Antibiotic-Like Behaviour of Polyoxometalates

In vitro comparative study: seven polyoxotungstates – nine antibiotics against gram-positive and gram-negative bacteria

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Knowing acquired antibiotic resistance of bacteria, discovery of new antibacterial compounds is an important goal. Herein, seven polyoxometalates with tungsten were synthesized by a single-step self-assembling method. The pseudo-Keggin/sandwich series of tungstobismuthates were characterized by analytical techniques including atomic absorption, thermal analysis, UV and FT-IR, spectroscopy. Their antibacterial activity were investigated against Gram-positive and Gram-negative bacteria, and against a Methicillin-Resistant Staphylococcus aureus strain isolated by us, comparatively with nine classic antibiotics. We found that five polyoxotungstates had significant antibacterial activity at the tested concentrations. Our results demonstrate antibiotic-like behaviors of these nanocompounds and their strong antimicrobial activity, and recommend them as alternative chemotherapeutics agents.

Keywords: polyoxometalates, tungsten, gram-positive and gram-negative bacteria, MRSA, transition metal cations

Known for over 200 years, [1] polyoxometalates (POMs) are metal-oxygen clusters whose synthesis requires self-assembling mechanisms into captivating archetypal structures (such as Keggin, Wells-Dawson, Anderson-Evans, etc.) [2, 3]. They can be divided in two categories: isopolyanions – $[M_mO_y]^{p-}$, without heteroatoms and heteropolyanions – $[X_xM_mO_y]^{q-}$, with heteroatoms (X) like Si, Ge, P, As, Sb, Bi, etc [4-6]. The most common metals (M) forming these type of compounds are W, Mo and V, and sometimes Nb and Ta [6-8].

Polyoxometalates can self-assemble into saturated and lacunary structures, during a single step synthesis ("all in one pot") in which successive stoichiometric reactions occur in the same medium, in aqueous solution [7, 8]. For the synthesis, setting of different parameters is crucial in order to obtain the desired dimensions of new compounds.

A few important parameters affecting the reaction processes should be reminded: *p*H, concentration, temperature, type of metal oxide anion, and presence/absence of additional ligands, presence or absence of mixed addenda atoms, reducing agents, type of heteroatom and their concentration etc. [8, 9].

POMs properties including shape, size, stability, slight solubility in water, redox behaviors, determine their applications in various fields: material science [10-12], magnetism [13, 14], electrochemistry [15, 16], catalysis [17, 18], and more recently in biology [19] and medicine [20]. Biomedical applications of these nanocompounds that require attention [9] are: antiviral [20-23] and antitumoral activities [24-30], blocking prions molecules in less infectious structures [31, 32] – with implications in amyloidosis and Alzheimer's disease, and last but not least implications in scrapie, bovine spongiform encephalopathy (Creutzfeldt-Jakob disease) [32]. However, one of the most interesting applications of POMs is due to their antibiotic-like behaviors and antibacterial actions [33-35]. Bae and coworkers showed high photocatalytic inactivation of two bacterial strains (*Escherichia coli* and *Bacillus subtilis*) by homogeneous photocatalyst – polyoxometalates, comparatively with heterogeneous photocatalyst – TiO_2 [36].

Today, in polyoxometalates chemistry, creation of new nanocompounds compatible with life is a not very easy target, but still drugs delivery *via* functionalized/derivatized inorganic compounds remains of great interest. Knowing acquired antibiotic resistance of Gram-positive and Gram-negative bacteria, discovery of new antibacterial compounds is an important goal. On this perspective, polyoxometalates are alternative potential chemotherapeutics agents with strong antimicrobial activity.

In this paper, we aimed to analyze the antimicrobial activities of seven polyoxotungstates (never investigated in this respect), with *pseudo*-Keggin/sandwich structures (synthesized and characterized by us) comparatively with nine antibiotics, against six reference bacterial strains – two Gram-positive, four Gram-negative bacteria species, and against a MRSA (*Methicillin-Resistant Staphylococcus aureus*) strain isolated by us.

