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Original scientific paper

Antimicrobial, antioxidant and DNA-binding studies of palladium(II) complexes with different chelate ligands containing nitrogen donor atoms

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Abstract: The antimicrobial and antioxidant activities, as well as the DNA-binding of four square-planar Pd(II) complexes, $[Pd(terpy)Cl]^+$ (**C1**), $[Pd(en)Cl_2]$ (**C2**), $[Pd(DMEAIm^{iPr})Cl_2]$ (**C3**) and $[Pd(dach)Cl_2]$ (**C4**) (terpy = 2,2′:6′,2″-terpyridine, en = ethylenediamine, dach = *trans*-1,2-diaminocyclohexane and $DMEAIm^{iPr}$ = N^2-((1,3-dihydro-1,3-diisopropyl-4,5-dimethyl)-2*H*-imidazol-2-ylidene)-N^1,N^1-dimethyl-1,2-ethanediamine are reported. The antimicrobial activities of the Pd(II) complexes with the appropriate ligands were tested using the microdilution method against 18 strains of microorganisms, whereby the minimal inhibitory concentration (*MIC*) and the minimal microbicidal concentration (*MMC*) were determined. The antibiofilm activity of $[Pd(terpy)Cl]^+$ and the corresponding ligand were determined on a formed biofilm. The intensity of antimicrobial activity varied depending on the type of microorganism and the tested compound. The **C1** complex with the corresponding ligand demonstrated significantly greater overall antimicrobial activity than **C2**, **C3** and **C4**. The antibacterial activity of the **C1** complex was better than its antifungal activity that was overall greater than that of the positive control, fluconazole. The greatest sensitivity for **C1** and **L1** was with *Penicillium italicum* (*MIC* < 0.49 μg mL^{-1}) among the fungi, and with *Proteus mirabilis* ATCC 12453 (*MIC* = 0.98 μg mL^{-1}) among the tested bacteria. The tested compounds show low and moderate antibiofilm activity. The complexes showed weak antioxidant properties when tested using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. The interaction of the metal complexes **C1**–**C4** with calf thymus DNA (CT-DNA) was further examined by absorption (UV–Vis) and emission spectral studies (EthBr displacement studies). Overall, the investigated complexes exhibited good DNA interaction ability.

Keywords: palladium(II) complexes; biological activity; structure; reactivity.

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INTRODUCTION

Transition metal complexes in the past few decades have started to play an important role as pharmaceutical and diagnostic agents.[1] Everything started with the discovery of the antitumor characteristics of cisplatin,[2] followed by the discovery of a large number of other metallo-drugs, among which are the Paul Erlich organo-arsenic compound for the treatment of syphilis, antiarthritic gold preparations, and diagnostic agents for magnetic resonance imaging (Gd, Mn, Fe) among others.[3]

Today a large number of antibiotics and chemotherapeutics are accessible for treatment of infectious diseases. However the resistance of microorganisms to antibiotics and antifungals is a serious problem around the world. This indicates the necessity for the discovery of new compounds endowed with antimicrobial activity as soon as possible. Preferably, these new compounds should have different mechanisms of action, distinct from those of well-known classes of antimicrobial agents to which many clinically relevant pathogens are now resistant. Special attention is paid to the study of the antimicrobial activity of complex compounds with different metal ions.[4–7]

The chemical behaviour in solution of structurally analogous Pt(II) and Pd(II) complexes is very similar.[8] Since the antitumor activity of certain Pd(II) complexes has been demonstrated, the idea arose to examine several structurally different Pd(II) complexes for their antimicrobial activity as well as their interaction with calf thymus DNA (CT–DNA). The pharmacological activity of metal complexes is highly dependent on the nature of the metal ion and of the ligand, because different ligands exhibit different biological properties. Ideally, the new drug would have an increased spectrum of activity, reduced drug resistance, decreased required dose and reduced toxic side effects.

A review of the current literature showed that Pd(II) complexes with different ligands exhibit significant biological activity,[9] which leaves the possibility for further investigation of the complexes **C1–C4** with ligands such as: 2,2′:6′,2″-terpyridine, ethylenediamine,N^2-((1,3-dihydro-1,3-diisopropyl-4,5-dimethyl)-2*H*-imidazol-2-ylidene)-N^1,N^1-dimethyl-1,2-ethanediamine and *trans*-1,2-diaminocyclohexane, Fig. 1.

The complexes will probably react with a range of different microorganisms relevant for human health. However, the goal of this work was to show whether DNA could be considered as one of the targets.

