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Evaluation of DNA/BSA interaction and in vitro cell cytotoxicity of μ_2-oxido bridged divanadium(V) complexes containing ONO donor ligands

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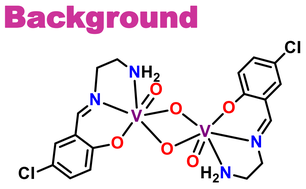
Introduction

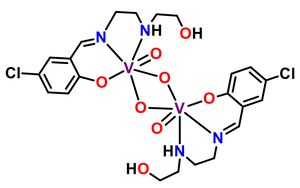
- Designing metal based drugs is an active area of research in medicinal inorganic chemistry to develop alternatives to platinum based anticancer drugs like cisplatin and its derivatives because of their limitations and disadvantages.
- Among the first-row transition metals, vanadium metal complexes have received a lot of attention due to their effective cytotoxic activity, proapoptotic effects. Also, DNA binding as well as photo-induced DNA cleavage activity.
- Moreover, several vanadium-based compounds have also been studied for their chemical transformation like hydrolysis and redox reactions in aqueous solution or cell culture media.
- So, keeping all these observations in mind, herein we have reported the synthesis of two new pentacoordinated μ_2-oxido bridged divanadium(V) complexes (1-2) by using ONO-donor ligands, their characterization, and also study of their DNA/BSA interaction along with antiproliferative activity and its mechanism.

Background



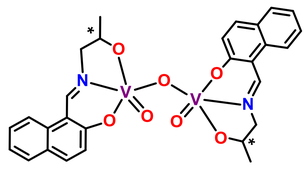
Insulin-enhancing activity and cytotoxicity

J. Inorg. Biochem. 104 (2010) 851–857



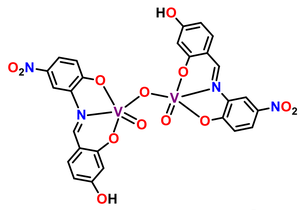
Insulin-enhancing activity and cytotoxicity

Eur. J. Med. Chem. 45 (2010) 6077–6084.



In vitro anticancer activity, DNA and HSA interaction

Eur. J. Med. Chem. 135 (2017) 230–240.



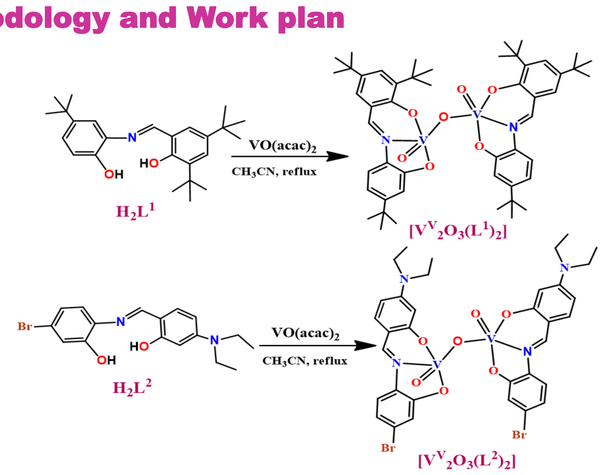
DNA Interaction and Cytotoxicity Activity

Inorganics 9 (2021) 66.

Objectives

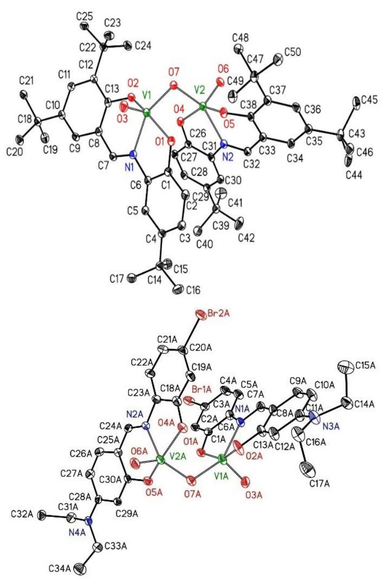
- Synthesis of two pentacoordinated μ_2-oxido bridged divanadium (V) complexes (**1-2**) by using bi-negative tridentate ONO-donor ligands.
- Spectral Characterization and structure determination by single crystal X-ray diffraction study and correlation of the structure with the observed properties. The aqueous phase stability of these complexes has been evaluated through HR-ESI-MS in CH_3CN:H_2O mixture.
- Exploring the biomolecular (DNA/BSA) interaction and evaluation of their *in vitro* cytotoxic activity against HeLa (cervical cancer) cancer cell and NIH-3T3 (normal mouse embryonic fibroblasts cells) cell line by MTT assay.
- Study the mechanism of cell death through nuclear staining, cell cycle and Annexin V/PI double staining apoptotic assay.