Experimental part

Materials

All chemical substances (high analytical purity) and HCl, used in the synthesis of nanocompounds, were purchased

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from Sigma-Aldrich (N.V./S.A., Bornem, Belgium) and Merck (KGaA, Darmstadt, Germany), respectively. *In vitro* susceptibility testing was conducted on reference microbial strains (*Staphylococcus aureus* ATCC 6538P, *Bacillus cereus* ATCC 14579, *Escherichia coli* ATCC 10536, *Salmonella enteritidis* ATCC 13076, *Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 27853) – obtained from American Type Culture Collection (ATCC, Manassas, VA, USA), as well as on a *MRSA* (*Methicillin-Resistant Staphylococcus aureus*) strain isolated by us (limb amputated and *MRSA* infected from a patient with critical ischemia).

Synthesis and analytical characterization of POMs

The *pseudo*-Keggin/sandwich series of tungsto-bismuthates taken into consideration in our work (to be tested against the various bacterial species) consisted of seven complexes with transition metal ions:

$Na_{12}[Cu_3(H_2O)_3(BiW_9O_{33})_2]\cdot 29H_2O$ (1),
$K_{12}[(VO)_3(BiW_9O_{33})_2]\cdot 36H_2O$ (2),
$Na_{12}[Ni_3(H_2O)_3(BiW_9O_{33})_2]\cdot 39H_2O$ (3),
$Na_{12}[Co_3(H_2O)_3(BiW_9O_{33})_2]\cdot 37H_2O$ (4),
$Na_{12}[Mn_3(H_2O)_3(BiW_9O_{33})_2]\cdot 43H_2O$ (5),
$Na_9[Fe_3^{III}(H_2O)_3(BiW_9O_{33})_2]\cdot 36H_2O$ (6),
$Na_{12}[Fe_3^{II}(H_2O)_3(BiW_9O_{33})_2]\cdot 34H_2O$ (7).

The nanocompounds were synthesized to form *pseudo*-Keggin type sandwich structures, in an *all in one pot* single step (*p*H- and temperature-dependent, in aqueous solution), by modifying methods previously mentioned in the literature [37-40].

Synthesis involved mixing, according to stoichiometric reaction, of: $Bi(NO_3)_3\cdot 5H_2O$, $Na_2WO_4\cdot 2H_2O$, HCl, a transition metal cation (TM) salt (added in the same solution), and finally NaCl for the all POMs, excepting the POM with vanadyl ions (in which KCl was added). The sandwiched resulted complexes were formed by coordination of the transition metal cations with the lacunary polyoxoanions. The salts of TM used were: $VOSO_4\cdot H_2O$; $MnCl_2\cdot 4H_2O$; $FeCl_3\cdot 6H_2O$; $FeCl_2\cdot 4H_2O$; $CoCl_2\cdot 6H_2O$; $NiCl_2\cdot 6H_2O$; and $CuCl_2\cdot 2H_2O$.

The general reaction for the nanocompounds obtaining, aside from POM 6, is shown below:

$18WO_4^{2-} + 2Bi^{3+} + 12H^+ + 3TM^{n+} \rightarrow [(TM)_3^{n+}(H_2O)_a(BiW_9O_{33})_2]^{(18-3n)-} + 3H_2O$, where n=2, 3; and a=0, 3; at a final *p*H between 6.00-7.00 and a temperature between 70-80°C.

General synthesis of POMs

0.97 g (2 millimoles) of $Bi(NO_3)_3\cdot 5H_2O$ dissolved in 1.5 mL of HCl 6 M, was added in small portions to a hot solution (80°C) of $Na_2WO_4\cdot 2H_2O$ (5.94 g, 18 millimoles, dissolved in 48 mL bidistilled water), to a *p*H of 7.50-8.00 under stirring. After one hour, the mixture was cooled to 50°C, and the TM salt solution (3 millimoles in 40 mL bidistilled water) was gradually added and a Bi:TM = 2:3 molar ratio was finally achieved. The stirring was further continued for 15 minutes at 50°C. The resulting solution was then cooled to room temperature and filtered under vacuum using a medium porosity frit. After several days at room temperature, the precipitates deposited of nano-compounds were filtered and washed with NaCl 2M solution, ethanol and ether, respectively. Only POM 2 was obtained by adding powered KCl. 0.7 millimoles of each POMs were obtained with a yield of synthesis at ~ 70%, based on the transitional metal content. POMs were recrystallized from a minimum amount of bidistilled water, resulting in crystals of various shapes and different colors.

