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Regular paper

The effects of nickel(II) complexes with imidazole derivatives on pyocyanin and pyoverdine production by *Pseudomonas aeruginosa* strains isolated from cystic fibrosis*

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***Pseudomonas aeruginosa* infection is problematic in patients with cystic fibrosis (CF). *P. aeruginosa* secretes a diversity of pigments, such as pyocyanin and pyoverdine. The aim of this study was to evaluate the effects of complexes of nickel(II) ($[Ni(iaa)_2(H_2O)_2]\cdot H_2O$ (iaa = imidazole-4-acetate anion), $[Ni(1\text{-allim})_6](NO_3)_2$ (1-allim = 1-allylimidazole) and $NiCl_2$ on pyocyanin and pyoverdine production by 23 strains of *P. aeruginosa* isolated from cystic fibrosis under growth conditions specific for the CF respiratory system. The antibacterial effects and biophysical properties of the tested substances were measured by spectrofluorometric techniques, as well as by laser interferometry, confocal and atomic force microscopy. The cytotoxic properties of all compounds were measured by Annexin/IP assay against A549 cells. All tested compounds have no effect on pyocyanin production and decrease the pyoverdine secretion in about 40% of tested *P. aeruginosa* strains at non-cytotoxic range of concentrations. Imidazole-4-acetate anion and 1-allylimidazole have good diffusion properties in the mature *P. aeruginosa* PAO1 biofilm. In conclusion, the tested nickel(II) complexes do not have clinical implications in *P. aeruginosa* eradication in cystic fibrosis. The diffusion properties of 1-allylimidazole and imidazole-4-acetate and their lack of effect on A549 cells suggest that they might be considered for chemical synthesis with other transition metals.**

Key words: *Pseudomonas* sp., cystic fibrosis, nickel complexes, pyocyanin, pyoverdine

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INTRODUCTION

Cystic fibrosis (CF) is a heritable disease, characterized by chronic inflammation of the airways, leading to bronchiectasis and respiratory failure. It causes accumulation of a thick, dehydrated mucus in the lungs which increases the patient's susceptibility to bacterial infections. It is an optimal niche for microorganisms which induce chronic lung diseases in children and young adults. These bacterial infections lead to progressive pulmonary damage and emphysema. Eradication of bacterial biofilms formed in mucus is a crucial problem, because the distribution of classic antibiotics in biofilm/mucus structure is weak and their antibacterial activity might stimulate drug resistance. Biophysical properties of biofilm structure exopolymer secretions (EPS) in gel-like mucus are directly associated with reduced susceptibility to antibiotics and limit the effective eradication of bacteria (Stewart & Costerton, 2003; Manago *et al.*, 2015). It suggests that not only antibacterial, but also diffusion parameters of new drugs should be considered in the eradication of biofilm-forming bacteria.

P. aeruginosa is a common opportunistic pathogen causing respiratory diseases in patients with CF, and produces a diversity of pigments, such as pyocyanin and pyoverdine (Kolpen *et al.*, 2014; Nguyen *et al.*, 2014; Muller & Merrett, 2015). Pyocyanin (1-hydroxy-5-methyl-phenazine) is a redox-active compound having the ability to accept and donate electrons. In the sputum of CF patients, pyocyanin is detected at 100 μM (Wilson *et al.*, 1988). In the cytoplasm of host cells, this pigment has the ability to transfer electrons from reduced compounds, e.g. glutathione, and leads to a reduction of antioxidants along with higher concentrations of strong oxidizing compounds, such as reactive oxygen species (ROS) and superoxide anions (Manago *et al.*, 2015). Pyocyanin is able to damage the cilia of respiratory epithelium, increases IL-8 secretion by epithelial cells, induces apoptosis and inhibits T-cell proliferation (Gloyne *et al.*, 2011). Pyoverdine, acting as a siderophore, binds and transports iron ions into bacterial cell. It was shown that pyoverdine plays a key role in biofilm formation regardless of the presence of iron (Meyer *et al.*, 1996; Nadal Jimenez *et al.*, 2010).

