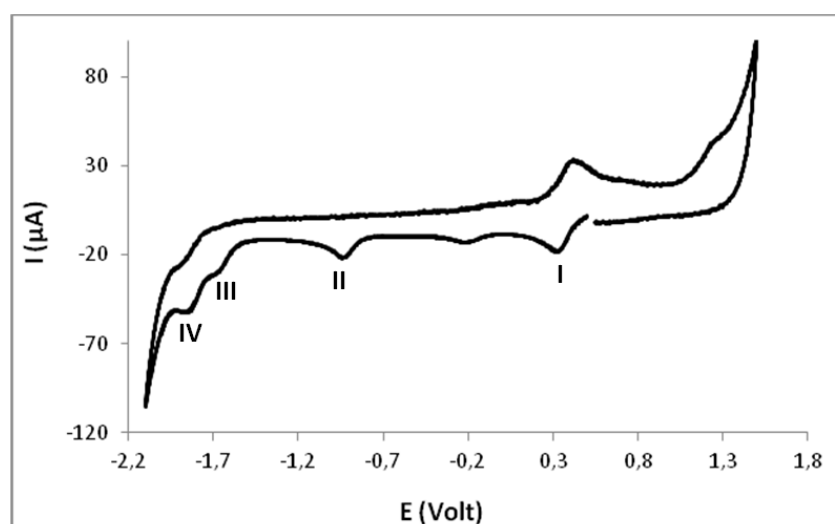
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1 Electrochemical properties

2 Complex **1** in DCM displays one anodic quasi-reversible process attributed to V(V)→V(IV)
3 reduction (wave **I**, **Figure 2**) at $E_{1/2}^{red} = 0.49$ V, which fits well in the range of potential
4 reported for oxidovanadium(V) complexes with ONO type ligands.^{33,35–37,42,43,45,97} Two
5 additional irreversible cathodic waves (**II**, $E_p^{red} = -0.99$ V, and **III**, $E_p^{red} = -1.57$ V, **Figure 2**)
6 and one lower potential reversible wave (**IV**, $E_{1/2}^{red} = -1.74$ V, **Figure 2**) are also observed. The
7 potential of the irreversible wave **III** is close to that of the free ligand ($E_p^{red} = -1.68$ V, **Figure**
8 **S13**), suggesting a ligand based process; while the remaining two observed cathodic waves
9 (**II** and **IV**) we could not able to assigned.

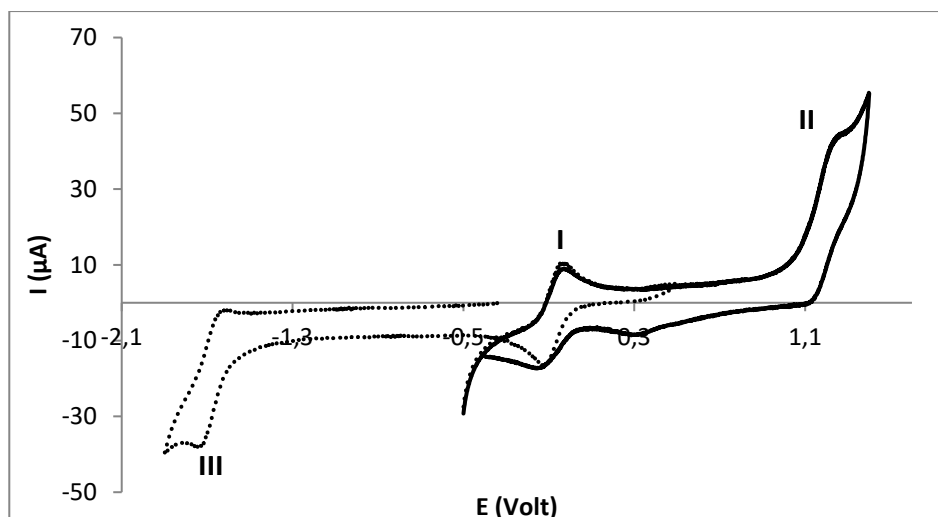


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11 **Figure 2.** Cyclic voltammogram of complex **1** (E values vs. SCE) measured in DCM at 200
12 mV/s using a Pt wire electrode.

13

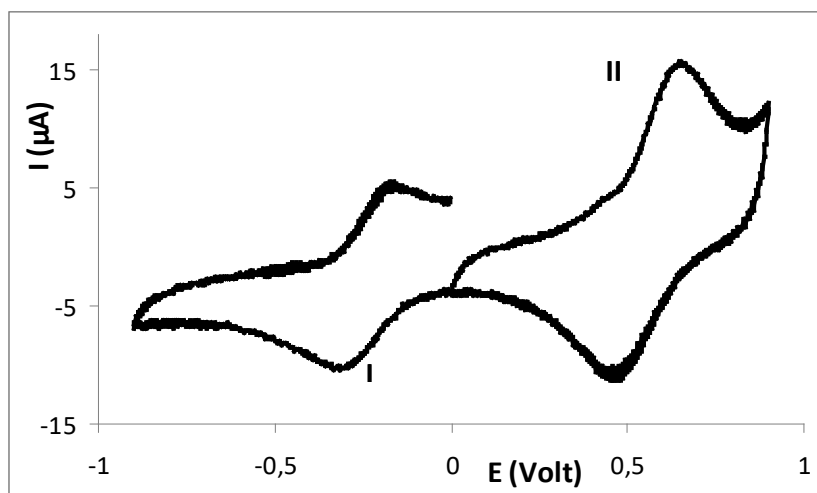
14 Non-oxidovanadium(IV) complexes **2** and **3** display similar electrochemical behavior in
15 dichloromethane. Both show *quasi*-reversible cathodic processes at close potentials (wave **I**,
16 **Figure 3** and **4**), which are attributed to V(IV)→V(III) reduction.



1

2 **Figure 3.** Cyclic voltammograms (E values vs. SCE) of complex **2**. Anodic scan (—) and
 3 anodic scan reversed to the cathodic region upon wave **I** (···). Data obtained in DCM at 200
 4 mV/s, using a Pt wire electrode.

5



6

7 **Figure 4.** Cyclic voltammogram (E values vs. SCE) of complex **3** measured in DCM at 200
 8 mV/s, using a Pt wire electrode.

