Scientific paper

Synthesis, Crystal Structures and Antibacterial Activities of *N,N'*-Ethylene-bis(3-bromosalicylaldimine) and Its Copper(II) and Cobalt(III) Complexes

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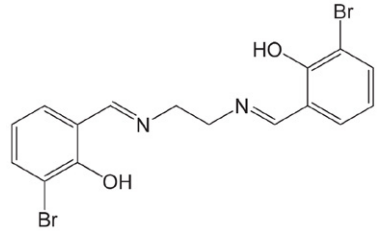
Abstract

A bis-Schiff base *N,N'*-ethylene-bis(3-bromosalicylaldimine) (H_2L) was prepared from 3-bromosalicylaldehyde and ethane-1,2-diamine. With H_2L as ligand, a new copper(II) complex [CuL] (**1**) and a new cobalt(III) complex [CoL(NCS)(DMF)] (**2**) were prepared and characterized by physico-chemical methods and single crystal X-ray analysis. X-ray analysis indicates that the Cu atom in complex **1** is in square planar coordination, and the Co atom in complex **2** is in octahedral coordination. The compounds were tested *in vitro* for their antibacterial activities on *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas fluorescens*. Both complexes have effective activities on the bacteria.

Keywords: Schiff base; copper complex; cobalt complex; crystal structure; antibacterial activity

1. Introduction

Schiff bases bearing typical group C=N are important ligands in coordination chemistry, which are readily prepared by the condensation reaction of carbonyl containing compounds with organic amines. Schiff bases with donor atoms (N, O, S) have structure similarities with natural biological systems and due to the presence of imine group, are utilized in biological systems.[1] A great number of literature reported that they have interesting antibacterial, antifungal and antitumor activities.[2] It is well known that some biological activities, when administered as metal complexes, are being increased.[3] Bis-Schiff bases derived from ethane-1,2-diamine, propane-1,3-diamine, propane-1,2-diamine and cyclohexyl-1,2-diamine with salicylaldehyde and its analogues are widely used tetradentate ligands.[4] It was reported that salicylaldehyde derivatives with halo atoms in the aromatic ring showed variety of biological activities, especially antibacterial activities.[5] Schiff base copper and cobalt complexes have shown remarkable antibacterial properties.[6] In addition, thiocyanate anion is a co-ligand in most Schiff base cobalt complexes.[7] In the present work, two new copper(II) and cobalt(III) complexes, [CuL] (**1**) and [CoL(NCS)(DMF)] (**2**), where L is the dianionic form of *N,N'*-ethylene-bis(3-bromosalicylaldimine) (H_2L), are prepared and characterized. The antibacterial activities against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas fluorescens*, were studied.



Scheme 1. The bis-Schiff base H_2L

2. Experimental

2. 1. Materials and Measurements

3-Bromosalicylaldehyde and ethane-1,2-diamine with AR grade were obtained from Aldrich and used as received. Copper nitrate and cobalt nitrate were purchased from TCI. Ammonium thiocyanate was purchased from Aladin Chemical Co. Ltd. Elemental analyses were performed using a Perkin-Elmer 240C analytical instrument. Infrared spectra were recorded on a Nicolet 5DX FT-IR spectrophotometer with KBr pellets. UV-Vis spectra were

recorded on a Lambda 35 spectrometer. Molar conductance was measured with a Shanghai DDS-11A conductometer.

2. 2. Synthesis of H_2L

3-Bromosalicylaldehyde (0.40 g, 2.0 mmol) dissolved in methanol (30 mL) was reacted with ethane-1,2-diamine (0.030 g, 1.0 mmol) diluted by methanol (10 mL). The mixture was stirred at reflux for 1 h and with three quarter of the solvent removed by distillation, to give yellow product. Yield: 0.37 g (88%). Anal. Calcd. for $C_{16}H_{14}Br_2N_2O_2$ (%): C, 45.10; H, 3.31; N, 6.57. Found: C, 44.92; H, 3.40; N, 6.71. IR data (KBr, cm^{-1}): 3438 (OH), 1628 (C=N), 1217 (Ar–O). UV-Vis data in methanol [λ_{max} (nm), ε (L mol^{-1} cm^{-1})]: 215, 22350; 260, 8940; 328, 2980; 415, 1935.

