NJC

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: D. Marques-da-Silva, G. Fraqueza, R. Lagoa, A. A. Vannathan, S. S. Mal and M. Aureliano, *New J. Chem.*, 2019, DOI: 10.1039/C9NJ01208G.

ROYAL SOCIETY OF CHEMISTRY

rsc.li/njc

ROYAL SOCIETY OF CHEMISTRY
View Article Online
DOI: 10.1039/C9NJ01208G

Journal Name

ARTICLE

Polyoxovanadates inhibition of *Escherichia coli* growth shows a reverse correlation with Ca^{2+}-ATPase inhibition

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Dorinda Marques-da-Silva[1,2], Gil Fraqueza[3,4], Ricardo Lagoa[1,2], Anjana Anandan Vannathan[5], Sib Sankar Mal[5*] and Manuel Aureliano[4,6*]

Recently, a global analysis of the structure-activity-relationship of a series of polyoxometalates (POMs) referred that the most active POMs were ascribed to be polyoxovanadates (POVs), especially decavanadate (V_{10}), which was very active against certain bacteria (Bijelic et al, Chem Comm, 2018). The present study explores this observation and compares the effects of three POVs namely MnV_{11}, MnV_{13} and V_{10} against *Escherichia coli* growth. It was observed that MnV_{11} presents the lowest growth inhibition (GI_{50}) value for *Escherichia coli* followed by MnV_{13} compound being about 2 times lower than V_{10}, respectively the values obtained were 0.21, 0.27 and 0.58 mM. All three compounds were more effective than vanadate alone (GI_{50} = 1.1 mM) and also than decaniobate, Nb_{10} (GI_{50} > 10 mM), a isostructural POM of V_{10}. However, the POVs exhibiting the highest antibacterial activity (MnV_{11}) shows to have the lowest Ca^{2+}- ATPase inhibitor capacity (IC_{50} = 58 µM) whereas decavanadate, which was also very active against this membranar ATPase (IC_{50}=15 µM), was the less active against bacteria growth suggesting that POVs inhibition of ion pumps might not be associate with the inhibition of *Escherichia coli* growth.

Introduction

Since the first paper published in the 70´s, today it can be found about 4,623 articles with the topic "Polyoxometalates". In the last seven years (2011-2017) the number has increase 1.5 times in comparison to the previous ones (2004-2010), from 1,446 to 2,228, after a quick research at the Web of Science. Polyoxometalates (POMs) present a diversity of different structure and size, with a versatile combination of metals having applications in several fields such as catalysis, prevention of corrosion, smart glasses, macromolecular crystallography, among others, besides being recently to be very relevant to the medicine field [1-6]. In fact, due to the rapid progress of the research field of POMs, POM-based hybrid and nanocomposite structures with antibacterial and anticancer activities, these topics have been recently reviewed [7,8].

As bacterial resistance is growing every year all around the world, together with the toxicity of chemotherapeutic agents, POMs have been selected by many researchers due to its promising results as alternative antibacterial substances [7]. In this fundamental review, it was established a future perspective of the application of POMs, POM-based hybrids and/or nanocomposites as antimicrobial agents [7]. Furthermore, a detailed analysis of the reported antibacterial activities of POMs in order to obtain a structure-activity-relationship, which was currently missing in this field, push forward the use of these metallodrugs in the combat of pathogenic microorganisms [7]. Besides the antibacterial activities, POMs applications as anticancer, antidiabetic and antiparasitic agents, among others, have also been described [7,8,9,10]. However, even if POMs's applications in medicine are extensive and well documented, the mechanism of action by which each POM exert its effects as potential inhibitors of bacteria and tumor proliferation is still not well understood [7,8]. For example, the antitumor activity

[1]ESTG, Polytechnic Institute of Leiria, Portugal; [2] UCIBIO, Faculty of Science and Technology, University NOVA of Lisbon, Portugal; [3]ISE, University of Algarve, 8005-139 Faro, Portugal; [4]CCMar, University of Algarve, 8005-139 Faro, Portugal; [5]Department of Chemistry, National Institute of Technology Karnataka, Mangalore 575025, Karnataka, India; [6]FCT, University of Algarve, 8005-139 Faro, Portugal

*Corresponding authors address: M. Aureliano (maalves@ualg.pt), Sib Sankar Mal (malss@nitk.edu.in)

† Footnotes relating to the title and/or authors should appear here.
Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

View Article Online
DOI: 10.1039/C9NJ00966G

enhanced by these versatile inorganic compounds it seems to be due, at least in part, to the inhibition of certain enzymes such as alkaline phosphatases, kinases, ecto-nucleotidases, and P-type ATPases [8,11-15]. Regarding the former type of enzymes, it was recently referred that several POMs inhibits not only *in vitro* but also *in vivo* P-type ATPases such as Ca^{2+}-ATPase and Na^{+}/K^{+}-ATPase [16]. In addition, several POMs, particularly decavanadate seems also to strongly interfere with several biochemical processes such as muscle contraction and mitochondria respiration [17,18,19], while others can exert a immunomodulatory activity [20]. POMs have also shown promising against Alzheimer's disease once it was referred to strongly inhibit amyloid aggregation associated with this disease [21,22].

Decavanadate, is an polyoxometalate with 10 octahedral vanadium(V) centres ($V_{10}O_{28}^{6-}$), known to interact with a wide range of biomolecules and exerts several biological activities, not only *in vitro* but also *in vivo*, by affecting, for example, mitochondrial function, increasing glucose uptake in adipocytes, inhibiting the process of muscle contraction as well as actin polymerization besides inducing changes in oxidative stress parameters differently from the monomeric oxovanadate [17,18,19,23-25]. So far, the most cited paper regarding decavanadate (V_{10}) in biology is the interaction of V_{10} in a spatially selective manner within the protein cages of virions [26]. Besides preventing the formation of virions, decavanadate is also able to inhibit the virus activities by preventing the virus-cell host binding [26]. In our laboratory there is a long tradition in the preparation of decavanadate solutions [16,17,18,23,24,25]. We started thirty five years ago, in 1984, during the study of the interactions of Mo, W and V oxoanions with monosaccharides by NMR spectroscopy. Currently, we are focused mainly on studies about the POMs interactions with P-type ATPases besides on antibacterial activities by polyoxovanadates (POVs) and polyoxotungstates (POTs) (Fig. 1). Several research kingdoms were crossed during this period of time, some much larger time than expected such as calcium ATPase domain (from 1991 to 2018) and actin (from 2006 to 2018) whereas others were smaller as expected such as myosin (1988 to 2007) and the *in vivo* studies (from 2000 to 2008) (Fig. 1).

Although V_{10} has been detected in some acid cell organelles, the processes of transport of intact decavanadate as well as others POMs and their permeation through cellular membrane is not known [23,25,27,28]. In addition, the existence of decavanadate in cells is still object of discussion [25,28]. Nevertheless, it was suggested that once formed it can interact with ATPases as well as others membrane associated enzymes including ion pumps, sialyl- and sulfo-transferases most likely from the extracellular side of the membrane. Thus, decavanadate and similarly others POMs could, at least partially, be responsible for the prevention of several processes such as respiration [17,18,19] (or other redox processes) and concomitantly for the observed antibacterial activity [7] as well as in the combat against cancer [8,29].

As microorganisms represent a serious threat to human health and have been the origin of different kinds of epidemics it is of great importance to push this field forward in order to better understand the potential significance of POMs in the combat against bacteria. Regarding the antibacterial activity of POMs, it was found that for *Helicobacter pylori*, POMs exhibiting the highest activity were mostly Keggin-type POTs, polyoxovanadotungstates and large highly negatively charged POMs, whereas in *Streptococcus pneumoniae*, the most active POMs were ascribed to be POVs, especially decavanadate, which was also very active against other bacteria [7]. It is well known that *Escherichia coli* (*E. coli*) infection is related with hygienic conditions inducing several diseases in humans such as urinary tract infections, colitis, and diarrhea [30]. In the present study, we report and compare the effects of decavanadate, decaniobate and two manganesepolyoxovanadates against *E. coli*. Furthermore, we analyzed the relationship between the inhibition of bacterial growth and the capacity of inhibiting the Ca^{2+}- ATPase by these POVs. It was observed that the POV exhibiting the highest antibacterial activity shows to have the lowest Ca^{2+}-ATPase inhibitor capacity whereas decavanadate, which was also very active against Ca^{2+}-ATPase was comparatively the less active against *E. coli.*

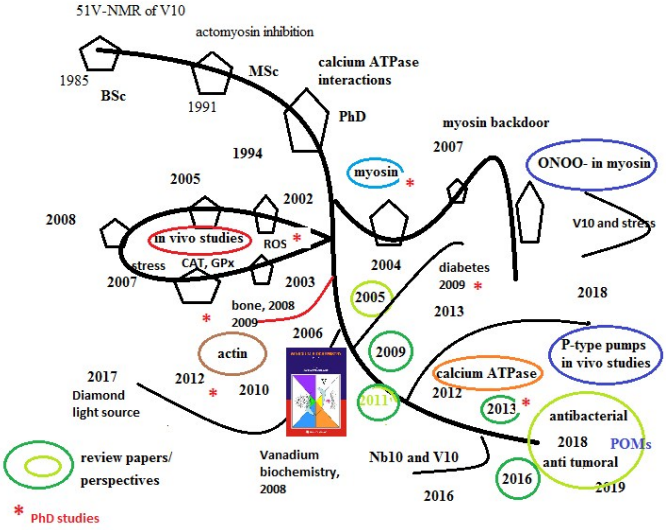


Figure 1. Timeline of Aureliano and co-workers decavanadate studies since 1985 to 2018. Several research kingdoms were cross some much larger than expected whereas others were smaller.

