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# Stereospecific ligands and their complexes. XI: Synthesis, characterization and antimicrobial activity of palladium(II) complexes with some alkyl esters of (*S,S*)-ethylenediamine-*N,N′*-di-2-(3-methyl)-butanoic acid ☆

Gordana P. Radić [a], Verica V. Glođović [a], Ivana D. Radojević [b], Olgica D. Stefanović [b], Ljiljana R. Čomić [b], Vesna M. Đinović [c], Srećko R. Trifunović [a,*]

[a] Department of Chemistry, Faculty of Science, University of Kragujevac, R. Domanovića 12, 34000 Kragujevac, Republic of Serbia
[b] Department of Biology and Ecology, Faculty of Science, University of Kragujevac, R. Domanovića 12, 34000 Kragujevac, Republic of Serbia
[c] Faculty of Chemistry, University of Belgrade, Studentski trg 16, 11000 Belgrade, Republic of Serbia

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ABSTRACT

Three new ligand precursors and their palladium(II) complexes of general formula [PdCl{(*S,S*)-(R)eddv}] (R = *n*-propyl, *n*-butyl and *n*-pentyl; *S,S*-eddv = (*S,S*)-*ethylenediamine-N,N′*-di-2-(3-methyl)-butanoate) have been synthesized and characterized by microanalysis, infrared, $^{1}H$ and $^{13}C$ NMR spectroscopy and mass spectrometry. *In vitro* antimicrobial activity for these ligands and complexes is investigated. Testing is performed by microdilution method and minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) have been determined. Testing is conducted against 15 microorganisms (five strains of pathogenic bacteria, three species of probiotic bacteria, two yeast species and five pathogenic fungi). Tested ligands, with a few exceptions, show very low antimicrobial activity. Palladium(II) complexes show selective and moderate activity. The difference in antimicrobial activity between ligands and corresponding palladium(II) complexes is noticed and it is always higher with palladium(II) complexes.



## 1. Introduction

In parallel with the rapid development of a wide range of antibacterial agents since the 1940s, bacteria have proved extremely adept at developing resistance to each new employed agent. The rapidly increasing incidence of bacterial resistance to antimicrobial agents has become a serious problem worldwide. Resistance mechanisms have been identified and described for all the known antibiotics currently available for clinical use [1].

In some previous papers synthesis and characterization of the palladium(II) complexes with $R_2$-*S,S*-eddp ligands (*S,S*-eddp = (*S,S*)-ethylenediamine-*N,N′*-di-2-propanoate, R = *n*-Pr [2], *n*-Bu [2], *n*-Pe [2], *i*-Pr [3a], *i*-Bu [3b], *c*-Pe [4], Cy [4]) were published. It was concluded from NMR spectra that the mixture of diastereoisomers of the [$PdCl_2$($R_2$-eddip)] complexes was obtained. The antitumoral investigations of some of these ligands and complexes were performed [4].

This study is focused on the synthesis, characterization and antimicrobial activity of four novel $R_2$edda-type ligand precursors: *O,O′*-diethyl- (**L1**) [5], *O,O′*-dipropyl- (**L2**), *O,O′*-dibutyl- (**L3**), *O,O′*-dipentyl-(*S,S*)-ethylenediamine-*N,N′*-di-2-(3-methyl)-butanoate dihydrochloride (**L4**) and their corresponding palladium(II) complexes: chlorido((*S,S*)-ethylenediamine-*N*-(*O*-ethyl-2-(3-methyl)-butanoate)-*N′*-2-(3-methyl) butanoato-palladium(II) [5], (**C1**), chlorido((*S,S*)-ethylenediamine-*N*-(*O*-propyl-2-(3-methyl)-butanoate)-*N′*-2-(3-methyl)butanoato-palladium(II) (**C2**), chlorido((*S,S*)-ethylenediamine-*N*-(*O*-butyl-2-(3-methyl)-butanoate)-*N′*-2-(3-methyl)butanoato-palladium(II) (**C3**), chlorido((*S,S*)-ethylenediamine-*N*-(*O*-pentyl-2-(3-methyl)-butanoate)-*N′*-2-(3-methyl)butanoato-palladium(II) (**C4**).

