Polyhedron 31 (2012) 69–76

Contents lists available at SciVerse ScienceDirect

Polyhedron

journal homepage: www.elsevier.com/locate/poly

Synthesis, characterization and antimicrobial activity of palladium(II) complexes with some alkyl derivates of thiosalicylic acids: Crystal structure of the *bis*(*S*-benzyl-thiosalicylate)–palladium(II) complex, [Pd(*S*-bz-thiosal)$_2$]

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ARTICLE INFO

Article history:
Received 24 May 2011
Accepted 24 August 2011
Available online 21 September 2011

Keywords:
Palladium(II) complexes
Crystal structure
IR, ^{1}H and ^{13}C NMR spectroscopy
Antimicrobial activity

ABSTRACT

S-Alkyl (R = benzyl, methyl, ethyl, propyl and butyl) derivatives of thiosalicylic acid and the corresponding palladium(II) complexes were prepared and their structures were proposed on the basis of infrared, ^{1}H and ^{13}C NMR spectroscopy. The *cis* geometrical configurations of the isolated complexes were proposed on the basis of an X-ray structural study of the *bis*(*S*-benzyl-thiosalicylate)–palladium(II), [Pd(*S*-bz-thiosal)$_2$] complex.

Antimicrobial activity of the tested compounds was evaluated by determining the minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) in relation to 26 species of microorganisms. The tested ligands, with a few exceptions, show low antimicrobial activity. The palladium(II) complexes, [Pd(*S*-R-thiosal)$_2$], have statistically significant higher activity than the corresponding ligands. The complexes [Pd(*S*-et-thiosal)$_2$] and [Pd(*S*-pro-thiosal)$_2$] displayed the strongest activity amongst the all tested compounds. The palladium(II) complexes show selective and moderate antibacterial activity and significant antifungal activity. The most sensitive were *Aspergillus fumigatus* and *Aspergillus flavus*.

1. Introduction

Thiosalicylic acid and its derivatives have many various applications: as reagents for metal determination [1,2], modificators for graphite paste electrodes [3], as photoinitiators for free radical polymerization [4] and in cosmetics in hair growth treatment [5]. They are useful in numerous disease treatments, in particular inflammatory, allergic and respiratory diseases [6] as well as Ras-tumor growth inhibitors [7]. Ketones derived from thiosalicylic acids have application as bile acid transport inhibitors [8].

The synthesis and evaluation of the biological activity of new metal-based compounds are fields of growing interest. Numerous complexes based on the palladium(II) ion have been synthesized and their different biological activities have been documented [9–11]. The impact of different palladium complexes on the growth and metabolism of various groups of microorganisms has been studied. Garoufis et al. [12] reviewed numerous scientific papers on anti-viral, 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). There are other papers in the literature showing different intensities of palladium complex activity on various species of bacteria and fungi [13–20].

S-Alkyl (alkyl = benzyl, methyl, ethyl, propyl and butyl) derivatives of thiosalicylic acid have already been prepared and characterized using IR and elemental microanalysis [21–24], and the *S*-methyl derivate has also been characterized using NMR spectroscopy [22,23].

Thiosalicylic acid has been used for the synthesis of palladium(II) complexes [25–28], but corresponding *S*-alkyl derivatives have not. Our investigations are focused on the synthesis of the corresponding Pd(II) complexes of *S*-alkyl derivatives as well as the *in vitro* antimicrobial activity of the ligands and the complexes. The structures of the isolated complexes are proposed on the basis of their infrared, ^{1}H and ^{13}C NMR spectra. The structures as well as the *cis* geometrical configurations of the isolated complexes are proposed on the basis of an X-ray structural study of the *cis*-S-*cis*-O *bis*(*S*-benzyl-thiosalicylate)–palladium(II) [Pd(*S*-bz-thiosal)$_2$] complex. Our investigations are focused on the impact of the newly synthesized Pd(II) complexes on probiotics, since they are used as supplements and they play a significant role in the protection and maintenance of the balance of intestinal microflora during antibiotic therapy.

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doi:10.1016/j.poly.2011.08.042

2. Experimental

2.1. Chemistry

2.1.1. Reagents and instruments

All chemicals were obtained commercially and used without further purification. For the infrared spectra, a Perkin–Elmer Spectrum One FT-IR spectrometer was employed. Elemental microanalyses for C, H and S were performed by standard methods on a Vario III CHNS Elemental Analyzer, Elemental Analysensysteme GmbH.

2.1.2. General procedure for the synthesis of S-alkyl thiosalicylic acids (*L1*)–(*L5*)

The thioacid ligands **L1**–**L5** were prepared by alkylation of thiosalicylic acid by means of the corresponding alkyl halogenides in alkaline water–ethanol solution.

Thiosalicylic acid (1 mmol) was added to a 100 cm^3 round bottom flask containing 50 cm^3 of 3a 0% solution of ethanol in water and stirred. A solution of NaOH (2 mmol in 5 cm^3 of water) was added to the acid suspension, whereupon the solution became clear. The corresponding alkyl halogenide (2 mmol) was dissolved in 5 cm^3 of ethanol and transferred to the stirred solution. The resulting mixture was kept overnight at 60 °C. The reaction mixture was transferred into a beaker and ethanol was evaporated off on a water bath. Diluted hydrochloric acid (2 mol/dm^3) was added to the resulting water solution and *S*-alkyl thiosalicylic acid was precipitated as a white powder. The liberated acid was filtered off and washed with plenty of distilled water. The product was dried under vacuum overnight. Yield: 85–95%.