2. Experimental section

2.1. Preparation of polyoxometalatates

Manganesepolyoxovanadates $K_5MnV_{11}O_{33}{\cdot}10H_2O$ (abbreviated MnV_{11}) and $K_7MnV_{13}O_{38}{\cdot}18H_2O$ (abbreviated MnV_{13}) were synthesized according to published procedures [31,32]. The two compounds were crystalline in nature. We have confirmed those compounds by FTIR (Fig. S1 and Fig. S2). Aqueous stock solutions of these POVs were prepared in concentrations up to 1 or 4 mM stock solution in water considering a M_r of 1518.92 g/mol for MnV_{11} and 1923.12 g/mol for MnV_{13}, respectively. Solutions of MnV_{11} and

MnV_{13} were prepared daily wherever adequate by dissolution of the solid compounds in water and kept on ice during the utilization to avoid putative POM decomposition. Other common analytical solutions described below for the preparation of the calcium pump vesicles and for the kinetic studies, were prepared from reagents obtained from Sigma-Aldrich (Portugal). Ammonium metavanadate (99.9% NH_4VO_3) was also purchased from Sigma-Aldrich. Pale yellow stock solutions of ammonium metavanadate (50 mM) were prepared in water after solubilization. The pH of this solution was then adjusted with NaOH to 10.5 and heated until we obtain a collorless solution. After cooling, to half of this solution of vanadate, HCl was added until pH 4.0 resulting an orange colored solution of decavanadate with 5 mM concentration.

Therefore, with the same "mother" vanadate solution we obtained two working solutions: vanadate (50 mM V_1, pH 10.5) and decavanadate (5 mM V_{10} (50 mM V-atoms), pH 4.0), similarly as previously described in studies of V_{10} interactions with proteins and also in *in vivo* studies [17,18,33,34]. Decaniobate solutions (100 mM Nb_{10}) were prepared in water from tetramethylammonium decaniobate $[N(CH3)_4]_6[Nb_{10}O_{28}]\cdot 6H_2O$, M_r of 1930.01 g/mol, that was synthesized according to methods described elsewhere [35]. Here, we are analysing the antibacterial effects of POMs belonging to common representative structure of isopolyanions such as V_{10}, (and also for Nb_{10}) as well as POVs which contain one additional element (heteropolyanions) such as MnV_{11} and MnV_{13}, belonging these vanadomanganates, at least the latter one to the lacunary Keggin archetype.

2.2. Antibacterial action

Inhibition of bacterial growth by the compounds in this work was studied with cultures of *E. coli* (ATCC 25922) in LB medium, by two experimental approaches. Minimum inhibitory concentrations (MIC) were measured by the serial two-fold dilution method. Dilutions of each compound in LB broth (final volume 100 ml) were prepared in the range 64 to 4096 µg/ml (anion mass), inoculated (5 ml) and incubated 18 hours at 35 ºC. Manganesepolyoxovanadates MnV_{11} and MnV_{13} were tested only up to 2048 µg/ml concentration because of their limited solubility. After incubation, occurrence or absence of bacterial growth was visually examined. The pH of the culture media with the compounds was checked at end of incubations and all values were approximately equal to 6, except the cultures with Nb_{10} at the higher concentration that showed a slight alkalinisation to pH 8. In other set of experiments, the inhibitory potency of low milimolar concentrations of the compounds against *E. coli* growth was evaluated in 3-hour cultures. Compounds were added to LB medium (final volume 1200 ml), that was inoculated with 5 µl of an overnight culture of bacteria and incubated at 37 °C for 3 hours. Vanadate (50 mM, pH 10.5) and decavanadate (5 mM V_{10} (50 mM V-atoms), pH 4.0) stock solutions were added to the bacterial assay media. Control cultures were run in parallel in the same conditions but only deionized water added to medium.

Bacterial growth was assessed by the optical density (OD) of cultures at 600 nm, and absorbance of the culture supernatant after centrifugation (10000 g for 10 min) was also measured for possible changes in the optical absorbance of media. Inhibition of bacterial growth was obtained from the increase of optical density at 600 nm of cultures in the presence of tested compound normalized to the increase observed in the control culture in the absence of the compounds and with the same starting bacterial culture. Data presented are the mean ± standard error from three independent experiments.

Stability studies using UV/Vis spectroscopy were performed for all the compounds in water and in the medium after 0 and 3 hours upon dilution. It was only observed changes in decavanadate UV/Vis spectra after 3 hours incubation on the medium as well as in water (Fig. S3). This observation is in agreement with previous studies where the half-life time of V_{10} decomposition were determined for several experimental conditions [17,18,33,34]. However, it has been referred that the rate of decavanadate decomposition is slow (half-life time of hours) enough to allow observing its effects [23,33,34]. Furthermore, it was suggested that decameric vanadate is stabilized upon interaction with proteins [36].

By analyzing the UV/Vis spectra at Fig S3, after 3 hours, decavanadate species that absorbs at 400 nm are still present in medium solution (Fig. S3B). As it can be observed in Fig. S3B, there is an evidence for a decrease in V_{10} content after 3 hours. In addition, it was observed that these V_{10} as well as the V_1 samples in the presence of the bacteria have a greenish colour after 3 hours incubation (Fig. S4). This observation is probably due to the reduction of the vanadium(V) to oxidovanadium(IV) which is blue, and that in combination with the yellow colour, due to the presence of decavanadate, results in a final green color solution (Fig. S4) after 3 hours incubation. Moreover, in Fig. 2 it can be observed that the yellow color of V_{10} persists even after 18 h incubation if bacteria is blocked, while the color changes to blue-green with bacterial growth. Conversely, in the presence of the colourless monomeric vanadate V_1 it was observed a blue colour due to the formation of oxidovanadium(IV) species bound to certain compounds or proteins present in the medium (Fig. S4).

Bactericidal action of the compounds was also investigated by inoculating fresh media with a small volume of cultures from the previous growth inhibition studies. Growth was observed after 24 hours incubation in all cases. It was observed that the presence of the each of MnV_{11}, MnV_{13}, V_{10} , Nb_{10} and V_1 do not impede the normal growth of *E. coli* discarding a bactericidal effect of the compounds in the conditions studied.

2.3 Preparation of sarcoplasmic reticulum Ca^2-ATPase vesicles

Isolated sarcoplasmic reticulum (SR) vesicles prepared from rabbit skeletal muscles as described elsewhere [37], were suspended in 0.1 M KCl, 10 mM HEPES (pH 7.0), diluted 1:1 with 2.0 M sucrose

and frozen in liquid nitrogen prior to storage at -80 °C. The protein concentration was determined spectrophotometrically at 595 nm, by the Bradford method, using bovine serum albumin as a standard and in the presence of 0.125% of sodium dodecyl sulphate (SDS). The percentage of each protein present in the SRVs preparations was determined through densitometry analysis of SDS-polyacrylamide gel electrophoresis (7.5% acrylamide). The SR Ca^{2+}-ATPase analysed by SDS polyacrylamide gel electrophoresis comprised at least 70% of the total protein in the SR-vesicles and it was the only ATPase present.

We use this SR Ca^{2+}-ATPase preps since 1991 in previously described studies on the effects of vanadates and it represents an excellent model to study the effects of several compounds in calcium ATPase activity and in calcium homeostasis. Detailed description of experimental procedures necessary for inhibition studies, characterization and preparation of sarcoplasmatic reticulum Ca^{2+}-ATPase vesicles can be found elsewhere [16,37]. The model used was previously characterized in previous papers and it maintains the adequate properties to analyze the effects of the POVs in enzyme activity, as previously ascertain [16,37].