All seven nanocompounds were analytically characterized by atomic absorption (OES-ICP) using the ICP 2070 BIRD spectrometer (Bird, Netherlands), and their thermal stability was also investigated. A Mettler-Toledo TG/S DTA 851 thermogravimeter (Mettler-Toledo, Schweiz, GmbH, Greinfensee, Switzerland) was used for thermal analysis of each POM. A Shimadzu SPECORD UV-VIS-75 (Shimadzu Europa GmbH, Duisburg, Germany) was used for recording UV electronic spectra of POMs in aqueous solution (in quartz cells, 1 cm path length).

FT-IR absorption spectra in KBr pellets, were obtained with a Jasco 610 FT-IR spectrophotometer (Jasco Int. Co. Ltd., Gross-Umstadt, Germany), at a resolution of 0.5 cm^{-1}, and with measurable W.N. range between 4000-350 cm^{-1}. Jasco Spectra Manager Version 2.05.03 software (Jasco Int. Co. Ltd., Gross-Umstadt, Germany) was used for the FT-IR spectra analysis.

Evaluation of the antibacterial activity of POMs

Disk diffusion methods

The potential antibacterial activity of the POM compounds was determined using the disk diffusion method [41], adapted for this experiment, according to the proposed recommendations by Clinical and Laboratory Standards Institute [42]. The inoculum was similar to that prepared for the antibiotic susceptibility test (turbidity corresponding to a concentration of approximately 1.5×10^8 CFU/mL); therefore comparisons in terms of sensitivity between regular antibiotics and the POM compounds were possible.

In vitro susceptibility testing was conducted on reference microbial strains (two Gram-positive bacteria species: *S. aureus*, *B. cereus*, and four Gram-negative bacteria species: *E. coli*, *S. enteritidis*, *S. typhimurium, P. aeruginosa*) and as well as on a *MRSA* strain of 'human' origin.

For the preparation of inoculum, microbial strains of 18-24 h were required. 6 identical colonies were suspended for 24 h in sterile saline solution (representative inoculum) to match the turbidity of the 0.5 McFarland barium sulfate standards. Afterwards, Müller Hinton agar plates (*Merck KGaA*, Darmstadt, Germany) were inoculated with the different strains. After the plates were dried for 15 min , wells were then punched, taking care that they were at approximately 15 mm from the periphery of the plate and, respectively, at 30 mm from one another. The each nanocompound (POMs) or antibiotic was distributed in the wells and were let to diffuse into the agar plates for 15 min. Then the plates were incubated 24 h at 37±2°C, and after this the results were read and interpreted (readings were conducted by measuring the diameter of the inhibition area, in mm). Germ sensitivity for a specific concentration of the POM compounds was estimated by comparing the diameter of the inhibition area to that generated by certain antibiotics with known inhibition values (and meaning that as the test substance was more active, the inhibition of microbial growth was more extensive). All tests were duplicated and the diameters of the inhibition area values were expressed as mean ± standard deviation.

Determination of the minimum inhibitory concentration

Values of the minimum inhibitory concentration (MIC), defined as the lowest concentration of antimicrobial substance that prevents visible growth of germs, were determined using the serial micro-broth dilution method with Müller-Hinton broth, described by Carson et al. [43]. Testing was conducted on the same Gram-positive (*S. aureus*, *B. cereus*) and Gram-negative (*E. coli*, *S. enteritidis*, *S. typhimurium, P. aeruginosa*) species. In this case, we

considered as necessary to be tested three POMs dilutions: of 1.6 mg/mL (**D1**), 0.8 mg/mL (**D2**) and 0.4 mg/mL (**D3**), in Müller-Hinton broth. From these solutions were inoculate ten successive serial micro-dilutions, starting with 40 µL from each of D1, D2 and D3 and 40 µL from each inoculum. The microplates thus prepared were incubated at 37±2ºC for 24 h. The maximal dilution for which the tested compounds inhibited bacterial growth was established. All tests were duplicated and the MIC values were expressed as mean ± standard deviation.

Results and discussions

Chemistry of polyoxotungstates

All POMs, obtained with a yield synthesis ~70%, were analytically characterized by elemental analysis, thermal analysis, and UV and FT-IR spectroscopy.