The aim of this study was to evaluate the effects of complexes of nickel(II) ($[Ni(iaa)_2(H_2O)_2]\times H_2O$ (iaa = imidazole-4-acetate anion), $[Ni(1\text{-allim})_6](NO_3)_2$ (1-allim = 1-allylimidazole) and $NiCl_2$ on pyocyanin and pyoverdine production by 23 strains of *P. aeruginosa* isolated from CF at non-cytotoxic range of concentrations against A549 cells. Moreover, the diffusion parameters of tested substances through *P. aeruginosa* PAO1 biofilm (used as a model of *Pseudomonas*

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Abbreviations: AFM, atomic force microscopy; C, control group; CBL, concentration boundary layer; CF, cystic fibrosis; EPR, electron paramagnetic resonance; EPS, exopolymer secretions; G, liquid culture density; IR, infrared spectroscopy; PBS, phosphate buffered saline; P, pyocyanin or pyoverdine; PET, polyethylene terephthalate; PI, propidium iodide; ROS, reactive oxygen species; T, treatment group; TSB, tryptic soy broth; UV-Vis, ultraviolet–visible spectroscopy

sp.) were determined by laser interferometry and confocal microscopy. The physical parameters of PAO1 biofilm were determined by atomic force microscopy.

MATERIALS AND METHODS

Bacterial strains and eukaryotic cell line. The antibacterial properties of nickel(II) complexes with imidazole derivatives were tested on 23 strains of *Pseudomonas aeruginosa* isolated from patients with CF and derived from BCCM/LMG Bacteria Collection, Gent, Belgium (De Soyza *et al*., 2013); no. 1 — LES B58, no. 2 — LES 400, no. 3 — C3719, no. 4 — *P.* DK2, no. 4 — AES-1R, no. 5 — AUS23, no. 6 — AUS52, no. 7 — AA2, no. 8 — AA43, no. 9 — AA44, no. 10 — AMT0023-30, no. 11 — AMT0023-34, no. 12 — AMT0060-1, no. 13 — AMT0060-2, no. 14 — AMT0060-3, no. 15 — CHA, no. 16 — NN2, no. 17 — IST 27 mucoid, no. 18 — 2192, no. 19 — NH57388A, no. 20 — U018A, no. 21 — RP1, no. 22 — KK1, no. 23 — TBCF10839. The biophysical properties of bacterial biofilm were tested on *P. aeruginosa* ATCC 15692 (PAO1) as a model of *Pseudomonas* sp. The cytotoxicity of nickel(II) complexes with imidazole derivatives were analyzed on A549 line used as a model of the eukaryotic cells.

Nickel complexes. Nickel(II) complexes with 1-allylimidazole (1-allim) and imidazole-4-acetate anion (iaa) with the stoichiometry $[Ni(1\text{-allim})_6](NO_3)_2$ and $[Ni(iaa)_2(H_2O)_2]H_2O$ were synthesized according to the previously described procedure (Kurdziel & Głowiak, 2000). The structures of the obtained nickel(II) complexes were characterized by IR, farIR, UV-Vis, EPR spectroscopy, and the magnetic moment and thermal stability were determined. Compounds which used for the synthesis were: imidazole-4-acetic acid-sodium salt (Sigma-Aldrich Chemical, St. Louis, MO, USA.), 1-allylimidazole (Sigma-Aldrich Chemical, St. Louis, MO, USA.), $Ni(NO_3)_2{\times}6H_2O$ (POCH, Poland), propan-2-ol (POCH Poland) and trimethyl orthoformate (Sigma-Aldrich Chemical, St. Louis, MO, USA.).

Pyocyanin and pyoverdine production by clinical strains of *P. aeruginosa*. Clinical *P. aeruginosa* strains were grown in Tryptic Soy Broth (TSB) in the presence of metal complexes $[Ni(1\text{-allim})_6](NO_3)_2$, $[Ni(iaa)_2(H_2O)_2]{\times}H_2O$, or their ligands, at concentrations in the range from 7–500 μM, overnight (16–18 h) at 37°C under microaerophilic conditions (5% CO_2) in stationary culture. The absorbance of pyocyanin was determined spectrophotometrically at 691 nm in TSB growth medium (200 μl of bacterial cell free supernatant) from the culture described above. The fluorescence of pyoverdine was determined ($\lambda_{ex}=398$ nm; $\lambda_{em}=460$ nm) in growth medium under the same conditions with a Microplate Reader Infinite 200 PRO (TECAN, Switzerland). All samples were measured in one repetition, in two independent experiments.

The level of pyocyanin or pyoverdine production in the presence of tested substances, as well as non-treated control, was normalized (ND_t or ND_c, respectively) against the culture density measured spectrophotometrically at 600 nm according to the formulas:

$$ND_t=P_t/G_t \quad (1)$$

and

$$ND_c=P_c/G_c, \quad (2)$$

where P is pyocyanin or pyoverdine absorbance/fluorescence, G is the liquid culture density respectively for the treatment group (T) and the control group (C).