Diffraction quality yellow single crystals were obtained by slow evaporation of the methanol solution of the product.

2. 3. Synthesis of [CuL] (1)

H_2L (42 mg, 0.10 mmol) and copper nitrate trihydrate (24 mg, 0.10 mmol) were dissolved in methanol (20 mL). A brown solution was formed immediately. After 30 min stirring, the solution was filtered and the filtrate was kept for slow evaporation. The diffraction quality brown single crystals that deposited over a period of 5 days were collected by filtration and washed with methanol. Yield: 22 mg (45%). Anal. Calcd. for $C_{16}H_{12}Br_2CuN_2O_2$ (%): C, 39.41; H, 2.48; N, 5.74. Found: C, 39.26; H, 2.37; N, 5.85. IR data (KBr, cm^{-1}): 1632 (C=N), 1180 (Ar–O). UV-Vis data in methanol [λ_{max} (nm), ε (L mol^{-1} cm^{-1})]: 270, 16720; 368, 6270. Λ_M (10^{-3} mol L^{-1} in DMSO/H_2O): 41 Ω^{-1} cm^2 mol^{-1}.

2. 4. Synthesis of [CoL(NCS)(DMF)] (2)

H_2L (42 mg, 0.10 mmol) and cobalt nitrate hexahydrate (29 mg, 0.10 mmol) were dissolved in methanol (20 mL) and DMF (5 mL). A deep red solution was formed immediately. After 30 min stirring, the solution was filtered and the filtrate was kept for slow evaporation. The diffraction quality red single crystals that deposited over a period of 27 days were collected by filtration and washed with methanol. Yield: 18 mg (29%). Anal. Calcd. for $C_{20}H_{19}Br_2CoN_4O_3S$ (%): C, 39.11; H, 3.12; N, 9.12. Found: C, 39.27; H, 3.21; N, 9.03. IR data (KBr, cm^{-1}): 1641 (C=O), 1633 (C=N), 1183 (Ar–O). UV-Vis data in methanol [λ_{max} (nm), ε (L mol^{-1} cm^{-1})]: 267, 15560; 380, 3315. Λ_M (10^{-3} mol L^{-1} in DMSO/H_2O): 33 Ω^{-1} cm^2 mol^{-1}.

2. 5. X-Ray Crystallography

Suitable single crystals of the complexes were selected and mounted on a Bruker Smart 1000 CCD area-detector diffractometer with graphite monochromatized Mo-Kα radiation (λ = 0.71073 Å). Diffraction data for the compounds were collected by ω scan mode at 298(2) K. Data reduction and cell refinement were performed by the SMART and SAINT programs.[8] Empirical absorption correction was applied by using SADABS.[9] The structures were solved by direct methods and refined with the full-matrix least-squares technique using SHELXL.[10] The non-H atoms in the structures were subjected to refined anisotropic refinement. The hydrogen atoms were located

Table 1. Crystallographic data and refinement details for the compounds

	H_2L	1	2
Molecular formula	$C_{16}H_{14}Br_2N_2O_2$	$C_{16}H_{12}Br_2CuN_2O_2$	$C_{20}H_{19}Br_2CoN_4O_3S$
Molecular weight	426.11	487.64	614.20
Crystal system	Monoclinic	Orthorhombic	Monoclinic
Space group	$P2_1/n$	$Pbca$	$P2_1/c$
a, Å	8.3320(5)	10.6159(8)	11.1597(6)
b, Å	10.1997(7)	12.6984(9)	16.6043(8)
c, Å	9.5717(7)	24.0006(12)	13.3480(7)
β, °	101.332(2)	90	109.9890(10
V, Å^3	797.58(9)	3235.4(4)	2324.4(2)
Z	2	8	4
ρ_{calcd}, g cm^{-3}	1.774	2.002	1.755
μ, mm^{-1}	5.090	6.299	4.295
Reflections collected	7634	16247	26163
Unique reflections	1730	3009	4330
Observed reflections ($I \geq 2\sigma(I)$)	1344	2320	3243
Data/restraints/parameters	1730/0/101	3009/0/208	4330/0/282
R_{int}	0.0329	0.0538	0.0365
GOOF on F^2	1.055	1.035	1.015
R_1, wR_2 ($I \geq 2\sigma(I)$)	0.0328, 0.0724	0.0272, 0.0552	0.0360, 0.0766
R_1, wR_2 (all data)	0.0496, 0.0812	0.0446, 0.0605	0.0571, 0.0861

in geometrically and treated with the riding mode. Crystallographic data and experimental details for the compounds are summarized in Table 1.

