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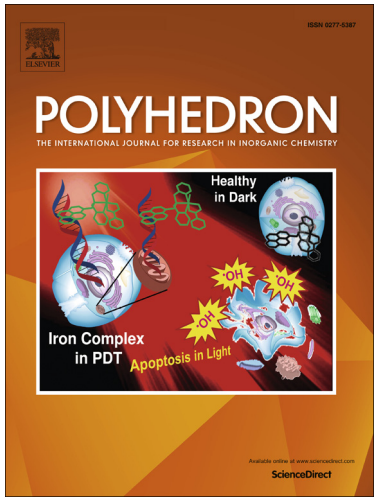

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Synthesis, characterization and antimicrobial activity of copper(II) complexes with some S-alkyl derivatives of thiosalicylic acid. Crystal structure of the binuclear copper(II) complex with S-methyl derivative of thiosalicylic acid

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Abstract

The five new copper(II) complexes with some S-alkyl derivatives of thiosalicylic acid (alkyl = = benzyl (**L1**), methyl (**L2**), ethyl (**L3**), propyl (**L4**), butyl (**L5**)) have been synthesized and characterized by microanalysis and infrared spectra. The spectroscopically predicted structure of the obtained binuclear copper(II) complex with S-methyl derivative of thiosalicylic acid was confirmed by X-ray analysis. Single crystals suitable for X-ray measurements were obtained by slow crystallization from a water solution. The compound crystallizes with two binuclear Cu(II) complex molecules in the

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asymmetric unit. Both molecules have typical paddle-wheel structure with apical positions occupied by water molecules. The independent molecules showed slight difference in configuration mainly reflected in the different orientation of the phenyl rings relating to their carboxylate groups. Antimicrobial activity of these complexes was tested by microdilution method and both minimal inhibitory and microbicidal concentration were determined. The intensity of the antimicrobial activity varied depending on the species of microorganism and the compound type. In general, the activity of the complexes was higher than or similar to the corresponding ligands. All the tested complexes demonstrated moderate or selective antibacterial activity and low antifungal activity.

Keywords: S-alkyl derivatives of thiosalicylic acid; copper(II) complexes; infrared spectra; crystal structure; antimicrobial activity

1. Introduction

The complex forming ability of thiosalicylic acid with many metal ions has been the subject of several investigations [1-6]. Also, detailed studies on the complexation equilibria of copper(II) with thiosalicylic acid have been previously published [7]. The synthesis and characterization of copper(II) complexes with thiosalicylic acid proceeded the studies on the interaction of metals with ligands containing biological and pharmacological activities. The antimicrobial activity of dinuclear and mononuclear copper(II) complexes with the COO group coordinated to Cu for some bacteria, yeast and mold was demonstrated [8-16]. The previous work reported the formation of a light blue complex containing copper(II) with thiosalicylic acid in which the metal ion occupied only one of two potential sites of the ligand: the –SH group [17]. Then, Ferrer et. al. reported synthesis and characterization of a new green dimeric complex copper(II) with thiosalicylic acid and pyridine [18].

Our investigations presented in this paper are focused on the synthesis of the corresponding copper(II) complexes of S-alkyl derivatives as well as *in vitro* antimicrobial activity of the ligands and the complexes. The preparation and spectral characterization of S-alkyl derivatives of thiosalicylic acid were published earlier [19-23]. The structures of the isolated complexes are proposed on the basis of elemental microanalysis and infrared spectra. The dimeric structures of isolated copper(II) complexes are confirmed on the basis of an X-ray structural study of copper(II) complex with S-methyl derivative of thiosalicylic acid, $[Cu_2(S\text{-met-thiosal})_4(H_2O)_2]$. Our research is are related to the impact of the newly synthesized copper(II) complexes on some pathogenic bacteria and fungi, in particular on probiotics, since being used as supplements they play a significant role in protection and maintenance of the balance of intestinal microflora during antibiotic therapy.

2. Experimental

2.1. Materials and measurements

The reagents were obtained commercially and used without further purification. Elemental analyses were done on a Vario III CHNOS Elemental Analyzer, Elemental Analysensysteme GmbH. For infrared spectra a Perkin-Elmer FTIR 31725-X spectrophotometer and KBr pellet technique were employed. The magnetic measurements of synthesized complexes were performed at 294 K by the Evans' method using a MSB-MK1 balance (Sherwood Scientific Ltd.) with $Hg[Co(SCN)_4]$ as calibrant; diamagnetic corrections were calculated from Pascal's constants.

2.2. Syntheses

*2.2.1. General procedure for the synthesis of S-alkyl derivatives of thiosalicylic acid (**L1**)–(**L5**)*

The S-alkyl derivatives of thiosalicylic acid ligands (alkyl = benzyl (**L1**), methyl (**L2**), ethyl (**L3**), propyl (**L4**), butyl (**L5**)) were prepared [19] by alkylation of thiosalicylic acid by means of the corresponding alkyl halides in alkaline water-ethanol solution.

*2.2.2. Preparation of copper(II) complex with S-benzyl derivative of thiosalicylic acid, $[Cu_2(S\text{-}bz\text{-}thiosal)_4(H_2O)_2]$ (**C1**)*

Copper(II)-nitrate trihydrate (0.1000 g, 0.4139 mmol) was dissolved in 10.0 mL of water on a steam bath and S-benzyl thiosalicylate (0.2022 g, 0.8278 mmol) was added. The reaction mixture was heated for 3 h and during this period 10.0 mL of LiOH water solution (0.0348 g, 0.8278 mmol) was added in small portions and the solution was filtered and evaporated to small volume. The blue precipitate of copper(II) complex was separated by filtration, washed with cold water and air-dried. Yield: 0.1910 g (81.21 %). *Anal.* Calc. for $[Cu_2(S\text{-bz-thiosal})_4(H_2O)_2] = Cu_2C_{56}H_{48}O_{10}S_4$ (M_r = 1136.308): C, 59.19; H, 4.26; S, 11.29. Found: C, 59.01; H, 4.18; S, 11.14. μ(294 K) = 1.56 μ_B

*2.2.3. Preparation of copper(II) complex with S-methyl derivative of thiosalicylic acid, $[Cu_2(S\text{-met-thiosal})_4(H_2O)_2]$ (**C2**)*