9

10 Complex **2** reduces at $E_{1/2}^{red} = -0.012$ V (**Figure 3**, wave **I**), and complex **3** at a slightly lower
 11 potential ($E_{1/2}^{red} = -0.18$ V) (**Figure 4**, wave **I**) and both values are well in the range of

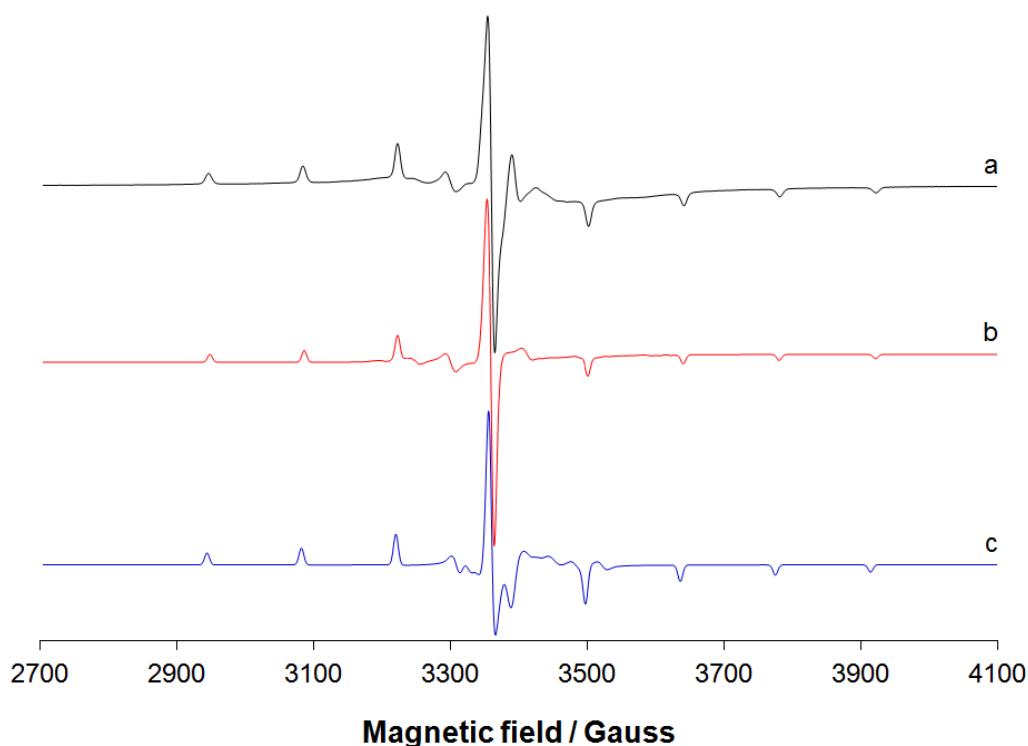
1 potentials observed for V(IV) non-oxido complexes.^{44,45,75} Additionally, **2** displays a
2 reversible cathodic wave at a much lower potential ($E_{1/2}^{red} = -1.62$ V; wave **III**, **Figure 3**)
3 which is attributed to a ligand based process. No related process was detected for complex **3**.
4 Both complexes **2** and **3** display quasi-reversible anodic waves attributed to the V(IV)→V(V)
5 oxidation. However, the potential of the anodic processes display considerably different
6 values at the two complexes, *i.e.* complex **3** oxidizes at a considerably lower potential ($E_{1/2}^{ox} =$
7 0.61 V) than complex **2** ($E_{1/2}^{ox} = 1.20$ V). The relatively low oxidation potential measured for
8 complex **3** (0.61 V) was unexpected for a neutral V^{IV} complex, since values in that range
9 have been reported for the V(IV)→V(V) oxidation of the anionic complexes.^{44,97} However,
10 the considerably low Hammett σ parameter for the -NH₂ substituent (-0.66) at the aromatic
11 ring evidences its high electron releasing effect (considerably higher than for the -OH
12 substituent, -0.37), thus a higher electron density exists at the V^{IV} site,⁹⁸ this enabling a
13 relatively low V(IV)→V(V) potential which tends to those observed in anionic complexes.

14

15 **EPR spectroscopy**

16 The anisotropic EPR spectra of the non-oxido complexes **2** and **3** were recorded in DCM at
17 liquid nitrogen temperature (77 K). The spin Hamiltonian parameters (g and A tensors) were
18 extracted generating the experimental spectra with WinEPR software⁵³ and are reported in
19 **Table 2**. The experimental and generated spectra of **2** are depicted in **Figure 5**.

20



1
2 **Figure 5.** First derivative X-band anisotropic EPR spectra of $[V^{IV}(L^2)_2]$ (**2**): (a) experimental
3 in DCM at 77 K, (b) generated by WinEPR software to extract the spin Hamiltonian
4 parameters and (c) calculated by DFT methods.

5
6 **Table 2.** Experimental and DFT calculated spin Hamiltonian parameters (g and A values)
7 complexes **2** and **3** in DCM.^a

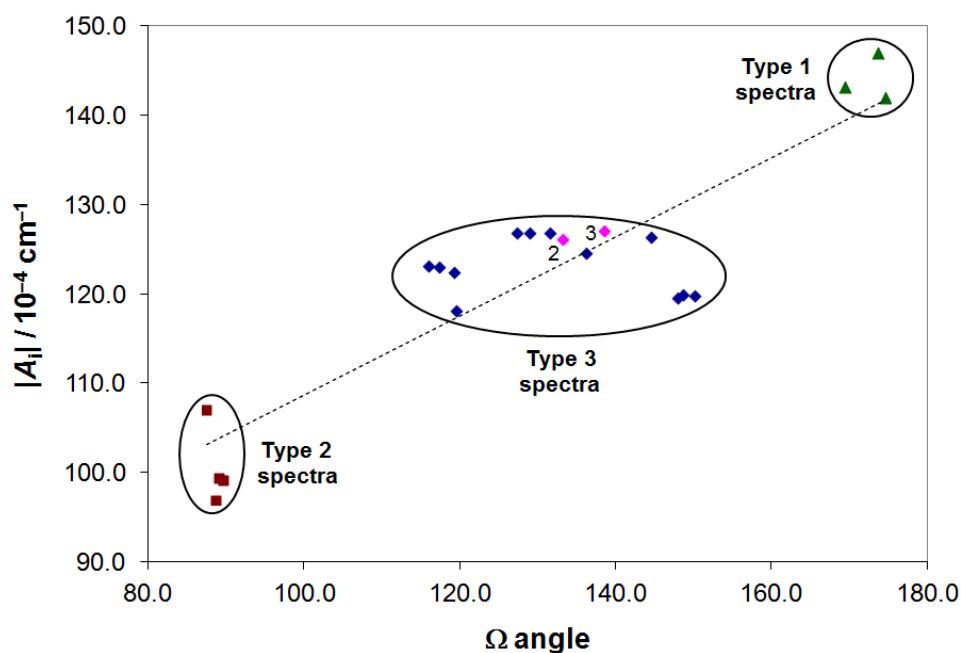
Parameter	Complex 2		Complex 3	
	Experimental	DFT calculated ^b	Experimental	DFT calculated ^b
g_x	1.986	1.980	1.986	1.981
g_y	1.975	1.959	1.974	1.961
g_z	1.958	1.957	1.957	1.957
A_x	-12.0	-18.2	-12.0	-18.3
A_y	-50.0	-27.9	-53.0	-27.6
A_z	-126.2	-125.7	-127.1	-126.6
PD (A_z)	-0.4		-0.4	

8 ^a Values of A reported in 10^{-4} cm^{-1} units. ^b Values calculated with B2PLYP functional and
9 VTZ basis set on the optimized structures (see "Experimental and Computational" section).