*S-Benzyl-thiosalicylic acid (**L1**):* M.p. 179–180 °C, white powder. IR (KBr, cm^{-1}): 3414, 3061, 2920, 2648, 2559, 1674, 1584, 1562, 1463, 1412, 1317, 1272, 1255, 1154, 1062, 1046, 897, 743, 711, 652, 551. ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 4.17 (s, 2H, CH_2), 7.21–8.14 (m, 9H, Ar and bz). ^{13}C NMR (50 MHz, DMSO-d_6, δ ppm): 35.9 (CH_2), 124.1, 125.9, 126.7, 127.3, 127.9, 128.3, 128.6, 129.3, 131.0, 132.4, 136.8, 141.3 (Ar and bz), 167.5 (COOH).

*S-Methyl-thiosalicylic acid (**L2**):* M.p. 165–166 °C, white powder. IR (KBr, cm^{-1}): 3446, 3068, 2916, 2652, 2560, 1674, 1586, 1561, 1466, 1412, 1308, 1291, 1270, 1255, 1151, 1062, 1048, 892, 743, 699, 652, 556. ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 2.48 (s, 3H, CH_3), 7.16–8.18 (m, 4H, Ar). ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 15.6 (CH_3), 123.5, 124.4, 125.4, 132.5, 133.6, 144.4 (Ar), 171.6 (COOH).

*S-Ethyl-thiosalicylic acid (**L3**):* M.p. 133–134 °C, white powder. IR (KBr, cm^{-1}): 3435, 3066, 2972, 2652, 2562, 1682, 1588, 1563, 1466, 1414, 1315, 1275, 1252, 1152, 1063, 1049, 884, 740, 704, 690, 651, 550. ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 1.42 (t, 3H, CH_3), 2.97 (q, 2H, CH_2), 7.16–8.17 (m, 4H, Ar). ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 13.1 (CH_3), 26.2 (CH_2), 124.0, 125.9, 126.4, 132.6, 133.2, 142.9 (Ar), 171.4 (COOH).

*S-Propyl-thiosalicylic acid (**L4**):* M.p. 104 °C, white powder. IR (KBr, cm^{-1}): 3414, 3056, 2979, 2641, 2555, 1678, 1588, 1562, 1462, 1405, 1310, 1271, 1257, 1150, 1062, 1053, 811, 740, 704, 691, 653, 554. ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 1.1 (t, 3H, CH_3), 1.74 (m, 2H, CH_2), 2.92 (t, 2H, CH_2), 7.15–8.15 (m, 4H, Ar). ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 13.8 (CH_3), 21.6 (CH_2), 34.1 (CH_2), 123.8, 125.6, 126.2, 132.5, 133.1, 143.1 (Ar), 171.6 (COOH).

*S-Butyl-thiosalicylic acid (**L5**):* M.p. 82–83 °C, white powder. IR (KBr, cm^{-1}): 3420, 2955, 2869, 2641, 2556, 1674, 1586, 1560, 1462, 1408, 1320, 1270, 1250, 1153, 1060, 1048, 924, 810, 738, 704, 651, 553. ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 0.96 (t, 3H, CH_3), 1.46 (m, 2H, CH_2), 1.78 (m, 2H, CH_2), 2.94 (t, 2H, CH_2), 7.15–8.16 (m, 4H, Ar). NMR (50 MHz, $CDCl_3$, δ ppm): 13.7 (CH_3), 22.3 (CH_2), 30.2 (CH_2), 31.9 (CH_2), 123.8, 125.7, 126.3, 132.5, 133.1, 143.1 (Ar), 171.4 (COOH).

2.1.3. Preparation of the bis(S-benzyl-thiosalicylate)–palladium(II) complex, [Pd(S-bz-thiosal)$_2$] (*C1*)

$K_2[PdCl_4]$ (0.100 g, 0.3065 mmol) was dissolved in 10 cm^3 of water on a steam bath and (*S*-benzyl)-2-thiosalicylic acid (0.1497 g, 0.613 mmol) was added into the solution. The resulting mixture was stirred for 2 h and during this time an aqueous solution of LiOH (0.0256 g, 0.613 mmol in 10 cm^3 of water) was introduced. The complex [Pd(*S*-bz-thiosal)$_2$] (**C1**) as a yellow precipitate was filtered, washed with water and air-dried. Yield: 0.11 g (58.70%). *Anal.* Calc. for $C_{28}H_{22}O_4S_2Pd$ (M_r = 592.98): C, 56.71; H, 3.74; S, 10.82. Found: C, 56.43; H, 3.85; S, 10.75%. IR (KBr, cm^{-1}): 3420, 3057, 1634, 1616, 1562, 1327, 1146, 753, 708, 698. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 4.05 (s, 4H, CH_2), 7.08–8.10 (m, 9H, Ar and bz). ^{13}C NMR (50 MHz, DMSO-d_6, δ ppm): 25.9 (CH_2), 124.1, 125.6, 125.7, 126.2, 126.3, 126.8, 127.3, 127.8, 129.5, 133.2, 136.2, 139.7 (Ar and bz), 171.5 (COO^-).

2.1.4. Preparation of the bis(S-methyl-thiosalicylate)–palladium(II) complex, [Pd(S-met-thiosal)$_2$] (*C2*)

The complex [Pd(*S*-met-thiosal)$_2$] (**C2**) was prepared as described in Section 2.1.3 using (*S*-methyl)-2-thiosalicylic acid (0.103 g, 0.613 mmol) instead of (*S*-benzyl)-2-thiosalicylic acid. Yield: 0.08 g (59.80%). *Anal.* Calc. for $C_{16}H_{14}O_4S_2Pd$ (M_r = 440.672): C, 43.61; H, 3.20; S, 14.52. Found: C, 43.41; H, 3.39; S, 14.21%. IR (KBr, cm^{-1}): 3419, 1619, 1597, 1399, 1385, 1332, 1306, 1142, 960, 865, 741, 693, 654. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 2.35 (s, 6H, CH_3), 7.19–8.08 (m, 8H, Ar). ^{13}C NMR (50 MHz, DMSO-d_6, δ ppm): 14.6 (CH_3), 123.6, 125.1, 125.2, 129.0, 132.7, 135.7, (Ar), 171.8 (COO^-).