The estimation of the nickel ions, complexes or their ligands, effects (E_s) on pyocyanin and pyoverdine production and bacterial growth were based on the measurement developed by the authors, according to the formula:

$$E_s= ND_t/ND_c \quad (3)$$

Theoretically, one of three kinds of effects might be observed: $E_s>1$ (increase of the pyocyanin or pyoverdine production); $E_s=1$ (no effect on the pyocyanin or pyoverdine production) and $E_s<1$ (decrease of the pyocyanin or pyoverdine production).

The statistical significance of E_s was estimated by the Wilcoxon matched-pairs signed rank test of paired *T*-test, nonparametric. A *P* value of <0.05 was considered significant. Statistical analyses were performed using GraphPad Prism version 6 (GraphPad Software, USA).

Statistical analyses were carried out to check the strength of the E_s values of all the substance concentrations, in comparison to the absolute value 1 taken as no effect. If the E_s values of all substance concentrations were significantly below or above the value of 1, it meant that E_s is statistically significant for all substance concentrations for a particular bacterial strain.

Cytotoxicity of Ni(II) complexes with imidazole derivatives. A549 cells (adenocarcinoma human alveolar basal epithelial cells) were cultured at 37°C, in a humidified 5% CO_2 atmosphere, in plastic dishes, in F-12K medium supplemented with 10% heat inactivated fetal calf serum, 2 mM L-glutamine and antibiotics (100 units/ml penicillin and 100 μg/ml streptomycin). A549 cells were treated with $[Ni(iaa)_2(H_2O)_2]{\times}H_2O$, $[Ni(1\text{-allim})_6](NO_3)_2$ and their ligands at concentrations of 125 μM or 250 μM, for 48 hours. After treatment, the frequencies of early apoptotic, late apoptotic and necrotic cells were evaluated with the Annexin V-FITC apoptosis detection Kit I (BD Pharmingen, USA), described elsewhere (Darzynkiewicz, 2003). The cells were briefly washed two times with cold PBS, and then resuspended in a 1× binding buffer at a concentration of $1{\times}10^6$ cells/ml. An aliquot of 125 μl of the cell suspension was incubated with 5 μl of annexin V-FITC, and 5 μl of propidium iodide (PI), at room temperature for 15 min in the dark. The cells were resuspended in 400 μl of 1× binding buffer. The fluorescence was determined using an LSR II flow cytometer (Becton Dickinson, USA). CellQuest Pro (Becton Dickinson, USA) was used for data acquisition and analysis. Data for 20 000 events were stored. A cell gate containing A549 was established on the basis of forward and side light scatter. Four different populations of cells were detected with the annexin V-FITC kit: normal cells that are annexin-negative and PI-negative and express no fluorescence, early apoptotic cells that are annexin-positive and PI-negative and express green fluorescence, late apoptotic/necrotic cells that are annexin-positive and PI-positive and express green and orange fluorescence, and necrotic cells that are annexin-negative and PI-positive and express orange fluorescence.

Atomic Force Microscopy of PAO1 biofilm. The PAO1 biofilm was formed for 72 h, at 37°C, in TSB medium on polyethylene terephthalate (PET) membrane with pore diameter of 1 μm, as an element of the Cell Culture Inserts (BD Biosciences, USA). The study of surface topography was performed using atomic force microscopy NanoCompact AFM (PHYWE, Germany). It was measured with the tapping mode, using the probe Tap190Al-G (BudgetSensors, Bulgaria). The mean surface roughness (S_a), root mean square (S_q), average value (S_m), valley depth (S_v), maximum peak (S_p) and height

(S_y) were determined. Roughness parameters were determined using the Measure Nano software dedicated to this model of the microscope.

