Peroxoniobium inhibit leukemia cell growth

Supporting Information

EXPERIMENTAL

Cells and culture

The K562 cell line was purchased from the Rio de Janeiro Cell Bank (number CR083 of the RJCB collection). This cell line was established from the pleural effusion of a 53 year-old female with chronic myelogenous leukemia in terminal blast crisis. Cells were cultured in RPMI 1640 (Sigma Chemical Co.) medium supplemented with 10% fetal calf serum (CULTILAB, São Paulo, Brazil) at 37°C in a humidified 5% CO_2 atmosphere. Cultures grow exponentially from 10^5 cells/mL to about 8×10^5 cells/mL in 3 days. Cell viability was checked by Trypan Blue exclusion (Sigma Chemical Co) using optical microscopy. The cell number was determined by analysis in a Neubauer chamber. For cytotoxicity assessment, 1×10^5 cells/mL were cultured for 72 h in the absence and presence of a range of concentrations of tested compound. The sensitivity to the compound was evaluated by the concentration that inhibits cell growth by 50%, IC_{50}. Stock solutions of the compound were prepared in dimethyl sulfoxide. Other solutions of these complexes were prepared from stock solutions in water. The final concentration of dimethylsulfoxide in the culture medium was less than 0.1%

Photocytotoxicity

For photocytotoxicity assessment, 1×10^5 cells/mL were cultured for 4 h in the absence and presence of a range of concentrations of tested compound. After this time, the cells were washed three times with cold isotonic buffer, and resuspended in the same buffer to be subjected to irradiation for 5 minutes with UV-A light (365 nm, 610 $\mu W\ cm^{-2}$). Then, the isotonic buffer was replaced by RPMI 1640 medium supplemented with 10% fetal calf serum, and cells were incubated for 72 hours at 37°C in a humidified 5% CO_2 atmosphere. The sensitivity to compound was evaluated by the concentration that inhibits cell growth by 50%, IC_{50}. For comparison, cytotoxicity assays were performed with the same protocol, but without exposure of cells to radiation. Cell viability was checked by Trypan Blue exclusion using optical microscopy and the cell number was determined by analysis in a Neubauer chamber.

Control tests were performed by irradiating the cells in isotonic buffer for 5 minutes with UV-A light (365 nm, 610 $\mu W\ cm^{-2}$). Under this condition, irradiation did not affect cell growth or viability.

^{31}P NMR Analyses

^{31}P NMR spectra were acquired on a spectrometer with a 400 MHz field using phosphoric acid as standard. For the preparation of the guanosine sodium 5-monophosphate (5-GMP) samples, a solution was prepared by dissolving 20mg of the sample in 600µL of D_2O. Subsequently, a solution containing 5-GMP polyoxoniobate was prepared and 200µL of this solution was added 400µL of D_2O. Another sample was prepared following the same procedure described above, and this was subjected to radiation at a wavelength of 365nm for 7 min. All 3 samples were submitted to ^{31}P NMR experiment.

^{31}P experiments were performed at 20◦C on an RMN spectrometer Avance DRX 200 at the High Resolution Magnetic Resonance Laboratory of UFMG (Laremar). With a 5 nm multinuclear probe and procedure to optimize the field homogeneity (shimming), as well as probe tuning in the frequency of ^{31}P, in order to have optimal resolution and signal detection.

Computational methodology

1. Semi-empirical methods

The semi-empirical methods emerged in 1931 with the studies of Michael Polanyl and Henry Eyring, (Morgon & Coutinho, 2007), which address the quantum theory coupled to empirical results, producing satisfactory data. This category of methods enables the approach of large systems with many atoms, and it is easily used today. As such, it is possible to work with proteins, DNA, enzymes, and other molecular systems with tens of thousands of atoms. The PM6 semi-empirical method has shown to be appropriate for performing the calculations of the Nb complex binding energies in the Dickerson-Drew Dodecamer B-DNA.

Summarizing, properties such as enthalpy of formation, dipole moment, ionization potential, angles, dihedral, and bond lengths are included. The reference database should be extended to a representative group of molecules that present good accuracy in the empirical results of its properties (Morgon & Coutinho, 2007).