The representation of *pseudo*-Keggin sandwich structures of POMs is showed in figure 1.

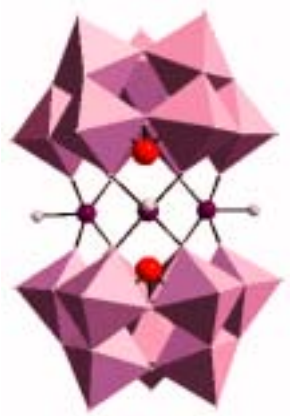

Fig. 1. Polyhedral and ball and stick representation of the *pseudo*-Keggin sandwich structures of POMs. (WO_6 octahedrons in light purple, Bi atoms in red, coordination water molecules in white and transition metals in dark purple, modified after [37])

Thermal analysis revealed the behavior of these nanocompounds: they were dehydrated and decomposed in wide temperature ranges, depending on the type of polyoxometalates. The POMs lost in two steps their water, both crystallization (lattice) and coordination water molecules. The latter process occurred in parallel with the decomposition of the compounds (by polymorphic transformations) [2, 5, 8]. However, the dehydration process was performed in two successive phases. In the first stage, the process was conducted at full speed at temperature ranging between 100-150ºC, and mass loss corresponded to the lattice water. In the second stage, more slowly, dehydration was performed at lower speed as compared to the first stage, and between 260-305ºC. The weight loss, which corresponded to the lost of the three-coordinating water molecules from polyoxotungstate complexes, was associated with POMs decomposition.

UV electronic spectra of all nanocompounds exhibited characteristic bands for POMs structures, namely two charge-transfer bands [2, 44]. UV spectra of POMs showed very similar allure, and this was in agreement with coordination of the transition metalic ions in the three vacances filled to form the sandwiched polyoxotungstate structures (by the oxygen atoms, O_c and O_e). O-$_{c,e}$ are the oxygen atoms which connect corner and edge sharing octahedral, O-$_t$ is a terminal oxygen and O_b are oxygen atoms which link the two units to finally form sandwiched *pseudo*-Keggin structure. The first broader band, ν_1, in UV electronic spectra of all POMs, corresponded to the $p_\delta(O_{c,e}) \rightarrow d_{\delta^*}(W)$ charge transfer transition in the tricentric W-O$_{c,e}$-W bonds, and the second band, more intense – ν_2, consistent with the $p_\delta(O_t) \rightarrow d_\delta(W)$ transition was centered at ~194 nm [44-46]. In the UV electronic spectra, the band at ~50 000cm^{-1} (ν_2) was not shifted in complexes spectra, in comparison with the corresponding band in the UV ligand spectrum ($Na_9[(BiW_9O_{33}]\cdot 14H_2O$, as was reported in the literature data), which is consistent with the non-participation of terminal oxygen atoms into TM cations coordination [37, 47]. While the band at ~ 38 000cm^{-1} (ν_1) was strong shifted in complexes spectra, comparatively to the ligand spectrum (centered at 39 231 cm^{-1}) [37], which was correlated with TM cations coordination by O_c and O_e oxygen atoms [47].

Vibrational spectra were recorded for all complexes of Mn (II), Fe (III, II), Co (II), Ni (II) and Cu (II) complexes as the sodium salts, and the (VO) (II) as potassium salt respectively. From the total (4000-400 cm^{-1}) area in there FT-IR absorption spectra of heteropolyoxotungstates, the interval between 1200-400 cm^{-1} was considered of interest for the field of polyoxometalates [5, 48-50]. We aimed to locate "polyoxometalic bands" for identifying specific vibrations of bonds.

All the nanocompounds synthesized and analytically characterized are shown below.