Laser interferometry. The measurements of 1-allylimidazole or imidazole-4-acetate anion diffusion through PAO1 biofilm was performed by the laser interferometry system (Arabski *et al.*, 2007; 2009a; 2009b; 2013b; Danis-Wlodarczyk *et al.*, 2015). The system under study consists of two glass cuvettes separated by the horizontally located membrane (Nephrophane membrane with PAO1 biofilm formed for 72 h). The lower cuvette was filled with an aqueous 1-allylimidazole or imidazole-4-acetate anion solution at an initial concentration of 100 mmol/L, while in the upper cuvette there was pure water. With such a configuration of the measurement system, the solution in the upper cuvette remains undisturbed and a stable concentration boundary layer (CBL) of *d* thickness is created. The 1-allylimidazole or imidazole-4-acetate anion concentration is uniform in the planes parallel to the biofilm-solution interface and concentration gradients occur only in the vertical direction. The interferograms, which appear due to the interference of two laser beams, are determined by the refraction coefficient of the solute, which in turn depends on the concentration of the 1-allylimidazole or imidazole-4-acetate anion solution. With recording the interferograms with a given time-step (Dt=2 min.), one can reconstruct after computer analysis the interference fringe course of the spatio-temporal concentration distribution (i.e. concentration profile C(x,t)). The amount (mol) of 1-allylimidazole or imidazole-4-acetate anion, N(t), which diffuses in time t through PAO1 biofilm formed on Nephrophane membrane to water was calculated by integrating the concentration profile C(x,t) in the upper cuvette according to:

$$N(t) = S\int_0^{\delta} C(x,t)dx, \tag{4}$$

where S denotes the surface of the biofilm-water interface ($S=7\times10^{-5}$ m^2), and d the CBL thickness (determined interferometrically). All experiments were performed at a temperature of 37°C. The diffusion coeffi-