1
2 The g and ^{51}V A tensors for **2** and **3** were calculated by DFT simulations with the double
3 hybrid B2PLYP functional and VTZ basis set on the optimized structures. ORCA software
4 was used since it includes the SO contribution, which is fundamental to correctly predict the
5 values of A , in particular the largest value of the diagonalized tensor A (A_x or A_z). This
6 computational protocol allows calculating A_z with a percentage deviation below 4%.⁷³ For **2**
7 and **3**, both the values of percent deviation (PD), defined in the "Experimental and
8 Computational" section, for A_z are -0.4%. The contribution of second-order SO term is 6.9%
9 for both the species and obviously cannot be neglected. The spectrum generated using the
10 DFT data is shown in trace (c) of **Figure 5**.

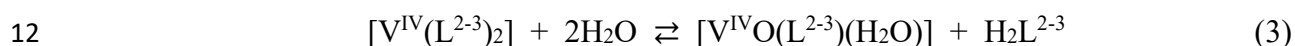
11 In a series of studies,^{73,99,100} some of us demonstrated that EPR spectra of non-oxido V^{IV} -
12 complexes can be grouped in three different types depending on the angle Ω formed by the
13 two external donors of the tridentate ligand: **type 1** spectra for species with geometry close to
14 the octahedron and $|A_z|$ in the range $(135-155)\times 10^{-4} \text{ cm}^{-1}$; **type 2** spectra for complexes with
15 geometry close to the trigonal prism and $|A_x| \sim |A_y|$ in the range $(90-120)\times 10^{-4} \text{ cm}^{-1}$; **type 3**
16 for compounds with an intermediate geometry and intermediate values of $|A_x|$ or $|A_z|$, in the
17 range $(120-135)\times 10^{-4} \text{ cm}^{-1}$. In **Figure 6** the data of the complexes **2** and **3** were added to
18 those found for other hexa-coordinated 'bare' V^{IV} -complexes;^{45,75,92,99-102} it emerges that the
19 spectra of **2** and **3** belongs to the **type 3** group, corresponding to the open-chain tridentate
20 ligands, for which a singly occupied molecular orbital (SOMO) based on $\text{V}-d_{xy}$ is expected.⁷³
21 This suggests that the geometry in the solid state, intermediate between the octahedral and the
22 trigonal prismatic, is retained by compounds **2** and **3** in DCM solution.

23



1
2 **Figure 6.** Experimental $|A_i|$ values for non-oxido V^{IV} -complexes as a function of the
3 experimental Ω angles. The regions corresponding to the **type 1** (rigid ligands, green
4 triangles), **type 2** (cyclic ligands, brown squares) and **type 3** spectra (open-chain non rigid
5 ligands, blue rhombi). The pink rhombi in the **type 3** spectra region denote complexes **2** and
6 **3** of this study. $|A_i|$ ($i = x$ or z) are the largest values of the ^{51}V A tensor. The dotted line
7 represents the best linear fitting for the twenty-one points considered.

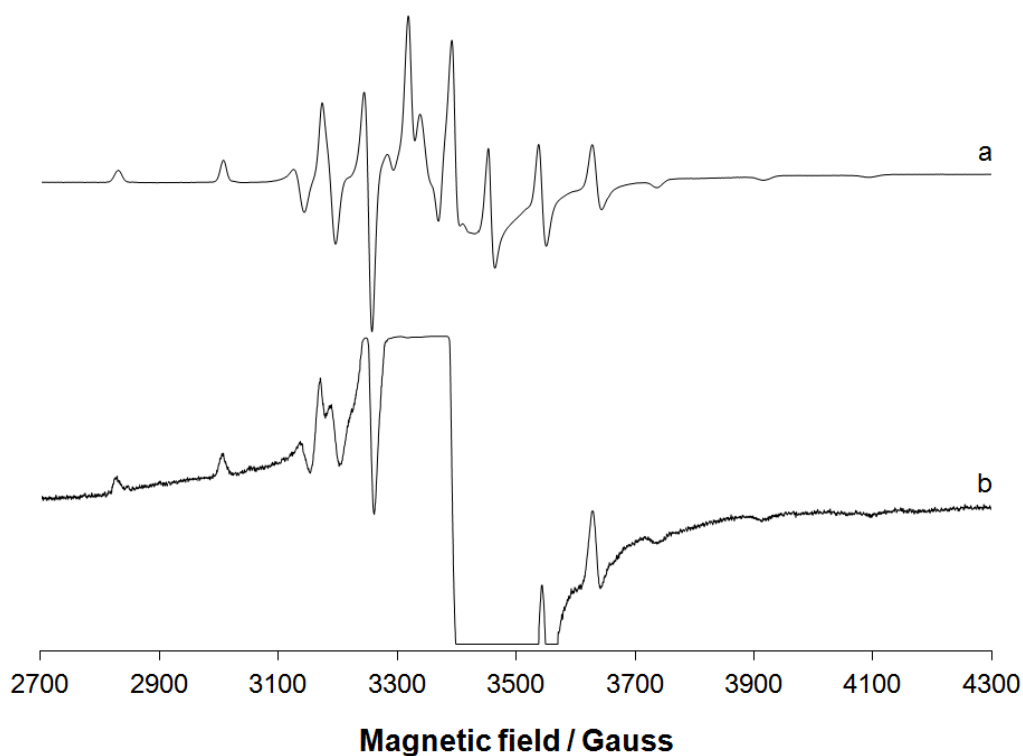
8
9 When **2** and **3** were dissolved in water or in phosphate buffer (see the EPR spectra in **Figure**
10 **7**), the transformation to an oxidovanadium(IV) partly occurs according to the reaction (3), as
11 substantiated by ESI-MS measurements:



13 The species $[V^{IV}O(L^2)(H_2O)]$ and $[V^{IV}O(L^3)(H_2O)]$ are abbreviated as **2'** and **3'**. The g_z and
14 $|A_z|$ for $[V^{IV}O(L^2)(H_2O)]$ are 1.949 and $164.5 \times 10^{-4} \text{ cm}^{-1}$ (H_2O , pH 5) and 1.948 and
15 $164.9 \times 10^{-4} \text{ cm}^{-1}$ (phosphate buffer); for $[V^{IV}O(L^3)(H_2O)]$ they are 1.948 and $164.4 \times 10^{-4} \text{ cm}^{-1}$
16 (H_2O , pH 5) and 1.948 and $164.7 \times 10^{-4} \text{ cm}^{-1}$ (phosphate buffer). The bianionic ligands $(L^2)^{2-}$

1 and $(L^3)^{2-}$ bind $V^{IV}O^{2+}$ in a tridentate fashion with the (O^-, N, O^-) donors on the equatorial
2 plane, the remaining position being occupied probably by a solvent molecule. The spin
3 Hamiltonian parameters are comparable to those measured for other 1:1 $V^{IV}O$ species formed
4 by tridentate ligands with a similar coordination mode.^{40,99,100,103,104}

5



6

7 **Figure 7.** First derivative X-band anisotropic EPR spectra recorded in the systems where
8 $[V^{IV}(L^3)_2]$ (**3**) is dissolved in: (a) phosphate buffer (pH 7.4) and (b) in water (pH 5.0).

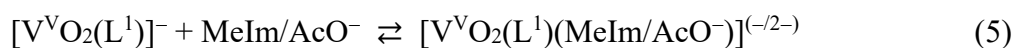
9

10 **Study of protein interaction through docking analysis**

11 The docking analysis for the covalent binding was carried out for the V^VO_2 species
12 $[V^VO_2(L^1)]^-$ (**1'**) and $V^{IV}O$ species $[V^{IV}O(L^2)(H_2O)]$ (**2'**) and $[V^{IV}O(L^3)(H_2O)]$ (**3'**),
13 postulating that proteins (ubiquitin and lysozyme) replace the monodentate water ligand
14 through an amino acid side-chain donor forming a coordination bond, according to the
15 reaction (4):



The possibility that $[\text{V}^{\text{V}}\text{O}_2(\text{L}^1)]^-$ could bind to proteins through a covalent bond to give a hexa-coordinated adduct $\text{V}^{\text{V}}\text{O}_2(\text{L}^1)(\text{Protein})$, has been taken into account computationally evaluating – at DFT level of theory and including the solvent effects with SMD model ⁶⁹ – the relative stability of $[\text{V}^{\text{V}}\text{O}_2(\text{L}^1)]^-$ and of the species $[\text{V}^{\text{V}}\text{O}_2(\text{L}^1)(\text{MeIm})]^-$ and $[\text{V}^{\text{V}}\text{O}_2(\text{L}^1)(\text{AcO})]^{2-}$, where MeIm and AcO^- represent 1-methylimidazole and acetate ion, as models for the binding of His-N or Asp/Glu-COO (eq. 5).



Only for the complex formed by MeIm it was possible to find an energy minimum, while AcO^- is not stable at the axial site, as it was determined for water (see "ESI Mass Spectrometry" section). However, ΔG_{aq} for the formation of $[\text{V}^{\text{V}}\text{O}_2(\text{L}^1)(\text{MeIm})]^-$ is 15.7 kcal/mol, indicating that, for MeIm as well, the possible formation of the 6-coordinated species in solution must be discarded. These data allow to exclude the direct coordination, through a covalent bond, of a protein donor to $[\text{V}^{\text{V}}\text{O}_2(\text{L}^1)]^-$ to give a hexa-coordinated species; so, only the non-covalent bond of $[\text{V}^{\text{V}}\text{O}_2(\text{L}^1)]^-$ with a closed coordination sphere was considered during the docking calculations (see below).

With Ub, the results indicated that there are several solvent exposed side-chain donors potentially able to bind vanadium. The scoring F and population, suggested by docking methods for the potential adducts, are listed in **Table 3**.

Table 3. Proposed binding sites for the covalent binding to ubiquitin of $[\text{V}^{\text{IV}}\text{O}(\text{L}^2)(\text{H}_2\text{O})]$ (**2'**) and $[\text{V}^{\text{IV}}\text{O}(\text{L}^3)(\text{H}_2\text{O})]$ (**3'**) suggested by docking methods.^a

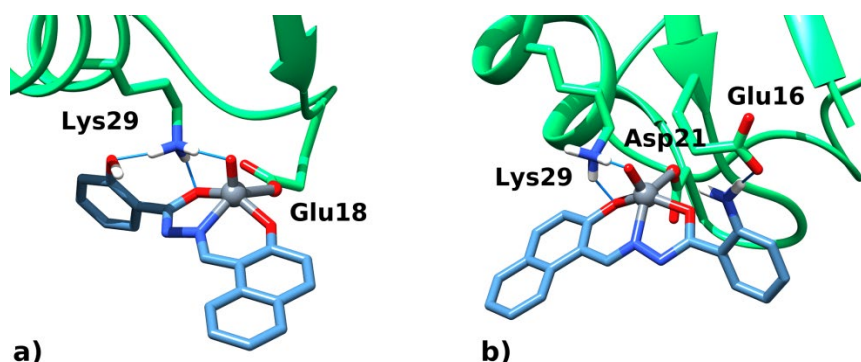
Complex	Residue	V–D/Å	Second interactions	F_{\max}^b	F_{mean}^c	Pop./% ^d
[V ^{IV} O(L ²)(H ₂ O)]	Glu18^e	2.450	Lys29···VO, Lys29···OH	59.1	54.6	18.2
	Asp21^e	2.110	Lys29···VO, Lys29···OH	56.4	55.3	1.7
	Glu16 ^e	2.290	Lys29···VO, Lys29···OH	50.8	51.1	34.8
	Glu51^f	2.440	Arg72···OH	48.3	41.3	21.0
	His68	2.280	Thr12···OH	47.1	42.4	14.4
	Asp52 ^f	2.150	Arg72···OH	40.3	39.7	9.9
[V ^{IV} O(L ³)(H ₂ O)]	Asp21^e	2.424	Lys29···VO, Glu16···H ₂ N	53.8	46.0	20.3
	His68	1.968	–	52.3	41.2	11.5
	Glu51^f	2.198	Gln49···N–N	46.7	41.3	32.4
	Glu16 ^e	2.508	Lys29···VO	45.0	44.1	16.9
	Glu18 ^e	2.259	Lys29···VO, Glu16···NH ₂	44.1	46.5	16.2
	Asp52 ^f	1.941	Glu51···NH ₂	37.8	35.1	2.7

1 ^a The preferential binding sites are highlighted in boldface. ^b GoldScore *Fitness* value
2 obtained for the most stable pose of each cluster (F_{\max}). ^c Average value of GoldScore *Fitness*
3 for each cluster (F_{mean}). ^d Percentage computed considering the total of the solutions reported
4 (numbers of solutions per cluster). ^e The binding of Glu16, Glu18 and Asp21 is incompatible
5 with each other. ^f The binding of Glu51 and Asp52 is incompatible with each other.