1. $Na_{12}[Cu_3(H_2O)_3(BiW_9O_{33})_2]\cdot 29H_2O$ (yellow crystals): M=5826.05; Elemental analysis and TG data (found (calcd.)): Na (4.78 (4.74)); Cu (3.31 (3.27)); Bi (7.13 (7.17)); W (56.84 (56.80)); H_2O (9.93 (9.89)); UV (H_2O) in nm/cm^{-1}: ν_2 194/51480 and ν_1 =289/34519; FT-IR bands (ν_{max}, cm^{-1}): 943 (s, ν_{as}(W=O$_t$)); 891 (s, b, ν_{as}(W-O$_c$-W)); 835 (m, sp, ν_{as}(Bi-O$_i$)); 772 (vs, ν_{as}(W-O$_e$-W)); 723 (vs, ν_{as}(W-O$_e$-W)); 723 (vs, ν_{as}(W-O$_b$-W)); 506 (w, d(W-O-W)); 436 (m, sp, ν_{as}(TM-O)).

2. $K_{12}[(VO)_3(BiW_9O_{33})_2]\cdot 36H_2O$ (dark brown crystals): M=6101.59; Elemental analysis and TG data (found (calcd.)): K (7.71 (7.69)); V (2.53 (2.50)); Bi (6.82 (6.85)); W (54.25 (54.23)); H_2O (10.67 (10.63)); UV (H_2O) in nm/cm^{-1}: ν_2 194/51450 and ν_1 = 257/38889; FT-IR bands (ν_{max}, cm^{-1}): 949 (s, b, ν_{as}(W=O$_t$)); 906 (vs, sh, ν_{as}(W-O$_c$-W)); 893 (vs, sh, ν_{as}(W-O$_c$-W)); 835 (vs, sp, ν_{as}(Bi-O$_i$)); 806 (vs, b, ν_{as}(W-O$_e$-W)); 724 (vs, b, ν_{as}(W-O$_e$-W)); 724 (vs, b, ν_{as}(W-O$_b$-W)); 513 (w, d(W-O-W)); 960 (s, ν_{as}(TM-O)).

3. $Na_{12}[Ni_3(H_2O)_3(BiW_9O_{33})_2]\cdot 39H_2O$ (yellow-greenish crystals): M=5991.64; Elemental analysis and TG data (found (calcd.)): Na (4.63 (4.60)); Ni (2.97 (2.94)); Bi (6.94 (6.98)); W (55.28 (55.23)); H_2O (12.67 (12.63)); UV (H_2O) in nm/cm^{-1}: ν_2 194/51520 and ν_1 = 259/38676; FT-IR bands (ν_{max}, cm^{-1}): 945 (s, ν_{as}(W=O$_t$)); 868 (s, b, ν_{as}(W-O$_c$-W)); 833 (sh, ν_{as}(Bi-O$_i$)); 795 (vs, ν_{as}(W-O$_e$-W)); 753 (vs, ν_{as}(W-O$_e$-W)); 720 (s, sh, ν_{as}(W-O$_b$-W)); 512 (w, d(W-O-W)).

4. $Na_{12}[Co_3(H_2O)_3(BiW_9O_{33})_2]\cdot 37H_2O$ (blue-violet crystals): M=5956.33; Elemental analysis and TG data (found (calcd.)): Na (4.66 (4.63)); Co (2.98 (2.97)); Bi (7.00 (7.02)); W (55.60 (55.56)); H_2O (12.15 (12.10)); UV (H_2O) in nm/cm^{-1}: ν_2 194/51500 and ν_1 = 256/38991; FT-IR bands (ν_{max}, cm^{-1}): 946 (s, ν_{as}(W=O$_t$)); 867 (vs, vb, ν_{as}(W-O$_c$-W)); 839 (s, sp, ν_{as}(Bi-O$_i$)); 795 (vs, ν_{as}(W-O$_e$-W)); 740 (s, b, ν_{as}(W-O$_e$-W)); 740 (s, b, ν_{as}(W-O$_b$-W)); 508 (w, d(W-O-W)).

5. $Na_{12}[Mn_3(H_2O)_3(BiW_9O_{33})_2]\cdot 43H_2O$ (orange crystals): M=6052.44; Elemental analysis and TG data (found (calcd.)): Na (4.59 (4.56)); Mn (2.75 (2.72)); Bi (6.88 (6.91)); W (54.73 (54.67)); H_2O (13.72 (13.69)); UV (H_2O) in nm/cm^{-1}: ν_2 194/51470 and n$_1$ = 271/36890; FT-IR bands (ν_{max}, cm^{-1}): 941 (s, b, ν_{as}(W=O$_t$)); 875 (s, b, ν_{as}(W-O$_c$-W)); 840 (s, ν_{as}(Bi-O$_i$)); 760 (vs, sh, ν_{as}(W-O$_e$-W)); 720 (vs, sh, ν_{as}(W-O$_e$-W)); 720 (vs, sh, ν_{as}(W-O$_b$-W)); 510 (w, d(W-O-W)).