2.1.5. Preparation of the bis(S-ethyl-2-thiosalicylate)–palladium(II) complex, [Pd(S-et-thiosal)$_2$] (*C3*)

The complex [Pd(*S*-et-thiosal)$_2$] (**C3**) was prepared as described in Section 2.1.3 using (*S*-ethyl)-2-thiosalicylic acid (0.1117 g, 0.613 mmol) instead of (*S*-benzyl)-2-thiosalicylic acid. Yield: 0.0832 g (57.90%). *Anal.* Calc. for $C_{18}H_{18}O_4S_2Pd$ (M_r = 468.856): C, 46.11; H, 3.87; S, 13.68. Found: C, 45.97; H, 3.93; S, 13.54%. IR (KBr, cm^{-1}): 1436, 1587, 1518, 1393, 752. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 1.27 (t, 6H, CH_3), 2.83 (q, 4H, CH_2), 7.11–8.08 (m, 8H, Ar). ^{13}C NMR (50 MHz, DMSO-d_6, δ ppm): 14.4 (CH_3), 13.8 (CH_2), 124.8, 125.3, 126.1, 128.7, 133.2, 135.9 (Ar), 172.0 (COO^-).

2.1.6. Preparation of the bis(S-propyl-2-thiosalicylate)–palladium(II) complex, [Pd(S-pro-thiosal)$_2$] (*C4*)

The complex [Pd(*S*-pro-thiosal)$_2$] (**C4**) was prepared as described in Section 2.1.3 using (*S*-propyl)-2-thiosalicylic acid (0.1203 g, 0.613 mmol) instead of (*S*-benzyl)-2-thiosalicylic acid. Yield: 0.0889 g (58.40%). *Anal.* Calc. for $C_{20}H_{22}O_4S_2Pd$ (M_r = 496.908): C, 48.34; H, 4.46; S, 12.91. Found: C, 48.52; H, 4.11; S, 12.73%. IR (KBr, cm^{-1}): 1421, 1589, 1541, 1520, 1397, 752. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 0.98 (t, 6H, CH_3), 1.76 (m, 4H, CH_2), 2.84 (t, 4H, CH_2), 7.20–8.25 (m, 8H, Ar). ^{13}C NMR (50 MHz, DMSO-d_6, δ ppm): 13.2 (CH_3), 22.0 (CH_2), 27.6 (CH_2), 125.1, 126.6, 126.7, 130.5, 134.2, 137.2 (Ar), 172.5 (COO^-).

2.1.7. Preparation of the bis(S-butyl-2-thiosalicylate)–palladium(II) complex, [Pd(S-bu-thiosal)$_2$] (*C5*)

The complex [Pd(*S*-bu-thiosal)$_2$] (**C5**) was prepared as described in Section 2.1.3 using (*S*-butyl)-2-thiosalicylic acid, (0.1289 g, 0.613 mmol) instead of (*S*-benzyl)-2-thiosalicylic acid. Yield: 0.0941 g (58.43%). *Anal.* Calc. for $C_{22}H_{26}O_4S_2Pd$ (M_r = 524.960): C, 50.33; H, 4.99; S, 12.22. Found: C, 50.52; H, 4.51; S, 12.56%. IR (KBr, cm^{-1}): 3420, 1634, 1616, 1561, 1327, 1146, 753, 698. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 0.95 (t, 6H, CH_3), 1.33 (m, 4H, CH_2), 1.62 (m, 4H, CH_2), 2.79 (t, 4H, CH_2), 7.24–8.19 (m, 8H, Ar).

Table 1
Crystal data and structure refinement for the [Pd(*S*-bz-thiosal)$_2$] complex (**C1**).

Identification code	CCDC 824331
Empirical formula	$C_{28}H_{22}O_4S_2Pd$
Formula weight	592.98
T (K)	123(2)
λ (Å)	0.71073
Crystal system	monoclinic
Space group	$P2_1/c$
Unit cell dimensions	
a (Å)	12.0280(5)
b (Å)	21.0330(8)
c (Å)	9.5049(4)
α (°)	90
β (°)	92.578(2)
γ (°)	90
V (Å^3)	2402.16(17)
Z	4
D_{calc} (g/cm^3)	1.640
Absorption coefficient (mm^{-1})	0.981
F(000)	1200
Crystal size (mm^3)	0.16 × 0.04 × 0.02
Theta range for data collection	2.96–25.02°
Index ranges	$-13 \leqslant h \leqslant 14$, $-23 \leqslant k \leqslant 25$, $-11 \leqslant l \leqslant 11$
Reflections collected	13559
Independent reflections	4212 [R_{int} = 0.1276]
Completeness to θ = 25.02°	99.3%
Absorption correction	multi-scan
Maximum and minimum transmission	0.9807 and 0.8589
Refinement method	full-matrix least-squares on F^2
Data/restraints/parameters	4212/78/316
Goodness-of-fit (GOF) on F^2	0.981
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.0624, wR_2 = 0.1034
R indices (all data)	R_1 = 0.1215, wR_2 = 0.1179
Largest difference in peak and hole (e Å^{-3})	0.587 and −0.637

^{13}C NMR (50 MHz, DMSO-d_6, δ ppm): 13.4 (CH_3), 20.5 (CH_2), 21.6 (CH_2), 33.7 (CH_2), 123.8, 124.4, 126.3, 130.2, 131.9, 135.9 (Ar), 171.9 (COO^-).