Copper(II)-nitrate trihydrate (0.1000 g, 0.4139 mmol) was dissolved in 10.0 mL of water on a steam bath and S-methyl derivative of thiosalicylic acid (0.1392 g, 0.8278 mmol) was added. The reaction mixture was heated for 3 h and during this period 10.0 mL of LiOH water solution (0.0347 g, 0.8278 mmol) was added in small portions and the solution was filtered. Single crystals of $[Cu_2(S\text{-met-thiosal})_4(H_2O)_2]$, (**C2**) suitable for X-ray measurements were obtained by slow crystallization from a water solution by evaporation. Yield: 0.1970 g (81.74 %). *Anal.* Calc. for $[Cu_2(S\text{-met-thiosal})_4(H_2O)_2] = Cu_2C_{32}H_{32}O_{10}S_4$ (M_r = 831.932): C, 46.20; H, 3.88; S, 15.42. Found: C, 46.17; H, 3.69; S, 15.39. μ(294 K) = 2.30 μ_B.

*2.2.4. Preparation of copper(II) complex with S-ethyl derivative of thiosalicylic acid, $[Cu_2(S\text{-et-thiosal})_4(H_2O)_2]$ (**C3**)*

Copper(II)-nitrate trihydrate (0.1000 g, 0.4139 mmol) was dissolved in 10.0 mL of water on a steam bath and S-ethyl derivative of thiosalicylic acid (0.1509 g, 0.8278 mmol) was added. The reaction mixture was heated for 3 h and during this period 10.0 mL of LiOH water solution (0.0347 g, 0.8278 mmol) was added in small portions and the solution was filtered and evaporated to small volume. The blue precipitate of copper(II) complex was separated by filtration, washed with cold water and air-dried. Yield: 0.1490 g (81.07 %). *Anal.* Calc. for $[Cu_2(S\text{-et-thiosal})_4(H_2O)_2] = Cu_2C_{36}H_{40}O_{10}S_4$ (M_r = 888.036): C, 48.69; H, 4.54; S, 14.44. Found: C, 48.55; H, 4.39; S, 14.28. μ(294 K) = 1.99 μ_B.

*2.2.5. Preparation of copper(II) complex with S-propyl derivative of thiosalicylic acid, $[Cu_2(S\text{-pr-thiosal})_4(H_2O)_2]$ (**C4**)*

Copper(II)-nitrate trihydrate (0.1000 g, 0.4139 mmol) was dissolved in 10.0 mL of water on a steam bath and S-propyl derivative of thiosalicylic acid (0.1625 g, 0.8278 mmol) was added. The reaction mixture was heated for 3 h and during this period 10.0 mL of LiOH water solution (0.0347 g, 0.8278 mmol) was added in small portions and the solution was filtered and evaporated to small volume. The blue precipitate of copper(II) complex was separated by filtration, washed with cold water and air-dried. Yield: 0.1590 g (81.37 %). *Anal.* Calc. for $[Cu_2(S\text{-pr-thiosal})_4(H_2O)_2] = Cu_2C_{40}H_{48}O_{10}S_4$ (M_r = 944.140): C, 50.88; H, 5.12; S, 13.58. Found: C, 50.71; H, 5.04; S, 13.48. μ(294 K) = 1.85 μ_B

*2.2.6. Preparation of copper(II) complex with S-butyl derivative of thiosalicylic acid, $[Cu_2(S\text{-bu-thiosal})_4(H_2O)_2]$ (**C5**)*

Copper(II)-nitrate trihydrate (0.1000 g, 0.4139 mmol) was dissolved in 10.0 mL of water on a steam bath and S-butyl derivative of thiosalicylic acid (0.1741 g, 0.8278 mmol) was added. The reaction mixture was heated for 3 h and during this period 10.0 mL of LiOH water solution (0.0347 g, 0.8278 mmol) was added in small portions and the solution was filtered and evaporated to small volume. The blue precipitate of copper(II) complex was separated by filtration, washed with cold water and air-dried. Yield: 0.1680 g (81.20 %). *Anal.* Calc. for $[Cu_2(S\text{-bu-thiosal})_4(H_2O)_2] = Cu_2C_{44}H_{56}O_{10}S_4$ (M_r = 1000.244): C, 52.83; H, 5.64; S, 12.82. Found: C, 52.75; H, 5.58; S, 12.71. μ(294 K) = 1.80 μ_B

2.3. Single crystal X-ray crystallography

Single crystals of $[Cu_2(S\text{-met-thiosal})_4(H_2O)_2]$, (**C2**) suitable for X-ray measurements were obtained by slow crystallization from a water solution. Single-crystal diffraction data for **C2** were collected at room temperature on an Agilent Gemini S diffractometer equipped with Cu$K\alpha$ radiation (λ = 1.5418 Å). Data reduction and empirical absorption corrections were accomplished using CrysAlisPro [24]. Crystal structure was solved by direct methods, using SIR2002 [25] and refined using SHELXL program [26]. The hydrogen atoms attached to C atoms were placed at geometrically idealized positions with C–H distances fixed to 0.93 and 0.96 Å from phenyl and methyl C atoms, respectively. Their isotropic displacement parameters were set equal to $1.2U_{eq}$ and $1.5U_{eq}$ of the parent phenyl and methyl C atoms. The hydrogen atoms of the apical water ligands were located in difference Fourier map and refined isotropically. The crystallographic data are listed in Table 1. The PARST [27] and PLATON [28] programs were used to perform geometrical calculation and the programs ORTEP [29] and Mercury [30] were employed for molecular graphics.

176

177 *2.4. In vitro antimicrobial assay*

178

179 *2.4.1. Test substances*

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

185

186 *2.4.2. Test microorganisms*

187 The antimicrobial activity of the copper(II) complexes **C1**–**C5** was tested against 18
188 microorganisms. The experiment involved 8 strains of pathogenic bacteria, including two
189 standard strains (*Escherichia coli* ATCC 25922 and *Bacillus subtilis* ATCC 6633) and six
190 clinical isolates (*Escherichia coli, Enterococcus faecalis*, *Bacillus subtilis, Proteus mirabilis,*
191 *Salmonella enterica, Salmonella typhimurium*). Also, three species of probiotic bacteria
192 (*Lactobacillus plantarum* PMFKG-P31, *Bacillus subtilis* IP 5832 PMFKG-P32,
193 *Bifidobacterium animalis subsp. lactis* PMFKG-P33), three mould species (*Aspergillus niger*
194 ATCC 16404, *Aspergillus flavus* PMFKG-F24, *Botrytis cinerea* PMFKG-F33) and three yeast
195 species (*Candida albicans* ATCC 10231, *Candida albicans,* clinical isolate, *Rhodotorula* sp.
196 PMFKG-F27, *Saccharomyces boulardii* PMFKG-P34) were tested. All clinical isolates were
197 a generous gift from the Institute of Public Health, Kragujevac. The other microorganisms
198 were provided from the collection held by the Microbiology Laboratory Faculty of Science,
199 University of Kragujevac.