2. DFT Calculations

Density functional calculations were performed using the PBE exchange/correlation functional as implemented in Gaussian 09 suite package. The niobium atoms were described by LanL2DZV effective potential and the oxygen atoms by the aug-cc-pVDZ basis sets. The Lindqvist and Nyman structures were optimized and the harmonic frequencies calculated using the analytical second derivatives. Real frequencies assure that a minimum at the potential energy surface (PES) was obtained. The solvent effects were included using the polarizable continuum model PCM for water solvent (ε=78.3553) and the parameter α set to 1.4.

Results and Discussion

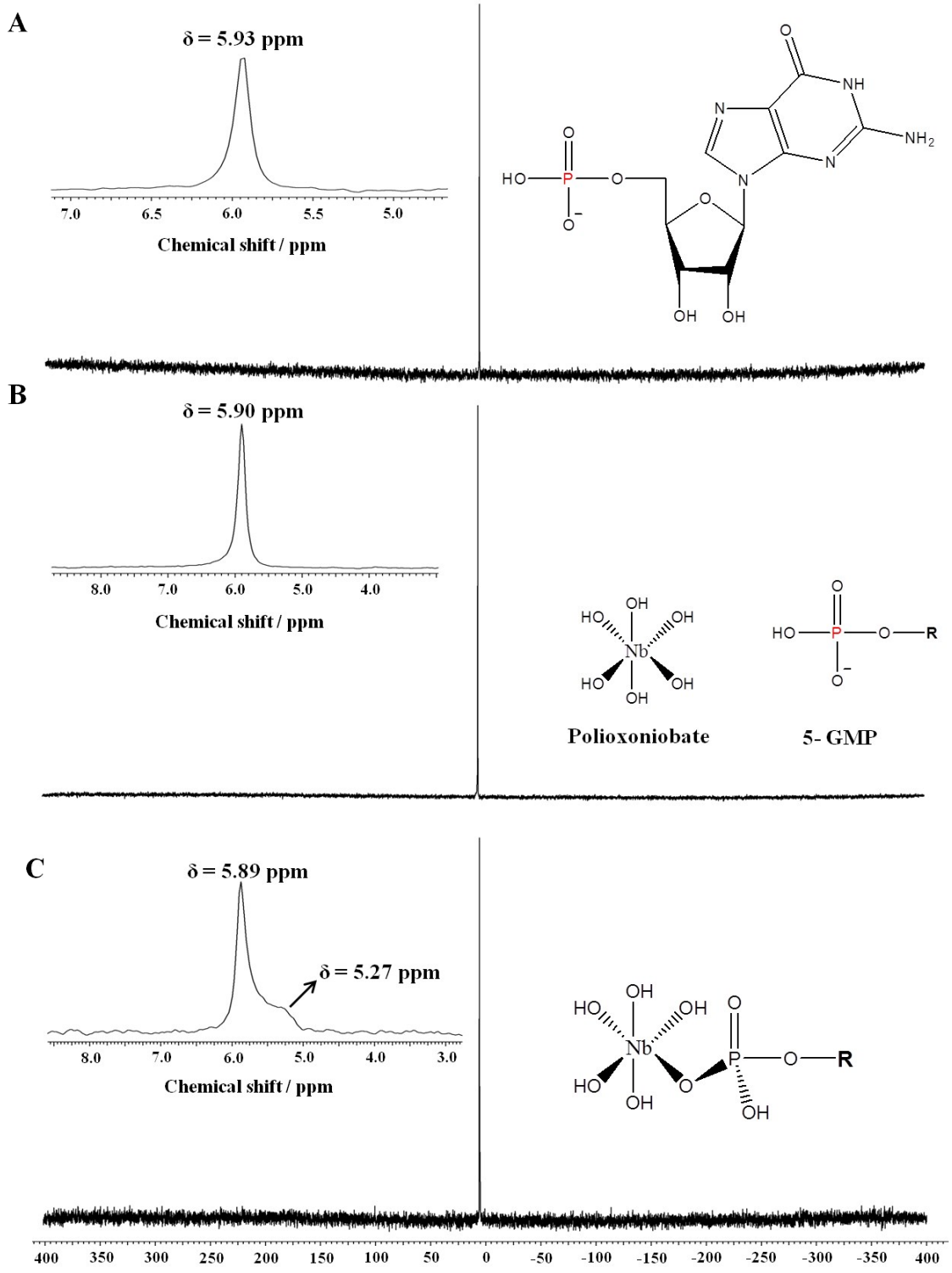

Figure S1: Spectra of 31P acquired on a 400MHz spectrometer for A) sodium 5-monophosphate (5-GMP), B) mixture of 5-GMP and polyoxoniobate and C) mixture of 5-GMP and polyoxoniobate after radiation.