2.4. Effects of POVs in the ATP hydrolysis by the SR Ca^{2+}-ATPase

Steady-state assays of the sarcoplasmic reticulum (SR) Ca^{2+}-ATPase were measured spectrophotometrically at 22 ˚C using the coupled enzyme pyruvate kinase/lactate dehydrogenase assay, as described elsewhere [16,37] under the following conditions: 25 mM HEPES (pH 7.0), 100 mM KCl, 5 mM $MgCl_2$, 50 µM $CaCl_2$, 2.5 mM ATP, 0.42 mM phosphoenolpyruvate, 0.25 mM NADH, 18 IU lactate dehydrogenase and 7.5 IU pyruvate kinase, with or without each of the several POMs. The experiments were initiated by the addition of 10 µg/ml calcium ATPase, in the presence and absence of 4% (w/w) of calcium ionophore A23187, and followed for 5 minutes, as briefly described below. Freshly prepared POVs solutions were added to the medium immediately prior to sarcoplasmic reticulum Ca^{2+}-ATPase addition. The ATPase activity and the inhibition was measured taken into consideration the decrease of the OD per minute in the absence (100%) and in the presence of the POVs [16,37]. After the addition of the enzymes to the medium, NADH was added followed by the vesicles containing Ca^{2+}-ATPase. Finally, after the addition of ATP the absorbance was recorded during about 1 minute (basal activity) and then the ionophore was added the following decreased of the absorbance was measured during about 2 minutes (uncoupled ATPase activity). All experiments were performed at least in triplicate. The inhibitory power of the investigated POV was evaluated determining IC_{50} values meaning the POM concentration inducing 50% of Ca^{2+}-ATPase inhibition of the enzyme activity.

3. Results and Discussion

3.1. Inhibition of bacterial growth by polyoxovanadates

Growth inhibition experiments were designed to measure the effects of three polyoxovanadates, namely MnV_{11}, MnV_{13} and V_{10} and also to measure the effects of Nb_{10} and V_1 on *E. coli* growth. The values of MIC measured by the broth dilution method suggested the compounds have a low activity against the Gram negative *E. coli*. Because of the limited solubility, MnV_{11} and MnV_{13} were tested in concentrations up to 2048 µg/ml, which were unable to prevent growth of the bacteria (MIC of these compounds >2048 µg/ml). MIC values for V_{10} and V_1 were equal to 4096 µg/ml, while Nb_{10} showed an inferior antibacterial activity (Fig. 2). However, in some cultures with the higher concentrations of the compounds not blocking growth of the bacteria, it was visible a decrease in the culture turbidity, suggestive of growth inhibition (Fig. 2). In addition, it was evident in the assays with V_{10} that conversion to a green-blue solution occurred on bacterial growth through the 18 hours incubation time (Fig. 2). Therefore, an additional approach was used to further assess the antibacterial activity of the compounds in a shorter culture time.

Vanadate solutions are yellow and Fig. 2 shows the yellow color of V_{10} (at high concentration inhibiting bacterial growth) persists even after 18 h incubation. With V_{10} concentration 2048 ug/mL, a greenish colour appears, probably due to bacteria-mediated reduction of the vanadium(V) to oxidovanadium(IV) which is blue, and that in combination with the yellow colour, due to the presence of decavanadate, results in a final green colour solution. With 1024 ug/mL of V_{10}, the colours are paler, and only a light blue-green colour is apparent, also interfered by the higher turbidity of this suspension with increased bacterial growth.

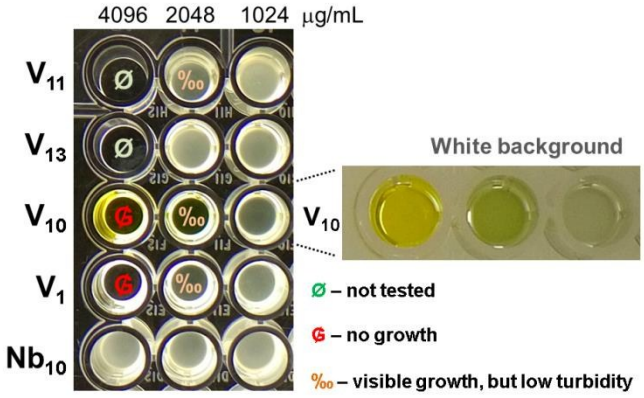


Figure 2. Representative microplate from the assays for determination of minimum inhibitory concentrations of the 5 compounds against *Escherichia coli*. After the 18 hours incubation, the microplate was photographed over a black background to evidence the turbidity in each well, and over a white background to confirm the colours of decavanadate wells.

The bacterial growth after 3 hour incubation was evaluated using absorbance at 600 nm and the concentration of vanadium compounds used was up to 1 mM for MnV_{11}, MnV_{13} (Fig. 3) and V_{10}

(Fig. 4A) and up to 10 mM for the monomeric vanadate V_1 (Fig. 4C) and also for decaniobate, Nb_{10} (Fig. 4B). The GI_{50} (concentration for 50% of maximal inhibition of bacterial growth) values were calculated for the different POVs. The GI_{50} values obtained were 210 μM for V_{11}, 270 μM for V_{13} whereas for the V_{10} experiment a value of 580 μM was found. Regarding Nb_{10} experiment, the maximum growth inhibition observed was about 30% for 10 mM concentration, meaning that the GI_{50} value is greater than 10 mM (Fig. 4B).

In fact, after *E. coli* incubation during 3 hours with increasing concentrations of the different polyoxovanadates (POVs) and also with Nb_{10} and V_1 it was observed distinct inhibitory curves for the three different POVs as well as for Nb_{10} and V_1 on the bacterial growth (Fig. 3 and Fig. 4). MnV_{11} presents the lowest GI_{50} value of bacterial inhibition (Fig. 3A) being a similar value verified for the MnV_{13} compound (Fig. 3B) which is about 2 times lower than the one observed for V_{10} (Fig. 4A). To assure that the inhibition growth effect of MnV_{11} and MnV_{13} was not due to manganese (Mn) a control test was made in the presence of $MnSO_4$ and the results showed no inhibition of *E. coli* growth by concentrations up to 2 mM of $MnSO_4$.

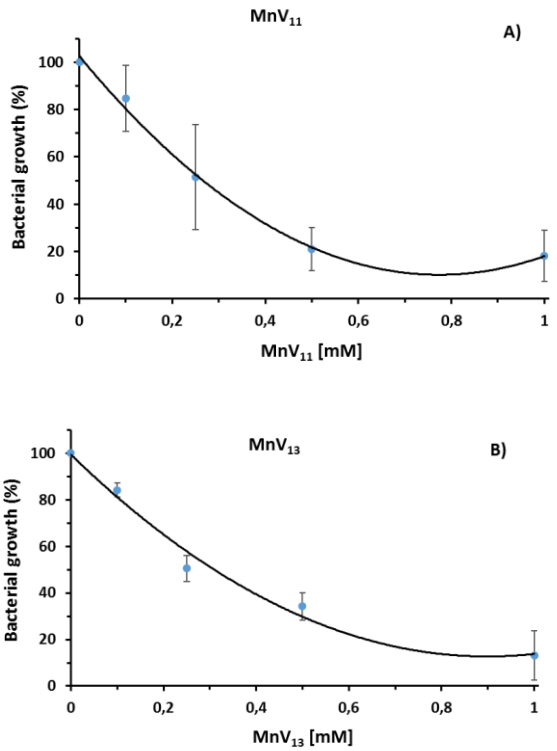


Figure 3. Inhibition of *Escherichia coli* growth in liquid medium by the two manganesepolioxovanadates: A) MnV_{11}, B) MnV_{13}. The measurements monitor growth using absorbance at 600 nm and concentrations up to 1 mM for MnV_{11} and MnV_{13} were used.

For V_{10} experiment, it was observed that the inhibition curve is different and not reaching a steady state for higher concentrations as observed for the others POVs (Fig. 4A). However, if upon 3 hours incubation at 37 ºC, all the V_{10} would totally decomposed into V_1 we should have obtained an similar curve as observed with V_1 concentrations up to 10 mM (Fig. 4C) and not the curve that it was obtained up to 1 mM of V_{10} (Fig. 4A). As it can be also observed in Fig. 4C, when the *E. coli* was incubated with V_1 a higher GI_{50} value was calculated for the V_1 experiment that is 1.1 mM. Thus, the more potent inhibition by V_{10} compared to V_1 shows that the V_{10} is inducing an inhibition, and that the *E. coli* inhibition is not due to the V_1 formed from hydrolyzed V_{10}.