200

2.4.3. Suspension preparation

Bacterial and yeast suspensions were prepared by the direct colony method. The colonies were taken directly from the plate and suspended in 5 mL of sterile 0.85 % saline. The turbidity of the initial suspension was adjusted by comparing it with 0.5 McFarland's [31]. When adjusted to the turbidity of the 0.5 McFarland's standard, the bacterium suspension contains about 10^8 colony forming units (CFU)/mL and the suspension of yeast contains 10^6 CFU/mL. 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.

2.4.4. 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 [32]. 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/mL) 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 7.8 μg/mL. The method is described in detail in the reported paper [19].

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. 10 % DMSO was recorded not to inhibit the growth of microorganisms. Also, the concentration of DMSO in the experiment 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 the 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.

3. Results and discussion

3.1. Synthesis and chemical characterization

S-alkyl (R = benzyl (**L1**), methyl (**L2**), ethyl (**L3**), propyl (**L4**) and butyl (**L5**)) derivatives of thiosalicylic acid were prepared [19] by alkylation of thiosalicylic acid by means of the corresponding alkyl halogenides in alkaline water-ethanol solution (Scheme 1). The corresponding $[Cu_2(S\text{-}R\text{-}thiosal)_4(H_2O)_2]$ complexes were obtained by direct reaction of $Cu(NO_3)_2$ with S-alkyl derivatives of thiosalicylic acid (molar ratio 1:2) in water solution with satisfactory yields (more then 80 %) (Scheme 2).

Infrared spectra of the isolated complexes were measured in order to find coordination mode of the S-alkyl derivatives of thiosalicylic acid. The asymmetric stretching frequencies of carboxylic group were specially used to determine whether this carboxylic group was coordinated (the absorption bands are located in the region 1600-1650 cm^{-1}) or uncoordinated (the absorption bands are located in the region 1700-1750 cm^{-1}) to the metal ion [33-35].

The isolated $[Cu_2(S\text{-}R\text{-}thiosal)_4(H_2O)_2]$ complexes show double sharp and strong asymmetric stretching frequencies of the carboxylic groups of the coordinated S-alkyl derivatives of thiosalicylic acid to Cu(II) ion at about 1565-1620 cm^{-1} (Table 2). The observed

clear double bands for isolated complexes suggest the small differences in the coordination of carboxylic groups of the ligands to the Cu(II) ion. Based on the content of the complexes and the shape of thier IR spectra it can not be concluded with certainty how S-alkyl derivatives of thiosalicylic acid are coordinated to Cu(II) ion. Although we expected the same coordination type of S-alkyl derivatives of thiosalicylic to the Cu(II) as to Pd(II) ion [19], the proper molecular structure was obtained on the basis of X-ray analysis of $[Cu_2(S\text{-met-thiosal})_4(H_2O)_2]$ complex. Also the strong sharp single symmetric stretching bands of the coordinated carboxylic groups of the S-alkyl derivatives of thiosalicylic acid lie in the expected region (about 1400 cm^{-1}) [34,35].

3.2. Magnetic measurements

Dinuclear cupper(II) carboxylates complexes (18, 36) are stable in dimeric form. The low value of μ_{eff} at room temperature (1.20-2.30 BM) is indicative of an antiferromagnetic interaction between the two metal centers typical of binuclear carboxylates of copper(II) of the type: $[Cu(R\sim COO)_2L]_2$ (37-39). The main factor determining the magnitude of the antiferromagnetic interaction in the dimeric copper(II) carboxylates is the electronic structure of the bridging OCO moiety as published earlier (18, 36-39).

3.3. Structural description of the complex $[Cu_2(S\text{-met-thiosal})_4(H_2O)_2]$, ***(C2)***

The crystal structure of $[Cu_2(S\text{-met-thiosal})_4(H_2O)_2]$ complex consists of two crystallographically independent dinuclear Cu(II) complex molecules (A and B), each with inversion center located between the metal ions. In both molecules the pairs of Cu(II) are bridged by four S-methyl-thiosalicylate ligands forming a so-called “paddle-wheel” type

structure. Fig. 1 shows the molecular structure and atom numbering scheme of molecule A. The structure of molecule B and equivalent numbering scheme are given in Fig. S1 (Supplementary Material file). The distances between the Cu atoms in A and B are 2.6220(6) and 2.6180(6) Å, respectively which is closely comparable to the Cu…Cu distance found in dimeric copper(II) acetate (2.614 Å) [40]. Both Cu centers (Cu1 and Cu2) display a square-pyramidal coordination geometry with the four oxygen atoms from symmetry related carboxylate ligands placed at the corners of the basal plane. In both molecules the apical positions of the copper coordination polyhedron are occupied by the water O atoms. The Cu1 and Cu2 atoms deviate from the basal plane towards the apical ligand for 0.2011(8) and 0.1964(8) Å, respectively.

The geometrical parameters listed in Table 3 show slight differences in conformation of the A and B molecules. The different distortion in the square-pyramidal environment of each Cu(II) is evidenced from the range of coordination angles [86.80(7)-101.87(7)° in A and 88.03(8)-99.71(8)° in B] as well as from variation in lengths for the basal Cu1−O and Cu2−O bonds [1.952(2)-1.984(2) Å in A and 1.948(2)-1.975(2) Å in B]. The bonds to the axial water molecules have nearly the same lengths (Table 3); these ligands however, show slightly different bending with regard to Cu…Cu direction, resulting in O1w−Cu1−$Cu1^{i}$ and O2w−Cu2−$Cu2^{ii}$ angles of 172.39(6) and 168.15(5)° (symmetry codes: (i) −x+1, −y, −z+1; (ii) −x+1, −y+1, −z). The geometrical parameters in both molecules are within those found in corresponding O-methyl derivative [41].