6

7 The best docking proposals were found for the coordination of Asp/Glu-O and His-N of: i)
8 Glu18 or Asp21 and Glu51 or His68 for **2'**; and ii) Glu16 or Asp21 and His68 for **3'**. The best
9 solutions for each complex are shown in **Figure 8**. It is relevant to note that the preferred
10 binding sites are different for the two moieties: Glu18 for V^{IV}O(L²) and Asp21 for V^{IV}O(L³),
11 respectively (**Figure 8**). Moreover, because of the three-dimensional disposition of amino
12 acids and large size of the complexes, the simultaneous binding of Glu16, Glu18 or Asp21
13 residues on one hand, and Glu51 and Asp52 on the other are incompatible. These sites are the
14 same individuated recently for the interaction of V^{IV}OL₂ potential drugs with Ub.^{25,80} Each
15 adduct appears to be stabilized by at least one hydrogen bond with the surrounding amino
16 acids; in particular, the –NH₃⁺ of Lys, –NH of Arg, –OH of Thr and –COO[–] of Glu are
17 engaged in contacts with the V=O group or –OH, –N–N and –NH₂ functionalities of the

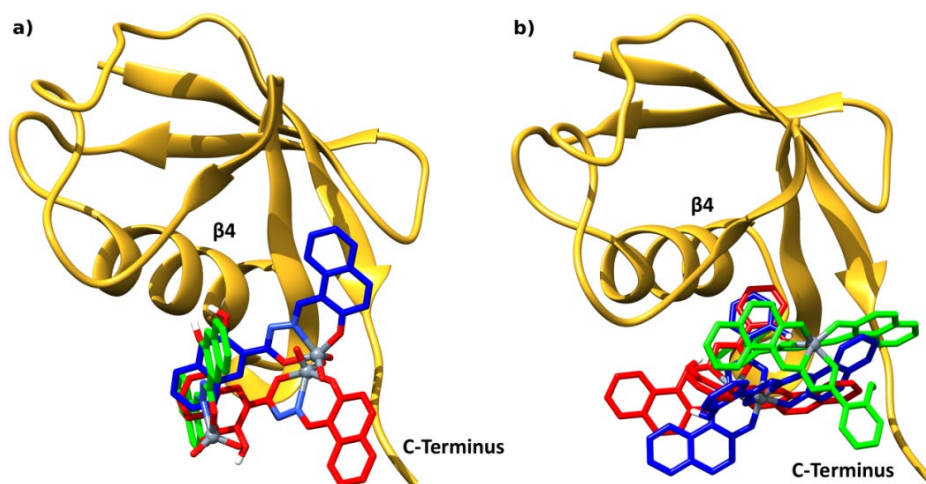
1 ligands.



2
3 **Figure 8.** Best docking solutions for the covalent binding of V^{IV}O-moieties derived from
4 complexes 2-3 to ubiquitin: (a) V^{IV}O(L²) (2') and (b) V^{IV}O(L³) (3'). Glu18 and Asp21
5 replaces the water ligand of [V^{IV}O(L²⁻³)(H₂O)] (2' and 3'). V atom is shown with the grey
6 ball, while hydrogen bonds are depicted as blue thin lines.

7
8 Concerning the non-covalent interaction with ubiquitin of penta-coordinated [V^VO₂(L¹)]⁻ (1')
9 and hexa-coordinated non-oxido V^{IV} complexes [V^{IV}(L²⁻³)₂] (2 and 3), only for 1' and 2 a
10 stable interaction is expected. For these complexes, in fact, F_{\max} of the solutions at the
11 interface between the α 1, β 3, β 4 and the C-terminus subunits reaches values higher than 15
12 GoldScore units. In a recent paper, some of us demonstrated that – using a modified version
13 of GoldScore – only for values larger than 16-17 units the complexes are blocked with a
14 strong interaction (for V^{IV}O-flavonoid species it is strong enough to cause the transition from
15 an isotropic to an anisotropic EPR signal at room temperature).¹⁸ The structures, depicted in
16 **Figure 9**, are stabilized by several hydrogen bonds between i) the aza functionality of L² and
17 the charged Arg72 side chain, ii) the oxido ligands of 1' and the charged Arg72, Arg42,
18 Lys26 or Gln49 side chains and iii) the non-coordinating phenolic group of L¹⁻² and the
19 carboxylate group of Glu 51 or quaternary nitrogen of Lys27 (**Figure 9**).

20



1
2 **Figure 9.** Best representative solutions of the three first clusters (in red, green and blue) for
3 the non-covalent interaction with ubiquitin of: (a) [V^VO₂(L¹)]⁻ (**1'**) and (b) [V^{IV}(L²)₂] (**2**).

4
5 Similarly to ubiquitin, lysozyme presents several exposed amino acids that can potentially act
6 as coordinating residues to bind covalently to the oxidovanadium(IV) species **2'** and **3'**. The
7 docking solutions, summarized in **Table 4**, show appreciable F_{\max} values and population.