EXPERIMENTAL

Material and methods

The reagents were obtained commercially and used without further purification. Elemental analyses were performed on a Vario III C, H, N and S elemental analyser in the CHS mode. Infrared spectra were recorded on a Perkin–Elmer FTIR 31725-X spectrophotometer using the KBr pellet technique. The UV–Vis spectra were obtained on a Perkin–Elmer

Lambda 35 or 25 double beam spectrophotometer, using 1.0 cm path-length quartz cuvettes (3.0 mL). Fluorescence measurements were run on a RF-1501 PC spectrofluorometer (Shimadzu, Japan). The palladium(II) complexes **C1**–**C4** with the above-mentioned ligands (**L1**––**L4**) were synthesized by modification of a previously described method.[10-13] The ligand, DMEAIm$^{i\mathrm{Pr}}$, was prepared according to a literature procedure.[12]

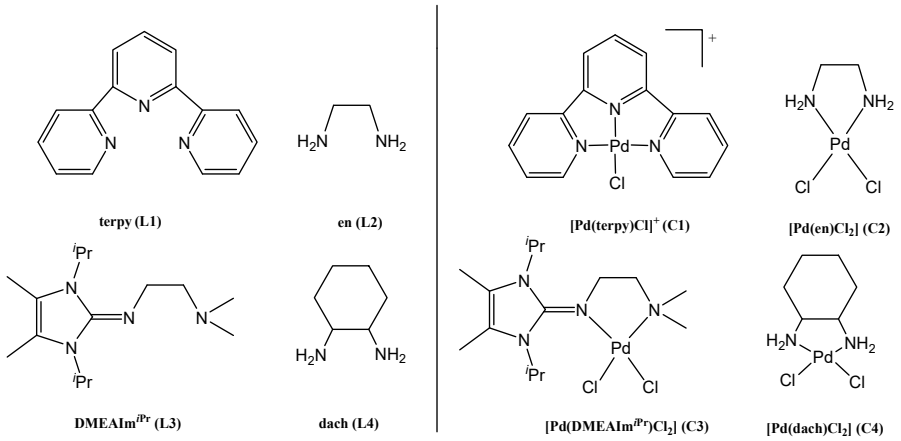

Fig. 1. a) The structural formulae of the ligands **L1**–**L4** and b) the structural formulae of the Pd(II) complexes **C1**–**C4**.

*Synthesis of the palladium(II) complexes **C1–C4**[10-13]*

*[Pd(terpy)Cl]Cl·3H$_2$O (**C1**), [Pd(en)Cl$_2$] (**C2**) and [Pd(dach)Cl$_2$] (**C4**)*. $PdCl_2$ (0.5 g, 2.8 mmol) was dissolved under reflux in a mixture of 10 mL H_2O and 3 mL of concentrated HCl. The clear solution was filtered, and a solution of terpy (0.653 g; 2.8 mmol in 10 mL methanol) or en (0.168 g; 2.8 mmol in 10 mL methanol) or dach (2.8 mmol in 10 mL methanol) was added dropwise to the warm solution of $[PdCl_4]^{2-}$. The pH of the solution was carefully adjusted to 4.5–5.0 by the addition of NaOH. The resulting yellow solution was stirred for a few hours at 50 °C. The obtained solution was left at ambient temperature and a yellow solid was obtained.

*[Pd(DMEAIm$^{i\mathrm{Pr}}$)Cl$_2$] (**C3**)*. Dichloro[(1,2,5,6-η)-1,5-cyclooctadiene]palladium [Pd(COD)Cl_2] (0.100 g, 0.338 mmol) was added to 0.95 g (0.22 mmol) of DMEAIm$^{i\mathrm{Pr}}$ ligand in 12 mL of THF. The reaction mixture was stirred overnight at 40 °C affording a red precipitate. The precipitate was filtered and dried *in vacuo*. The product was obtained as a deep-red solid.

In vitro *antimicrobial activity*

The tested compounds were first dissolved in dimethyl sulfoxide and then diluted with nutrient liquid medium resulting in 10 % solutions. Antibiotics, tetracycline (Galenika A.D., Belgrade), ceftriaxone (Galenika A.D., Belgrade), and vancomycin (Lek farmaceutska družba, D.D., Slovenia) were dissolved in Mueller–Hinton broth (Torlak, Belgrade), while antifungal drugs fluconazole (Pfizer Inc., USA), ketoconazole (Hemofarm A.D., Serbia) and amphotericin B (Chiesi Pharmaceuticals GmbH, Austria) were dissolved in Sabouraud dextrose broth (Torlak, Belgrade).

