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Evaluation of cellular uptake, cytotoxicity and cellular ultrastructural effects of heteroleptic oxidovanadium(IV) complexes of salicylaldimines and polypyridyl ligands

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ABSTRACT

Searching for prospective vanadium-based drugs for cancer treatment, a new series of structurally related [$V^{IV}O$(L−2H)(NN)] compounds (**1**–**8**) was developed. They include a double deprotonated salicylaldimine Schiff base ligand (L-2H) and different NN-polypyridyl co-ligands having DNA intercalating capacity. Compounds were characterized in solid state and in solution. EPR spectroscopy suggests that the NN ligands act as bidentate and bind through both nitrogen donor atoms in an axial-equatorial mode. The cytotoxicity was evaluated in human tumoral cells (ovarian A2780, breast MCF7, prostate PC3). The cytotoxic activity was dependent on type of cell and incubation time. At 24 h PC3 cells presented low sensitivity, but at 72 h all complexes showed high cytotoxic activity in all cells. Human kidney HEK293 and ovarian cisplatin resistant A2780cisR cells were also included to evaluate selectivity towards cancer cells and potency to overcome cisplatin resistance, respectively. Most complexes showed no detectable interaction with plasmid DNA, except **2** and **7** which depicted low ability to induce single strand breaks in supercoiled DNA. Based on the overall cytotoxic profile, complexes with 2,2′-bipyridine and 1,10-phenanthroline ligands (**1** and **2**) were selected for further studies, which consisted on cellular distribution and ultrastructural analyses. In the A2780 cells both depicted different distribution profiles; the former accumulates mostly at the membrane and the latter in the cytoskeleton. Morphology of treated cells showed nuclear atypia and membrane alterations, more severe for **1**. Complexes induce different cell death pathways, predominantly necrosis for **1** and apoptosis for **2**. Complexes alternative mode of cell death motivates the possibility for further developments.

1. Introduction

The recognition of the relevance of vanadium in several biological processes has led to increasing research on the potential medicinal uses of its compounds [1,2]. Although traditional research on vanadium medicinal chemistry has been mainly focused on improving biodistribution and tolerability of the vanadium insulin-enhancing moiety or on the development of anti-tumoral compounds, vanadium complexes have also been proposed for the treatment of other diseases, such as those caused by parasites. Nevertheless, no vanadium compound is currently used in the clinical setting [3–8].

Chemo-preventive and anti-tumoral effects of vanadium compounds have been widely investigated on various types of tumor cell lines and on experimental animal models, but the underlying mechanisms of action remain not fully understood. Vanadium compounds have shown ability to disrupt the cellular metabolism through generation of radical oxygen species (ROS) [9], changes in cellular organelles such as lysosomes, mitochondria, and proteins such as actin and tubulin [10–12], and effects on signal transduction pathways, cyclins and caspases, which play a role in cell cycle arrest and apoptosis [13,14]. Additionally, cell proliferation may be also affected via DNA damage [1,15].

Vanadium-*N*-salicylideneamino acidato compounds have attracted the attention of different research groups during decades. They mimic enzymatic systems that involve the formation of a Schiff base by condensation of an aromatic aldehyde (pyridoxal) and an amino acid, with the metal ion promoting the preservation of the planarity of the conjugated system through chelate ring formation [16]. Although, the first objective was to prove the functionality of oxidovanadium(IV/V) *N*-salicylideneamino acidato compounds as models of the reactions catalyzed by pyridoxal-containing enzymes, fostering insight on the mechanisms involved [16,17], ternary compounds, including NN planar

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polypyridyl co-ligands, were lately developed as prospective metal-based drugs [8,18,19]. The inclusion of the NN heterocyclic base renders the complexes with the ability to bind DNA through intercalation or surface and/or groove binding [20,21].

Searching for prospective vanadium-based drugs for the treatment of cancer, a new series of eight structurally related heteroleptic [$V^{IV}O$(L-2H)(NN)] compounds was developed. These include a double deprotonated salicylaldimine ligand (L-2H), derived from glycine and salicylaldehyde or 5-bromo-salicylaldehyde, and different NN-polypyridyl co-ligands having DNA intercalating capacity (Fig. 1). The compounds were characterized in the solid state and in solution.

Moreover, to pursuit our goals on developing prospective candidates as anticancer drugs we further explored the biological activity of the compounds in a set of human tumoral cells and plasmid DNA models. How the compounds are distributed inside the cells, the identification of potential targets and attempts to elucidate the mechanisms of cell death may allow to improve our knowledge of such critical cellular events and, also help to achieve a more rational approach in the design of V anticancer drugs.