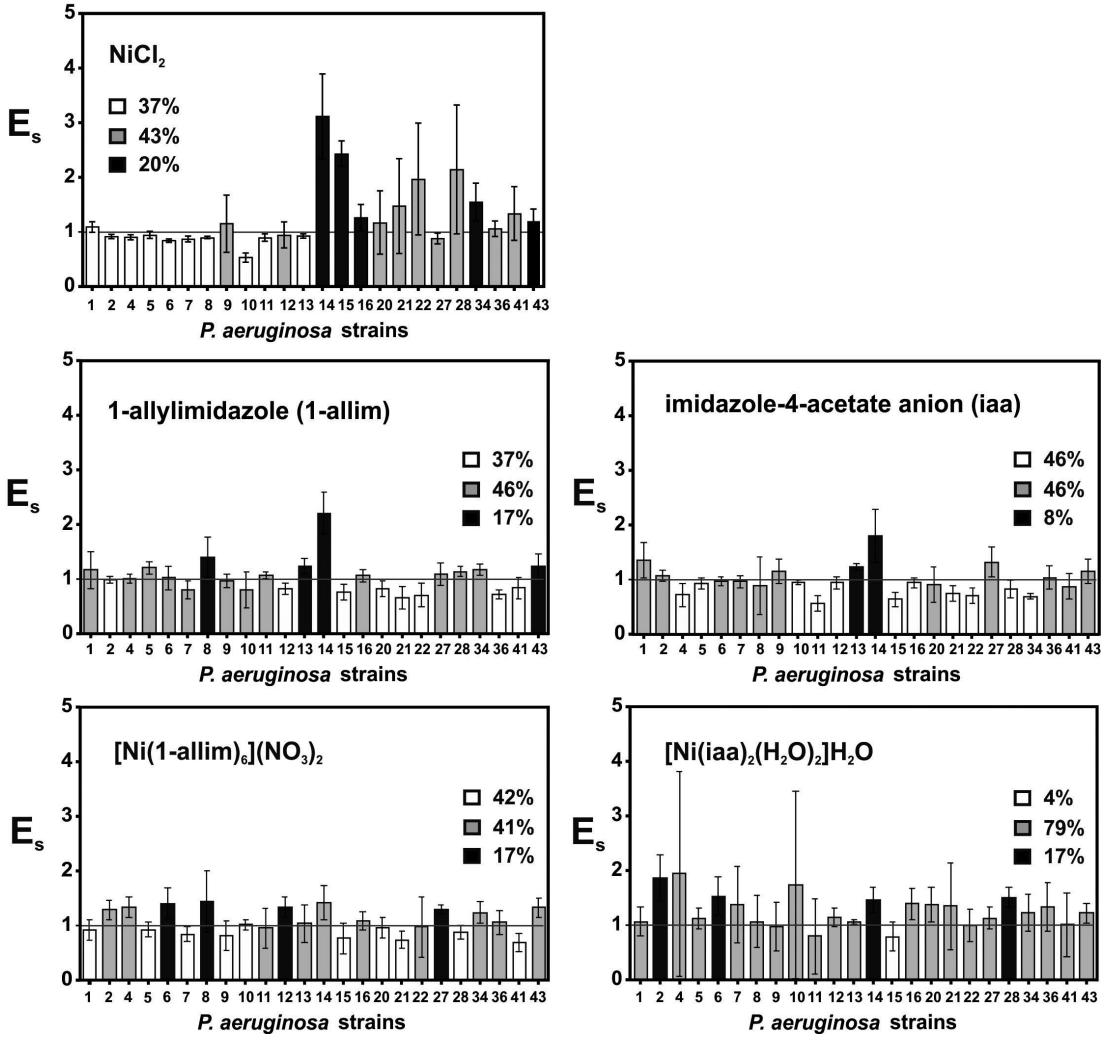


Figure 1. The effects of nickel(II) complexes with 1-allylimidazole or imidazole-4-acetate anion, and their ligands alone, on *P. aeruginosa* growth in correlation with pyoverdine production.
White bars denote that the tested compound decreased ($p<0.05$) the secretion of pyoverdine in comparison to untreated control, independent of bacterial growth ($E_s<1$). Black bars denote that the tested compound increased ($p<0.05$) the secretion of pyoverdine in comparision to untreated control, independent of bacterial growth ($E_s>1$). Grey bars denote no effect on pyoverdine level in growth medium in the presence of the tested compounds. The percent (%) of bacterial strains with proper effect on pyoverdine level are presented.

cient D of 1-allylimidazole or imidazole-4-acetate anion in PAO1 biofilm as well as in water was determined on the basis of Fick's first law and the second Kedem-Katchalsky equation (Arabski *et al.*, 2007; Slezak *et al.*, 2005).

Confocal microscopy of *P. aeruginosa* PAO1 biofilm. The thickness of the biofilm formed on the Nephrophane membrane was analyzed on the basis of pyoverdine fluorescence released into the matrix of PAO1 biofilm formed 72 h at 37°C. The membrane with formed biofilm was placed on a microscopic slide and analyzed by confocal microscopy. The value of the biofilm thickness was used for calculation of diffusion parameters of imidazole derivatives through biofilm (obtained by laser interferometry). The fluorescence analysis of PAO1 biofilm was performed by using confocal microscope A1R (Nikon, Japan). The specimen underwent excitation with laser beam having a wavelength equal to 405 and 488 nm. Images was taken with PlanAPO 10× DIC-L lens. Resolution of DU4 12-bit photomultiplier tube was set to X:1024 px Y:1024 px and the resolving power of microscope was 0.62 μm/px. Image acquisition was obtained within the range of 85 μm. The thickness of PAO1 biofilm was measured in 18 steps with Z-step (5 μm). The fluorescence intensity in the area of the biofilm was measured as the average value along a randomly chosen thin plane of biofilm. It was measured through all layers collected in the Z — axis scan.

RESULTS

Effect of nickel complexes on *P. aeruginosa* strains

Studies were carried out under microaerophilic conditions specific for the CF respiratory system. Statistical studies enabled the independent assessment of the growth and production of pigments: pyocyanin and pyoverdine by *Pseudomonas aeruginosa* in the presence of nickel(II) complexes and their ligands. The growth of all tested *P. aeruginosa* strains was positively correlated with pyocyanin production, in contrast to diversified secretion of pyoverdine by bacteria after 24 h, at 37°C under microaerophilic conditions (5% CO_2) (results not shown). The nickel(II) complexes and their ligands had no effect on pyocyanin production by all tested bacterial strains (results not shown). Figure 1 shows the summary of effects of nickel(II) complexes with 1-allylimidazole or imidazole-4-acetate anion (% of strains; $p<0.05$) on *P. aeruginosa* growth in correlation with pyoverdine production. The presence of nickel ions(II) or imidazole-4-acetate anion, in contrast to their complexes, resulted in a decrease in pyoverdine secretion in 37% and 46% of tested *P. aeruginosa* strains, respectively. The ligand 1-allylimidazole alone and in the $[Ni(1\text{-allim})_6](NO_3)_2$ complex, inhibited pyoverdine production in 42% and 37% of bacterial strains, respectively.

Cytotoxicity of nickel(II) complex

The Annexin/IP assay allowed to determine the frequency of apoptotic and necrotic A549 cells in the presence of nickel(II) complexes or their ligands. Table 1 shows that $[Ni(iaa)_2(H_2O)_2]H_2O$ induced late apoptosis, as well as necrosis, at the concentration of 250 μM in A549 cells after 48 hours, in contrast to the $[Ni(1\text{-allim})_6](NO_3)_2$ complex. Both tested nickel (II) complexes at the concentrations of 125 μM, as well as their ligands, did not induce apoptosis in A549 cells.