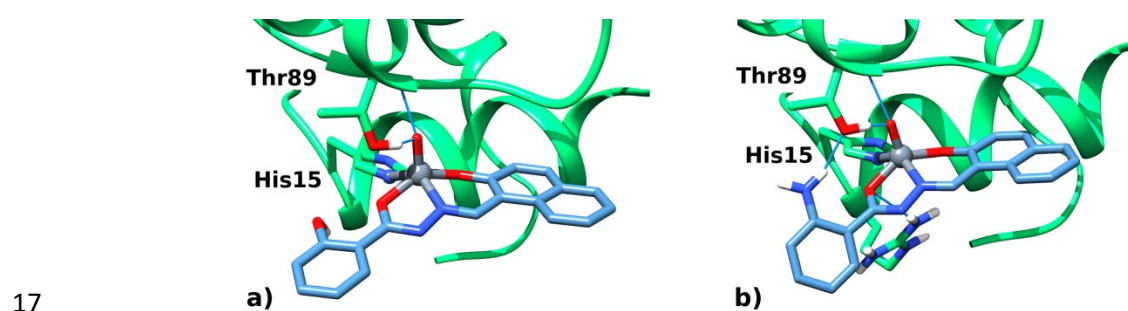
8
9 **Table 4.** Proposed binding sites for the covalent binding to lysozyme of [V^{IV}O(L²)(H₂O)] (**2'**)
10 and [V^{IV}O(L³)(H₂O)] (**3'**) suggested by docking methods.^a

Complex	Residue	V-D/Å	Second interactions	F_{\max} ^b	F_{mean} ^c	Pop./% ^d
[V ^{IV} O(L ²)(H ₂ O)]	His15	2.206	Thr89···VO	63.1	53.2	40.0
	Glu35	1.888	Asp52···OH	57.5	53.5	60.0
[V ^{IV} O(L ³)(H ₂ O)]	His15	2.273	Arg14···O	64.9	56.3	41.5
	Asp52^e	1.808	Val109···N-N	50.4	50.3	1.2
	Glu35^e	1.931	Asp52···NH ₂	58.7	54.5	57.3

11 ^a The preferential binding sites are highlighted in boldface. ^b GoldScore *Fitness* value
12 obtained for the most stable pose of each cluster (F_{\max}). ^c Average value of GoldScore *Fitness*
13 for each cluster (F_{mean}). ^d Percentage computed considering the total of the solutions reported
14 (numbers of solutions per cluster). ^e The binding of Glu35 and Asp52 is incompatible with
15 each other.

16

1 On the basis of the docking solutions, the preferential binding sites for the two moieties
2 $V^{IV}O(L^2)$ and $V^{IV}O(L^3)$ should involve the coordination to the V center of the imidazole-N of
3 His15 and of carboxylate-O of Glu35 or Asp52. Similarly to ubiquitin, these adducts are
4 stabilized by at least one hydrogen bond. The most important secondary interactions are
5 between the functionalities NH_2 of Arg or carboxylate of Glu with the $V=O$ or OH groups of
6 the ligands. The best docking solution for each complex is reported in **Figure 10**.
7 Differently from ubiquitin, the most stable adducts of $V^{IV}O(L^2)$ and $V^{IV}O(L^3)$ with lysozyme
8 are formed upon the coordination of only one residue (His15) and with the binding of a
9 nitrogen donor instead of a carboxylate oxygen. Another interesting feature is that lysozyme
10 would bind these $V^{IV}O$ moieties with a mono-chelated (eq-eq-eq) ligand through His15,
11 while it binds the more crowded bis-chelated $V^{IV}O$ fragments having an (eq-eq); (eq-ax)
12 arrangement of the two ligands (for example, picolinate and maltolate) with accessible
13 carboxylate-containing side chains (Asp52, Glu35 or Glu7).^{21,105} The reason lies in the fact
14 that His15 is not completely exposed on the protein surface and the steric hindrance of the
15 ligand(s) hinders its binding to vanadium; in contrast, the fourth equatorial position of
16 $V^{IV}O(L^2)$ and $V^{IV}O(L^3)$ is less crowded and the approach of His15 becomes possible.

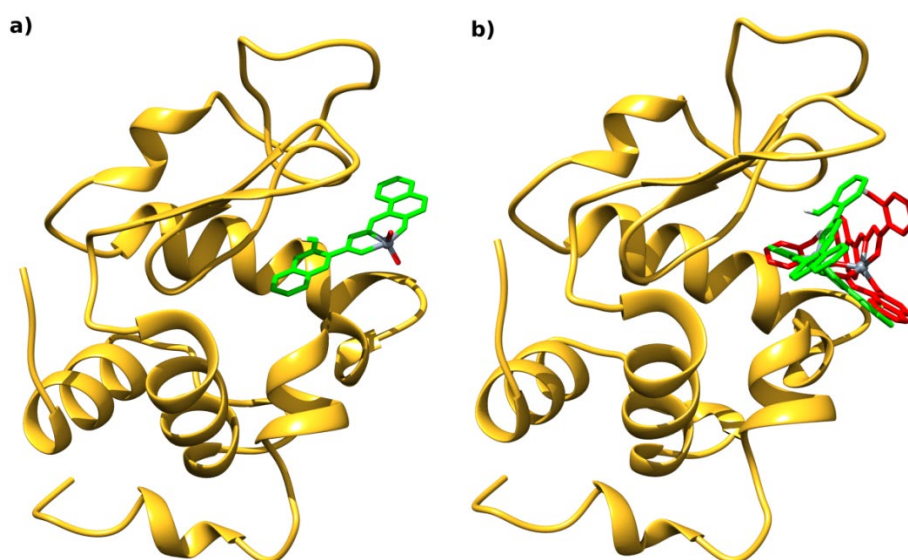


18 **Figure 10.** Best docking solutions for the covalent binding of $V^{IV}O$ moieties derived from
19 complexes **2-3** with lysozyme: (a) $V^{IV}O(L^2)$ (**2'**) and (b) $V^{IV}O(L^3)$ (**3'**). His15 replaces the
20 water ligand of $[V^{IV}O(L^{2-3})(H_2O)]$ (**2'** and **3'**). V atom is shown with the grey ball, while
21 hydrogen bonds are depicted as blue thin lines.

1

2 As in the systems with ubiquitin, the analysis of the non-covalent surface interaction of the
3 oxido V^V **1'** and non-oxido V^{IV} complexes **2** and **3** suggested that only **1'** and **2** form stable
4 adducts. In fact, F_{max} values higher than 15 GoldScore units were obtained. The best docking
5 solutions, depicted in **Figure 11**, are stabilized by hydrogen bonds between the NH group of
6 Trp62 and Trp63, NH_2 of Arg112, CO of Asn109 and COO^- of Asp52 with the phenolic
7 function of the $L^1(2-)$ and $L^2(2-)$ ligands.

8



9

10 **Figure 11.** Best representative solutions of the two first clusters (in green and red,
11 respectively) for the non-covalent interaction with lysozyme of: (a) $[V^VO_2(L^1)]^-$ (**1'**) and (b)
12 $[V^{IV}(L^2)_2]$ (**2**).