Nevertheless, as analyzed by UV/Vis spectra, there is an evidence for a slow decrease in V_{10} content as the experiment progressed up to 3 hours (Fig. S3). Although it is suggested that is really decavanadate as well as the others POVs which are causing the inhibitory growth effect, rather than other smaller species, we cannot rule out the possibility the effect is caused by hydrolysis products, or some mechanism in which POVs delivers vanadium atoms to the cells. Moreover, some of the components of the medium may form complexes with vanadate as well as with POV. Examples of candidates for complex formation are phosphate, peptides and proteins [38, 39].

All the three POVs shows to be more effective than vanadate alone (IC_{50} 1.1 mM) and by comparing the inhibition values the effects in *E. coli* growth are not due to the final product of these POVs putative decomposition. Therefore, the fact that all the POVs have a greater effect than V_1 (or 10 V_1 molecules regarding V_{10}) supports the interpretation that the growth inhibitory effects observed are induced by each of the added POVs. Finally, it was observed that the *E. coli* incubation with decaniobate (Nb_{10}), on contrary to the effects observed for the isostructural compound decavanadate (V_{10}), only induce a partial inhibition of bacterial growth with GI_{50} values higher as 10 mM (Fig. 4B). This lack of effect might be due to the stability of Nb_{10} regarding the oxidation reduction reactions. Conversely, decavanadate is well now to induce oxidation of certain proteins whereas vanadyl formation can be observed [23,25,36,37].

The putative reduction of V_{10} to vanadyl (V^{IV}), that, for instance, we previously observed with actin, after prolonged period of time. In fact, it was needed to wait for 1 hour incubation and to use huge amounts of the protein. Still in the presence of the natural ligand (ATP) the reduction was not observed. For EPR measurements we also need higher amount of the protein and vanadyl than we used in the kinetic studies. Under such conditions the vanadyl signal was only detected after 60 to 90 minutes of incubation [23,25,36,37]. So, at the experimental conditions the redox stability of V_{10} and the other POVs during the biological measurements was not possible to be determined. Still, in several papers it has been referred that V(V) can be reduced to V(IV) in the cellular environment with the consequence that also V(IV) species could contribute to the inhibitory action [23,25,40]. However, little is known about the speciation of aqueous vanadyl at neutral pH, mainly because at this pH there is no electron paramagnetic resonance (EPR) signal,

presumably because of the dimerization/oligomerization of the vanadyl species or oxidation to vanadate [40].

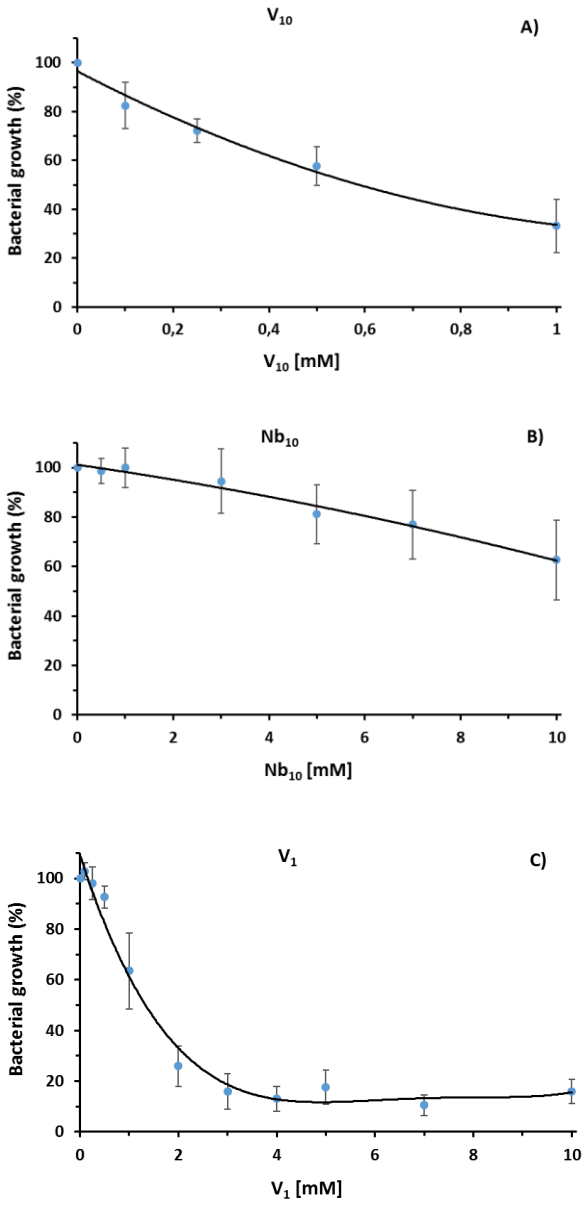


Figure 4. Inhibition of *Escherichia coli* growth in liquid medium by the two isopolyoxometalates, namely; A) V_{10}, B) Nb_{10} and C) V_1 on *Escherichia coli*. The measurements monitor growth using absorbance at 600 nm and concentrations of V_{10} up to 1 mM and up to 10 mM for Nb_{10} and monomeric vanadate V_1 were used.

If we estimated an GI_{50} value about 12.6 mM, it means that Nb_{10} is about 20 times less potent than V_{10} in preventing *E. coli* growth. This totally different result for V_{10} and Nb_{10} on the inhibition the *E. coli* growth may be probably due to the polyoxovanadates redox chemistry once polyoxoniobates are electrochemically more inert. Besides, it was observed that although the solutions of V_{10} and Nb_{10} are both kinetically stable under basic pH conditions for at least two weeks and at moderate temperature, V_{10} dissociates at most pH values into smaller tetrahedral vanadate oligomers such as V_1 and V_2, whereas Nb_{10} dissociates into Nb_6 under mildly ($10 > pH > 7.6$) or highly alkaline conditions [41].

On the other hand, the different inhibitory activity on bacterial growth for V_{10} and Nb_{10} point out that not always the structure and charge features will help to clarify and justify the biological activity of certain POMs. For instance, analysis of the structure–activity-relationship of a series of POMs against two bacteria, namely *Helicobacter pylori* and *Streptococcus pneumoniae*, indicated that isostructural POMs, despite having the same size and similar charge, can be split into two groups according to their activity [7]. Nevertheless, regarding bacteria more studies are needed to better understand the structure–activity-relationship of POMs.

To our knowledge, there are not many studies on the *E. coli* growth inhibitory effects by decavanadate and concomitantly very few GI_{50} values were found in the literature. Recently, it was described that the lack of the chemoprotective effects of some POVs in *E. coli* cultures were due to the low stability of some POVs that decompose into some products such as V_{10} known for several biological activities, and potentially toxic for the *E.coli* [42]. These authors referred that PV_{14} $[H_6V_{14}O_{38}(PO_4)]^{5-}$ was toxic against *E. coli* because it decomposes into V_{10}. On the other hand V_{15} $[V_{15}O_{36}(Cl)]^{6-}$ once it decomposes to give V_1, among others oligovanadates, was not toxic. More recently, the same research group further explored some of these observations, particularly the V_{10} effects [43]. However, in their studies, the treatment of the *E. coli* cultures with sodium salt of V_{10} only resulted in significant inhibition at the highest concentration tested (GI_{50} of 1.8 mM). Yet, V_{10} association with nicotinamide and isonicotinamide compounds, showed higher toxicity toward *E. coli* (GI_{50} of 0.47 and 0.67 mM, respectively) compared to V_{10} [43]. It was suggested that the *E.coli* V_{10} cytotoxic activity appears to be potentialized by association of V_{10} with nontoxic organic components [41]. However, the value found in these studies [43] for V_{10} (1.8 mM) is more close to the one that we found here in the present paper for V_1 (1.1 mM) than for V_{10} (0.58 mM) probably because the experimental conditions were different or decavanadate solutions were not stable to induce its effects.