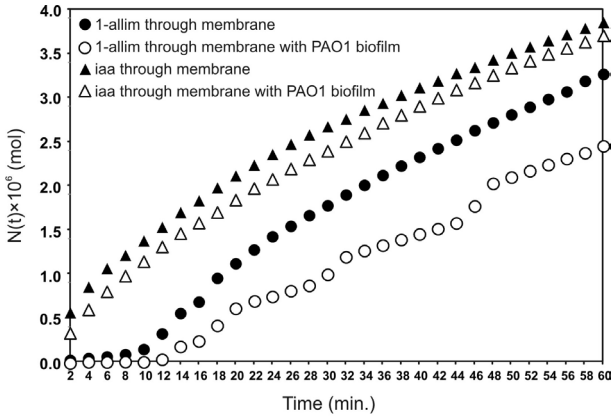
Diffusion of ligands through PAO1 biofilm

Figure 2 shows the laser interferometry analysis of 1-allylimidazole or imidazole-4-acetate anion diffusion through PAO1 biofilm formed on Nephrophane membrane for 72 h at 37°C, 5% CO_2. The amount of imidazole-4-acetate anion transported through *P. aeruginosa* PAO1 biofilm formed on the membrane (3.80×10^{-6} mol) was similar to 1-allylimidazole (2.55×10^{-6} mol) at initial concentration of 100 mmol/L after 60 min. It seems that this small difference is determined by specific diffusion properties of the membrane alone for each ligand. For 1-allylimidazole, it was observed that the membrane is saturated with this compound after 12 min, in contrast to imidazole-4-acetate anion. It was decided to calculate the diffusion coefficients for the elimination of the membrane effect (scaffold for biofilm). On the basis of confocal microscopy analysis (Fig. 3) of PAO1 biofilm thickness (65 μm), the diffusion coefficients of

Table 1. Percentage of early and late apoptotic and necrotic A549 cells treated with nickel complexes and their ligands; the mean of two independent experiments ± S.D. Cytotoxic activity calculated by ANOVA test.

Substance [mM]	Normal cells (Annexin-/IP-)	Apoptosis		Necrosis (Annexin-/IP+)
		Early (Annexin+/IP-)	Late (Annexin+/IP+)	
Control	93.05±2.71	3.15±2.76	3.63±1.77	0.19±0.21
10 camptothecin*	81.57±1.38	11.47±0.15	6.87±0.25	0.20±0.18
125 $NiCl_2$	96.80±4.32	0.80±2.21	2.00±0.97	0.30±1.38
250 $NiCl_2$*	77.88±0.73	0.40±0.28	10.28±4.93	11.38±4.41
125 $[Ni(iaa)_2(H_2O)_2]H_2O$	83.47±4.62	2.89±0.34	11.24±2.46	2.40±1.59
125 Na (iaa)	96.80±0.53	1.77±0.06	0.80±0.23	0.67±0.55
125 $[Ni(1\text{-allim})_6](NO_3)_2$	97.33±1.23	0.63±0.38	1.17±0.61	0.82±0.26
125 1-allim	94.93±1.53	2.03±1.00	2.16±0.64	0.87±0.55
250 $[Ni(iaa)_2(H_2O)_2]H_2O$*	61.35±6.99	1.07±0.24	22.73±5.14	14.82±3.13
250 Na (iaa)	95.50±1.47	2.30±0.52	1.33±0.64	0.87±0.49
250 $[Ni(1\text{-allim})_6](NO_3)_2$	85.00±4.88	1.12±0.87	8.93±2.88	4.93±4.86
250 1-allim	95.70±1.47	1.93±0.58	1.13±0.58	1.23±0.95

*$p<0.05$



diffusion coefficient of 1-allim in water	6.0×10^{-10} m²/s
diffusion coefficient of 1-allim in membrane	5.6×10^{-10} m²/s
diffusion coefficient of 1-allim in PAO1 biofilm	1.1×10^{-10} m²/s
diffusion coefficient of iaa in water	8.2×10^{-10} m²/s
diffusion coefficient of iaa in membrane	7.6×10^{-10} m²/s
diffusion coefficient of iaa in PAO1 biofilm	5.9×10^{-10} m²/s

Figure 2. The laser interferometry analysis of 1-allylimidazole or imidazole-4-acetate anion diffusion through PAO1 biofilm formed on Nephrophane membrane for 72 h, at 37°C, 5% CO_2, as well as the native membrane.