1.0 DFT calculations

DFT techniques have been widespread, showing good performance for complex systems, such as biomolecules[1,2]. The calculations were based on the generalized gradient approximation functional proposed by Gustin et al[3]. This relationship between density functional and basis set has been tested for similar systems[4, 5].

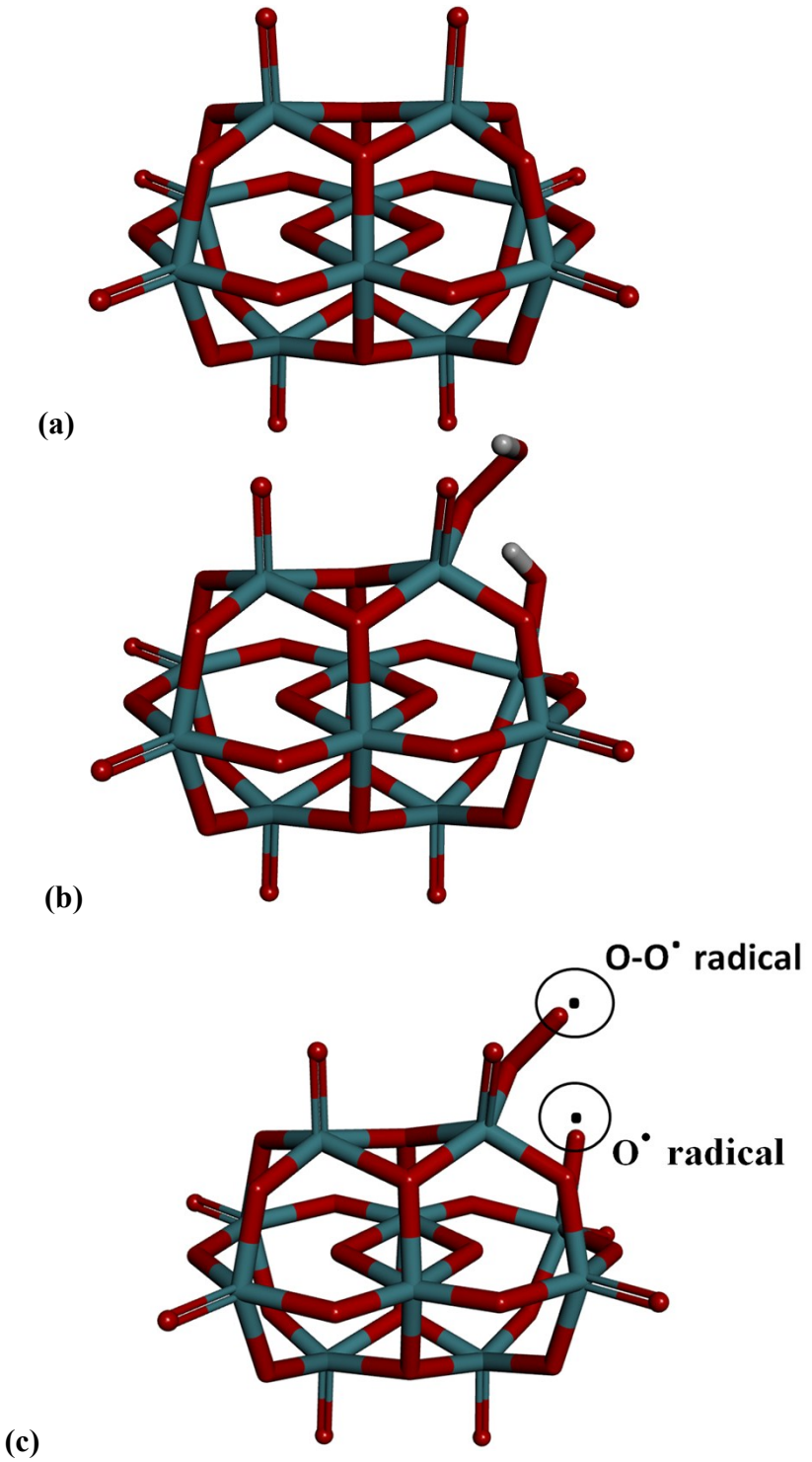

Figure S2: Nb complexes structures: (a) no radicals group, (b) protonated structure and (c) radicals formation.