The antibacterial activity was previously described for several inorganic POVs about twenty years ago against *Staphylococcus aureus* [44]. However, it was verified that polyoxotungstates (POTs) and polyoxomolybdates (POMos) are less active and with minimum inhibitory concentration (MIC) values between 128 and 8000 µg/ml, whereas all the tested POVs showed high antibacterial activities with MIC values in the range of 4 - 32 µg/ml. It was suggested that POMs severely affect cellular ion gradients and concomitantly lead to the cell death of the organism [44]. In fact, it is well known that certain POVs such as decavanadate $[V_{10}O_{28}]^{6-}$ interacts with P-type ATPases e.g. Ca^{2+}-ATPase [16,37]. More recently, in another study, a series of three organoantimony(III)-containing hybrid POMs namely $[(PhSb^{III})_4(A\text{-}\alpha\text{-}Ge^{IV}W_9O_{34})_2]^{12-}$, $[(PhSb^{III})_4(A\text{-}\alpha\text{-}P^{V}W_9O_{34})_2]^{10-}$ and $[\{2\text{-}(Me_2NCH_2C_6H_4)Sb^{III}\}_3(B\text{-}\alpha\text{-}As^{III}W_9O_{33})]^{3-}$ (Phe, phenyl group) were tested their antibacterial activity on a variety of bacterial

strains including *E. coli* [43]. All three hybrid POMs were stable in aqueous media at physiological pH and showed promising antibacterial activity against *Escherichia coli* (MIC values ranging from 80 - 130 μg/ml) [45]. These observations were further explored by the same groups using other organoantimony(III)-based POTs [44]. It was referred that by increasing the number of attached {$PhSb^{III}$} groups the antibacterial activity (MIC) was also enhanced from 500 to 125 μg/ml [46]. One of the most effective organic-inorganic antibacterial hybrid complex consisting of the Keggin structure $[HSiW_{12}O_{40}]^{3-}$, cobalt and the clinical antibacterial agent gatifloxacin ($C_{19}FH_{22}N_3O_4$), $[Co^{II}(C_{19}FH_{22}N_3O_4)_3][C_{19}FH_{23}N_3O_4][HSiW_{12}O_{40}]$ showed a higher activity against *E. coli* with a MIC value of 2.42 μg/ml [47].

Also recently, Chen et al. have studied the anti-bacterial activity of a chitosan-V_{10} complex against *Escherichia coli* and *Staphylococcus aureus* [48]. The complex demonstrated the same anti-bacterial activity with a minimum inhibitory concentration (MIC) of 12.5 μg/ml against both bacteria. Last decade, nanohybrid membranes consisting of the Keggin structure $H_5PV_2Mo_{12}O_{40}$ and poly(vinylalcohol)/polyethylenamine (PVA/PEI) were shown to exhibited antibacterial activity against *E. coli* [49]. The bioactivity increased with increasing $H_5PV_2Mo_{12}O_{40}$ content within the PVA/PEI membrane exhibiting MIC values of 2 μg/ml. With similar MIC values, another nanocomposite system is that of the same Keggin structure $H_5PV_2Mo_{12}O_{40}$ and bamboo charcoal (BC) [50]. Recently, multilayer films based on the Keggin structures $[SiW_{12}O_{40}]^{4-}$ and $[PMo_{12}O_{40}]^{3-}$ within dye methylene blue as well as the antibacterial potential of POM ionic liquids (POM-ILs) were reported to be active against *E. coli,* although with lower MIC values in comparison with the one referred above [51]. The MIC values for the above described antibacterial activity of POMs alone, POM-hybrids and nanocomposites against *E. coli* can be compare in Table S1.

Besides the studies in bacteria several others recent studies showing polyoxovanadate activity against others pathogenic microorganisms are emerging, pointing out once again to decavanadate and decavanadate compounds to future applications as antimicrobial agents [7,52,53]. Thus, a new decavanadate functionalized by Zn-fluconazole complexes shows antifungal activity against 19 *Candida* species with MIC values as low as 4 μg/ml were described [50]. Also very recently, Crans et al, have investigated the effect of vanadate and decavanadate on the growth of *Mycobacterium tuberculosis* [53]. Whereas GI_{50} value calculated for the V_1 experiment was 2.0 mM for the V_{10} experiment 29 μM (0.29 mM V-atoms) were found point out once again to the specific ability of decavanadate in inducing antimicrobial activity. Moreover, it was suggested that mycobacteria or some component excreted by the mycobacteria catalyses the hydrolysis of V_{10} thus favouring the transport of vanadate by vanadate transporters into the cell [53]. This point of view is particularly interesting because it was also previously suggested that *in vivo* exposition to decavanadate induced an increase of the amount of vanadium particularly in mitochondria in comparison with the exposition to vanadate alone [19,21,32]. In fact in these *in vivo* studies it was verified that for hepatic, cardiac and renal tissues decavanadate solutions causes higher vanadium accumulation in mitochondria than vanadate solutions, and that this is independent of the mode of administration [19,23,34]. Regarding mitochondria, probably one of the most potent effects for decavanadate so far described is the inhibition of mitochondrial oxygen consumption showing an IC_{50} as low as 99 nM whereas lower values were found for mitochondrial membrane depolarization (IC_{50} = 40 nM) [17,18].

Putting it all together, and taken in account the similarities between mitochondria and bacteria we cannot totally exclude that V_{10} interaction with the bacteria surface of the bacteria, might favours the delivering a vanadium atoms to the bacterium inducing several changes in several oxidative stress parameters, such as GSH depletion, ROS production and concomitantly cell death [19]. In previous *in vivo* animal studies the global tendency is that decavanadate clearly induces much more changes in mitochondrial antioxidant enzymes activities in comparison to vanadate. However, no correlation was found between the levels of vanadium in mitochondria and antioxidant enzyme activities, upon decavanadate exposure [19].

3.2 Inhibition of sarcoplasmic reticulum Ca^{2+}-ATPase activity by manganesepolyoxovanadates

In parallel with the *E. coli* growth studies performed in the presence of several POVs and also Nb_{10} and V_1, the effects of the two manganesepolyoxovanadates namely MnV_{11} and MnV_{13} on the activity of sarcoplasmic reticulum Ca^{2+}-ATPase obtained from skeletal muscle were also investigated. It was observed that both POVs inhibits the Ca^{2+}-ATPase activity, expressed as a percentage of the control enzyme value obtained without inhibitor, in a concentration dependent manner (Fig. 5).

The inhibitory power of MnV_{11} and MnV_{13} were evaluated using IC_{50} values meaning the POV concentration inducing 50% of Ca^{2+}-ATPase inhibition of the enzyme activity. IC_{50} values of 31 and 58 μM were determined, respectively for MnV_{13} and MnV_{11} (Fig. 5). Ca^{2+}-ATPase IC_{50} values of inhibition for decavanadate $[V_{10}O_{28}]^{6-}$ (IC_{50} = 15 μM) and decaniobate $[Nb_{10}O_{28}]^{6-}$ (IC_{50} = 35 μM) (Nb_{10}) were previously described [37]. Using exactly the same experimental conditions as described in the present paper regarding to this Ca^{2+}-ATPase, IC_{50} values below 1 μM were found for some POTs such as P_2W_{18} (0.6 μM) and Se_2W_{29} (IC_{50} = 0.3 μM) whereas a lowest potency were observed for TeW_6 (IC_{50} = 200 μM) [16]. In the range of inhibitory power (IC_{50}) from 1 to 35 μM, besides the V_{10} and Nb_{10} referred above, Keggin-based POTs such as mono-substituted CoW_{11} (IC_{50} = 4 μM), tri-lacunary α-SiW_9O_{34} (IC_{50} =16 μM) and α-AsW_9O_{33} (20 μM), lacunary Dawson type $P_2W_{12}O_{62}$ (11 μM) and $As_2W_{19}O_{67}$ (28 μM) were also described [16,37].

View Article Online
DOI: 10.1039/C9NJ01208G

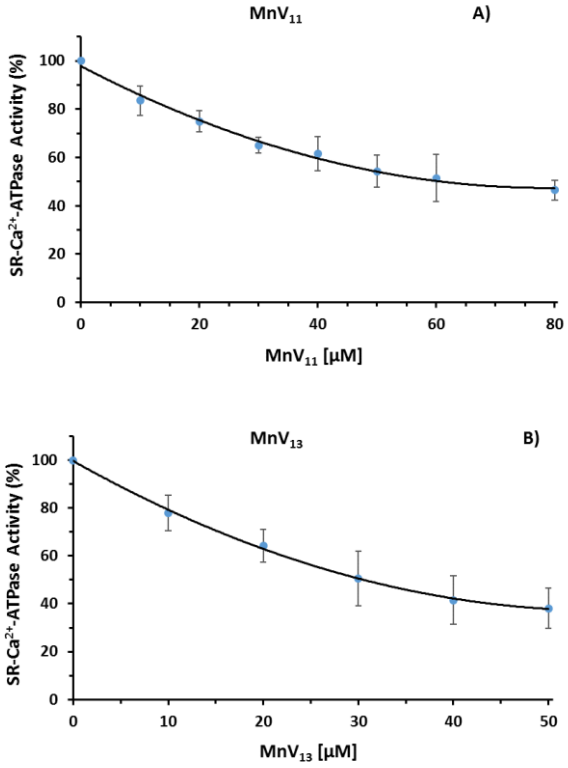


Figure 5. Inhibition of Ca^{2+}-ATPase activity by the polyoxometalate MnV_{11} (A) and MnV_{13} (B) Ca^{2+}-ATPase was measured spectrophotometrically at 340 nm and 25 °C, using the coupled enzyme pyruvate kinase/lactate dehydrogenase assay. Data are plotted as means ± SD. The results shown are the average of triplicate experiments.