2. Materials and methods

2.1. Materials

All common laboratory chemicals were purchased from commercial sources and were used without further purification. Salicylaldimine ligands L (Fig. 1) were synthesized in situ from an equimolar mixture of the corresponding aldehyde and glycine as described below (L1 = 5Brsal-Gly = *N*-5-bromosalicylidene-glycinate; L2 = sal-Gly = *N*-salicylidene-glycinate).

2.2. Syntheses of the oxidovanadium(IV) complexes, [$V^{IV}O$(L-2H))(NN)], 1–8

The [$V^{IV}O$(L-2H)(NN)] complexes, where L = 5-bromosalicylaldehyde glycine derivative (L1) or salicylaldehyde glycine derivative (L2) and NN = 2,2′-bipyridine (bipy), 1,10-phenanthroline (phen), 5-amine-1,10-phenanthroline (aminophen), 5,6-epoxy-5,6-dihydro-1,10-phenanthroline (epoxyphen), dipyrido[3,2-*a*: 2′,3′-*c*]phenazine (dppz) or [1,2,5]thiadiazolo[3,4-*f*][1,10]phenanthroline (tdzp), were synthesized using the following general procedure: 0.50 mmol of glycine (42 mg) and 1.0 mmol of sodium acetate were dissolved in 2 mL of distilled water. 0.50 mmol of 5-Br-salicylaldehyde (100 mg) or salicilaldehyde (61 mg) and 0.50 mmol of NN (78 mg bipy, 98 mg phen, 98 mg aminophen, 98 mg epoxyphen, 141 mg dppz or 120 mg tdzp) were dissolved in 3 mL EtOH (MeOH for dppz). Both solutions were mixed; to this mixture a solution of 0.43 mmol $V^{IV}OSO_4$ (108 mg) in 1 mL H_2O was added dropwise. The reaction mixture was stirred at room temperature for 30 min. The orange-brown solids were separated by centrifugation, washed five times with 2 mL portions of H_2O and dried under vacuum.

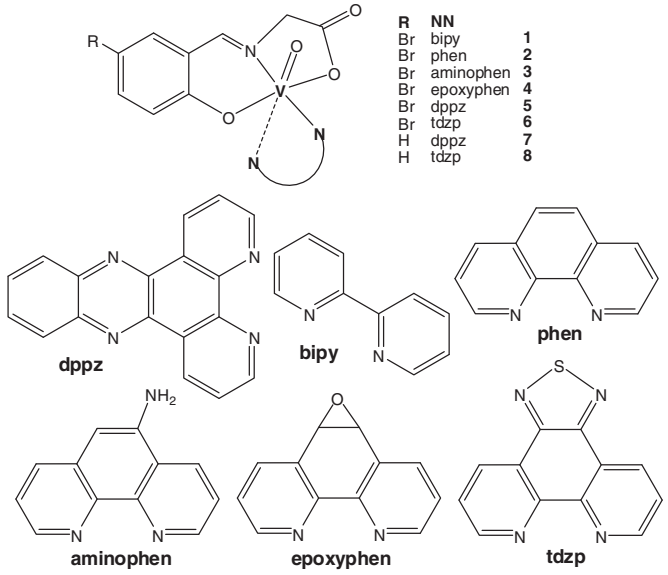

Fig. 1. Selected NN polypyridyl co-ligands and molecular structure of the oxidovanadium(IV) complexes with tridentate *N*-salicylidene-glycinato ligands studied in this work (dppz = dipyrido[3,2-*a*: 2′,3′-*c*]phenazine, bipy = 2.2′-bipyridine, phen = 1.10-phenanthroline, aminophen = 5-amine-1,10-phenanthroline, epoxyphen = 5.6-epoxy-5,6-dihydro-1,10-phenanthroline and tdzp = [1,2,5]thiadiazolo[3,4-*f*][1,10]phenanthroline).

2.2.1. [$V^{IV}O$(L1 −2H)(bipy)]·2H_2O, 1

Yield: 57 mg, 22%. Anal. calc. for $C_{19}H_{14}BrN_3O_4V\cdot 2H_2O$: C, 44.3; H, 3.5; N, 8.2. Found: C, 44.0; H, 3.6; N, 8.1. Electrospray ionization mass spectra (ESI-MS) (MeOH) m/z [Found (Calcd)]: 157.1 (157.07) (40%) $[bipy + H]^+$; 478.9 (478.96) (20%) (Br isotopic pattern) $[M + H]^+$, 523.7 (522.93) (80%) (Br isotopic pattern) $[M + 2Na\text{-}H]^+$. Thermogravimetric analysis (TGA) weight loss below 100 °C [Found (Calcd)]: 3.4, 3.2% (7.0%), two weight losses.