Antimicrobial activity of palladium(II) complex was tested against 18 microorganisms. All the tested microorganisms are presented in Table I. Clinical isolates of the pathogenic bacteria were donated by the Institute of Public Health, Kragujevac, Serbia. The other microorganisms were provided by the Laboratory of Microbiology, Faculty of Sciences, University of Kragujevac.

The bacterial and fungi suspensions were prepared by taking colonies directly from the surface and suspending them in 5 mL of sterile saline solution. The turbidity of the initial suspension was adjusted using a densitometer (DEN-1, BioSan), and comparing it with 0.5 McFarland's standard.[14] When adjusted to the turbidity of the 0.5 McFarland's standard, the bacteria suspensions contained around 10^8 CFU mL^{-1} and yeast suspension around 10^6 CFU mL^{-1} (colony-forming unit). Fungi spore suspensions were prepared by careful removal of spores from the mycelium. The initial suspensions were additionally diluted in sterile 0.85 % saline solution in 1:100 ratios for bacteria and yeast, and in 1:1000 for filamentous fungi.

The antimicrobial activities of the ligands and their complexes with palladium(II) were tested by determining the minimum inhibitory concentration (*MIC*) and minimum microbicidal concentration (*MMC*) using microtiter plates with resazurin.[15] Tetracycline, ceftriaxone, vancomycin, fluconazole, ketoconazole and amphotericin B were used as positive controls. In order to exclude the effect of the solvent (dimethyl sulfoxide) on the growth of the microorganisms, its influence was followed in a concentration of 10 %.

A microtiter plate with 96 wells was filled with 100 μL of nutrient surface in each well. One hundred μL of each tested compound with a starting concentration of 2000 μg mL^{-1} was added to the first row of the microtiter plate. Twofold dilutions were made in a concentration range from 1000 μg mL^{-1} in the first row to 7.81 μg mL^{-1} in the last row of the plate. After that, 10 μL of suspensions of bacteria, yeast, or filamentous fungi spores were added to the appropriate wells. The tested compounds were examined with a bacterial concentration of 10^5 CFU mL^{-1} and a yeast and fungi spores concentration of 10^3 CFU mL^{-1}.[16] Finally, resazurin, as an indicator of cell growth, was added to each well. The microtiter plates were incubated at 37 °C for 24 h for the bacteria, at 28 °C for 48 h for the yeast, and 72 h for the filamentous fungi. The *MIC* is defined as the lowest concentration of the tested substance preventing resazurin colour change from blue to pink. With the filamentous fungi, the *MIC* represents the lowest concentration of the tested substances that prevents mycelium growth. The results were read visually.

The *MMC* were determined by placing 10 μL of samples from the wells where no indicator colour change was recorded onto nutrient agar medium. At the end of the incubation period, the lowest concentration with no growth (no colony) was defined as the *MMC*.

Antibiofilm activity

Antibiofilm activities were determined using the method previously described by Christensen *et al.*[17] It is the most widely used method for examining antibiofilm activity on a formed biofilm. Each test included a biofilm formation control. Bacterial biofilm formation properties were well described by O'Toole and Kolter.[18]

Polystyrene plates with a flat bottom and with 96 wells (Sarstedt, Germany) were prepared by dispensing 100 μL of nutrient broth into each well. Ten μL of a fresh bacterial suspension was added to each well. The inoculated microtiter plates were incubated at 37 °C for 24 h for the Gram-negative bacteria and 48 h for the Gram-positive bacteria. One hundred μL of the stock solution of the tested complexes (concentration of 2000 μg mL^{-1}) were added into the first row of the plate. Then, twofold serial dilutions were made for each following row using a multichannel pipette. After 24 h incubation, the content of the plates was removed and

then the wells were washed with 200 μL of buffer solution (0.15 mol L^{-1} ammonium sulphate, 0.1 mol L^{-1} potassium dihydrogen phosphate, 0.034 mol L^{-1} sodium dihydrogen citrate and 0.001 mol L^{-1} magnesium sulphate) to remove free-floating bacteria. The biofilms formed of adherent cells in the plate were stained with crystal violet (0.1 %) and incubated at room temperature for 20 min. The excess stain was rinsed off with deionised water and the microtiter plates were fixed with 200 μL of ethanol–acetone solution (4:1). The optical densities (*OD*) of the stained adherent bacteria were determined by a micro ELISA plate reader at a wavelength of 630 nm. The biofilm inhibitory concentration (*BIC*) was defined as the lowest concentration of each complex that led to the formation of dispersed bacterial biofilms. Broth alone or broth with complexes solutions served as a control for the sterility check and non-specific binding of the media. All tests were performed in duplicate. Tetracycline, vancomycin and ceftriaxone were used as positive controls.