Here, we show that MnV_{13} presents a 2-fold stronger inhibition of the calcium pump (IC_{50} = 31 µM) than the Keggin type MnV_{11} (IC_{50} = 58 µM). Both MnV_{11} and MnV_{13} compounds showed to be a Ca^{2+}-ATPase mixed type inhibitors regarding the natural ligand MgATP, as it can be observed in Figure 6, for MnV_{13} (Fig S5 for MnV_{11}). This mixed type inhibition was also previously observed for $P_2W_{18}O_{62}$ and TeW_6O_{24} [16,54] suggested that these POVs and POTs can interact with the Ca^{2+}-ATPase whether or not the enzyme has already bound substrate. Previously, for both Nb_{10} and V_{10}, it was showed a Ca^{2+}-ATPase non-competitive type of inhibition [37].

Only for V_{10} a binding site in the Ca^{2+}-ATPase was previously described, involving at least three protein domains including the phosphorylation and the nucleotide binding sites [55]. To our knowledge, studies about the type of inhibition of others POVs as well as mode of interaction with this or others P-type ATPases are still to be determined [8,16,38]. P-type ATPases plays a crucial role in cellular ion homeostasis and have been described as potential molecular targets of polyoxometalates [7,8,16]. It was suggested that decavanadate interacts with the ion pumps without needing to cross the membrane, which is from the extracellular side [24].

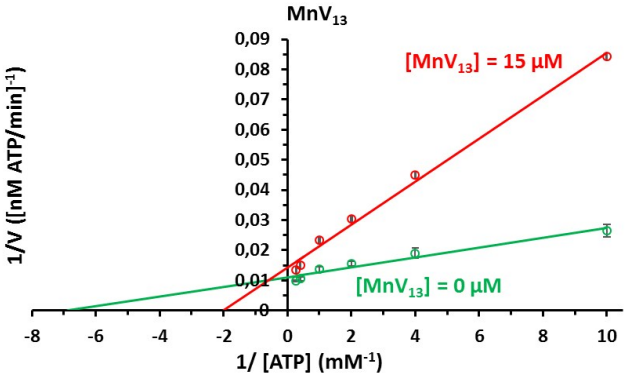


Figure 6. Lineweaver-Burk plot of Ca^{2+}-ATPase activity in the absence (green) and in the presence (orange) of 15 µM of the polyoxometalate MnV_{13}, used for determining the type of enzyme inhibition. The POV presented a mixed type of inhibition. Data are plotted as means ± SD. The results shown are the average of triplicate experiments.

Therefore, decavanadate interaction with ion pumps from the outside can more rapidly induce changes in cellular ion homeostasis with implications in, for example, ROS production and bacterial death [7]. P-type ATPase was recently identified in bacteria for the supply of Ca^{2+} for growth and important for the integrity of the cell envelope [56]. Besides the P-type ATPases, POMs can also affect bacterial cells in changing their morphological structure leading them to death. Inoue *et al.* have reported that POMs such as $As_4W_{40}O_{140}$ and Sb_9W_{21} could enable morphological changes of *H. pyroli* from bacillary to a U-shaped or coccoid form [57]. Fiorani *et al.* have also revealed extensive degradation of an *E. coli* strain in the disruption of their rod-shaped morphology by a complex formed of a chitosan compound with V_2Mo_{10} [58].

Regarding V_{10}, it was suggested that V_{10} interacts with G-actin polymerization/depolymerization dynamics in skeletal muscle cells [36,59], which can lead to actin cytoskeleton damage and cellular death processes [7,8]. Actin is one of the most abundant proteins in cells, being involved in many cellular and biological processes. However, studies between POMs and cytoskeleton structures are scarce and only V_{10}–actin interactions having been so far described [59]. Meanwhile XANES and EXAFS studies allowed to confirmed previous studies [36] that decavanadate interacts with G-actin but not with F-actin whereas oxidovanadium(IV)-species are detected within G-actin/decavanadate interactions that induces oxidation of the cysteine core residues but not oxidation of the so-called cysteine "fast" residues [60].

3.3 Inhibition of *Escherichia coli* growth by polyoxovanadates shows a reverse correlation with Ca^{2+}-ATPase inhibition

The ATPase from sarcoplasmic reticulum was used as a model for P-type ATPases. This specific type of ATPase was found important for

bacterial calcium regulation and growth [56]. Recently, it was described a structure-activity relationships for high affinity towards POTs (IC_{50} < 16 μM) indicating that the inhibition potential of the POMs for the Ca^{2+}-ATPase inhibition can be correlated with their charge density [16]. Conversely, as referred above, for bacteria it was possible to determine that isostructural POMs, despite having the same size and similar charge, can be split into two groups according to their antibacterial activity, considering the MIC values for the different POMs. Herein, in order to decipher others specific features for these three POVs responsible for the inhibition of *E.coli* growth we analyzed a different approach, that is, a putative correlation between the IC_{50} values of inhibition for Ca^{2+}-ATPase, and their activity against the bacteria growth, i.e. the GI_{50} values. Apparently, for the range of POVs studied, exhibiting IC_{50} values from 15 up to 58 μM, we observed a reverse correlation between their activity (IC_{50} value) and their GI_{50} values obtained for *E. coli* (Fig. 7).

Thus, for the analyzed POVs it was observed a reverse correlation between the Ca^{2+}- ATPase IC_{50} values and the *Escherichia coli* GI_{50} values suggesting that decavanadate and others POVs inhibition of ion pumps cannot be directly associate with the inhibition of *Escherichia coli* growth. Being effective against *Escherichia coli* and not against the Ca^{2+}- ATPase is not really bad news once the antibacterial therapeutic drugs requires high efficacy against bacteria coupled to a low toxicity against normal cells. On the other hand, we might suggest that to produce POMs more effective against *Escherichia coli* they should have specific features that are not P-type ATPase inhibitors and thus POMs present advantages as a drug in that their structure can be modified in order to produce the desire compound.

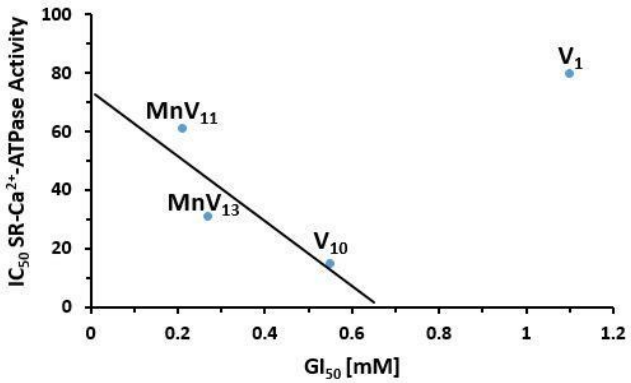


Figure 7. Reverse correlation between the IC_{50} values found for MnV_{11}, MnV_{13} and V_{10} for Ca^{2+}-ATPase inhibition with their growth inhibition capacity (GI_{50}) on *E. coli*.

Apparently, POVs can inhibit Escherichia coli growth, and shows a reverse correlation with Ca^{2+}-ATPase inhibition. The putative POVs interaction with ion pumps from the outside can more rapidly induce changes in cellular ion homeostasis with implications in, for example, ROS production and bacterial death [7]. However, the antibacterial mechanisms of POMs is not clear, and should be further studied [6,7]. On the other hand, once POM is redox active, it can induce alone or due to its decomposition production of ROS with bactericidal activity against Escherichia coli growth [7,61]. Moreover, even if specific POMs can target several processes and mechanisms that can be responsible for cell death the question remains to clearly understand which one is the early event affected by the POM and/or if the simultaneous effects observed are due to a putative decomposition within the interaction with the cell [7].