2.2. Crystal structure determination

Cystals of the [Pd(*S*-bz-thiosal)$_2$] complex (**C1**) suitable for X-ray determination were obtained by slow crystallization from a MSO-water system. The structural data were collected by a Bruker-Nonius Kappa CCD diffractometer equipped with an APEXII detector using graphite monochromatised Mo Kα radiation. The COLLECT [29] data collection software was used and obtained data were processed with DENZO-SMN [30]. The structures were solved by direct methods, using SIR-2004 [31], and refined on F^2, using SHELXL-97 [32]. The reflections were corrected for Lorenz-polarization effects and multi-scan absorption correction was applied [33]. The hydrogen atoms were inserted at their calculated positions with isotropic temperature factors [U_{iso}(H) factors of 1.2 times U_{eq}(C)] and refined as riding atoms. The figure was drawn with ORTEP-3 [34]. Other experimental X-ray data are shown in Table 1.

2.3. In vitro antimicrobial assay

2.3.1. Test substances

The tested compounds were dissolved in DMSO and then diluted into nutrient liquid medium to achieve a concentration of 10%. An antibiotic, doxycycline (Galenika A.D., Belgrade), was dissolved in nutrient liquid medium, a Mueller–Hinton broth (Torlak, Belgrade), while an antimycotic, fluconazole (Pfizer Inc., USA), was dissolved in Sabouraud dextrose broth (Torlak, Belgrade).

2.3.2. Test microorganisms

The antimicrobial activity of the ligands **L1**–**L5** and the corresponding palladium(II) complexes **C1**–**C5** was tested against 26 microorganisms. The experiment involved 14 strains of pathogenic bacteria, including five standard strains (*Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus ATCC* 25923, *Bacillus subtilis* ATCC 6633) and nine clinical isolates (*Escherichia coli*, *Enterococcus faecalis*, *P. aeruginosa*, *S. aureus*, *Sarcina lutea*, *Bacillus subtilis*, *Proteus mirabilis*, *Salmonella enterica*, *Salmonella typhimurium*). Also, four species of probiotic bacteria (*Lactobacillus plantarum* PMFKG-P31, *Bacillus subtilis* IP 5832 PMFKG-P32, *Bifidobacterium animalis* subsp. *lactis* PMFKG-P33, *Lactobacillus rhamnosus* PMFKG-P35), five species of mould (*Aspergillus niger* ATCC 16404, *Aspergillus fumigatus* PMFKG-F23, *Aspergillus flavus* PMFKG-F24, *Aspergillus restrictus* PMFKG-F25, *A. niger* PMFKG-F26) and three yeast species (*Candida albicans* (clinical isolate), *Rhodotorula* sp. PMFKG-F27, *Saccharomyces boulardii* PMFKG-P34) were tested. All clinical isolates were a generous gift from the Institute of Public Health, Kragujevac. The other microorganisms were provided from a collection held by the Microbiology Laboratory Faculty of Science, University of Kragujevac.

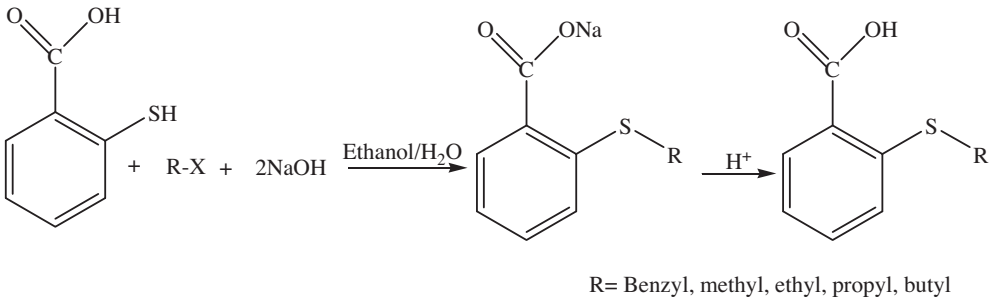
2.3.3. Suspension preparation

Bacterial and yeast suspensions were prepared by the direct colony method. The colonies were taken directly from the plate and were suspended in 5 cm^3 of sterile 0.85% saline. The turbidity of the initial suspension was adjusted by comparing it with 0.5 McFarland's standard (0.5 cm^3 1.17% w/v $BaCl_2 \times 2H_2O$ + 99.5 cm^3 1% w/v H_2SO_4) [35]. When adjusted to the turbidity of the 0.5 McFarland's standard, the bacteria suspension contains about 10^8 colony forming units (CFU)/cm^3 and a suspension of yeast contains 10^6 CFU/cm^3. Ten-fold dilutions of the initial suspension were additionally prepared into sterile 0.85% saline. The suspensions of fungal spores were prepared by gentle stripping of spore from slopes with growing *aspergilli*. The resulting suspensions were 1:1000 diluted in sterile 0.85% saline.