The most evident structural difference between two independent molecules of **C2** includes their S-methyl-thiosalicylate ligands with phenyl rings displaying different rotation with respect to the corresponding carboxylate groups (Fig. S2). Thus, in molecule A the rotation angles of the C2a/C7a and C2b/C7b rings with regard to their COO moieties are 7.9(3) and 17.1(4)°, while in molecule B the corresponding rotation angles of the C2c/C7c

and C2d/C7d rings are 24.3(2) and 5.8(2)°. Another noticeable difference between the A and B molecules regards their paddle wheel cores, $Cu_2C_4O_8$, which seems to present a different degree distortion. Bearing in mind the fine differences in coordination environment of Cu1 and Cu2 this is not unexpected. One should however point to the pronounced dissimilarity in the pairs of Cu–O–C angles formed by bridging coordination of carboxylate ligand to the pair of Cu atoms. Thus, in A molecule the coordination of one S-methyl-thiosalicylate ligand (ring C2a/C7a) gives the pair of angles, Cu1–O1a–C1a and $Cu1^{i}$–O2a–C1a, with the very dissimilar values of 116.6(1) and 130.1(1)°, respectively whereas the coordination of the second ligand (ring C2b/C7b) results in Cu1–O1b–C1b and $Cu1^{i}$–O2b–C1b angles with values of 126.2(1) and 120.1(1)°. For B molecule these differences are less visible, and the angles Cu2–O1c–C1c and $Cu2^{ii}$–O2c–C1c have the values of 127.4(2) and 119.3(2)°, while the angles Cu2–O1d–C1d and $Cu2^{ii}$–O2d–C1d are equal to 124.9(1) and 122.2(1)°, respectively. These noticeable variations in the pairs of Cu–O–C angles (from 2.7 to 14.4°) formed by the same type of ligand indicate a considerable influence of the crystal packing on the shape and deformation of two paddle wheel cores of A and B.

The examination of the crystal packing of **C2**, as expected, shows the dominance of O–H…O hydrogen bonds which involve the axial ligands as donors and the carboxilic oxygens as acceptors. Three interactions, O1w–H11…O1c, O1w–H12…O1d and O2w–H21…O1b (Table 4), interconnect the independent molecules to form an infinite ABAB chain (Fig. 2). The connection between the A and B molecules within the chain is reinforced by two significant O–H…S interactions, engaging the S acceptors to form the phenyl substituents (Table 4). The formed ABAB chain (Fig. 2) could be considered as the main structural motif of **C2**. It can be noted that the O–H…O interactions do not involve O1a and O2a atoms suggesting that the distortion in the peddle wheel of A (especially visible in angles involving O1a and O2a) could not be directly related to the strong hydrogen bonds. In

order to search for other potential causes of peddle wheel deformation in **C2** the similar crystal structures found in Cambridge Structural Databank (CSD) have also been analyzed [42].

A CSD search for Cu(II) paddle wheel complexes comprising aromatic carboxylate (ArCOO) and water ligands, $[Cu_2(ArCOO)_4(H_2O)_2]$, resulted in 26 examples. The geometrical properties of the **C2** compound are in good agreement with those observed for the Cu(II) complexes extracted from CSD. One can suggest several common features in the crystal structures of $[Cu_2(ArCOO)_4(H_2O)_2]$ complexes: (i) as in **C2** the substituted phenyl rings of the different carboxylate ligands in 26 crystal structures display a free rotation with respect to their COO moiety resulting in a wide range of corresponding dihedral angles. In accordance with the previous findings [43] the rotation of phenyl ring increases in the case of the structures with *ortho*-substituted aromatic ligands compared to those with *meta*- and *para*-substituted ligands (Fig. S3); (ii) the paddle wheel cores, $Cu_2C_4O_8$, permit a considerable degree of distortion. This is mostly reflected in visible differences of two Cu−O−C angles formed by a bridging carboxylate ligand. Thus the intetrakis(μ_2-2-Hydroxycarbamoylbenzoato-O,O')-diaqua-di-copper(II) complex [44] (CSD refcode VEYCUY) the Cu1−O1−C1 and Cu1−O2−C1angles differ for 2.5°, whereas in the tetrakis(μ_2-4-Hydroxy-3-methoxybenzoato-O,O')-diaqua-di-copper(II) complex [45] (CSD refcode GUSCAY01) these two angles differ for 12°. At the same time the corresponding pairs of Cu−O bonds have nearly the same length; (iii) among the 26 extracted $[Cu_2(ArCOO)_4(H_2O)_2]$ peddle wheel complexes 15 display the chain-like structural motif similar to that observed in **C2** (Fig. 2). The axial water ligands take place in the region of carboxyl O atoms of the neighboring molecule and form at least one pair of O−H…O hydrogen bonds eventually producing a chain of molecules (Fig. S3). This arrangement can be found even in structures containing solvents or additional acceptors on phenyl substituents.

One can also observe that the connection of complex molecules into the chain requires successive rotation of their closely adjacent substituted rings in order to reduce the potential steric hindrance (Fig. S3). In contrast to other examples, the chain formed in **C2** (Fig. 2) includes two types of symmetrically independent molecules. While in molecule B the ring C2c/C7c shows the largest twisting with respect to its COO group [24.3(2)°], the rotation of the adjacent C2a/C7a ring in molecule A [7.9(3)°] is partly prevented by the relatively strong O–H…S hydrogen bond. Therefore the visible distortion of the paddle wheel core observed in A molecule could serve in additionally separating the adjacent phenyl rings (C2a/C7a and C2c/C7c in Fig. 2 denoted as *a* and *c*) and decreasing their potential repulsion.

Apart from the hydrogen bonding arranging the molecules into ABAB chain there are no further significant interactions between A and B molecules. The three-dimensional crystal packing of **C2** viewed down the *b* axis shows the separate blocks of A and B molecules parallel to (001), (Fig. 3a). Inside the corresponding blocks the A molecules mutually interact by means of C–H…π interactions, while B molecules employ their least rotated C2d/C7d rings [5.8(2)°] to interconnect by weak π…π interaction (Fig. 3b,c).