Antioxidant activity

The ability of palladium(II) complexes to scavenge DPPH free radicals was assessed using the method described by Takao *et al.*[19] The test is based on the exchange of hydrogen atoms or electrons between antioxidant molecules of a chemical complex and DPPH radicals in solution.[20] A methanolic DPPH solution (2 mL, 20 μg mL^{-1}) was added to sample solutions in methanol (2 mL) at various concentrations (62.5–1000 μg mL^{-1}). After 30 min in the dark at room temperature, the absorbance was read in a spectrophotometer at 517 nm. Methanol was used as a blank and ascorbic acid as a positive control. The experiment was performed in triplicate and the results were expressed as arithmetic mean ± standard deviation. Antioxidant activity was expressed as the inhibition percentage, which was calculated using the following equation:

$$\text{Scavenging activity, } \% = 100\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}$$

where A_{control} is the absorbance of the control and A_{sample} is the absorbance of the sample.

The EC_{50} value is an effective concentration of a chemical substance in which 50 % of DPPH radicals were scavenged. A low EC_{50} value indicates strong ability of a compound to act as a DPPH scavenger.

DNA interactions

A stock solution of CT-DNA was prepared in PBS buffer, which gave a ratio of UV absorbance at 260 and 280 nm (A_{260}/A_{280}) of *ca.* 1.8–1.9, indicating that the DNA was sufficiently free of protein and the concentration was determined by the UV absorbance at 260 nm (ε = 6600 dm^2 mol^{-1}).[21] The UV–Vis spectra were obtained on a Perkin–Elmer Lambda 35 or 25 double beam spectrophotometer, using 1.0 cm path-length quartz cuvettes (3.0 mL). Fluorescence measurements were run on a RF-1501 PC spectrofluorometer (Shimadzu, Japan). The fluorescence spectra were recorded in the range 550–750 nm upon excitation at 527 nm in all cases. The excitation and emission bandwidths were both 10 nm.

UV–Vis absorption studies

In order to compare quantitatively the binding strength of the complexes, the intrinsic binding constants K_b were determined by monitoring the changes in absorption at the metal-to--ligand-charge-transfer (MLCT) band with increasing concentration of CT-DNA using Eq. (1):

$$\frac{c_{\text{DNA}}}{(\varepsilon_{\text{A}} - \varepsilon_{\text{f}})} = \frac{c_{\text{DNA}}}{(\varepsilon_{\text{b}} - \varepsilon_{\text{f}})} + \frac{1}{\left[K_{\text{b}}(\varepsilon_{\text{b}} - \varepsilon_{\text{f}})\right]} \quad (1)$$

where K_b is given by the ratio of slope to the y intercept in plots $c_{DNA}/(\varepsilon_A - \varepsilon_f)$ *vs.* c_{DNA}, where c_{DNA} is the concentration of DNA in base pairs, $\varepsilon_A = A_{obsd}/[complex]$, ε_f is the extinction coefficient for the unbound complex and ε_b is the extinction coefficient for the complex in the fully bound form.

Ethidium bromide (EthBr) displacement studies

The relative binding of complexes to CT-DNA was determined by calculating the quenching constant (K_{sv}) from the slopes of straight lines obtained from the Stern–Volmer equation (Eq. (2)):

$$I_0/I = 1 + K_{sv}[Q] \quad (2)$$

where I_0 and I are the emission intensities in the absence and the presence of the quencher (**C1**), respectively, $[Q]$ is the total concentration of quencher and K_{sv} is the Stern–Volmer quenching constant, which can be obtained from the slope of the plot of I_0/I *vs.* $[Q]$.

RESULTS AND DISCUSSION

Four square-planar Pd(II) complexes, $[Pd(terpy)Cl]^+$ (**C1**), $[Pd(en)Cl_2]$ (**C2**), $[Pd(DMEAIm^{iPr})Cl_2]$ (**C3**) and $[Pd(dach)Cl_2]$ (**C4**) (terpy = 2,2′:6′,2 ″-terpyridine, en = ethylenediamine, dach = *trans*-1,2-diaminocyclohexane and $ImCH_3$ = N^2--((1,3-dihydro-1,3-diisopropyl-4,5-dimethyl)-2H-imidazol-2-ylidene)-N^1,N^1-dimethyl-1,2-ethanediamine were synthesized by modification of a procedure previously reported in the literature (Fig. 1).[10–13] The structure of these complexes was confirmed by elemental microanalyses and IR, ^{1}H-NMR and UV–Vis spectroscopy. These data, given in the Supplementary material to this paper, are in accordance with those reported in the literature.[10–13] The antimicrobial activity of **C1**–**C4** complexes was tested against 18 strains of microorganisms determining the *MIC* and the *MMC* using the microdilution method. The antibiofilm activity of selected Pd(II) complexes and corresponding ligand were determined on a formed biofilm. The antioxidant activity was tested using the DPPH method. Furthermore, the interactions of these complexes with CT-DNA were investigated using UV–Vis and fluorescence emission spectroscopic techniques.

