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## Biological studies of organoselenium trans- palladium(II) complexes

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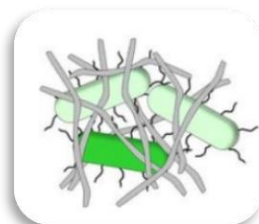
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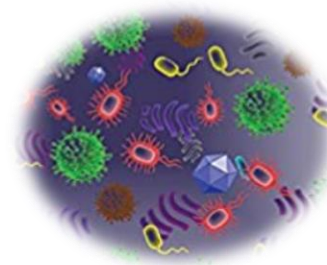
# Biological studies of organoselenium trans-palladium(II) complexes



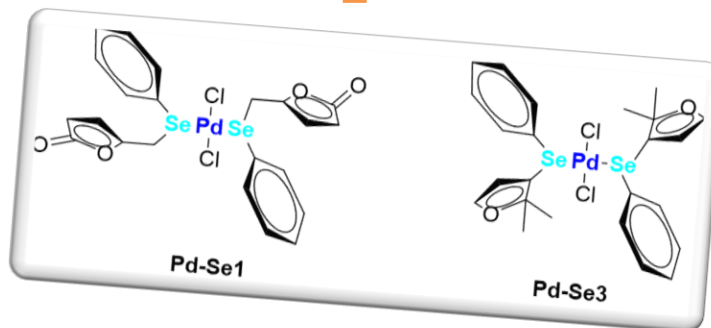
Antioxidant activity



Antibiofilm activity



Antimicrobial activity



Organoselenium trans-palladium(II) complexes

**Keywords:** Antimicrobial activity, Antioxidant activity, Biofilm, Palladium, Selenium.



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# Abstract:

## Introduction

The emergence of Multi-Drug-Resistance (MDR) has become a major global clinical problem. The emergence of an innovative bioorganic antimicrobial platform is an interesting alternative to combat microbial resistance, and therefore transition metal complexes are being investigated

## Methodology

In this work, two complexes of trans-palladium(II) with organoselenium compounds as their ligands were investigated., **PdSe1**, [Pd(L1)2Cl2], (**L1** = 5- (phenylselanylmethyl) -dihydrofuran-2 (3H) -one)) and **PdSe3**, [Pd(L3)2Cl2], (**L3** =2,2-dimethyl-3-(phenylselanylmethyl)-tetrahydro-2H-pyran). Their antimicrobial and antioxidant activity as well as the effect of the formed biofilm of selected bacteria were studied. Antimicrobial activity was tested by determining the minimum inhibitory concentrations (MIC) and minimum microbicidal concentration (MMC) using the resazurin microdilution plate method The effect of complexes and ligands on formed biofilm of *S. aureus* ATCC 25923, *S. aureus*, and *P. aeruginosa* was determined. The *in vitro* antioxidant activity of the complexes was determined based on the neutralizing capacity of DPPH radicals expressed as EK50.

## Results and Discussion

The compounds showed different degrees of antimicrobial activity. MICs and MMCs values were in a range from 15.63 to >1000 µg/mL. The investigated complexes showed the most significant activity on *Pseudomonas aeruginosa*. Complex **Pd-Se1** showed a better effect on the tested biofilm of *S. aureus*, while complex **Pd-Se3** showed a significant effect on the tested biofilm of *P. aeruginosa*. The antioxidant activity of all organoselenium trans-palladium(II) complexes is significant, with the **PdSe1** complex being more active. These complexes have the potential to be further investigated as metallodrugs.