3.4. Microbiology

The results of *in vitro* testing of antimicrobial activities for the five new copper(II) complexes are shown in Tables 5 and 6. For comparison, MIC and MMC values of the corresponding ligands [19] and doxycycline and fluconazole are also listed in the same tables. 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 the tested compound type. MIC and MMC values for complexes were in range 31.3 to >1000 μg/mL. In general, the activity of the complexes was higher than or

similar to the corresponding ligands. The exceptions are filamentous fungi, particularly *Aspergillus flavus*, where the ligands have higher activity.

Overall the copper(II) complexes showed low antifungal activity. 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/mL, except for the complexes **C2** and **C3** against *Rhodotorula* sp., where the MIC was 250 μg/mL.

All the tested complexes demonstrated moderate or selective antibacterial activity. The probiotics showed sensitivity similar to the sensitivity of the other Gram-positive bacteria. *Lactobacillus plantarum* showed somewhat higher resistance to the tested complexes. The Gram-positive bacteria *Bacillus subtilis* and probiotics *Bif. animalis subsp. lactis, Bacillus subtilis* IP 5832, were more sensitive than the other Gram-positive and Gram-negative bacteria. The most sensitive was *Bif. animalis subsp. lactis* with a MIC value of 31.3 μg/mL for the complexes **C2** and **C4**. The MICs for Gram-negative bacteria were in range 250 to > 1000 μg/mL. The tested complex **C2** exhibited somewhat stronger antibacterial activity to *Escherichia coli, E. coli* ATCC 25922 and *Salmonella enterica* (MIC = 250 μg/mL).

The previous research of antimicrobial activity of dinuclear and mononuclear copper(II) complexes with the COO group coordinated to Cu provides diverse conclusions. Basically, in most studies, as in ours weak antifungal activity is observed.

In some studies [8] the lack of specificity (and of activity) of the Cu(II) complex against all the tested Gram(−) and Gram(+) bacteria suggests that the sensitivity of test organisms to test compounds is not associated to the different cell wall structures.

Some research suggests that the Cu(II) complexes exhibit mild antimicrobial activities. The result revealed that copper complexes displayed inhibition still significantly lower than the standard drugs [10]. The results of the antibacterial activity of the tested Cu(II) complexes showed moderate activity against *E. coli* and *S. aureus* when compared to the standard drug,

401 tetracycline [11]. The same Cu(II) complexes either demonstrate the limited activity against
402 *A. flavus* and *C. albican* or not [11]. The water-insoluble Cu(II) dicarboxylate complex and
403 the water–soluble Cu(II) simple salts were inactive against all of the microorganisms. These
404 data indicate that the decrease of the ligand solubility (via complex formation) lowers the
405 bioavailablility of the dicarboxylates. Some Cu(II) complexes demonstrated no significant
406 activity whilst the phen adducts were active against *S. aureus* MRSA, *E. coli* and *Patonea*
407 *agglumerans*. Against *C. albicans* the phen-containing Cu(II) complexes had significantly
408 lower antifungal activity comparable to those of the commercial antifungal agent
409 ketoconazole. Thus, unlike the antibacterial studies, whereby formation of a Cu(II) phen
410 complex enhances the antibacterial activity of the phen ligand, the anti-Candida activity is
411 reduced upon complex formation [12]. The Cu(sparfloxacinato)(N-donor)Cl complexes are
412 among the most active ones against *Escherichia coli, Pseudomonas aeruginosa* and
413 *Staphylococcus aureus*, when compared to other corresponding copper–quinolone complexes
414 and their antimicrobial activity is increased in the order bipyam < bipy = phen [13].

415 While some binary Cu(II) complexes have a low effect on *A. flavus* and *C. albicans*,
416 ternary do not have it at all. The same binary and ternary Cu(II) complexes act on bacteria
417 better than tetracycline [14].

418 Some studies show the good antimicrobial activity of Cu(II) complexes in range of
419 standard drugs (ciprofloksacin/griseofulvin) [9,15]. Some Cu(II) complexes are good
420 antimicrobial agents (in the range of standard drugs such as ciprofloxacin/griseofulvin) [16]
421 compared to those reported for analogous ternary complexes [9,46].

422

423 **4. Conclusion**

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The five new copper(II) complexes with some S-alkyl derivatives of thiosalicylic acid (alkyl = benzyl, methyl, ethyl, propyl, butyl) have been synthesized and characterized by microanalysis and infrared spectra. Both independent paddle-wheel complex molecules (A and B) in the crystal structure of $[Cu_2(S\text{-met-thiosal})_4(H_2O)_2]$ interact by means of O–H…O and O–H…S interactions and form ABAB chain. In crystal packing, A molecules develop extensive C–H…π interconnections, while B molecules form the distinct chain by π…π stacking interactions. The intensity of the antimicrobial activity varied depending on the species of microorganism and the tested compound type. All the tested complexes demonstrated moderate or selective antibacterial activity and low antifungal activity.

Appendix A. Supplementary data

CCDC 974908 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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452 **References**

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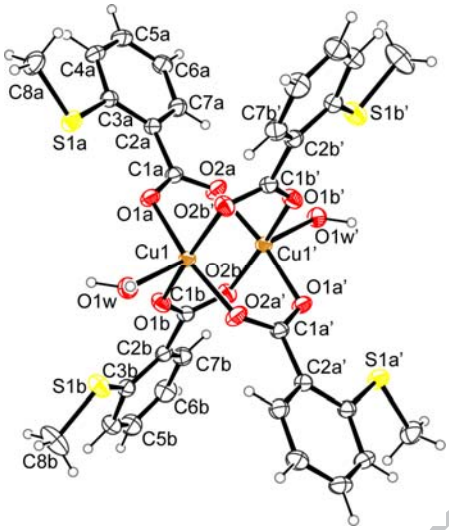


Fig. 1. Crystal structure and the atom numbering scheme of A molecule. Structure of B molecule and equivalent numbering scheme are given in Fig. S1