The synthesis and evaluation of biological activity of the new metal-based compounds has been the field of growing interest. Numerous complexes based on palladium(II)-ion have been synthesized and their different biological activities have been documented [6–8]. The impact of different palladium complexes on the growth and metabolism of various groups of microorganisms has been studied. Garoufis et al. [9] reviewed numerous scientific papers on antiviral, antibacterial and antifungal activity of palladium(II) complexes with different types of ligands (sulfur and nitrogen donor ligands, Schiff base ligands and drugs as ligands). Guerra et al. [10] synthesized three palladium complexes with antibiotics of the tetracycline family and they tested their effects on tetracycline sensitive and resistant bacterial strains. The palladium complex with tetracycline was 16 times stronger than

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* Corresponding author. Tel.: +381 34 300263; fax: +381 34 335040.
*E-mail address:* srecko@kg.ac.rs (S.R. Trifunović).

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the tetracycline itself against resistant strain. Vieira et al. [11] prepared new palladium(II) and platinum(II) complexes with fluoroquinolones which showed activity to *Mycobacterium tuberculosis*. There are other papers in the literature showing different intensity of palladium complexes activity on various species of bacteria and fungi [12–17].

The aim of this paper is to synthesize new palladium complexes and to research *in vitro* their antibacterial and antifungal activities. The second aim is to investigate the impact of newly synthesized palladium complexes on probiotics, since they are used as supplements and they play significant role in protection and maintenance of balance in intestinal microflora during the use of the antibiotic therapy.

## 2. Experimental

### 2.1. Chemistry

#### 2.1.1. Reagents and instruments

(*S,S*)-Ethylenediamine-*N,N′*-di-2-(3-methyl)-butanoic acid, ($H_2$-(*S,S*)-eddv) was prepared using similar methods described in literature [18]. $K_2[PdCl_4]$ was purchased from Merck and used without further purification. Alcohols were dried by standard methods. Infrared spectra were recorded by Perkin-Elmer Spectrum One FT-IR spectrometer using the KBr pellet technique (4000–400 $cm^{-1}$) and mass spectra were recorded by Agilent 5973B. $^1H$ and $^{13}C$ NMR (Fig. 1) spectra were recorded by Varian Gemini-2000 (200 MHz) spectrometer in $D_2O$ (ligand precursors) and $CDCl_3$ (palladium(II) complexes) using tetramethylsilane as internal standard and in DMSO-$d_6$ (ligands and complexes). Elemental microanalyses for C, H and N were performed by standard methods by Vario EL *III* C, H, N Elemental Analyzer.

#### 2.1.2. Preparation of *O,O′*-dialkyl esters of the (*S,S*)-ethylenediamine-*N,N′*-di-2-(3-methyl)-butanoic acid dihydrochloride, $R_2$-*S,S*-eddv·2HCl

In 50 mL of dry alcohol (ethanol, 1-propanol, 1-butanol or 1-pentanol), saturated with gas HCl, 2.50 g (7.5 mmol) of ($H_2$-(*S,S*)-eddv) was added and the mixture was refluxed for 12 h. The mixture was filtered off and the filtrate was left for a few days in the refrigerator. The esters were recrystallized from hot alcohol used for each reaction.

*O,O′*-Diethyl-(*S,S*)-ethylenediamine-*N,N′*-di-2-(3-methyl)-butanoate dihydrochloride det-*S,S*-eddv·2HCl (**L1**) was previously synthesized [5].