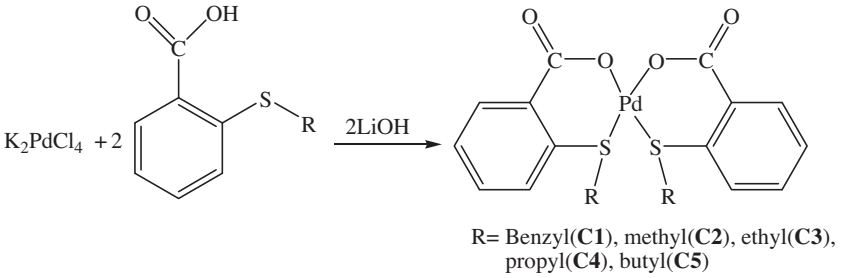
2.3.4. Microdilution method

Antimicrobial activity was tested by determining the minimum inhibitory concentrations (MIC) and minimum microbicidal concentration (MMC) using the microdilution plate method with resazurin [36]. The 96-well plates were prepared by dispensing 100 μL of nutrient broth, Mueller–Hinton broth for bacteria and Sabouraud dextrose broth for fungi and yeasts, into each well. A 100 μL aliquot from the stock solution of the tested compound (with a concentration of 2000 μg/cm^3) was added into the first row of the plate. Then, twofold, serial dilutions were performed by using a multichannel pipette. The obtained concentration range was from 1000 to 7.81 μg/cm^3. A 10 μL aliquot of diluted bacterial yeast suspension and suspension of spores were added to each well to give a final concentration of 5×10^5 CFU/cm^3 for bacteria and 5×10^3 CFU/cm^3 for fungi and yeast. Finally, 10 μL resazurin solution was added to each well inoculated with bacteria and yeast. Resazurin is an oxidation–reduction indicator used for the evaluation of microbial growth. It is a blue non-fluorescent dye that becomes pink and fluorescent when reduced to resorufin by oxidoreductases within viable cells [37]. The inoculated plates were incubated at 37 °C for 24 h for bacteria, 28 °C for 48 h for the yeast and 28 °C for 72 h for fungi. The MIC was defined as the lowest concentration of the tested substance that prevented the resazurin color change from blue to pink. For fungi, the MIC values of the tested substances were determined as the lowest concentration that visibly inhibited mycelia growth.

Doxycycline and fluconazole were used as a positive control. A solvent control test was performed to study the effect of 10% DMSO on the growth of microorganisms. It was observed that 10% DMSO



Scheme 1. The preparation of the benzyl, methyl, ethyl, propyl and butyl esters of 2-thiosalicylic acid.



Scheme 2. The preparation of the [Pd(*S*-R-thiosal)$_2$].

did not inhibit the growth of microorganisms. Also, in the experiment, the concentration of DMSO was additionally decreased because of the twofold serial dilution assay (the working concentration was 5% and lower). Each test included growth control and sterility control. All tests were performed in duplicate and the MICs were constant. Minimum bactericidal and fungicidal concentrations were determined by plating 10 μL of samples from wells where no indicator color change was recorded, on nutrient agar medium. At the end of the incubation period the lowest concentration with no growth (no colony) was defined as the minimum microbicidal concentration.

2.3.5. Statistical analysis

All statistical analyses were performed using SPSS package. Mean differences were established by the Student's *t*-test. Data were analyzed using one-way analysis of variance (ANOVA). In all cases P values <0.05 were considered statistically significant.

3. Results and discussion

3.1. Synthesis and chemical characterization

S-Alkyl (R = benzyl, methyl, ethyl, propyl and butyl) derivatives of thiosalicylic acid were prepared by the alkylation of thiosalicylic acid using the corresponding alkyl halogenides in alkaline water–ethanol solution (Scheme 1). The corresponding [Pd(*S*-R-thiosal)$_2$] complexes were obtained by the direct reaction of $K_2[PdCl_4]$ with the *S*-alkyl derivative of thiosalicylic acid (molar ratio 1:2) in water solution with satisfactory yields (more than 50%) (Scheme 2).

A bidentate S–O coordination of the ligands **L1**–**L5** to the Pd(II) ion is expected. The lack of S–H stretching absorption bands in the complexes **C1**–**C5** in the range 2600–2550 cm^{-1} [38] (2559 cm^{-1} for **L1**, 2560 cm^{-1} for **L2**, 2562 cm^{-1} for **L3**, 2555 cm^{-1} for **L4** and 2556 cm^{-1} for **L5**) suggests the deprotonation of the S–H groups of the ligands and their coordination to the Pd(II) ion in the complexes. The carboxylate asymmetric stretching bands of ligands (1674 cm^{-1} for **L1** and **L2**, 1682 cm^{-1} for **L3**, 1678 cm^{-1} for **L4** and 1674 cm^{-1} for **L5**) are located at lower energies than expected (1700–1750 cm^{-1}) [38,39]. This could be explained by the presence of big R–S groups in the *ortho* position. The positions of these bands in corresponding **C1**–**C5** complexes are in the expected region (1600–1650 cm^{-1}) [18] (1633 and 1616 cm^{-1} for **C1**, 1619 cm^{-1} for **C2**, 1587 cm^{-1} for **C3**, 1589 cm^{-1} for **C4** and 1633 and 1615 cm^{-1} for **C5**), confirming their deprotonation and coordination in the complexes. The presence of two bands for **C1** and **C5** suggests small energy differences. These differences could be explained due to the presence of large benzyl and butyl groups and their steric impact to the phenyl part of the thiosalicylic acid.

Chemical shifts arising from carbon and hydrogen atoms of this type of thioether and the corresponding palladium(II) complexes are found at the expected, and almost the same positions. Some differences in the chemical shifts of the carbon atoms of the carboxylic groups of the *S*-benzyl, *S*-methyl, *S*-ethyl, *S*-propyl and *S*-

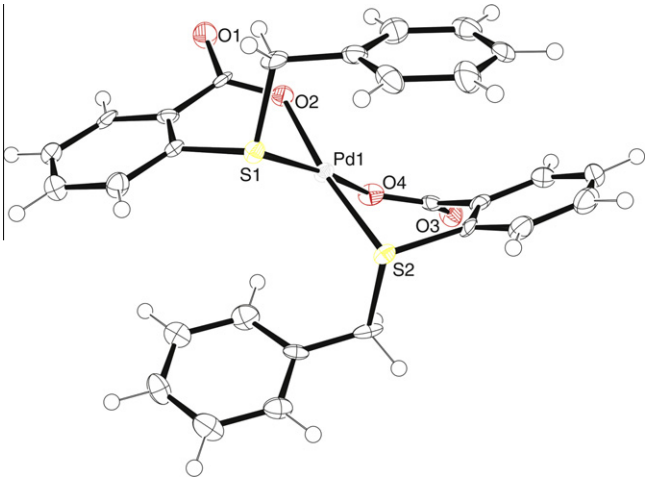


Fig. 1. Molecular structure of the [Pd(*S*-benzyl)$_2$] complex (**C1**) (heteroatoms are shown with an octant shaded mode).