6. $Na_9[Fe^{III}_3(H_2O)_3(BiW_9O_{33})_2]\cdot 36H_2O$ (dark yellow crystals): M=5860.08; Elemental analysis and TG data (found (calcd.)): Na (3.56 (3.53)); Fe (2.89 (2.86)); Bi (7.10 (7.13)); W (56.52 (56.47)); H_2O (12.05 (11.99)); UV (H_2O) in nm/cm^{-1}: ν_2 193/51550 and ν_1 = 270/37000; FT-IR bands (ν_{max}, cm^{-1}): 945 (s, ν_{as}(W=O$_t$)); 888 (s, vb, ν_{as}(W-O$_c$-W));

Table 1
EVALUATION OF THE ANTIBACTERIAL POTENTIAL BY DISK DIFFUSION METHOD: DIAMETERS OF THE INHIBITION AREAS (mm)

Bacterial strains	Antibiotics									POM compounds						
	S	L	TE	K	AMC	AMP	GEN	CIP	NOR	1	2	3	4	5	6	7
Staphylococcus aureus ATCC 6538P	20	25	22	21	30	25	26	22	17	15	16	16	16	13	TR	TR
MRSA ISOLATED STRAINS	TR	TR	TR	TR	11	TR	TR	TR	TR	15.5	14	12.5	13	13.5	TR	TR
Bacillus cereus ATCC14579	23	25	26	28	22	15	29	36	37	20.5	18	18.5	20	19.5	11	TR
Escherichia coli ATCC 10536	13	TR	16	18	20	18	15	24	22	16	14	12.5	14	11.5	TR	TR
Salmonella enteritidis ATCC 13076	RC	TR	20	11	25	22	8	20	20	13	14	8.5	15.5	11.5	TR	TR
Salmonella typhimurium ATCC 14028	13	TR	17	20	20	15	15	20	23	12.5	16	16	13	14	TR	TR
Pseudomonas aeruginosa ATCC 27853	12	TR	TR	TR	TR	TR	18	29	25	18.5	18	16	17.5	18	17	TR

S-streptomycin, L-lincomycin, TE-tetracycline, K-kanamycin, AMC-amoxicillin with clavulanic acid, AMP-ampicillin, GEN-gentamicin, CIP-ciprofloxacin, NOR-norfloxacin, TR-total resistance, RC-resistant colonies

833 (sh, ν_{as}(Bi-O_i)); 807 (vs, b, ν_{as}(W-O_e-W)); 770 (vs, b, ν_{as}(W-O_e-W)); 720 (s, sh, ν_{as}(W-O_b-W)); 508 (w, d(W-O-W)).

7. $Na_{12}[Fe_2^{II}(H_2O)_3(BiW_9O_{33})_2]\cdot 34H_2O$ (light brown crystals): M=5893.02; Elemental analysis and TG data (found (calcd.)): Na (4.71 (4.68)); Fe (2.87 (2.84)); Bi (7.07 (7.09)); W (56.21 (56.15)); H_2O (11.45 (11.31)); UV (H_2O) in nm/cm^{-1}: ν_2 194/51550 and n_1 = 280/35750; FT-IR bands (ν_{max}, cm^{-1}): 944 (s, ν_{as}(W=O_t)); 887 (s, b, ν_{as}(W-O_c-W)); 835 (sh, ν_{as}(Bi-O_i)); 809 (vs, b, ν_{as}(W-O_e-W)); 772 (s, b, ν_{as}(W-O_e-W)); 722 (s, sh, ν_{as}(W-O_b-W)); 505 (w, d(W-O-W)).