13

14 ***In vitro* cytotoxicity studies**

15 The cytotoxic activity of ligand precursors (H_2L^{1-3}) and complexes **1-3** was evaluated against
16 the ovarian A2780 and prostate PC3 cancer cells. Concerning pharmacological applications, a
17 relevant characteristic for a prospective anticancer drug is its high degree of specificity for
18 cancer cells, sparing normal cells.¹⁰⁶ In this work, V79 fibroblasts were chosen as a normal

1 cell line to evaluate the selectivity of the complexes against cancer cells (**Table 5**). As it can
2 be observed from **Table 5** and **Figure 12**, which depicts the dose response curves obtained,
3 the compounds displayed different cytotoxicity against the ovarian cancer cells upon 48 h of
4 exposure in the order **1** > **2** > **3**. Moreover, their cytotoxic activity was shown to be cell type
5 dependent, *i.e.*, they were more active in the ovarian cancer cells than the prostate cells. This
6 result was predictable taking into consideration the highly aggressive behavior of this prostate
7 cell line.¹⁰⁷ For H₂L¹, the coordination to vanadium did not improve its activity; in fact, H₂L¹
8 is more active than its corresponding complex. On the other hand compound **1** is much more
9 selective for A2780 cells than H₂L¹, IC₅₀: 1.1±0.6 μM and selectivity index (SI) = 19, thus
10 exhibiting important anti-proliferative activity associated with good SI when considering
11 normal fibroblasts. From this strict point of view, compound **1** is the most promising one for
12 A2780 cells. However, beside **1** being converted to **1'** once dissolved in incubation media, its
13 cytotoxicity is probably partly due to its ligand moiety, which has a much lower IC₅₀ and SI
14 against A2780 cells. For compounds **2** and **3**, the trend differs from **1**, since both complexes
15 were more active than their corresponding ligand precursors. H₂L³ was the least active
16 compound.

17 Recently the anti-proliferative activity of some non-oxidovanadium-catecholate complexes
18 was studied. Besides emphasizing the probable relation between their thermodynamic
19 stabilities and uptake by cells of the intact complex, the relevance of understanding their
20 hydrolysis in solution, and the determination of which is/are the active species for the
21 cytotoxicity was highlighted.⁴⁶ In the case of complexes **2–3**, in the above sections we
22 showed that there is significant transformation of the non-oxidovanadium complexes **2–3** into
23 their corresponding oxidovanadium(IV) species (**2'–3'**) in solution (eq. 3); V^{VO}-species were
24 also detected in ESI-MS. Therefore, we may predict that in incubation media all these species
25 may be present, and this mixture could be responsible for the cytotoxic activity in the

1 experiments with **2** and **3**.¹¹ Actually, the situation is more complex as the incubation media
 2 contains several potential ligands for vanadium.

3 Complexes **1** and **2** were less toxic to normal cells than **3**. Considering the data in **Table 5**,
 4 although compound **1** is more selective, the corresponding free ligand is quite toxic, thus
 5 globally **2** proved to be the most promising complex of the series and deserves to be further
 6 explored as anticancer agent, in particular for the ovarian cancer.

7 Nevertheless, the results of H₂L² and complex **2** with V79 fibroblasts are somewhat
 8 intriguing. The IC₅₀ for the ligand precursor is much lower than for complex **2**; yet, according
 9 to eq. 3, for each [V^{IV}O(L²)(H₂O)] formed, one free ligand is produced that should exert its
 10 toxic effect. The maintenance of most of [V^{IV}(L²)₂], due to its high stability, may probably
 11 explain this. When considering non-oxido vanadium complexes, their biological effects are
 12 normally attributed to their derivatives [V^{IV}O(L)(H₂O)] (or [V^VO_n(L)] with n = 1-2), formed
 13 from hydrolysis of the corresponding [V^{IV}(L)₂] precursors.¹⁵ Complex **2** may probably be an
 14 exception to this typical behavior. Other examples are amavadine¹⁰⁸ and previously reported
 15 V^{IV}-aroylhydrazonato complexes.⁴⁴

16

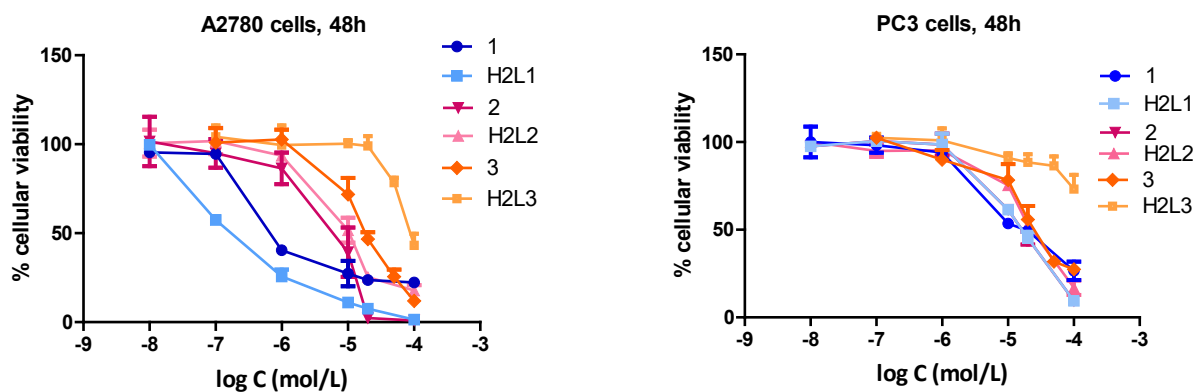
17 **Table 5.** IC₅₀ values (μM) calculated for the ligand precursors and corresponding vanadium
 18 complexes against the ovarian and prostate cancer cells, and the normal fibroblasts, upon 48 h
 19 of exposure. Results are the mean±SD of two independent experiments done with six
 20 replicates.

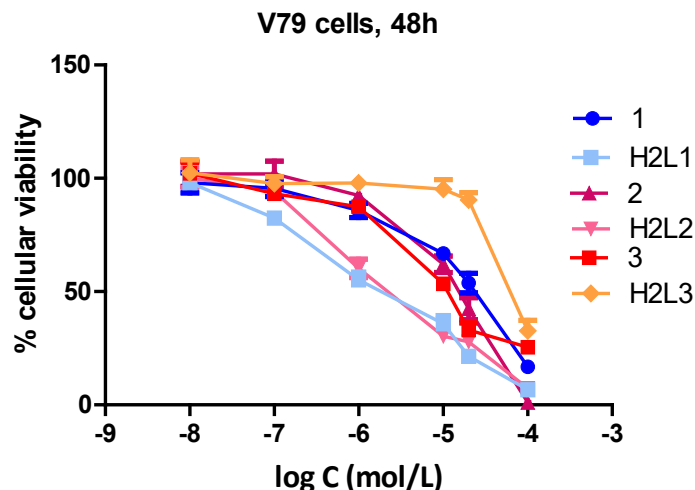
Compounds	A2780	PC3	V79 (normal cells)
H ₂ L ¹	0.2±0.1	8.7±0.4	2.4±1.1
1	1.1±0.6	17±1.0	21±3.1
H ₂ L ²	9.7±4.4	28±4.2	3.2±1.1
2	4.5±1.8	15±1.7	14±2.5
H ₂ L ³	>100	>100	73±18
3	20±3.0	29±5.0	12±2.3