Methodology and Work plan



Single crystal X-ray Diffraction Study

a)

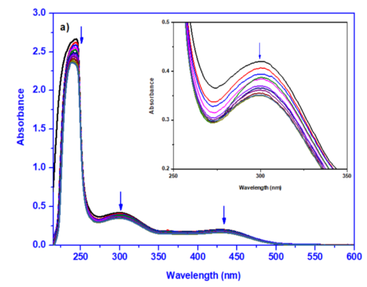


Complex	1	2
Crystal system	monoclinic	monoclinic
space group	P 2_1/c	P 2_1/c
a / Å	14.6419(6)	29.1710(7)
b / Å	26.0161(11)	10.2908(2)
c / Å	14.9570(6)	28.5104(7)
α /°	90	90
β /°	117.771(2)	118.1580(10)
γ /°	90	90
Z	4	8

b)

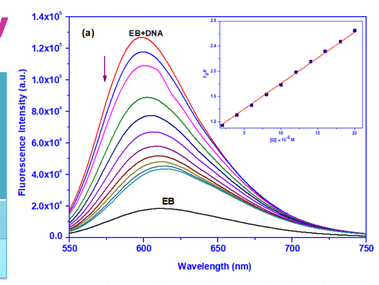
Molecular structure and atom labelling scheme of (a) $[V^V_2O_3(L^1)_2]$ (**1**), (b) the two crystallographic independent $[V^V_2O_3(L^2)_2]$ units of **2** with ellipsoids representing a probability of 30%, hydrogen atoms are neglected for the sake of clarity.

DNA Binding study



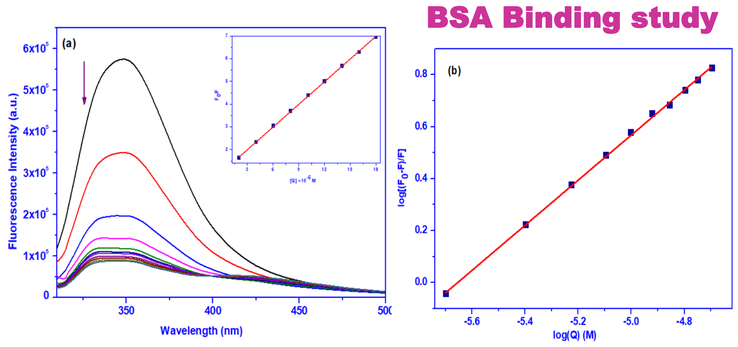
Electronic absorption spectrum of **1** (10 μM) upon titration with CT–DNA (0–50 μM) in Tris–HCl buffer (50 mM Tris, pH 7.4).

Complex	Binding constant (K_b) (M^{-1})
1	6.3×10^4
2	3.6×10^4



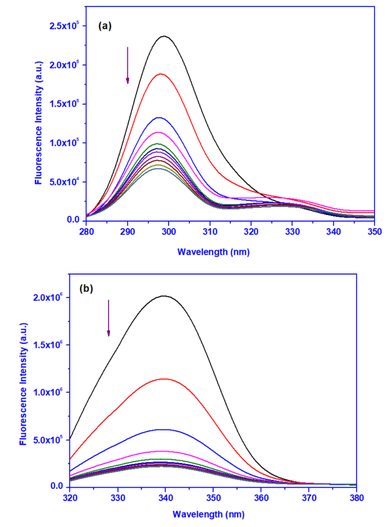
CT–DNA bound EB (5 μM) displacement assay by complex **1** upon increasing the concentrations (0–20 μM); inset showing the Stern–Volmer plot of complex **1**.

BSA Binding study



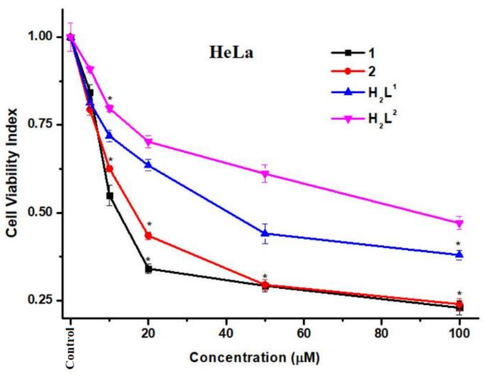
Fluorescence quenching spectra of BSA (2 μM) by complex **1** (0–10 μM); the Stern-Volmer plot is shown in inset (a), Scatchard plot of complex **1** (b).

Complex	K_{SV} (M^{-1})	k_q ($M^{-1}S^{-1}$)	K_a (M^{-1})	n
1	3.3×10^5	5.3×10^{13}	8.1×10^4	0.86
2	1.2×10^5	1.9×10^{13}	2.8×10^2	0.45

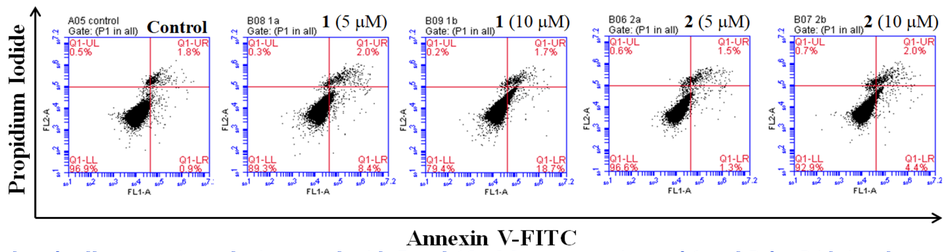


Synchronous fluorescence spectra of BSA (2 μM) by complex **1** (0–10 μM) at Δλ= 15 nm (a) and Δλ= 60 nm (b).