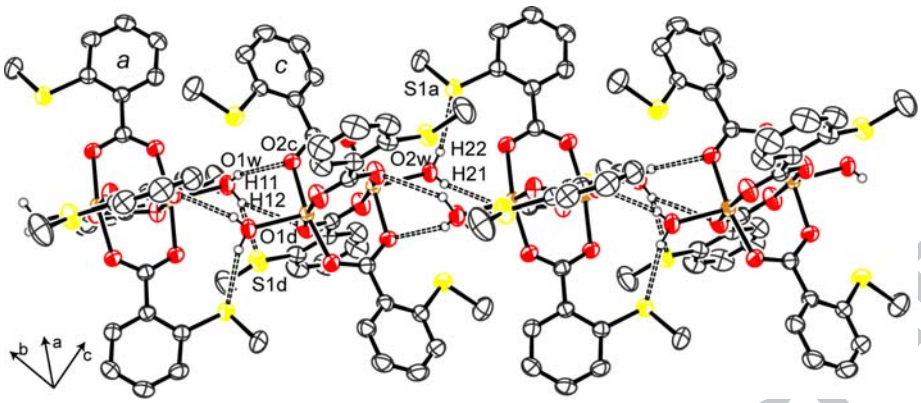


Fig. 2. A view of a chain consisting of hydrogen bonded A and B molecules

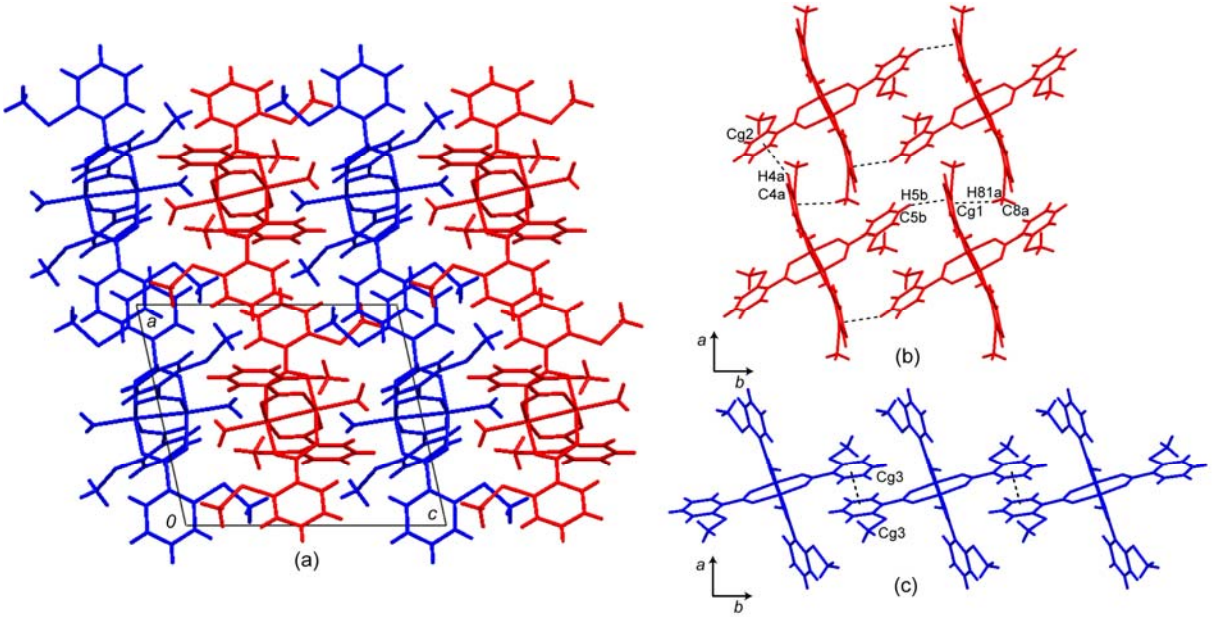
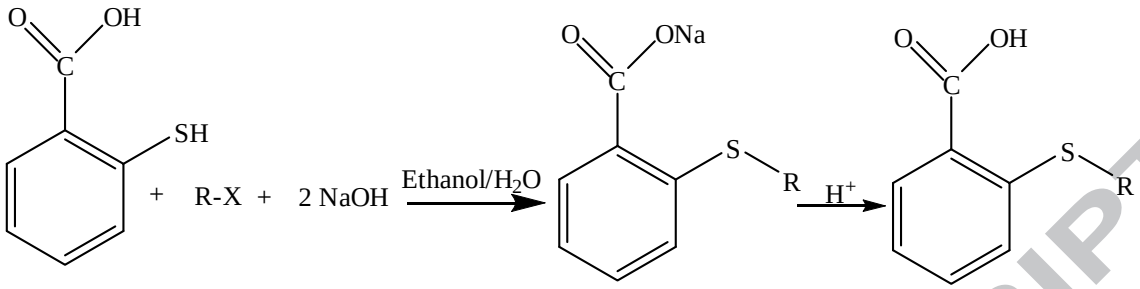
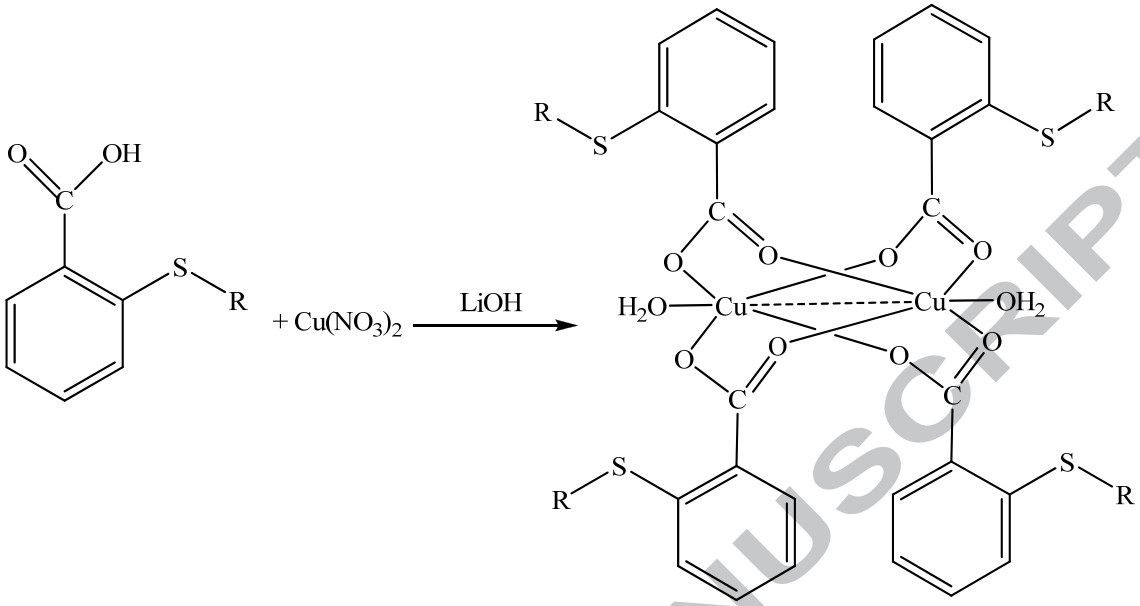


