Synthesis, Characterization, Antimicrobial and Time Killing Activities of New Sulfa-Derived Schiff Bases Coordinated with Cu(II)

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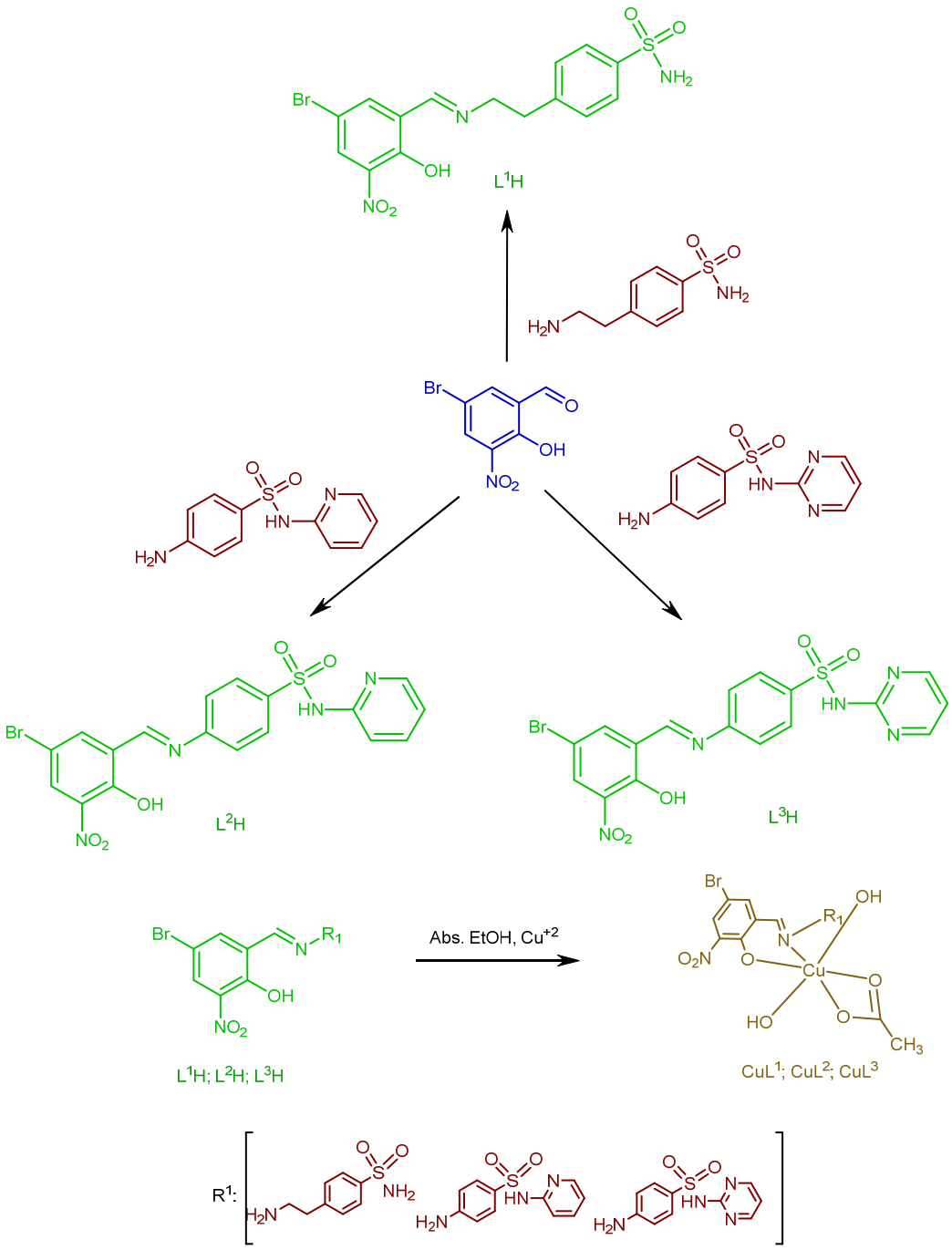
Abstract: *Synthesis of three Schiff bases of 5-bromo-3-nitro salicylaldehyde containing different sulfonamide group antibiotic compounds and their Cu(II) complexes was carried out. Structures of all compounds were characterized with spectroscopic methods, including Fourier transform infrared, proton nuclear magnetic resonance, and elemental analysis. The* in vitro *antimicrobial activity of ligands and complexes against Gram-negative and Gram-positive bacteria and the yeast* Candida albicans *was evaluated. It was determined that the ligand and complexes showed outstanding antimicrobial activity against almost all of the microorganisms tested. It has been observed that the newly synthesized complexes have more antimicrobial effects than the corresponding ligands. It has been determined that the newly synthesized complexes have more antimicrobial effects than the others (*E. coli*,* L. monocytogenes*, and* C. albicans*), especially on* Staphylococcus aureus *and* Pseudomonas aeruginosa.