*O,O′-Dipropyl-(S,S)-ethylenediamine-N,N′-di-2-(3-methyl)-butanoate dihydrochloride dpr-S,S-eddv·2HCl (**L2**).* Yield: 1.72 g (55%). *Anal.* Calc. for $C_{18}H_{38}Cl_2N_2O_4$ ($M_r$ = 417.404): C, 51.79; H, 9.18; N, 6.71. Found: C, 51.92; H, 9.06; N, 6.73%. 1H NMR (200 MHz, $D_2O$, $\delta$ ppm): 0.91 (t, 6H, $C^9H_3$), 1.21 (m, 12H, $C^{5,6}H_3$), 1.74 (m, 4H, $C^8H_2$), 2.37 (m, 4H, $C^1H_2$), 3.52 (m, 2H, $C^4H$), 4.11 (d, 2H, $C^2H$), 4.27 (q, 4H, $C^7H_2$). $^{13}C$ NMR (50 MHz, $D_2O$, $\delta$ ppm): 12.63 ($C^8H_3$), 19.47 ($C^5H_3$), 20.15 ($C^6H_3$), 24.22 ($C^8H_2$), 32.48 ($C^4H$), 46.48 ($C^1H_2$), 69.35 ($C^2H$), 72.21 ($C^7H_2$), 171.6 ($C^3$OOPr). $^1H$ NMR (200 MHz, DMSO-$d_6$, $\delta$ ppm): 0.86 (t, 6H, $C^9H_3$), 1.18 (m, 12H, $C^{5,6}H_3$), 1.58 (m, 4H, $C^8H_2$), 2.26 (m, 4H, $C^1H_2$), 3.46 (m, 2H, $C^4H$), 4.07 (d, 2H, $C^2H$), 4.37 (q, 4H, $C^7H_2$), 4.63 (2H, NH). $^{13}C$ NMR (50 MHz, DMSO-$d_6$, $\delta$ ppm): 12.48 ($C^8H_3$), 18.96 ($C^5H_3$), 20.17 ($C^6H_3$), 24.89 ($C^8H_2$), 33.03 ($C^4H$), 46.41 ($C^1H_2$), 68.97 ($C^2H$), 72.06 ($C^7H_2$), 170.9 ($C^3$OOPr).

*O,O′-dibutyl-(S,S)-ethylenediamine-N,N′-di-2-(3-methyl)-butanoate dihydrochloride dbu-S,S-eddv·2HCl (**L3**).* Yield: 1.74 g (52%). *Anal.* Calc. for $C_{20}H_{42}Cl_2N_2O_4$ ($M_r$ = 445.456): C, 53.92; H, 9.50; N, 6.29. Found: C, 53.48; H, 9.54; N, 6.48%. $^1H$ NMR (200 MHz, $D_2O$, $\delta$ ppm): 0.91 (t, 6H, $C^{10}H_3$), 1.13 (m, 12H, $C^{5,6}H_3$), 1.37 (m, 4H, $C^9H_2$), 1.70 (m, 4H, $C^8H_2$), 2.41 (m, 4H, $C^1H_2$), 3.56 (m, 2H, $C^4H$), 3.87 (d, 2H, $C^2H$), 4.34 (q, 4H, $C^7H_2$). $^{13}C$ NMR (50 MHz, $D_2O$, $\delta$ ppm): 15.80 ($C^{10}H_3$), 19.43 ($C^5H_3$), 19.76 ($C^6H_3$), 21.51 ($C^9H_2$), 32.60 ($C^4H$), 36.38 ($C^8H_2$), 46.19 ($C^1H_2$), 64.50 ($C^2H$), 70.49 ($C^7H_2$), 171.56 ($C^3$OOR). $^1H$ NMR (200 MHz, DMSO-$d_6$, $\delta$ ppm): 0.87 (t, 6H, $C^{10}H_3$), 1.17 (m, 12H, $C^{5,6}H_3$), 1.39 (m, 4H, $C^9H_2$), 1,66 (m, 4H, $C^8H_2$), 2.28 (m, 4H, $C^1H_2$), 3.48 (m, 2H, $C^4H$), 3.79 (d, 2H, $C^2H$), 4.26 (q, 4H, $C^7H_2$), 4.58 (2H, NH). $^{13}C$ NMR (50 MHz, DMSO-$d_6$, $\delta$ ppm): 14.99 ($C^{10}H_3$), 19.37 ($C^5H_3$), 19.58 ($C^6H_3$), 21.43 ($C^9H_2$), 32.54 ($C^4H$), 36.26 ($C^8H_2$), 46.03 ($C^1H_2$), 64.57 ($C^2H$), 70.48 ($C^7H_2$), 171.51($C^3$OOR).