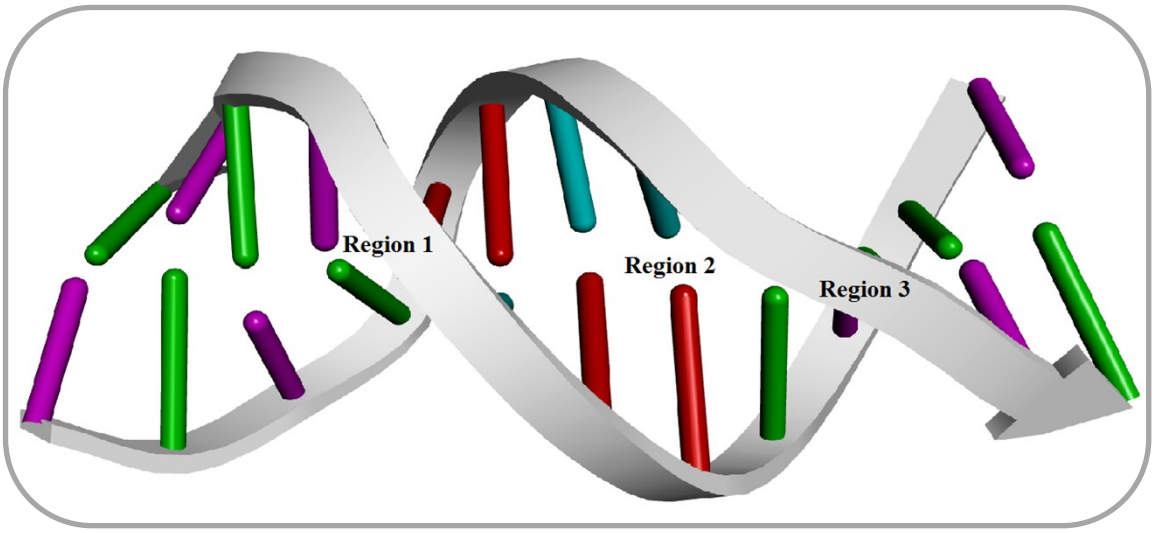

Figure S3: Regions in the B-DNA DODECAMER considered for this investigation.

Molecule: 5'-D(*CP*GP*CP*GP*AP*AP*TP*TP*CP*GP*CP*GP)-3'; Chains: A, B; Length: 12 [6].

Table S1: Relative interaction energy values from Nb complex (Complex **a**) in different regions of DODECAMER B-DNA.

DODECAMER B-DNA	**PM6** **$\Delta\Delta E^{\#}$ (kcal.mol^{-1})**
Region **1**	21.98
Region **2**	0.00
Region **3**	37.91

Table S2: Relative interaction energy values from Nb complexes (Figure S**2**) in DODECAMER B-DNA.

Nb Complex	**DFT** **$\Delta\Delta E^{\#}$ (kcal.mol^{-1})**
Complex **a**	2.88
Complex **b**	5.01
Complex **c**	0.00

Table S3: The mainly stretches and their features.

Nyman	
Wavenumber (cm^{-1})	Assignment
391	O_{bridge}-Nb-O_{bridge} symmetric bend
462-405	O_{bridge}-Nb-O_{bridge} symetric bend
524-508	Nb-O_{bridge}-Nb Asymmetric bend
550-761	Nb-O_{bridge}-Nb Asymmetric str
847-868	Nb-$O_{terminal}$ asymmetric str
Lindvqist	
Wavenumber (cm^{-1})	Assignment
375-378	Nb-$O_{central}$-Nb Asymmetric bend
492-489	Nb-O_{bridge}-Nb Asymmetric bend
645-643	Nb-O_{bridge}-Nb Asymmetric str
720-723	Nb-$O_{terminal}$ Asymmetric str

References

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