Antimicrobial activity

The results of testing the antimicrobial activity of the palladium(II) complexes are given in Table I. In the same table, the *MIC* and *MMC* values of terpy and dach that were used as ligands for the synthesis of palladium(II) complexes **C1** and **C4** are given. **L2** ligand was not tested due to its volatility, while **L3** ligand was not tested due to its air instability. Table S-I of the Supplementary material to this paper shows the values of *MIC* and *MMC* for the antibiotics tetracycline, ceftriaxone and vancomycin and the antifungal drugs fluconazole, ketoconazole and amphotericin B that were used as positive controls. A ten percent solution of dimethyl sulfoxide was used as the solvent for conducting the experiments and it was determined that it did not retard the growth of the tested microorganisms.

TABLE I. The results of antimicrobial activity (μg ml^{-1}) of ligands **L1** and **L4**, and palladium(II) complexes **C1**–**C4**

The tested species	**L1**		**C1**		**C2**		**C3**		**L4**		**C4**	
	MIC	*MMC*	*MIC*	*MMC*	*MIC*	*MMC*	*MIC*	*MMC*	*MIC*	*MMC*	*MIC*	*MMC*
Bacillus subtilis	7.81	7.81	15.62	15.62	500	500	250	500	>500	>500	500	500
Bacillus subtilis ATCC 6633	31.25	31.25	15.62	15.62	500	500	250	500	>500	>500	250	500
Staphylococcus aureus ATCC25923	7.81	31.25	15.62	62.5	250	>500	250	500	>500	>500	250	500
Proteus mirabilis ATCC 12453	0.98	125	0.98	125	500	500	500	500	>500	>500	500	500
Pseudomonas aeruginosa ATCC 27853	31.25	250	31.25	125	250	250	500	500	>500	>500	250	500
E. coli	15.63	31.25	31.25	62.55	500	500	500	500	>500	>500	500	500
Escherichia coli ATCC 25922	15.63	15.63	31.25	31.25	>500	>500	500	500	>500	>500	500	500
Salmonella enterica	31.25	62.5	31.25	31.25	250	250	500	500	>500	>500	500	500
Rhodotorula mucilaginosa	1.96	3.91	7.81	7.81	500	1000	250	1000	250	500	500	500
Candida albicans ATCC 10231	3.91	15.63	15.62	15.62	1000	>1000	500	1000	250	500	500	1000
Saccharomyces boulardii	3.91	3.91	7.81	7.81	500	1000	500	1000	125	250	125	500
Penicillium italicum	<0.49	<0.49	<0.49	<0.49	1000	1000	500	1000	250	250	500	500
Penicillium chrysogenum	1.96	1.96	15.63	31.25	500	1000	250	500	250	250	250	500
M. mucedo ATCC 20094	1.96	1.96	15.63	15.63	1000	>1000	1000	1000	500	500	500	1000
T. asperellum ATCC 13233	1.96	1.96	15.63	31.25	1000	1000	1000	1000	250	250	1000	1000
A. flavus ATCC 9170	0.98	1.96	15.63	31.25	1000	>1000	500	1000	500	500	500	500
A. fumigatus ATTC 204305	1.96	1.96	15.63	15.63	1000	1000	1000	1000	500	500	1000	1000
A. brasiliensis ATCC 16404	1.96	3.91	15.63	62.5	1000	1000	1000	>1000	500	500	500	>1000

The intensity of antimicrobial activity varies depending on the type of microorganism and the tested compound. Previous research have shown that palladium(II) complexes have significantly better antimicrobial activity than the appropriate ligands.[5,6,22] However, in the present study, the **L1** ligand showed the same or slightly better antimicrobial activity, especially towards yeast and filamentous fungi, than the **C1** complex. The **C4** complex showed better activity than the **L4** ligand when tested against bacteria, but the results were reversed for

the yeast and filamentous fungi. The **C2** and **C3** complexes could not be compared to the corresponding ligands.