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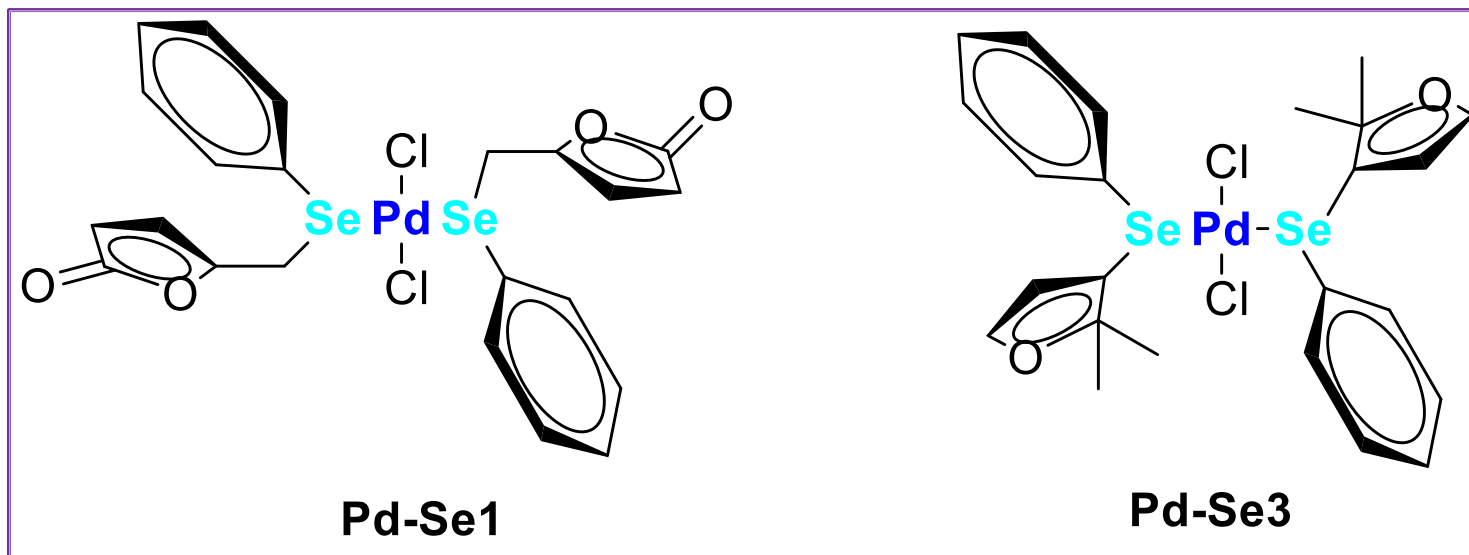
# Introduction

- ✓ Compounds with antioxidant potential protect the body from free radicals. These radicals react with various molecules in the cell and thus damage it. On the other hand, the resistance of microorganisms to antibiotics is a major public health problem. Due to all this, great research efforts are focused on the synthesis of new antimicrobial and antioxidant compounds [1].
- ✓ Innovative bioorganic antimicrobial substances are becoming an interesting alternative to combat microbial resistance. That is why metal complexes are often investigated [2].
- ✓ Selenium is an essential element and complexes of palladium and selenium compounds have proven antitumor, antimicrobial, and other pharmacological activities. [3]

1. Inorganica Chimica Acta. 442, 2016, 105–110.
2. Coordination Chemistry Reviews 351, 2017, 76–117.
3. Medicinal Chemistry, 2020 DOI: [10.2174/1573406416666200930112442](https://doi.org/10.2174/1573406416666200930112442)



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**Figure 1.** Structures of the investigated *trans*-Pd-Se complexes

✓ Complexes of *trans*-palladium(II) with organoselenium compounds were synthesized according to already published publication [3. 4]:

1. **PdSe1**,  $[\text{Pd}(\text{L}_1)_2\text{Cl}_2]$  ( $\text{L}_1$  = 5- (phenylselanylmethyl) -dihydrofuran-2 (3H) -one)
2. **PdSe3**  $[\text{Pd}(\text{L}_3)_2\text{Cl}_2]$  ( $\text{L}_3$  = 2,2-dimethyl-3- (phenylselanylmethyl) -tetrahydro-2H-pyran)

4. J Inorg Biochem, 143, 2015, 9-19.



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## Methodology

- ✓ **Antimicrobial activity** was tested by determining the minimum inhibitory concentrations (MIC) and minimum microbicidal concentration (MMC) using the resazurin microdilution plate method [5].
- ✓ The effect of complexes and ligands on the formed **biofilm** of *S. aureus* ATCC 25923, *S. aureus*, and *P. aeruginosa* was determined [3].
- ✓ The *in vitro* **antioxidant activity** of the complex was determined based on the neutralizing capacity of DPPH radicals [1].

5. Methods, 42(4), 2007, 321-324.



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# Antimicrobial activity

- ✓ The antimicrobial activity of the ligands and complexes were tested against **seventeen** microorganisms - **nine** strains of pathogenic bacteria (**five** standard strains and **four** clinical isolates), **five** mold species, and three yeast species.
- ✓ The bacterial suspensions were prepared by the direct colony method. Bacterial inocula were obtained from bacterial cultures incubated for 24 h at 37°C
- ✓ Suspensions of fungal spores were prepared from fresh mature (3-to 7-day-old) cultures that grew at 30°C on a PD (potato dextrose) agar substrate. [6]
- ✓ Antimicrobial activity was tested by determining the **minimum inhibitory concentrations (MIC)** and **minimum microbicidal concentration (MMC)** using the microdilution plate method with resazurin. [5]
- ✓ All the tests were performed in duplicate, and the MICs were constant