For certain chasing similar aims, very recently, it was suggested that besides POMs showed to be promising antibacterial agents against *Moraxella catarrhalis*, according to their MIC values POM activity mainly depends on composition, shape and size [62]. For the case of medium-size POTs it was referred that the antibacterial activity correlates with the total net charge [62]. Moreover, based on MIC values, it was showed that Dawson-type POMs exhibited highest activity and selectivity against *M. catarrhalis*. However, according to time-killing studies it seems clear that only the Preyssler anion shown a bacteriostatic effect against *M. catarrhalis* more close to azithromycin [62]. Finally, it was also referred that besides several POVs analyzed also the most active POM on *M. catarrhalis*, the Preyssler anion $P_5W_{30}^{14-}$ (MIC = 1μg/ml), were inactive against the Gram negative *E. Coli* (MIC > 256 μg/ml) [59]. This value in agreement with the MIC value we obtained here and very different from the one described before (Table 1). In fact, as referred above, for all the POMs analyzed in the present paper we obtained high MIC values against *E. Coli* for all the compounds, therefore suggesting based on this MIC value a clear ineffectiveness against this type of bacteria. However, more quantitative studies are needed to better decipher the structure-activity-relationship of antibacterial POMs as well as the mode of antibacterial action of POMs on both bacterial growth inhibition and cell death. Further understanding of the redox biochemistry and ROS production capacity of different POMs may lead to the development of more specific antibacterial agents [61]. Finally, in the future, it would be beneficial to establish the mode of antibacterial action by each POMs to each pathogenic microorganism.

4. Conclusions

The studies presented demonstrate that besides decavanadate (V_{10}), others two polyoxovanadates namely MnV_{11} and MnV_{13} inhibits the growth of *E. coli,* whereas the oxovanadates prepared from NH_4VO_3 inhibit growth with 2-6 fold less potency. The greater inhibitory selectivity of the MnV_{11}, MnV_{13} and V_{10} results are important because it demonstrates that besides the simple vanadate, polyoxovanadates can inhibit growth of *E. coli*. In addition, it was observed that conversely to V_{10}, Nb_{10} only induce residual effects on *E. coli* growth. Nb_{10} is a well know isostructural species of decavanadate, suggesting that the stronger decavanadate antibacterial activity is due to its specific versatility rather than solely its structure and/or charge features.

It was also observed that Ca^{2+}-ATPase activity from sarcoplasmic reticulum is inhibited by MnV_{11} and MnV_{13} which values of

inhibition, IC_{50} = 58 and 31 μM, respectively, were about 2 to 4 times less potent than the one previously described for V_{10} (15 μM). Conversely to Nb_{10} and V_{10}, known to be Ca^{2+}-ATPase non-competitive inhibitors, both MnV_{11} and MnV_{13} showed to be mixed type inhibitors regarding the natural ligand ATP. Finally, it was found that the POVs exhibiting the highest antibacterial activity (MnV_{11}) show to have the lowest Ca^{2+}- ATPase inhibitory capacity (IC_{50} = 58 μM) whereas decavanadate, which was also very active against this ATPase (IC_{50}=15 μM), was less active against *E. coli*.

Thus, for the analyzed POVs it was observed a reverse correlation between the Ca^{2+}- ATPase IC_{50} values and the *Escherichia coli* GI_{50} values suggesting that decavanadate and others POVs inhibitors of ion pumps cannot be directly associate with the inhibition of *Escherichia coli* growth. This type of association is novel and of general interest once POMs present advantages as a drug in that their structure can be modified to be more or less active against *E. coli*, and P-type calcium ATPases are emerging bacterial targets [56]. Nevertheless, although the biological processes affected in bacteria by each POMs are yet to be clarified ion pumps as well known molecular targets for drugs and polyoxometalates should be taken in consideration [7].

Acknowledgments

DMS and RL acknowledge ESTG-IPLeiria for the Microbiology laboratory facilities. MA thanks to national funds through FCT, Foundation for Science and Technology (UID/Multi/04326/2013). SSM thanks to Council of Scientific & Industrial Research (CSIR) for providing financial support (01(2906)/17/EMR-II). Decaniobate compound was previously supplied by a collaborator, the esteemed Professor William H. Casey from UC Davis, University of California, USA.

Conflict of Interest

The authors declare that the research was conducted without any commercial or financial conflict of interest.

Author Contributions

DMS and RL carried out the microbiological studies while GF carried out the ATPase kinetic studies and prepared the figures. SSM and AAV did the MnPOVs synthesis. MA combined the chemical and biological components of the work, advised and oversaw the studies and wrote the manuscript with the assistance of RL, GF and SSM. All authors contributed to the editing of the manuscript.

References

[1] Chen X, Yan S, Wang H, Hu Z, Wang X, Huo M. Carbohydr. Polym. 2015; 117:673–680.

[2] Wang SS, Yang GY. Chem. Rev. 2015; 115: 4893–4962.

[3] Mohapatra L, Parida KM, Phys. Chem.Chem.Phys. 2014; 16: 16985–16996.

[4] Moussawi MA, Leclerc-Laronze N, Floquet S, Abramov, Sokolov MN, Cordier S, Ponchel A, Monflier E, Bricout H, Landy D, Haouas M, Marrot J, Cadot E. J. Am. Chem. Soc. 2017; 139:12793-12803.

[5] Bijelic a, Rompel A. Acc. Chem. Res. 2017; 50:1441–1448.

[6] Rhule JT, Hill CL, Judd DA, Schinazi RF. Chem. Rev. 1998; 98:327-357.

[7] Bijelic A, Aureliano M, Rompel A., Chem. Comm. 2018; 54:1153-1169.

[8] Bijelic A, Aureliano M, Rompel A. Angew. Chem. Int. Ed. 2018; 57:2-21.

[9] Sánchez-Lara E, Treviño S, Sánchez-Gaytán BL, Sánchez-Mora E, Eugenia Castro M, Meléndez-Bustamante FJ, Méndez-Rojas MA, González-Vergara E., Front. Chem. 2018; 6 article 402, doi.org/10.3389/fchem.2018.00402

[10] Turner TL, Nguyen VH, McLauchlan CC, Dymon Z, Dorsey BM, Hooker JD, Jones MA, J. Inorg. Biochem. 2012; 108:96-104.

[11] Yatime L, Buch-Pedersen MJ, Musgaard M, Morth JP, Winther A-M L, Pedersen BP, Olesen C, Andersen JP, Vilsen B, Schiøtt B, Palmgre MG, Møller JV, Nissen P, Fedosova N. Biochim. Biophys. Acta. 2009; 1787:207–220.

[12] Stephan H, Kubeil M, Emmerling F, Mueller CE. Eur. J. Inorg. Chem. 2013;10–11:1585–1594.

[13] Lee SY, Fiene A, Li W, Hanck T, Brylev KA, Fedorov VE, Lecka J, Haider A, Pietzsch HJ, Zimmermann H, Sévigny J, Kortz U, Stephan H, Müller CE. Biochem. Pharmacol. 2015; 93:171–81.

[14] Bošnjaković-Pavlović N, Bajuk-Bogdanović D, Zakrzewska J, Yan Z, Holclajtner-Antunović I, Gillet JM, Spasojević-de Biré A. J. Inorg. Biochem. 2017; 176:90–99.

[15] Prudent R, Moucadel V, Laudet B, Barette C, Lafanechère L, Hasenknopf B, Li J, Bareyt S, Lacôte E, Thorimbert S, Malacria M, Gouzerh P, Cochet C. Chem. Biol. 2008; 15:683–692.

[16] Gumerova NI, Krivosudsky´ L, Fraqueza G, Breibeck J, Al-Sayed E, Tanuhadi E, Bijelic A, Fuentes J, Aureliano M, Rompel A. Metallomics. 2018; 10:287-295.

[17] Soares SS, Gutiérrez-Merino C, Aureliano M. Aquat. Toxicol. 2007; 83:1-9.

[18] Soares SS, Gutiérrez-Merino C, Aureliano M. J. Inorg. Biochem. 2007; 101:189-196.

[19] Aureliano M, Soares SS, Tiago T, Ramos S, Gutiérrez-Merino C. ACS Symposium Series. 2007; 249–263. doi:10.1021/bk-2007-0974.ch018

[20] Zhang B, Qiu J, Wu C, Li Y, Liu Z, Int. Immunopharmacol. 2015; 29:293-301.

[21] Geng J, Li M, Ren J, Wang E, Qu X, Angew. Chem. Int. Ed., 2011, 50: 4184-4188.