The **C1** complex demonstrated significantly greater antimicrobial activity than **C2**–**C4**. The antifungal activity of the **C1** complex was better than its antibacterial activity. The *MIC* values for the **C1** complex against yeast and filamentous fungi ranged from <0.49 to 15.63 μg mL^{-1}, while the *MIC* values for bacteria ranged between 0.98 and 31.25 μg mL^{-1}. The Gram-positive bacteria were more sensitive to the **C1** complex than the Gram-negative bacteria, with an *MIC* value of 15.62 and 31.25 μg mL^{-1}, respectively. The exception was *P. mirabilis* ATCC 12453 with an *MIC* 0.98 μg mL^{-1}. The **C2**–**C4** complexes had similar activity towards Gram-positive and Gram-negative bacteria. The results of previous studies showed that Gram-positive bacteria were more sensitive than Gram-negative bacteria.[23,24] Others, such as Ali *et al.* found antibacterial efficacy against Gram-positive and Gram-negative bacteria to be similar.[25]

Of the tested complexes, only **C1** demonstrated antimicrobial activity comparable to those of the positive controls. The **C1** complex with the corresponding ligand **L1** had better antimicrobial activity towards the standard and clinical strain of *B. subtilis* than ceftriaxone. This compound showed stronger activity towards *P. aeruginosa* ATCC 27853 and other Gram-negative bacteria than vancomycin (Tables I and S-I).

Apart from the **C1** complex, the antifungal activity of which matched that of fluconazole, the other complexes showed moderate and low activity towards most of yeast and filamentous fungi when compared to the positive controls. The most sensitive towards **C1** and **L1** was *P. italicum* (*MIC* < 0.49 μg mL^{-1}). Their effects against it matched that of ketoconazole (Tables I and S-I).

Previous research of antimicrobial activity led to different conclusions with most of the studies evidencing markedly lower antifungal activity,[7,26] although a study conducted in 2012 determined that palladium(II) complexes with a derivative of thiosalicylic acid as ligand showed significantly higher antifungal activity towards species from the *Aspergillus* genus (*A. fumigatus*, *A. flavus* and *A. restrictus*) than the positive control, fluconazole.[6] Garoufis *et al.* explored the antifungal and antibacterial activity of Pd(II) complexes with various ligands (sulphur and nitrogen donor ligands, Schiff bases and ligands used as drugs), and found that they had promising antibacterial but weak antifungal activity.[27]

Antibiofilm activity

C1 and **L1** were used for examining antibiofilm activity on a formed biofilm because they showed a significantly higher level of antimicrobial activity compared to the other tested complexes. The *in vitro* antibiofilm activity of palladium(II) complex was tested on three species of bacteria and the results are presented in Table S-II of the Supplementary material.

Based on the given results, it could be concluded that the tested compounds showed low and moderate antibiofilm activity. Palladium(II) complex with terpyridine as ligand showed higher activity than ceftriaxone towards *S. aureus* ATCC 25923 and vancomycin towards *P. mirabilis* ATCC 12453. Studies that tested antibiofilm activity of palladium(II) complexes were not found while examining the available literature.

Antioxidant activity

DPPH radicals scavenging activity. The results of DPPH radicals scavenging activity, expressed as EC_{50} values, showed the moderate activity of the tested compounds (Table II). The **C2** complex was not tested due to its low solubility in methanol, while the **L2** and **L3** ligands were not tested for already stated reasons.

The **C1** and **C3** complexes and the **L1** ligand demonstrated no anti-oxidant activity, while the **L4** ligand and **C4** showed weak antioxidant activity. The **C4** complex had significantly better antioxidant activity than **L4**, but it was lower than that of the positive control, ascorbic acid.

TABLE II. The radical scavenging capacity of ligand **L4**, palladium(II) complex **C4** and the positive control (ascorbic acid), expressed as EC_{50} values; each value is expressed as the arithmetic mean ± standard deviation

Tested compound	EC_{50} / μg mL^{-1}
L4	3050.58±42.86
C4	83.36±1.19
Ascorbic acid[a]	5.25