2. 6. Antibacterial Assay

Antibacterial activities of the compounds were tested *in vitro* against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas fluorescens* using MH medium (Mueller–Hinton medium: casein hydrolysate 17.5 g, soluble starch 1.5 g, beef extract 1000 mL). The minimum inhibitory concentrations (MIC) of the test compounds were determined by a colorimetric method using the dye MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide).[11] A solution of the compound (50 μg mL^{-1}) in DMSO was prepared and graded quantities of the assayed compounds were incorporated in specified quantity of sterilized liquid MH medium. A specified quantity of the medium containing the compound was poured into microtitration plates. Suspension of the microorganism was prepared to contain about 10^5 colony forming units cfu mL^{-1} and applied to micro-titration plates with serially diluted compounds in DMSO to be tested and incubated at 37 °C for 24 h. After the MICs were visually determined on each of the micro-titration plates, 50 μL of PBS (Phosphate Buffered Saline 0.01 mol L^{-1}, pH 7.4: $Na_2HPO_4{\cdot}12H_2O$ 2.9 g, KH_2PO_4 0.2 g, NaCl 8.0 g, KCl 0.2 g, distilled water 1000 mL) containing 2 mg of MTT was added to each well. Incubation was continued at room temperature for 4–5 h. The content of each well was removed, and 100 μL of isopropyl alcohol containing 5% 1.0 mol L^{-1} HCl was added to extract the dye. After 12 h of incubation at room temperature, the optical density (OD) was measured with a micro-plate reader at 550 nm.

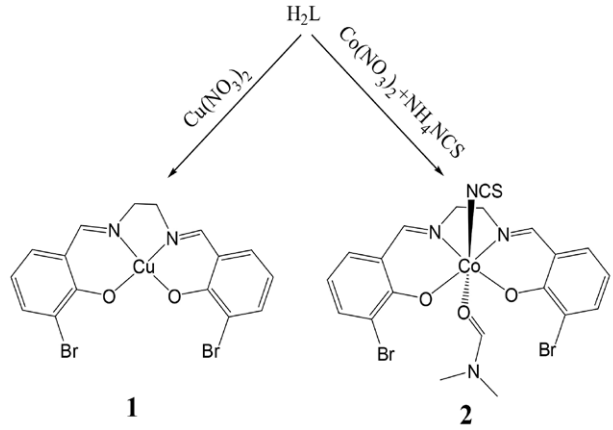
3. Results and Discussion

3. 1. Chemistry

The bis-Schiff base H_2L was prepared by 2:1 condensation reaction of 3-bromosalicylaldehyde with ethane-1,2-diamine in methanol (Scheme 2). Complexes **1** and **2** were facile prepared by the reaction of the bis-Schiff base with copper nitrate trihydrate and cobalt nitrate hexahydrate, respectively, in methanol. As usually observed for the preparation of cobalt complexes, Co^{II} in complex **2** underwent aerial oxidation to Co^{III} in the synthetic route. The molar conductivities of the complexes **1** and **2** measured in DMSO/H_2O (V:V = 1:9) at concentration of 10^{-3} mol L^{-1} are 41 and 33 Ω^{-1} cm^2 mol^{-1}, respectively, indicating the non-electrolytic nature of both complexes in such solution.[12]

3. 2. IR and Electronic Spectra

In the infrared spectrum of H_2L, the weak absorption at 3438 cm^{-1} is assigned to the O–H vibration of the phenol group, and the characteristic imine stretching is observed at 1628 cm^{-1} as a strong signal.[13] The spectra of complexes **1** and **2** show imine stretching at 1632–1633 cm^{-1}. The Schiff base ligands coordination is substantiated by the phenolic C–O stretching bands at 1217 cm^{-1} for H_2L, while 1180–1183 cm^{-1} for the complexes.[14] The intense absorption band at 2117 cm^{-1} in the spectrum of complex **2** can be assigned to the thiocyanate ligand.[15] Coordination of the Schiff base ligands is further confirmed by the appearance of weak bands in the low wave numbers 400–600 cm^{-1}, corresponding to ν(Cu/Co–N) and ν(Cu/Co–O).