6. NCCLS (National Committee for Clinical Laboratory Standards) : Proposed Standard M38-P. NCCLS, Wayne, PA, USA, 1998.



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## *In vitro* antibiofilm activity

- ✓ The effect of **PdSe1**, **PdSe3**, **L1** , and **L3** on formed biofilm of *S. aureus* ATCC 25923, *S. aureus* and *P. aeruginosa* was determined according to the method described in O'Toole and Kolter (1998), with some modifications [7]
- ✓ The inoculated microtiter plates were incubated at 37°C for 48 hours.
- ✓ After incubation, the content of each well was gently pulled out.
- ✓ Then, 100 µL of dissolved complexes **PdSe1** and **PdSe3**, and ligands **L1** and **L3** were added to each well, and the microtiter plates were incubated at 37°C for 24 hours.
- ✓ The concentration of complexes and ligands ranged from 1000-7.8 µg/mL.
- ✓ Biofilm inhibitory concentration required to reduce biofilm coverage by 50% (**BIC50**) was defined as the lowest concentration of extract that showed 50% inhibition of biofilm formation [3]

7. Mol Microbiol, 28, 1998, 449–461.



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# Antioxidant activity

- ✓ A **DPPH radical** neutralization capacity test was performed.
- ✓ The ability of palladium (II) complexes with some ligands to neutralize 2,2-diphenyl-1-picrylhydrazyl (**DPPH**) free radicals was assessed using this method [8].
- ✓ Methanolic DPPH solution (2 mL, 20 µg/mL) was added to the test solutions in methanol (2 mL) at various concentrations (62.5-1000 µg/mL).
- ✓ The solution has a dark pink color, and during the reaction, it changes color to lighter shades of pink, and even to yellow. The more pronounced the **color change**, the higher the antioxidant activity of the tested compound.
- ✓ After 30 minutes in the dark at room temperature, the absorbance was read on a spectrophotometer at 517 nm.
- ✓ **Methanol** was used as a control, while **ascorbic acid** was used as a standard.
- ✓ The **EC50 value** is the effective concentration of a chemical substance at which 50% of DPPH radicals have been neutralized [1].

8. Biosci. Biotechnol. Biochem. 58, 1994, 1780–1783.



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## Results and discussion



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# Antimicrobial activity

Tested substances	PdSe1		PdSe3		L1		L3		Doxycycline / Fluconazole	
	MIC <sup>a</sup>	MMC <sup>b</sup>	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MNC
<i>Bacillus subtilis</i> ATCC 6633	>1000	>1000	500	500	>1000	>1000	1000	1000	1.953	31.25
<i>Staphylococcus aureus</i>	>1000	>1000	125	500	1000	>1000	62.50	125	0.45	7.81
<i>S. aureus</i> ATCC 25923	>1000	>1000	125	250	>1000	>1000	62.50	125	0.224	3.75
<i>Pseudomonas aeruginosa</i>	1000	>1000	62.50	125	250	1000	15.63	62.50	250	1000
<i>P. aeruginosa</i> ATCC 27853	>1000	>1000	500	500	>1000	>1000	62.50	125	62.5	125
<i>Proteus mirabilis</i> ATCC 12453	1000	>1000	500	500	>1000	>1000	500	500	7.81	15.63
<i>Escherichia coli</i>	>1000	>1000	500	500	>1000	>1000	500	500	15.63	62.5
<i>E. coli</i> ATCC 25922	>1000	>1000	500	500	>1000	>1000	500	500	15.63	31.25
<i>Salmonella enterica</i>	>1000	>1000	500	500	>1000	>1000	1000	1000	15.63	31.25
<i>Rhodothorula mucilaginosa</i>	500	1000	250	500	500	1000	250	500	31.25	500
<i>Candida albicans</i> ATCC 10231	>1000	>1000	500	1000	>1000	>1000	250	1000	7.81	31.25
<i>Saccharomyces boulardii</i>	>1000	>1000	250	500	>1000	>1000	500	1000	31.25	62.5
<i>Mucor mucedo</i>	1000	1000	500	1000	1000	1000	1000	1000	250	250
<i>Trichoderma viridae</i> ATCC13233	1000	>1000	500	500	1000	>1000	1000	1000	500	1000
<i>Aspergillus flavus</i> ATCC 9170	1000	>1000	500	1000	1000	>1000	1000	1000	500	500
<i>A. fumigatus</i> ATCC 1022	1000	>1000	250	250	500	>1000	1000	>1000	1000	1000
<i>A. niger</i> ATCC 16404	1000	>1000	250	500	1000	>1000	1000	>1000	1000	1000