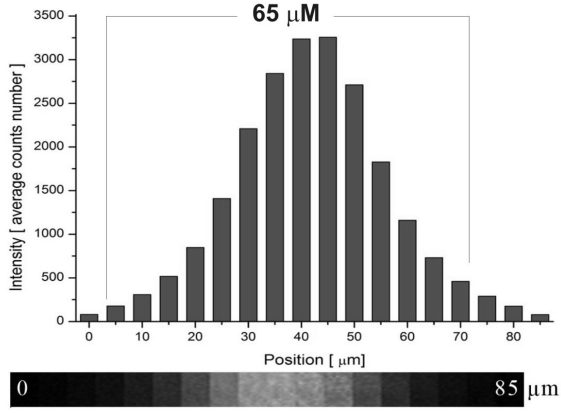


Figure 3. The confocal microscopy analysis of PAO1 biofilm thickness formed on Nephrophane membrane for 72 h, at 37°C, 5% CO_2.
The fluorescence intensity in the area of the biofilm was measured as the average value along a randomly chosen thin plane of biofilm (higher panel). The thickness of PAO1 biofilm was measured in 18 steps with Z-step (5 μm) (lower panel).

both tested imidazole derivatives were calculated. The diffusion coefficient of imidazole-4-acetate anion in PAO1 biofilm (2.1×10^{-11} m²/s) in PAO1 biofilm was twice as good as 1-allylimidazole (1.1×10^{-11} m²/s).

Analysis of PAO1 biofilm by AFM microscopy

The diffusion coefficients in PAO1 biofilm was measured in biofilm matrix formed on the Nephrophane membrane. For physical analysis of PAO1 biofilm structure formed for 72 h at 37°C, 5% CO_2, the PET membrane was chosen, from the methodological (AFM) point of view. Figure 4 shows the topography of the PET membrane alone and with PAO1 biofilm formed for 72 h at 37°C, 5% CO_2 measured by AFM. The PAO1 biofilm smooths the rough surface of the PET membrane (Table 2) and is formed on their surface (Danis-Wlodarczyk *et al.*, 2015).

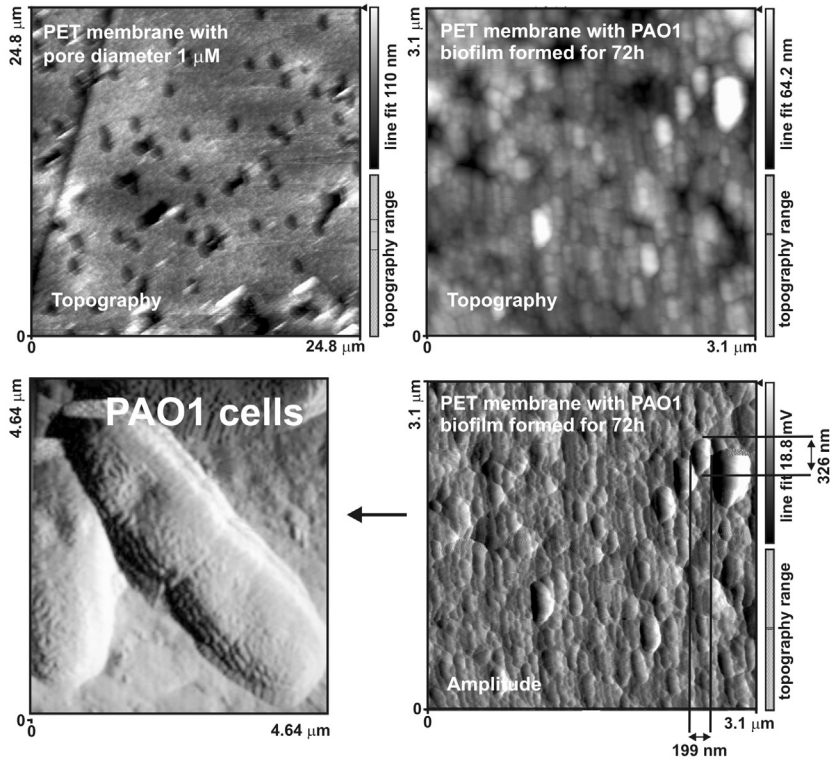


Figure 4. Topography and amplitude of PET membrane alone and with PAO1 biofilm formed for 72 h, at 37°C, 5% CO_2, measured by AFM microscopy.