Fig. 3. (a) Blocks of A (red) and B (blue) molecules viewed done b axis; (b) C−H…π interactions between the A molecules; (c) π…π interaction between the B molecules



R= Benzyl (**L1**), methyl (**L2**), ethyl (**L3**), propyl (**L4**), butyl (**L5**)

Scheme 1. The preparation of the benzyl (**L1**), methyl (**L2**), ethyl (**L3**), propyl (**L4**) and butyl (**L5**) derivatives of thiosalicylic acid



R= Benzyl (**C1**), methyl (**C2**), ethyl (**C3**), propyl (**C4**), butyl (**C5**)

Scheme 2. The preparation of the binuclear copper(II) complex with S-alky derivatives of thiosalicylic acid

525 **Table 1.** Crystallographic data for **C2**.

Empirical formula	$C_{32}H_{32}O_{10}Cu_2S_4$
Formula weight	831.90
Color, crystal shape	Green, prism
Crystal size (mm^3)	0.14 x 0.09 x 0.05
Temperature (K)	293(2)
Wavelength (Å)	1.5418
Crystal system	Triclinic
Space group	*P-1*
Unit cell dimensions	
a (Å)	11.3834(5)
b (Å)	11.8107(4)
c (Å)	13.9707(7)
α (°)	69.317(4)
β (°)	77.826(4)
γ (°)	88.925(3)
V ($Å^3$)	1714.54(13)
Z,Z'	2,1
D_{calc} (Mg/m^3)	1.611
μ (mm^{-1})	4.288
θ range for data collection (°)	3.47 to 72.30
Reflections collected	12035
Independent reflections, R_{int}	6612, 0.0218
Completeness (%) to θ = 67°	99.9
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	6612 / 0 / 453
Goodness-of-fit on F^2	1.041
Final R_1/wR_2 indices [$I > 2\sigma(I)$]	0.0328/ 0.0894
Final R_1/wR_2 indices (all data)	0.0384/ 0.0935
Largest diff. peak and hole (e $Å^{-3}$)	0.492/–0.276

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530 **Table 2.** The most important infrared bands (cm^{-1}) of the investigated compounds.

Compound	-S-R	$-COO^-$ (as)	$-COO^-$ (sim)
S-met-thiosal	699(m)	1672(s)	1412(s)
$[Cu_2(S\text{-met-thiosal})_4(H_2O)_2]$	697(m)	1596(s)	1411(s)
		1581(s)	
S-et-thiosal	704(m)	1682(s)	1414(s)
$[Cu_2(S\text{-et-thiosal})_4(H_2O)_2]$	697(m)	1619(s)	1399(s)
		1592(s)	
S-pr-thiosal	702(m)	1678(s)	1410(s)
$[Cu_2(S\text{-pr-thiosal})_4(H_2O)_2]$	704(m)	1614(s)	1401(s)
		1594(s)	
S-bu-thiosal	703(m)	1678(s)	1414(s)
$[Cu_2(S\text{-bu-thiosal})_4(H_2O)_2]$	696(m)	1590(s)	1403(s)
		1570(s)	
S-bz-thiosal	710(m)	1675(s)	1412(s)
$[Cu_2(S\text{-bz-thiosal})_4(H_2O)_2]$	696(m)	1589(s)	1403(s)
		1567(s)	

531 s-strong, m-medium
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537 **Table 3.** Selected bond lengths (Å) and angles (°) for two independent molecules of **C2.**
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A		B	
Cu1–Cu1^{i}	2.6220(6)	Cu2–Cu2^{ii}	2.6180(6)
Cu1–O2a^{i}	1.952(16)	Cu2–O2d^{ii}	1.9475(16)
Cu1–O1b	1.9641(16)	Cu2–O1d	1.9603(16)
Cu1–O1a	1.9817(15)	Cu2–O1c	1.9664(16)
Cu1–O2b^{i}	1.9845(15)	Cu2–O2c^{ii}	1.9751(16)
Cu1–O1w	2.1463(18)	Cu2–O2w	2.1453(18)
O1b–C1b	1.253(3)	O1c–C1c	1.256(3)
O2a–C1a	1.257(3)	O2c–C1c	1.256(3)
O1a–C1a	1.259(3)	O2d–C1d	1.258(3)
O2b–C1b	1.268(3)	O1d–C1d	1.259(3)
O1a–Cu1–O2b^{i}	86.80(7)	O1d–Cu2–O2c^{ii}	88.03(8)
O2a^{i}–Cu1–O1b	89.61(8)	O1d–Cu2–O1c	88.88(8)
O1b–Cu1–O1a	89.81(7)	O2d^{ii}–Cu2–O2c^{ii}	90.27(8)
O2a^{i}–Cu1–O1w	89.85(7)	O2d^{ii}–Cu2–O1c	90.52(8)
O2a^{i}–Cu1–O2b^{i}	91.39(8)	O2c^{ii}–Cu2–O2w	99.71(8)
O1b–Cu1–O1w	93.79(8)	O2d^{ii}–Cu2–O2w	91.79(8)
O2b^{i}–Cu1–O1w	98.10(7)	O1d–Cu2–O2w	97.26(7)
O1a–Cu1–O1w	101.87(7)	O1c–Cu2–O2w	99.71(8)
O1b–Cu1–O2b^{i}	168.06(7)	O1c–Cu2–O2c^{ii}	168.39(7)
O2a^{i}–Cu1–O1a	168.28(7)	O2d^{ii}–Cu2–O1d	168.45(7)

539 Symmetry codes: (i) −x+1, −y, −z+1; (ii) −x+1, −y+1, −z
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544 **Table 4.** Hydrogen bonding geometry and intermolecular interactions (Å,°) in the crystal
545 structure of **C2** (Cg is midpoint of corresponding phenyl ring).