Scheme 2. The synthetic procedure of the complexes

In the electronic spectra of H_2L and the complexes, the bands at 260–270 nm and 328 nm are attributed to the n–π^* transitions.[16] The bands at 360–380 nm in the complexes can be attributed to the ligand to metal charge transfer transition (LMCT).[17]

3. 3. Crystal Structure Description of H_2L

Molecular structure of H_2L is shown in Fig. 1. Selected bond lengths and angles for the compound are listed in Table 2. All the bond lengths in the compound are within normal ranges,[18] and comparable to those of the similar bis-Schiff bases.[19] The bond length of C7–N1 confirms it as a double bond. The two benzene rings form a dihedral angle of 0° due to the centrosymmetric symmetry. In the crystal structure of the compound, the molecules are linked through intermolecular hydrogen bonds of C–H···O (C7–H7 = 0.93 Å, H7···O1^i = 2.41 Å, C7···O1^i =

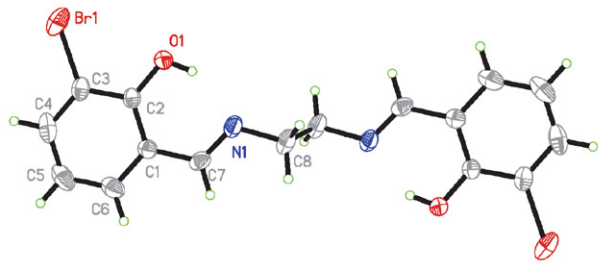


Fig. 1. Molecular structure of H_2L with thermal ellipsoids of 30% probability level.

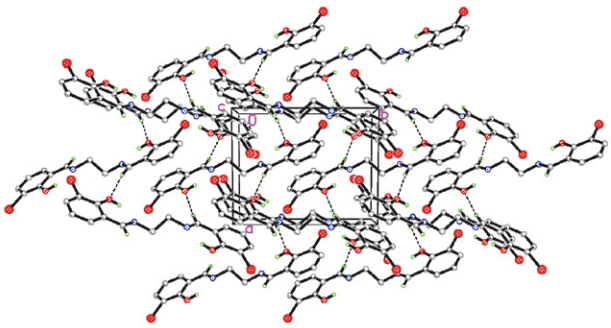


Fig. 2. Molecular packing diagram of H_2L, viewed along the *c* axis. Hydrogen bonds are shown as dashed lines.

3.328(3) Å, C7–H7···O1^{i} = 170°; symmetry code for i: ½ + *x*, ½ – *y*, ½ + *z*), to form two-dimensional sheets parallel to the *bc* plane (Fig. 2). Moreover, there are π–electron ring–π-electron ring interactions (4.209(3) Å) in the packing structure of the compound.

3. 4. Crystal Structure Description of Complex 1

Molecular structure of the mononuclear copper(II) complex **1** is shown in Fig. 3. Selected bond lengths and angles for the compound are listed in Table 2. The Cu atom is coordinated by two phenolate oxygen and two imino nitrogen of the bis-Schiff base ligand, forming square planar coordination. The square planar geometry is slightly distorted from ideal model, as evidenced by the bond angles. The angles in the coordination are in the ranges of 84.78(11)–92.96(10)° and 176.27(10)–177.64(10)°, respectively. The Cu–O and Cu–N bond lengths are comparable to those observed in Schiff base copper complexes.[20] The two benzene rings form a dihedral angle of 5.7(5)°.