[a]Positive control

Upon examining the literature, it was noticed that there were not many studies that tested the antioxidant activity of palladium(II) complexes. Previous studies showed that palladium(II) complexes have better antioxidant activities than nickel and platinum complexes.[28] Others found that Pd(II) complexes have a lower scavenging activity than their parent ligands.[29] Moreover, antioxidant activities close to that of ascorbic acid were demonstrated, particularly for palladium chloride complex with two *o*-chlorobenzylamine ligands, Pd2CBA ($C_{14}H_{15}N_2Cl_3Pd$).[30] Maskovic *et al.* found that Pd(II) complexes with *N*,*N*′-ligands have antioxidant activities higher than that of butylated hydroxytoluene but lower than that of ascorbic acid.[31] Pd(II) complex with 2-hydroxy-4-methoxybenzaldehyde-4-phenylthiosemicarbazone shows better antioxidant activity than Ni(II) and Cu(II) compexes with the same ligand, and vitamin C.[32] The Pd(II) complexes tested in the present study did not exhibit significant antioxidant activity, as opposed to the complexes that were investigated in above-mentioned studies.

DNA interactions

This part of the study was focused on the investigation of interactions of complexes **C1**–**C4** with DNA. DNA is a critical therapeutic target that is responsible for a wide variety of intracellular interactions.[33–35] Thus, knowledge of whether the studied complexes could interact with DNA to the desired extent enabled DNA to be considered as one of the targets for the complexes in the manifestation of their antimicrobial and antibiofilm activities.

UV–Vis absorption studies

Using complexes **C1**–**C4**, UV–Vis titrations were performed. The intrinsic equilibrium binding constant (K_b) was evaluated. The metal complex absorption titration studies were realized at room temperature using a fixed concentration of complex **C1**–**C4** (10 μmol L^{-1}) in PBS buffer, and varying the amount of CT-DNA (0–20 μmol L^{-1}).[36] The absorption intensity of the complex may decrease (hypochromism) or increase (hyperchromism) with a slight increase in the absorption wavelength (bathochromism) upon addition of DNA.[36,37] A studied system of adding CT-DNA to a solution of studied complex resulted in a significant hyperchromic effect with the appearance of a new band at 258 or 257 nm, but with only insignificant absorption changes in the region 300–500 nm, see Fig. 2. The other dependences were similar and they did not give a significant hyperchromic shift with the appearance of a new signal in the UV–Vis spectra, which clearly suggested an interaction between the complexes and CT-DNA.[37–39]

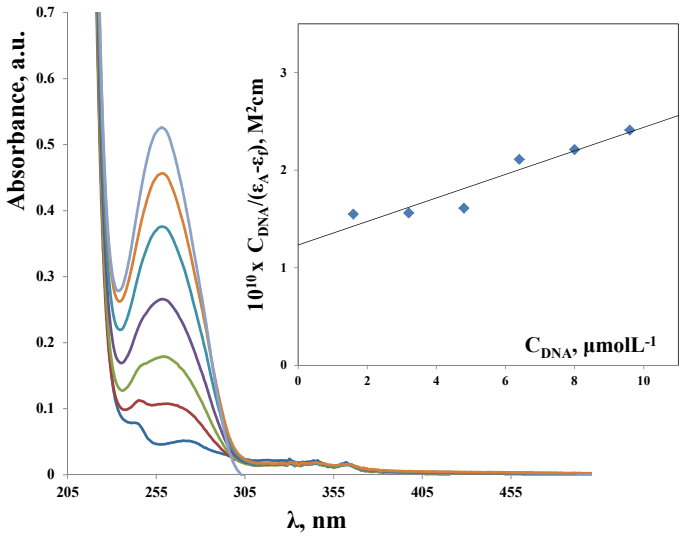

Fig. 2. UV–Vis titration spectra together with plots of $c_{DNA}/(\varepsilon_A-\varepsilon_f)$ *vs.* c_{DNA} of complex **C1** (10 μmol L^{-1}) in PBS buffer (phosphate buffer solution = 0.01 mol L^{-1}, c_{NaCl} = 0.137, c_{KCl} = 0.0027 mol L^{-1}, pH 7.4) with increasing concentration of CT-DNA (0–10 μmol L^{-1}).

Ethidium bromide (EthBr) displacement studies

The interaction of complexes **C1**–**C4** with CT-DNA has also been investigated by EthBr displacement studies, which provided strong evidence about competitive binding of drugs with CT-DNA. Ethidium bromide (EthBr) is a classical intercalator that gives significant fluorescence emission intensity when it intercalates into the base pairs of DNA. DNA-induced EthBr fluorescence emission could be quenched after addition of a complex capable of forming strong interactions with DNA, replacing EthBr.[40] The significant decrease in the intensity of the emission band at 612 nm after the addition of increasing amounts of complexes **C1–C4** shows competition of the studied complex in binding to DNA, Fig. S-1 of the Supplementary material. The Stern–Volmer quenching constant (K_{sv}) was calculated from the slopes of the plots I_0/I *vs.* [Q] using Eq. (2). Thus, complexes **C1**–**C4** are capable of displacing EthBr from the EthBr–DNA complex and could strongly interact with DNA binding sites.