D–H…A	D–H	D…A	H…A	D–H…A
O1w–H11…O2c	0.66(4)	2.991(3)	2.38(4)	155(4)
O1w–H12…S1d^{ii}	0.92(4)	3.247(2)	2.37(3)	157(4)
O1w–H12...O1d^{ii}	0.93(4)	3.193(2)	2.54(3)	127(3)
O2w–H21...O2b^{ii}	0.67(3)	2.882(2)	2.23(3)	162(4)
O2w–H22…S1a^{ii}	0.85(5)	3.228(3)	2.39(5)	166(4)
C4a-H4a…Cg2^{iii}	0.93	3.810(3)	3.03	142
C5b-H5b…Cg1 ^{iv}	0.93	3.738(3)	2.94	144
C8a-H81b…Cg1^{v}	0.96	3.770(4)	3.00	137
Cg3…Cg3^{vi}		3.784(2)		

546 Symmetry codes: (iii) x, y+1, z; (iv) −x+2, −y, −z+1; (v) −x+1, −y+1, −z+1; (vi) –x, –y+1, –z
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Table 5. *In vitro* antimicrobial activity of the ligands **L1**–**L3** [19] and the copper(II) complexes **C1**–**C3**.

	L1		**C1**		**L2**		**C2**		**L3**		**C3**	
Species	MIC[1]	MMC[2]	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC
Lactobacillus plantarum	500	1000	250	1000	500	500	500	1000	500	>1000	500	>1000
Bif. animalis subsp. lactis	500	500	125	250	500	500	31.3	125	1000	1000	62.5	250
Bacillus subtilis IP 5832	500	500	250	1000	500	500	500	1000	1000	>1000	500	1000
Bacillus subtilis	500	500	62.5	500	125	500	250	500	1000	>1000	125	500
B. subtilis ATCC 6633	500	500	1000	>1000	1000	1000	1000	1000	>1000	>1000	1000	>1000
Enterococcus faecalis	1000	>1000	1000	1000	1000	>1000	1000	1000	>1000	>1000	1000	1000
Escherichia coli	>1000	>1000	1000	1000	1000	>1000	250	500	1000	>1000	500	500
E. coli ATCC 25922	1000	>1000	500	1000	1000	>1000	250	250	>1000	>1000	250	500
Proteus mirabilis	1000	>1000	1000	>1000	1000	>1000	1000	1000	1000	>1000	1000	1000
Salmonella enterica	1000	>1000	1000	1000	1000	>1000	250	500	1000	>1000	500	1000
Salmonella typhimurium	1000	>1000	1000	1000	1000	>1000	500	1000	1000	1000	500	1000
Candida albicans	>1000	>1000	>1000	>1000	1000	1000	1000	1000	1000	1000	1000	>1000
C. albicans ATCC 10231	nt	nt	>1000	>1000	nt	nt	1000	1000	nt	nt	1000	1000
Rhodotorula sp.	>1000	>1000	>1000	>1000	500	1000	250	1000	500	1000	250	1000
Saccharomyces boulardii	1000	>1000	1000	1000	1000	>1000	1000	1000	1000	1000	1000	1000
Aspergillus flavus	31.3	250	>1000	>1000	1000	1000	1000	>1000	125	125	250	>1000
A. niger ATCC 16404	>1000	>1000	>1000	>1000	>1000	>1000	1000	1000	1000	>1000	>1000	>1000
Botrytis cynerea	nt	nt	500	500	nt	nt	500	500	nt	nt	1000	1000

[1]MIC values (µg/mL) – inhibitory activity; [2]MMC values (µg/mL) – microbicidal activity; nt - not tested

559 **Table 6.** *In vitro* antimicrobial activity of the ligands **L4**–**L5** [19] and the copper(II) complexes **C4**–**C5**.

Species	L4		C4		L5		C5		Doxycycline/ Fluconasole	
	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC
Lactobacillus plantarum	250	>1000	500	>1000	1000	>1000	1000	>1000	0.5	7.8
Bifido. anim. subsp. lactis	500	1000	31.3	250	1000	1000	250	500	31.3	62.5
Bacillus subtilis IP 5832	500	500	500	1000	1000	>1000	500	1000	2	15.6
Bacillus subtilis	500	1000	250	500	1000	>1000	500	>1000	0.1	2
B. subtilis ATCC 6633	1000	>1000	1000	1000	1000	>1000	>1000	>1000	2	31.3
Enterococcus faecalis	1000	>1000	1000	1000	>1000	>1000	>1000	>1000	7.8	62.5
Escherichia coli	1000	>1000	250	500	>1000	>1000	1000	>1000	7.8	15.6
E. coli ATCC 25922	1000	>1000	500	500	>1000	>1000	1000	1000	15.6	31.3
Proteus mirabilis	1000	>1000	1000	1000	>1000	>1000	1000	>1000	250	> 250
Salmonella enterica	1000	>1000	500	1000	>1000	>1000	500	1000	15.6	31.3
Salmonella typhimurium	1000	>1000	500	1000	>1000	>1000	1000	1000	15.6	125
Candida albicans	1000	1000	1000	1000	1000	1000	1000	1000	62.5	1000
C. albicans ATCC 10231	nt	nt	1000	1000	nt	nt	1000	1000	31.3	1000
Rhodotorula sp.	1000	1000	500	1000	500	1000	500	1000	62.5	1000
Saccharomyces boulardii	1000	1000	500	1000	500	1000	500	500	31.3	1000
Aspergillus flavus	125	500	1000	>1000	125	1000	500	1000	1000	1000
A. niger ATCC 16404	500	1000	1000	1000	1000	1000	>1000	>1000	62.5	62.5
Botrytis cynerea	nt	nt	1000	1000	nt	nt	250	250	31.3	500

560 [1]MIC values (µg/mL) – inhibitory activity; [2]MMC values (µg/mL) – microbicidal activity; nt - not tested
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The complexes have been obtained by direct reaction of copper(II)-nitrate trihydrate with S-alkyl derivatives of thiosalicylic acid (alkyl = benzyl (**L1**), methyl (**L2**), ethyl (**L3**), propyl (**L4**), butyl (**L5**)). The spectroscopically predicted structure of the obtained binuclear copper(II)--complex with S-methyl derivative of thiosalicylic acid was confirmed by X-ray analysis. Antimicrobial activity of these complexes was tested by microdilution method and both minimal inhibitory and microbicidal concentration were determined.