*O,O′-dipentyl-(S,S)-ethylenediamine-N,N′-di-2-(3-methyl)-butanoate dihydrochloride dpe-S,S-eddv·2HCl (**L4**). Yield: 1.82 g (51%). Anal. Calc. for $C_{22}H_{46}Cl_2N_2O_4$ ($M_r$ = 473.508): C, 55.80; H, 9.79; N, 5.92. Found: C,55.68; H, 9.76; N, 6.02%. $^1H$ NMR (200 MHz, $D_2O$, $\delta$ ppm): 0.90 (t, 6H, $C^{11}H_3$), 1.10 (m, 12H, $C^{5,6}H_3$), 1.37 (m, 8H, $C^{9,10}H_2$), 1.72 (m, 4H, $C^8H_2$), 2.40 (m, 4H, $C^1H_2$), 3.62 (m, 2H, $C^4H$), 4.09 (d, 2H, $C^2H$), 4.39 (q, 4H, $C^7H_2$). $^{13}C$ NMR (50 MHz, $D_2O$, $\delta$ ppm): 16.13 ($C^{11}H_3$), 19.48 ($C^5H_3$), 20.69 ($C^6H_3$), 24.52 ($C^{10}H_2$), 30.31 ($C^9H_2$), 32.66 ($C^8H_2$), 33.92 ($C^4H$), 46.29 ($C^1H_2$), 64.83 ($C^2H$), 70.74 ($C^7H_2$), 171.84 ($C^3$OOR). $^1H$ NMR (200 MHz, DMSO-$d_6$, $\delta$ ppm): 0.90 (t, 6H, $C^{11}H_3$), 1.12 (m, 12H, $C^{5,6}H_3$), 1.36 (m, 8H, $C^{9,10}H_2$), 1.68 (m, 4H, $C^8H_2$), 2.33 (m, 4H, $C^1H_2$), 3.64 (m, 2H, $C^4H$), 4.02 (d, 2H, $C^2H$), 4.21 (q, 4H, $C^7H_2$), 4.76 (2H, NH). $^{13}C$ NMR (50 MHz, DMSO-$d_6$, $\delta$ ppm): 16.06 ($C^{11}H_3$), 19.43 ($C^5H_3$), 20.32 ($C^6H_3$), 24.45 ($C^{10}H_2$), 30.29 ($C^9H_2$), 32.69 ($C^8H_2$), 33.88 ($C^4H$), 46.07 ($C^1H_2$), 64.12 ($C^2H$), 70.76 ($C^7H_2$), 171.34 ($C^3$OOR).*

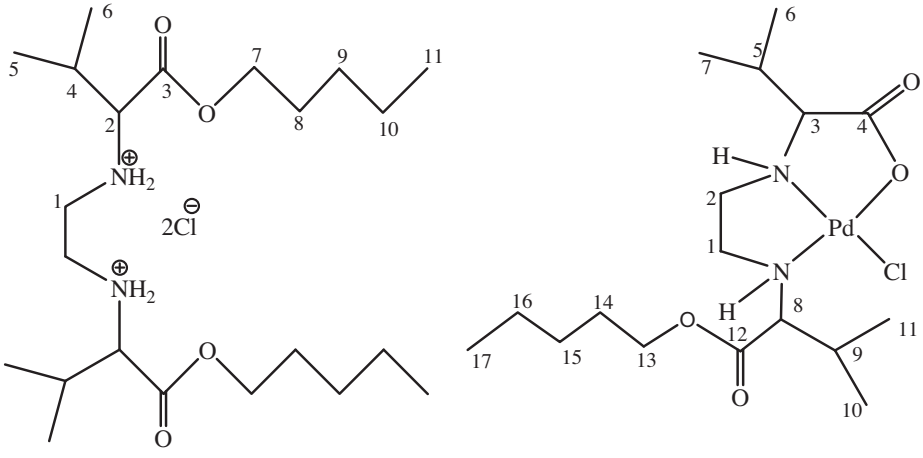


**Fig. 1.** Numbering of compounds used for NMR data.