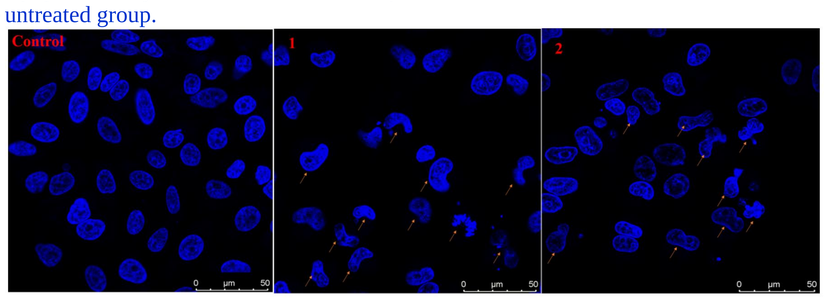
Study of Anticancer activity



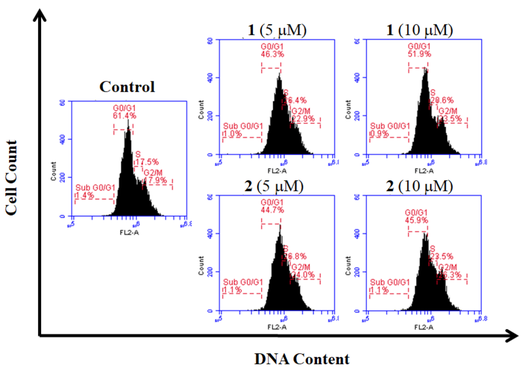
Cytotoxic profile of HeLa cells after treatment for 48 h with ligands $H_2L^{1,2}$ and complexes **1** and **2** (5, 10, 20, 50, 100 mM). *p < 0.05 statistical differences between treatment of **1** and **2**.



Quadrant graphs of cell apoptosis analysis treated with 5 and 10 μM concentrations of **1** and **2** for 24 h incubation time. Control being the untreated group.

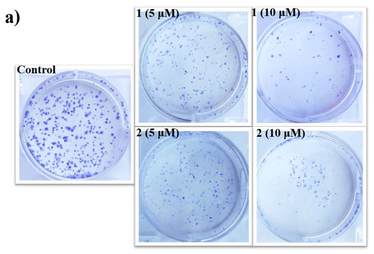


Morphologies of HeLa cells after treatment with **1** and **2** at 10 μM concentrations for 24 h.



Effect complexes **1** and **2** on cell cycle in HeLa cells determined by flow cytometer.

Complexes	IC_{50} (mM)		SI (Selectivity Index)
	HeLa	NIH–3T3	
1	13.57 ± 0.49	29 ± 0.95	2.13
2	16.62 ± 0.1	35.8 ± 0.3	2.15
H_2L^1	40.39 ± 0.03	71.2 ± 1.3	1.76
H_2L^2	88.9 ± 2.3	>100	1.21
Cisplatin	12.2	4.7	0.38

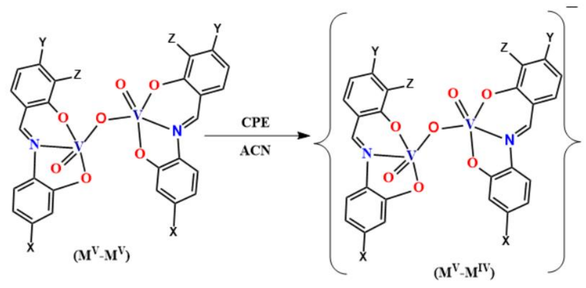


Representative images of colony formation by **1** and **2**.

Conclusion

- Two μ_2-oxido bridged divanadium(V) complexes $[V^V_2O_3(L^{1,2})_2]$ (**1** and **2**) by using bi-negative tridentate ONO-donor ligands were synthesized and characterized. The molecular structures of (**1**, **2**) were solved by single crystal X-ray diffraction analysis.
- The interaction of the complexes **1**, **2** with DNA/BSA were examined. The results show that the complexes bind with DNA in intercalation mode with binding constant in the order of 10^{-4} M^{-1}. In addition, **1** and **2** interact with BSA in ground state, showing static quenching phenomenon.
- Also, results of the antiproliferative activities of the synthesized complexes **1**, **2** suggested that they are significantly cytotoxic towards HeLa cell lines.
- Furthermore, the complexes showed the inhibitory effects on the S and G2M phase of cell cycle, which is an indication of apoptotic cell death. Also, the nuclear staining and Annexin V/PI double staining apoptotic assay unveil that both the complexes have the ability to induce apoptosis in HeLa cell lines.

Future work



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- A. Banerjee, S.P. Dash, M. Mohanty, G. Sahu, G. Sciortino, E. Garribba*, M.F.N. Carvalho, F. Marques, J. Costa Pessoa*, W. Kaminsky, K. Brzezinski, and R. Dinda*, **Inorg. Chem.** 59 (2020) 14042–14057.

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