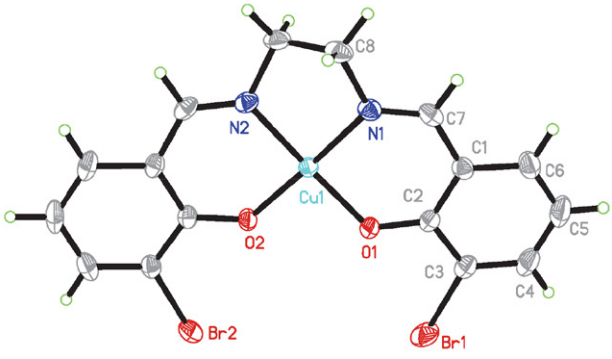


Fig. 3. Molecular structure of complex **1** with thermal ellipsoids of 30% probability level.

Table 2. Selected bond distances (Å) and angles (°) for the compounds

Bond	Å	Bond	Å
	H_2L		
C7–N1	1.269(4)	C8–N1	1.463(3)
C2–O1	1.319(3)		
	1		
C7–N1	1.279(4)	C8–N1	1.474(4)
C10–N2	1.279(4)	C9–N2	1.471(4)
C2–O1	1.301(3)	C12–O2	1.312(3)
Cu1–N1	1.940(2)	Cu1–N2	1.930(2)
Cu1–O1	1.896(2)	Cu1–O2	1.908(2)
O1–Cu1–N1	92.96(10)	O1–Cu1–N2	177.64(10)
O1–Cu1–O2	89.74(8)	N1–Cu1–N2	84.78(11)
N1–Cu1–O2	176.27(10)	N2–Cu1–O2	92.49(10)
	2		
C7–N1	1.271(5)	C8–N1	1.474(5)
C10–N2	1.276(5)	C9–N2	1.464(5)
C2–O1	1.302(4)	C12–O2	1.297(4)
Co1–O1	1.887(2)	Co1–N1	1.889(3)
Co1–N2	1.891(3)	Co1–O2	1.896(2)
Co1–N3	1.893(3)	Co1–O3	1.937(3)
O1–Co1–N1	94.70(12)	O1–Co1–N2	176.99(12)
O1–Co1–O2	87.30(11)	N1–Co1–N2	85.47(13)
N1–Co1–O2	177.45(12)	N2–Co1–O2	92.45(12)
N3–Co1–O1	92.68(13)	N3–Co1–O2	92.28(13)
N3–Co1–N1	89.21(13)	N3–Co1–N2	90.32(14)
O3–Co1–O1	87.40(11)	O3–Co1–O2	89.96(11)
O3–Co1–N1	88.56(12)	O3–Co1–N2	89.60(12)
N3–Co1–O3	177.76(13)		

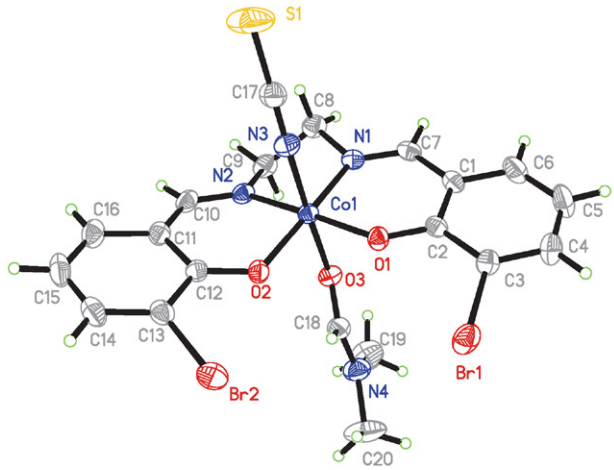


Fig. 4. Molecular structure of complex **2** with thermal ellipsoids of 30% probability level.

3. 5. Crystal Structure Description of Complex 2

Molecular structure of the mononuclear cobalt(III) complex **2** is shown in Fig. 4. Selected bond lengths and angles for the compound are listed in Table 2. The Co atom is coordinated by two phenolate oxygen and two imino nitrogen of the bis-Schiff base ligand, one thiocyanate nitrogen and one oxygen of a DMF ligand, forming octahedral coordination. The equatorial plane of the octahedral coordination is defined by the four donor atoms of the Schiff base ligand, and the axial positions are occupied by the donor atoms of thiocyanate and DMF ligands. The octahe-

dral geometry is distorted from ideal model, as evidenced by the bond angles. The bond angles in the coordination are in the ranges of 85.47(13)–94.70(12)° and 176.99(12)–177.76(13)°, respectively. The Co–O and Co–N bond lengths are comparable to those observed in Schiff base cobalt complexes.[21]

3. 6. Antibacterial Activities

The compounds were screened *in vitro* for antibacterial activities against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas fluorescens* by the MTT method. The MICs of the compounds against the bacteria are presented in Table 3. Penicillin was used as a reference.