In the FT-IR spectra of our studied complexes a strong shifting of vibration frequencies, that corresponded to the W-$O_{c,e}$-W bonds were observed, comparatively to the ligand, $Na_9[(BiW_9O_{33}]\cdot 14H_2O$, that was reported in the literature data [37]. This shifting might be correlated to transitional metal cations coordination by oxygen atoms of O_c and O_e types [49]. Comparing all complexes FT-IR spectra between them was observed that the bands due to vibrations of W=O_t and Bi-O_i bonds were insignificant shifted, which proved in fact that their oxygen atoms were not involved into coordination of transition metal cations [49, 51].

Bands due to "sandwich" bonds, W-O_b-W, were highlighted by the strong stretching vibrations recorded at ~720 cm^{-1} into iron (III, II) and nickel (II) complexes FT-IR spectra, and by excessive enlargement of the valence vibrations band of W-O_e-W bonds located in this area of FT-IR spectra, for other complexes studied, respectively [52].

Pharmacology – antimicrobial activity of polyoxotungstates

Today, is a fact already known that there are bacteria, including Methicillin-Resistant Staphylococcus aureus (MRSA) that developed multi-resistance to several antibiotics, and that they therefore cause many and various nosocomial infections [53-54]. This is why the discovery of new compounds with antimicrobial activity, based on new mechanisms of action meets this current challenge.

Five of the POM compounds tested showed antibacterial activity in variable degrees depending on POM type and microbial strain, respectively. Antimicrobial potential, as evidenced by the disk diffusion method in our research, matched that of conventional antibiotics (table 1 and fig. 2). By comparing the diameters of bacterial growth inhibition areas, the antimicrobial activity of nano-compounds 1 to 5 was evidenced. Of note, the largest inhibition areas (18-20.5 mm) were observed against *B. cereus*. The diameters of the inhibition areas were relatively lower for the tested POMs as compared to the classic antibiotics (table 1), excepting for the *MRSA* and *P. aeruginosa* strains.

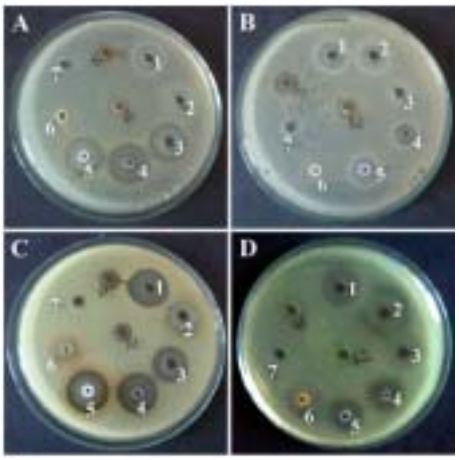

Fig. 2 *In vitro* susceptibility testing of polyoxotungstates by disk diffusion method

Effects of all tested nanocompounds (POMs 1-7) against: (A) *Staphylococcus aureus* ATCC 6538P; (B) *MRSA* (*Methicillin-Resistant Staphylococcus aureus*) strain of 'human' origin isolated by us; (C) *Bacillus cereus* ATCC 14579; (D) *Pseudomonas aeruginosa* ATCC 27853; with white Arabic numerals all POMs were marked.

The *MRSA* strain of *human* origin proved to be very resistant to antibiotics (total resistance to 8 out of the 9 antibiotics tested), but exhibited *in vitro* susceptibility to POMs 1-5 (the diameters of the inhibition areas ranging between 12.5 and 15.5 mm), the highest antimicrobial potential being observed for POMs 1 and 2. Similarly, the *P. aeruginosa* (ATCC 27853) reference strain proved to be multidrug resistant as five out of the nine antibiotics tested had no effect against it [55]. In contrast, POM compounds 1-6 exhibited antibacterial activity against *P. aeruginosa* similar to those of gentamicin.

Bacterial strains	MIC of POM compounds (mg/mL)				
	1	2	3	4	5
Staphylococcus aureus ATCC 6538P	1.6	1.6	1.6	1.6	1.6
MRSA ISOLATED STRAINS	1.6	1.6	1.6	1.6	1.6
Bacillus cereus ATCC14579	0.4	0.4	0.4	1.6	1.6
Escherichia coli ATCC 10536	1.6	1.6	1.6	1.6	1.6
Salmonella enteritidis ATCC 13076	1.6	1.6	1.6	1.6	1.6
Salmonella typhimurium ATCC 14028	1.6	1.6	1.6	1.6	1.6
Pseudomonas aeruginosa ATCC 27853	1.6	1.6	1.6	1.6	1.6

Table 2
MIC VALUES FOR THE TESTED POMs

The MIC value for the tested POMs was 1.6 mg/mL. The results are shown in tables 2, also mentioning the effects obtained against *B. cereus* of POMs 1-3, with MIC at 0.4 mg/mL.