Table 2
Selected bond lengths (Å) and angles (°) for the [Pd(*S*-benzyl)$_2$] complex (**C1**).

Bond lengths	(Å)	Bond angles	(°)
Pd(1)–O(2)	2.024(4)	O(2)–Pd(1)–O(4)	88.4(2)
Pd(1)–O(4)	2.034(4)	O(2)–Pd(1)–S(1)	89.84(14)
Pd(1)–S(1)	2.246(2)	O(4)–Pd(1)–S(1)	172.52(15)
Pd(1)–S(2)	2.254(2)	O(2)–Pd(1)–S(2)	172.77(14)
S(1)–C(3)	1.778(7)	O(4)–Pd(1)–S(2)	89.70(14)
S(1)–C(8)	1.829(7)	S(1)–Pd(1)–S(2)	92.94(7)
S(2)–C(17)	1.787(7)	C(3)–S(1)–C(8)	105.9(3)
S(2)–C(22)	1.830(7)	C(3)–S(1)–Pd(1)	100.0(2)
O(2)–C(1)	1.278(8)	C(8)–S(1)–Pd(1)	104.4(2)
O(4)–C(15)	1.279(8)	C(17)–S(2)–C(22)	105.9(3)
O(1)–C(1)	1.229(8)	C(17)–S(2)–Pd(1)	99.1(2)
O(3)–C(15)	1.235(8)	C(22)–S(2)–Pd(1)	105.7(2)
C(16)–C(15)	1.516(10)	C(1)–O(2)–Pd(1)	128.5(4)
C(2)–C(1)	1.518(9)	C(15)–O(4)–Pd(1)	127.5(5)
C(22)–C(23)	1.493(9)		
C(9)–C(8)	1.506(10)		

Short contacts	H···A (Å)	D···A (Å)	(°)	Dihedral angles	(°)
C6–H6···O4[a]		3.318(9)	125	Pd(1)–S(2)–C(22)–C(23)	−69.1(5)
C11–H11···O2[b]		3.414(9)	155	Pd(1)–S(1)–C(8)–C(9)	−52.0(5)
C14–H14···O3[c]		3.293(9)	156	Pd(1)–S(1)–C(3)–C(2)	−42.3(6)
C20–H20···O1[b]		3.305(9)	127	Pd(1)–S(2)–C(17)–C(16)	−40.5(6)
C22–H22B···O3[d]		3.380(9)	134	C(3)–C(2)–C(1)–O(2)	29.2(11)
C24–H24···O3[d]		3.471(9)	144	C(17)–C(16)–C(15)–O(4)	34.2(10)
C25–H25···O1[e]		3.388(9)	168		
C27–H27···O1[a]		3.223(10)	127		
C4–H4···π[d,g]	2.71	3.653(10)	174		
C18–H18···π[d,g]	2.71	3.617(10)	159		
C22–H22A···π[f,h]	2.87	3.778(10)	153		
π···π[d,h]		3.308(10)			

Symmetry operators:
[a] $-x, -y+1, -z$.
[b] $-x+1, -y+1, -z$.
[c] $x, y, z+1$.
[d] $x, -y+1/2, z+1/2$.
[e] $-x, y-1/2, -z+1/2$.
[f] $x, -y+1/2, z-1/2$.
[g] Distance to closest C atom.
[h] Distance from C to closest C atom.

butyl derivatives of thiosalicylic acid (167.5, 171.6, 171.4, 171.6, 171.4 ppm) and the corresponding palladium(II) complexes (171.5, 171.8, 172.0, 172.5, 171.9 ppm), respectively, can be observed. These differences could be explained by the coordination of the carboxylic group to the palladium(II) ion.

It can be concluded from IR and NMR spectra of the ligands and the corresponding complexes that the ligands are bidentately coordinated to the palladium(II) ion, but nothing can be concluded about geometry of complexes.

3.2. Description of the crystal structure

The [Pd(*S*-bz-thiosal)$_2$] complex (**C1**) crystallizes in the $P2_1/c$ space group of the monoclinic crystal system. The molecular structure of the **C1** complex is shown in Fig. 1 Selected geometric parameters are listed in Table 2. The crystal structure analysis shows the bidentate and a long *cis*-S–*cis*-O coordination (known for Pd-complexes) [40] of the *S*-benzyl-thiosalicylic acid ligand **L1** to the Pd(II) ion (Fig. 1.) and the expected square-planar geometry. All the bonds are in the expected region. But a small deformation of the geometry around Pd(II) [angles O(2)–Pd(1)–O(4) 88.37(19)° and S(1)–Pd(1)–S(2) 92.94(7)°] is expected and can be explained as a consequence of the presence of large S atoms in *cis* positions.