[22] Gao N, Sun H, Dong K, Ren J, Duan T, Xu C, Qu X, Nature Communications, 2014, 5:3422.

[23] Aureliano M, Crans DC. J. Inorg. Biochem. 2009; 103:536-546.

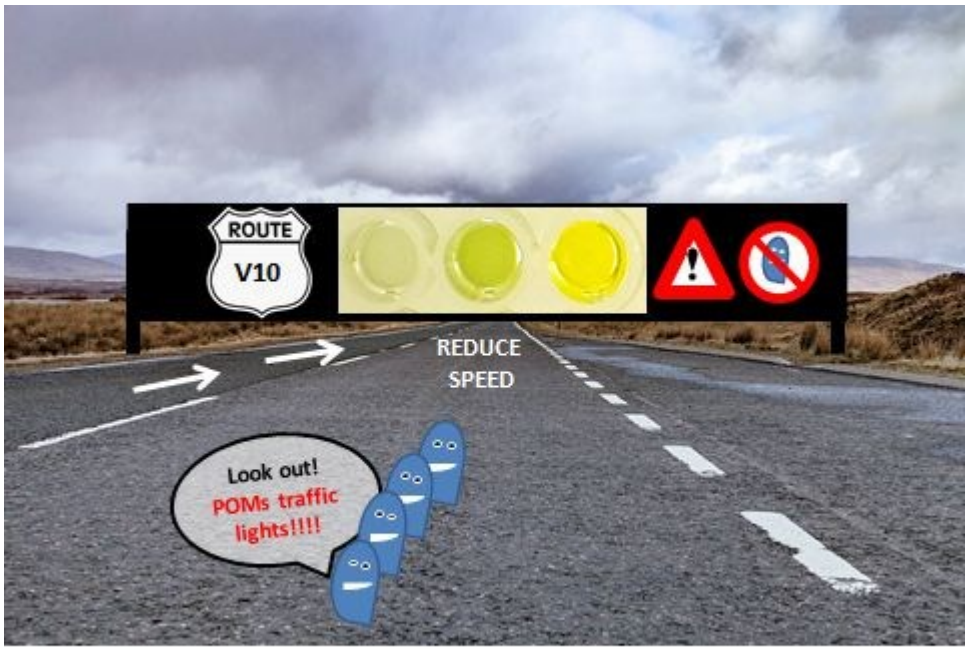
[24] Aureliano M, Fraqueza G, Ohlin CA. Dalton Trans. 2013; 42:11770–11777.

[25] Aureliano M. Oxid. Med. Cell. Longevity. 2016; doi: 10.1155/2016/6103457

[26] Douglas T, Young M. Nature. 1998; 393:152-155.

[27] Willsky GR, White DA, McCabe BC, J. Biol. Chem. 1984; 259:13273–13281.
[28] Samart N, Saeger J, Haller K, Aureliano M, Crans D. J. Mol. Eng. Mat. 2014; 2:1-21.
[29] Sun T, Cui W, Yan M, Qin G, Guo W, Gu H, Liu S, Wu Q. Adv. Mater. 2016; 28:7397–7404.
[30] Lu Z, Dockery CR, Crosby M, Chavarria K, Patterson B, Giedd M. Front. Microbiol. 2016; 7:01403.
[31] Flynn CM, Poper MT. Inorg. Chem. 1970, 9:2009-2014.
[32] Flynn CM, Poper M. T. J. Am. Chem. Soc. 1970; 92:85-90.
[33] Borges G, Mendonça P, Joaquim N, Coucelo JM, Aureliano M. Arch. Environ. Contam. Toxicol. 2003; 45:415–422.
[34] Gândara R, Soares SS, Martins H, Aureliano M, J. Inorganic Biochem. 2005; 99:2355-2361.
[35] Ohlin CA, Villa EM, Casey WH, Inorg. Chim. Acta. 2009; 362:1391–1392.
[36] Ramos S, Manuel M, Tiago T, Duarte R, Martins J, Gutiérrez-Merino C, Moura JJ, Aureliano M, J. Inorg. Biochem. 2006; 100:1734–1743.
[37] Fraqueza G, Ohlin CA, Casey WH, Aureliano M, J. Inorg. Biochem. 2012; 107:82–89.
[38] Andersson I, Gorzsás A, Kerezsi C, Tóth I, Pettersson L, Dalton Trans. 2005; 22:3658–3666.
[39] Arefian M, Mirzaei M, Eshtiagh-Hosseini H, Frontera A, Dalton Trans. 2017; 46:6812–6829.
[40] Treviño S, Díaz A, Sánchez-Lara E, Sanchez-Gaytan BL, Perez-Aguilar JM, González-Vergara E, Biological Trace Element Research 2019, 188: 68–98.
[41] Aureliano M, Ohlin CA, Vieira MO, Marques MPM, Casey WH, Batista de Carvalho LAE. Dalton Trans. 2016; 45:7391-7399.
[42] Postal K, Maluf DF, Valdameri G, Rudiger AL, Hughes DL, de Sa EL, Ribeiro RR, de Souza EM, Soares JF, Nunes GG, RSC Adv. 2016; 6:114955−114968.
[43] Missina JM, Gavinho B, Postal K, Santana FS, Valdameri G, de Souza EM, Hughes DL, Ramirez MI, Soares JF, Nunes GG, Inorg Chem. 2018; 57:11930-11941.
[44] Fukuda N, Yamase T, Tajima Y, Biol. Pharm. Bull. 1999; 22:463–470.
[45] Barsukova-Stuckart M, Piedra-Garza LF, Gautam B, Alfaro-Espinoza G, Izarova NV, Banerjee A, Bassil BS, Ullrich MS, Breunig HJ, Silvestru C, Kortz U, Inorg. Chem. 2012; 51:12015–12022.
[46] Yang P, Bassil BS, Lin Z, Haider A, Alfaro-Espinoza G, Ullrich MS, Silvestru C, Kortz U, Chem. 2015; 21:15600–15606.
[47] Liu H, Zou Y-L, Zhang L, Liu J-X, Song C-Y, Chai D-F, Gao G-G, Qiu Y-F. J. Coord. Chem. 2014; 67:2257–2270.
[48] Chen S, Wu G, Long D, Liu Y, Carbohydr. Polym. 2006; 64:92–97.
[49] Wu KH, Yu PY, Yang CC, Wang GP, Chao CM, Polym. Degrad. Stab. 2009; 94:1411–1418.
[50] Yang F-C, Wu K-H, Lin W-P, Hu M-K, Microporous Mesoporous Mater. 2009; 118:467–472.
[51] Kubo A-L, Kremer L, Herrmann S, Mitchell SG, Bondarenko OM, Kahru A, Streb C, ChemPlusChem. 2017; 82: 867–871.
[52] Guo S, Yang W, Zhao M, Tian R, Zhang B, Qi Y, Molecules, 2018; 23:1122; doi:10.3390/molecules23051122
[53] Samart N, Arhouma Z, Kumar S, Murakami HA, Crick DC, Crans DC, Front Chem 2018, DOI: 10.3389/fchem.2018.00519
[54] Fraqueza G, Fonseca C, Al-Sayed E, Rompel A, Aureliano M. AISANH, 2017; 5:17-20.
[55] Hua S, Inesi G, Toyoshima C. J. Biol. Chem. 2000; 275:30546–30550.
[56] Gupta HK, Shrivastava S, Sharma R. mBio. 2017; 26, 8 pii: e01388-17. doi: 10.1128/mBio.01388-17.
[57] Inoue M, Segawa K, Matsunaga S, Matsumoto N, Oda M, Yamase T. J. Inorg. Biochem. 2005; 99:1023–1031.
[58] Fiorani G, Saoncella O, Kaner P, Altinkaya SA, Figoli A, Bonchio M, Carraro MJ, Clust. Sci. 2014; 25:839–854.
[59] Ramos S, Duarte RO, Moura JJG, Aureliano M. Metallomics. 2012; 4:16-22.
[60] Marques MPM, Gianolio D, Ramos S , Batista de Carvalho LAE, Aureliano M. Inorg. Chem. 2017; 56:10893-10903.
[61] Chen Z, Wang Z, Ren J, Qu X, Acc. Chem. Res., 2018, *51*:789–799.
[62] Gumerova NI, Al-Sayed E, Krivosudský L, Čipčić-Paljetak H, Verbanac D, Rompel A. Front Chem. 2018; 6:336. doi: 10.3389/fchem.2018.00336.

View Article Online
DOI: 10.1039/C9NJ01208G



The road of bacterial/e.coli growth

Graphical abstract, Dorinda et al