<sup>a</sup>MIC- minimum inhibitory concentrations (µg/mL)

<sup>b</sup>MMC-minimum microbicidal concentration (µg/mL)

**Table 1.** Results of antimicrobial activity of *PdSe1*, and *PdSe3* complexes, ligands *L1* and *L3* and positive controls



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- ✓ The results of *in vitro* antimicrobial activity of studied compounds against 17 strains of bacteria and fungi.
- ✓ Generally, the compounds showed different degrees of antimicrobial activity.
- ✓ MICs and MMCs values were in a range from 15.63 to >1000 µg/mL, which agrees with previous research.
- ✓ There is no difference in the antimicrobial activity of the tested compounds between Gram-negative and Gram-positive bacteria.
- ✓ Complexes **Pd-Se1** and **Pd-Se3** showed the most significant activity on *Pseudomonas aeruginosa*.
- ✓ Activities of Pd-Se1 and Pd-Se3 were better than that of the positive control.
- ✓ The **Pd-Se3** complex also had significant activity on *P. aeruginosa* standard and *Staphylococcus aureus* standard and isolate.
- ✓ The **Pd-Se3** complex has significant activity on filamentous fungi.
- ✓ These results are from previously done research.[3,9]

9. Inorg Biochem, 143, 2015, 9-19.



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## *In vitro* antibiofilm activity

Species Tested compounds ( $\mu\text{g}/\text{mL}$ )	L1	PdSe1	L3	PdSe3	Tetracycline
<i>Staphylococcus aureus</i>	768,27	500	1000	>1000	156
<i>S. aureus</i> ATCC 25923	>1000	>1000	780	>1000	250
<i>Pseudomonas aeruginosa</i>	690,8	>1000	1000	125	746

**Table 2.** The effect of PdSe1, PdSe3, L1, and L3 on formed biofilm of selected bacteria. Values are given as BIC50.

- ✓ The best results showed complex **Pd-Se3** on biofilm of *P. aeruginosa* (BIC50 at 125  $\mu\text{g}/\text{ml}$ ). while complex **Pd-Se1** showed a significant effect on the biofilm of *S. aureus* (BIC50 at 500  $\mu\text{g}/\text{mL}$ ).
- ✓ **BIC50** for ligand **L3** was in the range of 780-1000  $\mu\text{g}/\text{mL}$ , while for ligand **L1**, was in the range of 690.8 - >1000  $\mu\text{g}/\text{mL}$ .
- ✓ Only *S. aureus* ATCC 25923 showed resistance to ligand **L1**.
- ✓ These results are from previously done research [3]



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# Antioxidant activity

Tested compounds ( $\mu\text{g/mL}$ )	EK50 <sup>a</sup>
<i>PdSe1</i>	79,75 $\pm$ 2,83
<i>PdSe3</i>	128,38 $\pm$ 2,82
Ascorbic acid	5,25

**Table 3.** Radical neutralization capacity of newly synthesized complexes (*PdSe1* and *PdSe3*) and positive controls expressed as EK50

- ✓ The values show moderate to significant activities depending on the examined complexes.
- ✓ The antioxidant activity of all organoselenium trans-palladium(II) complexes is significant, with the **PdSe1** complex being more active.
- ✓ The antioxidant activity of ligands **L1** and **L3** were examined, and they show antioxidant activity that is not dose-dependent, so EC50 could not be calculated.
- ✓ The dose at which they show 50% of their activity ranges from **62.50 to 250  $\mu\text{g/mL}$** .



# Conclusions

- ✓ The results of *in vitro* antimicrobial activity the investigated complexes showed significant activity on *Pseudomonas aeruginosa*. The **Pd-Se3** complex also had significant activity on *Staphylococcus aureus* standard and isolate.
- ✓ Complex **Pd-Se1** showed a better effect on the tested biofilm of *S. aureus*, while complex **Pd-Se3** showed a significant effect on the tested biofilm of *P. aeruginosa*.
- ✓ The antioxidant activity of all organoselenium trans-palladium(II) complexes is significant, with the **PdSe1** complex being more active.
- ✓ The antioxidant activity of ligands L1 and L3 were examined, and they show antioxidant activity that is not dose-dependent, so EC50 could not be calculated. The dose at which they show 50% of their activity ranges from 62.50 to 250 µg/mL.
- ✓ **These complexes have the potential to be further investigated as metallodrugs**



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