According to the values of constants presented in Table III, good interaction of the studied complexes with CT-DNA is evident. In addition, the results obtained by fluorescence spectroscopy are in excellent agreement with the UV–Vis spectral data, demonstrating that the studied complexes interact with CT-DNA.

TABLE III. The DNA-binding constants (K_b) and Stern–Volmer constants (K_{sv}) for complexes **C1**–**C4**

Complex	$K_b \times 10^{-4}$ / M^{-1}	$K_{sv} \times 10^{-4}$ / M^{-1}
C1	10±1	8.6±0.1
C2	8.7±0.1	5.3±0.1
C3[41]	1.0±0.1	1.7±0.1
C4	3.5±0.1	1.1±0.1

CONCLUSIONS

The tested ligands and complexes demonstrated selective and limited antimicrobial activity. The exception is the **C1** complex with the corresponding ligand, the effects of which on *B. subtilis* and *P. aeruginosa* ATCC 27853 were better than the positive controls. The same complex also showed significant activity towards *P. italicum*. The **C1** complex had higher antibiofilm activity than the positive control towards *S. aureus* ATCC 25923. Complex **C4** showed the best antioxidant activity, while the antioxidant activity of the other complexes was insignificant. Furthermore, the interaction of the Pd(II) metal complexes with calf thymus DNA (CT-DNA) was further examined by absorption (UV–Vis) and emission spectral studies (EthBr displacement studies). Overall, the studied complexes exhibited a good DNA interaction ability.

SUPPLEMENTARY MATERIAL

Additional data are available electronically at the pages of journal website: http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД

АНТИМИКРОБНА, АНТИОКСИДАТИВНА АКТИВНОСТ И ДНК ИНТЕРАКЦИЈЕ ПАЛАДИЈУМ(II) КОМПЛЕКСА СА РАЗЛИЧИТИМ ХЕЛАТНИМ ЛИГАНДИМА КОЈИ САДРЖЕ АТОМЕ АЗОТА КАО ДОНОРЕ

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У овом истраживању приказане су антимикробна и антиоксидативна активност, као и способност везивања за молекул ДНК четири квадратно-планарна кмплекса Pd(II), $[Pd(terpy)Cl]^+$ (**C1**), $[Pd(en)Cl_2]$ (**C2**), $[Pd(DMEAIm^{iPr})Cl_2]$ (**C3**) и $[Pd(dach)Cl_2]$ (**C4**) (terpy = 2,2′:6′,2″-терпиридин, en = етилендиамин, dach = *trans*-1,2-аминоцикохексан и $DMEAIm^{iPr}$ = N^2-((1,3-дихидро-1,3-диизопропил-4,5-диметил)-2*H*-имидазол-2-илиден)-N^1,N^1-диметил-1,2-етандиамин. Антимикробна активност Pd(II) комплекса са одговарајућим лигандима тестирана је микродилуционом методом на 18 врста микроорганизама, одређивањем минималне инхибиторне концентрације (*MIC*) и минималне микробицидне концентрације (*MMC*). Антибиофилм активност одабраног Pd(II) комплекса ($[Pd(terpy)Cl]^+$) и лиганда је тестирана на формирани бактеријски биофилм. Интензитет антимикробне активности варира у зависности од врсте микроорганизама и типа испитиваног једињења. Комплекс **C1** са одговарајућим лигандом је показао значајно бољу укупну активност од комплекса **C2**–**C4**. Антибактеријска активност комплекса **C1** била је боља од његове антигљивичне активности, а та активност је већа од позитивне контроле флуконазола. Највећу сензитивност према **C1** и **L1** међу гљивама имао је *Penicillium italicum* (*MIC* < 0,49 μg mL^{-1}), а међу бактеријама *Proteus mirabilis* ATCC 12453 (*MIC* = 0,98 μg mL^{-1}). Испитивана једињења показују ниску и умерену антибиофилм активност. Комплекси су показали слабу антиоксидативну активност коришћењем DPPH методе (2,2-дифенил-1-пикрилхидразил). Интеракција комплекса метала **C1**–**C4** са ДНК телећег тимуса (CT-DNA) је испитивана UV–Vis апсорпционом методом и емисионом методом (EthBr методом). Ипитивани комплекси су показали значајну интеракцију са молекулом CT-DNA.

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