The two six-membered rings involving the Pd atom are in skew-chair conformations in **C1** (Fig. 1) and are practically equal, as expected due to restrictions of the coordination geometry around Pd and the stiff thiosalicylic moieties. This is the reason for the orientation of benzyl groups, almost over the phenyl residues originating from another *S*-benzyl-thiosalicylic ligand. The non-parallel positions of the phenyl and benzyl groups, and therefore non-symmetric structure of the [Pd(*S*-bz-thiosal)$_2$] complex, can also be

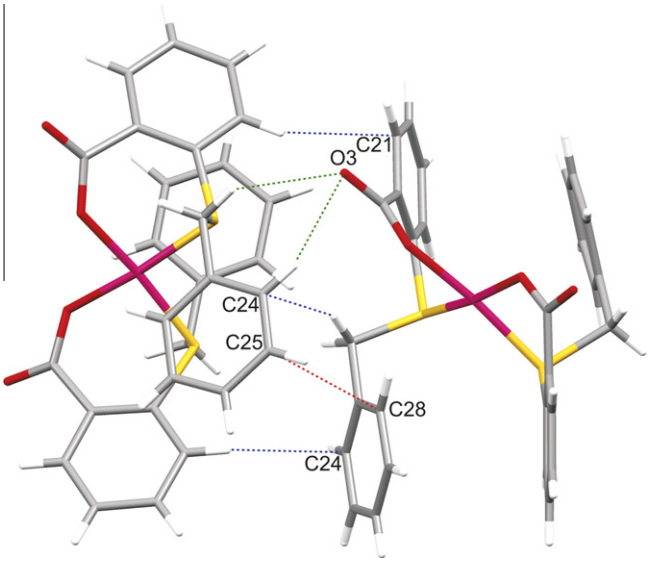


Fig. 2. A pair of **C1** complexes formed by intermolecular interactions: C–H···O (green, accepted by O3), π···π (red, closest C25···C28) and C–H···π (blue, closest accepted by C24 or C21). (Colour online.)

Table 3
In vitro antimicrobial activity of the tested ligands **L1–L5** and the corresponding palladium(II) complexes **C1–C5**.

Species	L1		C1		L2		C2		L3		C3		L4		C4		L5		C5		Doxycycline/ fluconazole	
	MIC[a]	MMC[b]	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC
Bifidobacterium animalis subsp. *lactis*	500	500	500	1000	500	500	1000	1000	1000	1000	500	500	500	1000	250	500	1000	1000	500	1000	31.25	62.5
Bacillus subtilis IP 5832	500	500	500	500	500	500	500	500	1000	>1000	250	500	500	500	250	250	1000	>1000	250	500	1.953	15.63
Lactobacillus plantarum	500	1000	250	500	500	500	500	500	500	>1000	250	500	250	>1000	250	500	1000	>1000	500	500	0.448	7.81
Lactobacillus rhamnosus	>1000	>1000	1000	1000	1000	>1000	500	1000	1000	1000	500	500	1000	>1000	500	>1000	>1000	>1000	1000	1000	7.81	31.25
Sarcina lutea	500	1000	250	500	1000	1000	500	500	500	>1000	250	250	500	>1000	250	250	1000	>1000	250	500	<0.448	3.75
Enterococcus faecalis	1000	>1000	1000	1000	1000	>1000	500	500	>1000	>1000	500	500	1000	>1000	500	500	>1000	>1000	1000	1000	7.81	62.5
E. faecalis ATCC 29212	1000	1000	500	500	1000	1000	500	1000	500	1000	250	500	500	1000	250	500	1000	1000	500	1000	7.81	62.5
Bacillus subtilis	500	500	250	500	125	500	500	500	1000	>1000	250	250	500	1000	250	250	1000	>1000	250	500	0.112	1.953
Bacillus subtilis ATCC 6633	500	500	250	500	1000	1000	500	500	>1000	>1000	250	250	1000	>1000	250	250	1000	>1000	250	500	1.953	31.25
Staphylococcus aureus	250	500	250	500	500	>1000	500	500	1000	>1000	250	250	250	1000	250	250	1000	>1000	125	500	0.448	7.81
S. aureus ATCC 25923	500	1000	500	1000	1000	1000	500	1000	1000	1000	500	500	500	1000	500	500	>1000	>1000	500	500	0.224	3.75
Escherichia coli	>1000	>1000	1000	1000	1000	>1000	500	500	1000	>1000	500	500	1000	>1000	500	500	>1000	>1000	1000	1000	7.81	15.63
Escherichia coli ATCC 25922	1000	>1000	1000	1000	1000	>1000	500	500	>1000	>1000	500	500	1000	>1000	500	500	>1000	>1000	1000	1000	15.625	31.25
Pseudomonas aeruginosa	1000	>1000	1000	1000	500	>1000	500	1000	1000	>1000	500	500	500	>1000	500	1000	1000	>1000	1000	1000	250	>250
P. aeruginosa ATCC 27853	500	>1000	500	1000	500	>1000	250	500	500	>1000	250	500	500	>1000	500	1000	500	>1000	500	1000	62.5	125
Proteus mirabilis	1000	>1000	500	1000	1000	>1000	500	1000	1000	>1000	500	500	1000	>1000	500	1000	>1000	>1000	500	1000	250	>250
Salmonella enterica	1000	>1000	1000	1000	1000	>1000	500	500	1000	>1000	500	500	1000	>1000	500	1000	>1000	>1000	1000	1000	15.625	31.25
Salmonella typhimurium	1000	>1000	1000	1000	1000	>1000	500	1000	1000	1000	500	500	1000	>1000	500	1000	>1000	>1000	1000	1000	15.625	125
Candida albicans	>1000	>1000	500	1000	1000	1000	1000	1000	1000	1000	500	1000	1000	1000	500	1000	1000	1000	500	1000	62.5	1000
Rhodotorula sp.	>1000	>1000	500	1000	500	1000	500	1000	500	1000	250	500	1000	1000	250	500	500	1000	500	1000	62.5	1000
Saccharomyces boulardii	1000	>1000	500	1000	1000	>1000	1000	1000	1000	1000	500	1000	1000	1000	500	1000	500	1000	500	1000	31.25	1000
Aspergillus niger	>1000	>1000	500	1000	500	500	500	1000	500	1000	500	1000	500	1000	250	500	1000	>1000	250	500	500	1000
Aspergillus niger ATCC 16404	>1000	>1000	500	1000	>1000	>1000	1000	1000	1000	>1000	500	1000	500	1000	500	500	1000	1000	500	1000	62.5	62.5
Aspergillus restrictus	500	>1000	125	250	500	1000	500	1000	500	1000	125	250	500	1000	31.3	125	250	250	31.3	250	500	2000
Aspergillus fumigatus	62.5	62.5	62.5	62.5	62.5	250	<7.8	15.68	15.68	15.68	<7.8	<7.8	15.68	125	<7.8	<7.8	62.5	62.5	31.3	31.25	500	1000
Aspergillus flavus	31.25	250	<7.8	<7.8	1000	1000	15.7	15.68	125	125	62.5	500	125	500	62.5	62.5	125	1000	125	250	1000	1000