Keywords: *Cu(II) complex; Schiff base; sulfonamide antibiotic; time killing*

■ INTRODUCTION

Schiff bases are considered to be one of the versatile classes of biologically active compounds due to their ability to interact well with periodic table transition elements. Schiff bases and complexes used as models for biological systems show antibacterial, anti-inflammatory, antifungal, herbicidal, and anti-cancer activities [1-2]. However, they are also widely used in various chemical and photochemical reactions due to their catalyst properties [3-4]. Schiff bases and their metal complexes affect electronic variations due to substituent effects [5]. In the presence of sulfur atoms in the compounds, biological activity is thought to improve due to its specific interaction with enzymes with sulfhydryl groups [6]. Sulfonamides are a class of therapeutic drugs used against infections caused by microbes. Sulfa drugs are important compounds used in pharmaceutical and agricultural fields due to their low toxicity, affordability, and significant activity profile. Since sulfonamides are structurally similar to *p*-aminobenzoic acid (PABA), a cofactor in folic acid synthesis by bacteria, they can compete with it efficiently to inhibit the synthesis of proteins and nucleic acids, resulting in the inhibition of various microorganisms. Also, it is a sulfonamide, versatile half for its various pharmacological activities, including antibacterial, antifungal, anti-inflammatory, anti-carbonic anhydrase, diuretic, hypoglycemic, antithyroid and antiproton activities, and enzyme inhibition [7-16]. Sulfonamides, which inhibit the growth of bacterial cells by blocking the synthesis of an important vitamin called folic acid, have also been the subject of many studies due to their significant antitumor activity both *in vitro* and *in vivo* [17-21]. Some of these derivatives are currently being evaluated in clinical trials, and there is much optimism that they could lead to new alternative anti-cancer drugs that are devoid of the side effects of current pharmacological agents [22].



Scheme 1. Synthetic routes of $L^{1-3}H$ and their Cu^{+2} complexes

In this study, the synthesis of three Schiff bases of 5-bromo-3-nitro salicylaldehyde containing different sulfonamide group compounds and their Cu(II) complexes was carried out. The structures of all compounds were characterized by spectroscopic methods such as Fourier transform infrared, proton nuclear magnetic resonance, and elemental analysis. *In vitro*, the antimicrobial activity of ligands and synthesized new complexes against Gram-negative and Gram-positive bacteria and yeast *Candida albicans* were evaluated. As a result of the antimicrobial study performed according to the microdilution method, the effects of the most effective complexes on the colony-forming abilities of the bacteria and their time-killing kinetics were determined.

■ EXPERIMENTAL SECTION

Materials

4-(2-Aminoethyl)benzenesulfonamide, sulfapyridine, sulfadiazine, 5-bromo-3-nitro salicylaldehyde, absolute ethanol (EtOH), copper(II) acetate hydrate ($Cu(CH_3CO_2)_2{\cdot}H_2O$) used for the synthesis and analysis were purchased from Merck and used without purifications. Nutrient and broth nutrient agar from Merck and DMSO (Sigma) were used in antimicrobial experiments.

Instrumentation

The FTIR spectra were carried out by a Shimadzu QATR-S (IR Spirit S1102SC) in the range of 4000–600 cm^{-1}. ^1H (400 MHz) NMR spectra were obtained in DMSO-d_6 solutions by Bruker DRX-400 high-performance digital FT-NMR spectrometer. C, H, N, and S content was determined by using a LECO CHNS-932 analyzer.

Procedure

Preparation and characterization

4-(2-{(*E*)-[(5-bromo-2-hydroxy-3-nitrophenyl)methylidene]amino}ethyl)benzene-1-sulfonamide (L^1H). To a solution of 4-(2-aminoethyl)benzenesulfonamide (0.600 g, 3 mmol) dissolved in 20 mL EtOH, 5-bromo-3-nitro salicylaldehyde (0.738 g, 3 mmol) dissolved in 15 mL EtOH was added dropwise. This mixture interacted at 200 °C for 12 h using the hydrothermal technique. The solid (powder) precipitate was filtered, washed with cold EtOH, and dried at room temperature to give a compound of L^1H as given in Scheme 1. (428.26 g/mol) Yield: 1.15 g (89%). m.p. 204 °C. Anal. Calc. for $C_{15}H_{14}BrN_3O_5S$: C, 42.07; H, 3.30; N, 9.81; S, 7.49%. Found: C, 41.58; H, 3.24; N, 9.68; S, 7.41.

FTIR (ATR, ν_{max}/cm^{-1}): 685, 762, 928, 973, 1089, 1130 ($-SO_2$ sym), 1276, 1324, 1361 ($-SO_2$ asym), 1452 (C–O), 1609 (C=N), 3295 (N–H, sym), 3421 (O–H); ^1H-NMR (DMSO-d_6, δ, ppm): 13.68 (s, 1H, O–H), 9.02 (s, 1H, CH=N), 8.34–6.04 (m, 6H, Ar–H), 7.65 (s, 2H, $-NH_2$), 2.54 (t, 2H, $-CH_2$), 2.36 (t, 2H, $-CH_2$). ^{13}C-NMR (DMSO-d_6, δ, ppm): 162.4 (C1), 160.2 (C7), 146.5 (C10), 142.6 (C13), 142.1 (C2), 134.2 (C11), 129.5 (C5), 124.6 (C3), 123.6 (C12), 117.9 (C6), 110.9 (C4), 44.5 (C9), 41.7 (C8).