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Prevalent chemotherapeutic drug such as cisplatin has shown comparable cytotoxic potential under identical conditions against A2780 and PC3 cancer cell lines with IC_{50} values of 22.4¹⁰⁹ and 51.0 μM ,⁸³ respectively. When the present compounds may be also compared with previously reported V^VO_2 -complexes of NNO donor Schiff base ligands, tested against A2780 and PC3 cancer cell lines (IC_{50} values in the range 7–22 μM),⁸³ or with with 8-hydroxyquinoline (8HQ) derived vanadium(IV,V) complexes, such as $[V^{IV}O(pic)(8HQ)]$ (pic = picolinate), $[V^{IV}O(sal-Gly)(8HQ)]$ (sal-Gly = N-salicylideneglycinato), $[V^VO(L-pheolnaph-im)(8HQ)]$, $[V^VO(L-pheolnaph-im)(5-Cl-8HQ)]$ (nath-Phe = N-naththalidene-L-phenylalaninato), and $[V^VO(MeO)(8HQ)_2]$ (IC_{50} values in the range 5.4–14.0 μM)¹⁰⁹ as well as with mixed ligand vanadium(IV) complexes of salicylaldimines $[VO(sal-Gly)(bipy)]$, $[VO(sal-Gly)(phen)]$ $[VO(sal-L-Phe)(H_2O)]$, $[VO(sal-L-Phe)(bipy)]$ and $[VO(sal-L-Phe)(phen)]$ (sal-Phe = N-salicylidene-L-phenylalaninato, bipy = 2,2'-bipyridine, phen = 1,10-phenanthroline) (IC_{50} values between 4.7 and 20.8 μM)¹¹⁰ have been tested. The complexes herein studied showed similar, or in some cases improved *in vitro* cytotoxicity, when compared with our previously reported vanadium systems against different cancer cell lines such as HeLa (cervical), HT-29 (colorectal) and A549 (lung).^{34,37–39,44}





1 **Figure 12.** Dose response curves obtained by the GraphPad Prism software (vs 5.0) for the
 2 effect of ligand precursors (H_2L^{1-3}) and respective complexes (**1-3**).

3

4 **CONCLUSIONS**

5 Three aroylhydrazones H_2L^{1-3} and corresponding vanadium complexes, one oxido
 6 $[V^V(O(L^1)(OEt))]$ (**1**) and two non-oxido compounds, $[V^{IV}(L^{2-3})]$ (**2** and **3**) were prepared and
 7 characterized by several physicochemical techniques, including single-crystal X-ray
 8 diffraction. DFT methodologies were used to model both their UV-Vis and EPR spectra;
 9 besides the good agreement between the experimental and DFT-calculated data, it was shown
 10 that the relatively strong bands observed for the non-oxido V^{IV} -complexes **2** and **3** in the
 11 visible range mainly correspond to LMCT transitions.

12 All complexes and free ligands were evaluated for their anti-proliferative activity against
 13 ovarian (A2780) and prostate (PC3) human cancer cells, as well as in normal fibroblasts
 14 (V79). While H_2L^1 is significantly more active than its corresponding complexes **1** (and/or
 15 **1'**), the non-oxido complexes **2** and **3**, and particularly **3** are more active than their ligand
 16 precursors H_2L^2 and H_2L^3 . In aqueous solution the non-oxido V^{IV} -complexes **2** and **3** are

1 partly converted to the corresponding $V^{IV}O$ -complexes $[V^{IV}O(L^{2-3})(H_2O)]$ (indicated with **2'**
2 and **3'**), and probably to a V^V -complex; therefore, the cytotoxicity measured for complexes **2**
3 and **3** is due to the presence of several species: the non-oxido complexes, their corresponding
4 $V^{IV,V}$ -complexes and the free ligands. Since any of these species may bind to components of
5 cell media, namely to BSA, thus the cytotoxic action may be due to many distinct factors.
6 Once uptaken by cells, the compounds may bind to enzymes/proteins somewhat affecting
7 their role in biological processes. By the use of docking procedures with lysozime and
8 ubiquitin, we showed that non-oxidovanadium complex **2**, as well as the
9 oxidovanadium(IV,V) derivatives (**1'**, **2'** and **3'**) may bind to proteins both with covalent
10 (coordination) bonds or non-covalent interactions on the protein surface. His-N and Asp/Glu-
11 COO^- are the preferential donors, mainly when the binding is stabilized by secondary
12 interactions.
13 Thus, interaction with proteins critical for the survival of the cells may be relevant to explain
14 the cytotoxic action of the compounds, these being factors that should be evaluated when
15 attempting to access mechanisms of biological action of vanadium compounds.

16

17 ASSOCIATED CONTENT

18 Supporting Information

19 The Supporting Information and crystallographic data in CIF format is available free of
20 charge at DOI: XXXXX.

21 Packing structure of molecules **1** (**Figure S1**), Crystal data and structure refinement details
22 for compounds **1-3** (**Table S1**) and their selected geometric parameters (**Table S2A** and
23 **S2B**). ESI-MS spectrum of **1** (**Figures S2-S3** and **Tables S3-S4**) and **2** (**Figures S4-S7** and
24 **Tables S5-S6**), UV-Vis spectra of **1-3** (**Figures S8-S9**), NMR spectra of **1** (**Figures S10-**
25 **S12**) and cathodic and anodic scans for H_2L^1 (**Figures S13**).

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16 The manuscript was written through contributions of all authors. All authors have given
17 approval to the final version of the manuscript.

18 **Notes**

19 The authors declare no competing financial interest.

20

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8

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SYNOPSIS

2 Characterization and solution behavior of one oxidoethoxidovanadium(V) and two non-
3 oxidovanadium(IV) complexes formed by three ONO donor aroylhydrazones are studied and
4 their anti-proliferative activity and selectivity evaluated. In aqueous solution the complexes
5 undergo partial change to V^VO_2 and $V^{IV}O$ complexes. Interactions of these V^VO_2 , $V^{IV}O$ and
6 V^{IV} species with proteins are investigated through docking approaches.

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