Table 3. Antibacterial activities (MIC (μg mL^{-1}))

	Bacillus subtilis	*Escherichia coli*	*Pseudomonas fluorescens*	*Staphylococcus aureus*
H_2L	12.5	25	25	6.25
1	0.78	3.12	12.5	1.56
2	6.25	6.25	3.12	3.12
Penicillin	1.3	> 100	> 100	2.1

The bis-Schiff base H_2L shows medium to weak activities against the bacteria. In general, both complexes have stronger activities against the bacteria than H_2L. Complex **1** showed strong activity against *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus*, and medium activity against *Pseudomonas fluorescens*. The complex has better activity against *Bacillus subtilis* than the polynuclear copper complex with the Schiff base ligand 2-hydroxy-5-methylbenzaldehyde oxime.[22] Complex **2** showed strong activity against *Pseudomonas fluorescens* and *Staphylococcus aureus*, and medium activity against *Bacillus subtilis* and *Escherichia coli*. Complex **1** has stronger activities against the bacteria than complex **2**, except for *Pseudomonas fluorescens*. Interestingly, complex **1** has stronger activities against the bacteria than Penicillin. The trends in the present work are in accordance with those in the literatures that metal complexes usually have stronger antibacterial activities than their corresponding ligands.[23] The copper complex has stronger activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* than the copper complex with the Schiff base 2-(2-(2,4-dinitrophenyl)hydrazono)-1,2-diphenylethanone.[24] The cobalt complex has stronger activity against *Staphylococcus aureus* than the cobalt complexes with the Schiff bases 4-X-2-{[2-(2-pyridine-2-yl-ethylsulfanyl)ethylimino]methyl}phenol (X = methoxy, phenylazo, bromo, nitro).[25] This enhanced antibacterial activity of the complexes can be explained by the coordination of the metal ions with the azomethine groups of the Schiff base ligands.[26] According to chelating theory,[27] chelation could enhance the lipophilic character of the central metal ions, which subsequently favor their permeation through the lipid layers of the cell membrane and blocking the metal binding sites on enzymes of microorganism. On chelation, the polarity of the metal ions decreases to a greater extent, due to the overlap of the ligand orbital and partial sharing of their positive charge with donor groups. In addition, it improves the π-electron delocalization on the whole chelating ring which affects the lipophilicity of the complexes.[28]

4. Conclusion

Two new copper(II) and cobalt(III) complexes have been prepared and characterized. The bis-Schiff base ligand coordinates to the metal atoms through phenolate oxygen and imino nitrogen. Structures of H_2L and the complexes were characterized by spectroscopic methods and confirmed by single crystal X-ray determination. The antibacterial activities of the bis-Schiff base and the complexes were assayed. The results indicated that both complexes are potential antibacterial agents.

Supplementary Material

CCDC reference numbers 969428 (H_2L), 2195359 (**1**) and 2195360 (**2**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk, or from Cambridge Crystallographic Data Center, 12, Union Road, Cambridge CB2 1EZ, UK; Fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk.

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Povzetek

Iz 3-bromosalicilaldehida in etan-1,2-diamina smo pripravili bis-Schiffovo bazo *N*,*N*'-etilen-bis(3-bromosalicilaldimin) (H_2L). Z H_2L kot ligandom smo pripravili nova kompleksa bakra(II) [CuL] (**1**) in kobalta(III) [CoL(NCS)(DMF)] (**2**), ki smo ju okarakterizirali s fizikalno-kemijskimi metodami in monokristalno rentgensko analizo. Rentgenska analiza kaže, da je atom Cu v kompleksu **1** v kvadratni planarni koordinaciji, atom Co v kompleksu **2** pa v oktaedrični koordinaciji. Spojine so bile testirane *in vitro* na antibakterijsko delovanje na *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* in *Pseudomonas fluorescens*. Oba kompleksa izkazujeta učinkovito delovanje na bakterije.