Chemical structure-antibacterial activity relationship

The effects of the POMs on bacterial sensitivity comparative with different antibiotics were relevant. The observation of growth inhibitory zone around the antibiotics/ polyoxotungstates disk indicated the different susceptibilities of the tested strains to antibiotics and to polyoxometalates. Other POMs previously tested showed similar antibacterial effects upon other bacterial strains [7, 24, 35].We found that the polyoxotungstates proved important antibacterial effects of POMs, and this can be attributed to: the heteroatoms (Bi) from their structures and the transition metalic cation types [56] from *pseudo*-Keggin sandwiched compounds.

Antibacterial activities exerted by polyoxotungstates were due to: a) ability of polyoxoanions to cross the peptidoglycan layer in a first stage [34]; b) bacterial membrane penetration by POMs perhaps *via* the high-affinity TupABC and WtpABC transporters (homologous ATP-binding cassette (ABC)-type transporters) similarly with sulfate [57] in second stage; c) disintegration/ dissolution of peptidoglycan layer of bacteria species and eventually of their membrane, in the third stage [35]. We found that suppression of cell proliferation was increased by increasing the polyoxotungstates concentration, and this was initially inoculation-dependent, according to literature data [24, 34]. It is known that several pathogenic bacteria acquires resistance to antibiotics due to PBP (penicillin-binding-protein) conformational changes in the PBP2' forms (this form has role in the construction of the cell wall of bacteria like regular PBP, but does not respond to any β-lactam antibiotics), such as *MRSA* and *P. aeruginosa* [58, 59]. POMs achieved their effects by inhibiting the transcription of *mec*A gene in mRNA or by inhibiting mRNA translation on the PBP2', leading to their synergic action of â-lactam antibiotics [24, 59]. As Yamase and coworkers stated, some characteristics of POMs, such as high chemical stability under physiological conditions, strong negative charges, and their redox behaviors, are responsible for their powerful antibacterial activity [24, 59]. The polyoxometalate tested here by us, proved to possess an important antibacterial action, especially against multidrug-resistant strain *MRSA* and *P. aeruginosa*.

Conclusions

All POMs synthesized and analyzed by us showed similar chemical features that were also specific for this class of compounds. The results of chemical elemental analysis were in good agreement with the calculated compositions, and with the proposed chemical formula. Thermal analysis of all POMs revealed the presence of two types of water molecules: three molecules of coordinating water (excepting the compound with vanadyl ions), and the remaining water (specific for the structure of each complex) consisted of lattice water molecules. In the UV and FT-IR spectra were found: strong shifted of both electronic bands (in the UV spectra) and the vibration frequencies bands (in the FT-IR spectra), corresponding of the tricentric W-$O_{c,e}$-W bonds, comparatively with specific bands of ligand spectrum from the literature data, and these can be attributed to the involvement of these types of oxygen atoms (O_c and O_e) in coordination of transition metalic cations. Among the seven POMs reported here, the first five ones proved concurrent antibacterial effect (in between and as compared to antibiotics), or even better than of the tested antibiotics. Our nanocompounds exhibited herein *in vitro* effects against both Gram-positive (*S. aureus* and *B. cereus*) and Gram-negative bacteria (*E. coli, S. enteritidis, S. typhimurium, P. aeruginosa*), and very important, against multidrug-resistant microbial strains (*MRSA* and *P. aeruginosa*). The antibacterial effects of POMs were related to their chemical structure: they were sandwiched polyoxotungstates, containing Bi as heteroatom and the three vacances filled with the different transition metal cations. Stability of the POMs in the bacteria culture media participated in their antibacterial action. Among the last two POMs, containing Fe, only POM 6 (with Fe^{3+}) was effective, but only upon *P. aeruginosa* and *B. cereus*, while POM 7 (with Fe^{2+}) did not display any antibacterial activity.

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Manuscript received: 16.06.2015