Table 2. The roughness parameters of the native PET membrane and covered with *P. aeruginosa* PAO1 biofilm, formed for 72 h, measured by AFM microscopy.

Parameters of surface roughness	PET membrane [nm]	PET membrane with PAO1 biofilm [nm]
S_a	13.156	8.031
S_q	18.448	10.975
S_y	276.350	113.110
S_p	139.761	57.103
S_v	−136.600	−56.005

DISCUSSION

Transition metal complexes with derivatives of imidazole might be considered as new antibacterial agents. The antibacterial properties of transition metals depend on the physical and chemical properties of such molecules, which may be transported through the cell membranes by diffusion, via calcium and iron channels (in interaction with pyoverdine as a siderophore) and by phagocytosis (Kasprzak *et al.*, 2003; Simonetti *et al.*, 2001). For example, the presence of imidazole-4-acetic acid anion in synthesized metal complexes may promote the effective transport of these molecules by changing the permeability of the cell membrane and making the bactericidal effect of antibiotics more intense. Moreover, metal complexes with imidazole, in contrast to metal ions alone, should (i) better diffuse in biofilm, because the neutral charge of metal complexes reduces their interaction with biofilm matrix components, like nucleic acids or proteins, in contrast to metal ions, (ii) reduce the redox potential in the environment of free radical reactions (chronic bacterial infections), and (iii) protect against formation of complexes with chloride ions, as it is important in the case of cystic fibrosis (Sekhon, 2010).

The aim of this study was to evaluate the effects of complexes of nickel(II) with imidazole derivatives and their ligands alone, on pyocyanin and pyoverdine production by 23 strains of *P. aeruginosa* isolated from CF, at non-cytotoxic concentration against eukaryotic cells. The antibacterial properties of the above newly synthesized complexes, were tested for the first time under conditions specific for eukaryotic cell line growth. The decrease of pyocyanin production was not observed in the presence of all tested compounds, as well as pyoverdine secretion by *P. aeruginosa* strains by $[Ni(iaa)_2(H_2O)_2]\times H_2O$ complex. $[Ni(1\text{-allim})_6](NO_3)_2$, nickel(II), and both imidazole ligands (imidazole-4-acetate anion and 1-allylimidazole), decreased the production of pyoverdine in c.a. 40% of the tested *P. aeruginosa* strains. The antibacterial effect of nickel(II) complexes with imidazole derivatives, as well as ligands alone, were tested at a non-cytotoxic range of concentrations (<125 μM) against A549 cells.

Scientists studying similar imidazole derivatives have observed bactericidal effect of the tested compounds on *P. aeruginosa* strains at much higher concentrations (Desai *et al.*, 2013; Vijesh *et al.*, 2011, 2013). The antibacterial properties of imidazole derivatives were previously determined, for example, for 1-alkylimidazole (Khabnadideh *et al.*, 2003), 2-(substituted phenyl)-1H-imidazole and (substituted phenyl)-[2-(substituted phenyl)-imidazol-1-yl]-methanone analogues (Sharma *et al.*, 2009), chloroaryloxyalkyl imidazole and benzimidazole derivatives (Khalafi-Nezhad *et al.*, 2005) or 2,4,5-trisubstituted imidazole derivatives (Khan *et al.*, 2008). The cytotoxic properties of the above imidazole derivatives against eukaryotic cells still remain open to analysis.

Imidazole-4-acetate anion and 1-allylimidazole have good diffusion properties through mature PAO1 biofilm, as measured by laser interferometry and confocal microscopy. The value of imidazole-4-acetate anion diffusion coefficient in biofilm was two-times higher than 1-allylimidazole, 2.1×10^{-11} m^2/s and 1.1×10^{-11} m^2/s, respectively. Moreover, the diffusion coefficient of ciprofloxacin in PAO1 biofilm, calculated on the basis of data from Arabski *et al.*, 2013a, is equal to 1.75×10^{-11} m^2/s. It indicates that diffusion properties of ciprofloxacin used in CF infection treatment and imidazole derivatives are similar, and all analyzed substances might penetrate the biofilm matrix at the same level. It was concluded that imidazole-4-acetate anion and 1-allylimidazole might be considered in chemical synthesis with other metals, for example with those that act as iron antagonists, like cobalt or gallium.

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