[a] MIC values ($\mu g/cm^3$) – means inhibitory activity.
[b] MMC values ($\mu g/cm^3$) – means microbicidal activity.

explained by a skew-chair conformation of the six-membered rings and, also, by the larger orientational freedom of the benzyl groups bonded to the S(1) and S(2) atoms. The spatial orientations of the benzyl groups are not similar, as can be seen from the values of the Pd(1)–S(1)–C(8)–C(9) and Pd(1)–S(2)–C(22)–C(23) dihedral angles (Table 2). Although the aromatic rings of the phenyl and benzyl groups seem to be over each other, the distance between them is too long for any reasonable intramolecular π···π interactions to occur. The deprotonated carboxyl groups are also twisted out of the adjacent aromatic plane, as can be seen from the C(3)–C(2)–C(1)–O(2) and C(17)–C(16)–C(15)–O(4) dihedral angles from Fig. 1.

The spatial orientation of the benzyl groups in the crystal structure is, of course, much more significantly defined by intermolecular interactions during the crystallization process, in which the attractive and repulsive contacts compete with each other and a stable balance between them must be achieved. The lack of strong hydrogen bond donors gives space for much weaker C–H···O interactions to dominate as attractive ones in the crystallization process of **C1**. Eight such interactions are found from the structure (Table 2). Three potential C–H···π-type interactions were also found. Two complexes seem to form a pair involving two C–H···O, all three C–H···π and one additional π···π contact in the x, $-y+½$, $z+½$ direction (Fig. 2).

Based on S,O-coordination of all the ligands and the crystal structure of the [Pd(*S*-bz-thiosal)$_2$] complex, it can be assumed that the other complexes occur in the form of a *cis*-S-*cis*-O geometric isomer.

3.3. Microbiology

The results of *in vitro* testing of antimicrobial activities for the five new palladium complexes are shown in Table 3. The solvent (10% DMSO) did not inhibit the growth of the tested microorganisms.

The intensity of the antimicrobial action varied depending on the species of microorganism and on the type of tested compound. In general, the activity of the complexes was higher than the corresponding ligands ($p < 0.05$). MICs and MMCs values for ligands were in range 15.68 to >1000 μg/cm^3, and for complexes <7.8 to 1000 μg/cm^3. The best effect was observed for **L3** and **L4** for the ligands, and **C3** and **C4** for the complexes ($p < 0.05$).

The palladium(II) complexes showed significant antifungal activity. The most sensitive was *A. fumigatus*, *A. flavus* and *A. restrictus*. The activity of the complexes was better than the positive control fluconazole ($p < 0.05$). The obtained concentrations of palladium(II) complexes which inhibit the growth of moulds were from <7.8 to 500 μg/cm^3. Antimicrobial testing of newly synthesized complexes of palladium(II), done by Vasić et al., led to similar results [41]. The standard and clinical strain of *A. niger* did not show sensitivity similar to that mentioned, where the MIC went from 250 to 1000 μg/cm^3.

The tested compounds did not affect the growth of yeasts or their activities were very low. The MIC and MMC values for yeasts were from 500 to >1000 μg/cm^3, except for the complexes **C3** and **C4** against *Rhodotorula* sp., where the MIC was 250 μg/cm^3.

All the tested compounds demonstrated weak and moderate antibacterial activity. The Gram-positive bacteria were more sensitive than the Gram-negative bacteria, especially for the activity of the complexes. The most sensitive was *S. aureus* with a MIC value of 125 μg/cm^3 for the complex **C5**. The MICs for Gram-negative bacteria were 500 and 1000 μg/cm^3. The tested complexes **C2** and **C3** exhibited somewhat stronger antibacterial activity towards P. aeruginosa ATCC 27853 (MIC = 250 μg/cm^3).

The probiotics showed sensitivity similar to the sensitivity of the other bacteria. The MICs were from 250 to >1000 μg/cm^3 and the MMCs were from 500 to >1000 μg/cm^3.

4. Conclusion

The results of antimicrobial activity showed that the tested ligands and the corresponding palladium(II) complexes showed different degrees of antimicrobial activity in relation to the tested species. The tested ligands, with few exceptions, showed low antimicrobial activity. The palladium(II) complexes showed selective and moderate activity. A difference in the antimicrobial activity was observed between the ligands and the corresponding palladium(II) complexes, with higher activities being displayed for the palladium(II) complexes. Interesting results were obtained for *Aspergillus* species, which are common in the environment and which cause the infection known as aspergillosis. The tested complexes reacted better than the positive control. The molecular structure in the crystalline state was obtained for complex **C1** and showed an unsymmetrical *cis* configuration around the Pd atom. The crystal structure was found to be stabilized by C–H···O, C–H···π-type and π···π interactions.

Acknowledgements

The authors are grateful for financial support from the Ministry of Education and Science of Republic of Serbia (Projects Nos. OI172016 and 41010) and the Academy of Finland (Project No. 122350).

Appendix A. Supplementary data

CCDC 824331 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223 336 033; or e-mail: deposit@ccdc.cam.ac.uk.

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