2.2.2. [$V^{IV}O$(L1-2H)(phen)], 2

Yield: 152 mg, 60%. Anal. calc. for $C_{21}H_{14}BrN_3O_4V$: C, 50.1; H, 2.8; N, 8.3. Found: C, 49.8; H, 2.9; N, 8.3. ESI-MS (MeOH) m/z [Found (Calcd)]: 168.8 (168.33) (42%) (Br isotopic pattern) $[M + 3H]^{3+}$; 181.2 (181.07) (10%) $[phen + H]^+$, 241.6 (241.14) (30%) [phen + isoprop + H]$^+$, 275.8 (275.01) (100%) (Br isotopic patterns) $[L1 + NH_4]^+$, 383.0 (383.13) (40%) $[2phen + Na]^+$. TGA: absense of crystallization solvent molecules.

2.2.3. [$V^{IV}O$(L1-2H)(aminophen)]·2H_2O, 3

Yield: 136 mg, 49%. Anal. calc. for $C_{21}H_{15}BrN_4O_4V\cdot 2H_2O$: C, 45.5; H, 3.5; N, 10.1. Found: C, 45.4; H, 3.6; N, 10.0. ESI-MS (MeOH) m/z [Found (Calcd)]: 196.2 (196.09) (32%) [aminophen + H]$^+$; 336.1 (335.99) (70%) (Br isotopic pattern) [L1 + DMSO + H]$^+$, 519.0 (517.98) (45%) (Br isotopic pattern) $[M + H]^+$, 1034.1 (1034.95) (35%) (Br isotopic pattern) $[2\ M + H]^+$. TGA weight loss below 100 °C [Found (Calcd)]: 7.1% (6.5%).

2.2.4. [$V^{IV}O$(L1-2H)(epoxyphen)]·2½H_2O, 4

Yield: 97 mg, 35%. Anal. calc. for $C_{21}H_{12}BrN_3O_5V\cdot 2½\ H_2O$: C, 44.8; H, 3.1; N, 7.5. Found: C, 44.7; H, 3.2; N, 7.4. ESI-MS (MeOH) m/z [Found (Calcd)]: 239.0 (239.02) (35%) [epoxyphen + 2Na-H]$^+$; 333.8 (333.89) (65%) [L1 + 2 K–H]$^+$, 577.0 (577.56) (25%) (Br isotopic pattern) [M + isoprop + H]$^+$. TGA weight loss below 100 °C [Found (Calcd)]: 6.9% (8.0%).

2.2.5. [$V^{IV}O$(L1-2H)(dppz)]·½H_2O, 5

Yield: 173 mg, 55%. Anal. calc. for $C_{27}H_{17}BrN_5O_4V\cdot ½H_2O$: C, 52.7; H, 2.8; N, 11.4. Found: C, 52.8; H, 2.9; N, 11.4. ESI-MS (MeOH) m/z [Found (Calcd)]: 283.3 (283.09) (45%) $[dppz + H]^+$; 587.0 (587.17) (30%) $[2dppz + Na]^+$. TGA weight loss below 100 °C [Found (Calcd)]: 1.9% (1.5%).

2.2.6. [$V^{IV}O$(L1-2H)(tdzp)]·2H_2O, 6

Yield: 80 mg, 28%. Anal. calc. for $C_{21}H_{12}BrN_5O_4SV\cdot 2H_2O$: C, 42.2; H, 2.7; N, 11.7; S, 5.4. Found: C, 42.2; H, 2.6; N, 11.6; S, 5.9. ESI-MS (MeOH) m/z [Found (Calcd)]: 168.8 (169.00) (45%) $[M + 2H + Na]^{3+}$; 241.5 (242.00) (35%) $[M + 2H]^{2+}$. TGA weight loss below 100 °C [Found (Calcd)]: 6.0% (6.4%).

2.2.7. [$V^{IV}O$(L2 −2H)(dppz)]·H_2O, 7

Yield: 170 mg, 63%. Anal. calc. for $C_{27}H_{17}N_5O_4V\cdot H_2O$: C, 59.6; H, 3.5; N, 12.9. Found: C, 59.5; H, 3.3; N, 12.9. ESI-MS (MeOH) m/z [Found