Preparation of the Cu(II)L1 complex

To a solution of L^1H ligand (0.428 g, 1 mmol) dissolved in 20 mL EtOH, $Cu(CH_3CO_2)_2{\cdot}H_2O$ (0.199 g, 1 mmol) dissolved in 15 mL absolute EtOH was added dropwise. This reaction mixture interacted at 200 °C for 12 h using the hydrothermal technique. The solid (powder) precipitate was filtered, washed with cold EtOH, and dried in an oven at 80 °C for 24 h to give a compound of CuL^1 (585.87 g/mol) as given in Scheme 1. Yield: 0.43 g (74%). Anal. Calc. for $C_{17}H_{20}BrN_3O_9SCu$: C, 34.85; H, 3.44; N, 7.17; S, 5.47%. Found: C, 34.52; H, 3.39; N, 7.06; S, 5.38.

FTIR (ATR, ν_{max}/cm^{-1}): 1126 ($-SO_2$ sym), 1355 ($-SO_2$ asym), 1441 (C–O), 1584 (–C=O, Acetate), 1604 (C=N), 3291 (N–H, sym), 3415 (H_2O).

4-{(*E*)-[(5-bromo-2-hydroxy-3-nitrophenyl)methylidene]amino}-N-(pyridin-2-yl)benzene-1-sulfonamide (L^2H). To a solution of sulfapyridine (0.747 g, 3 mmol) dissolved in 20 mL EtOH, 5-bromo-3-nitro salicylaldehyde (0.738 g, 3 mmol) dissolved in 15 mL EtOH was added dropwise. This mixture was reacted at 200 °C for 12 h using the hydrothermal technique. The solid (powder) precipitate was filtered, washed with cold EtOH, and dried at room temperature to give a compound of L^2H (477.29 g/mol) as given in Scheme 1. Yield: 1.26 g (88%). m.p. 218 °C. Anal. Calc. for $C_{18}H_{13}BrN_4O_5S$: C, 45.30; H, 2.75; N, 11.74; S, 6.72%. Found: C, 44.86; H, 2.71; N, 11.65; S, 6.64.

FTIR (ATR, ν_{max}/cm^{-1}): 692, 759, 926, 979, 1078, 1135 ($-SO_2$ sym), 1249, 1318, 1339 ($-SO_2$ asym), 1454 (C–O), 1613 (C=N), 3236 (N–H, sym), 3434 (O–H); ^1H-NMR (DMSO-d_6, δ, ppm): 13.74 (s, 1H, O–H), 11.68 (s, 1H, N–H), 8.96 (s, 1H, CH=N), 8.58–6.63 (m, 10H, Ar–H). ^{13}C-NMR (DMSO-d_6, δ, ppm): 162.6 (C1), 160.7 (C7), 159.3 (C8), 153.2 (C12), 151.1 (C16), 141.3 (C2), 139.9 (C11), 137.2 (C14), 130.5 (C10), 129.8 (C5), 124.8 (C9), 124.4 (C3), 119.3 (C15), 118.2 (C6), 112.8 (C13), 111.7 (C4).

Preparation of the Cu(II)L2 complex

To a solution of L^2H ligand (0.477 g, 1 mmol)

dissolved in 20 mL EtOH, $Cu(CH_3CO_2)_2{\cdot}H_2O$ (0.199 g, 1 mmol) dissolved in 15 mL EtOH was added dropwise. This mixture was reacted at 200 °C for 12 h using the hydrothermal technique. The solid (powder) precipitate was filtered, washed with cold EtOH, and dried in an oven at 80 °C for 24 h to give a compound of CuL^2 (634.90 g/mol) as given in Scheme 1. Yield: 0.45 g (71%). Anal. Calc. for $C_{20}H_{19}BrN_4O_9SCu$: C, 37.83; H, 3.02; N, 8.82; S, 5.05%. Found: C, 37.41; H, 2.97; N, 8.74; S, 4.99.

FTIR (ATR, ν_{max}/cm^{-1}): 1130 (–SO_2 sym), 1334 (–SO_2 asym), 1441 (C–O), 1585 (–C=O, Acetate) 1609 (C=N), 3231 (N–H, sym), 3414 (H_2O).

4-{(*E*)-[(5-bromo-(2-hydroxy-3-nitrophenyl)methylidene]amino}-N-(pyrimidin-2-yl)benzene-1-sulfonamide (L^3H). To a solution of sulfadiazine (0.750 g, 3 mmol) dissolved in 20 mL EtOH, 5-bromo-3-nitro salicylaldehyde (0.738 g, 3 mmol) dissolved in 15 mL EtOH was added dropwise. This mixture was reacted at 200 °C for 12 h using the hydrothermal technique. The solid (powder) precipitate was filtered, washed with cold EtOH, and dried at room temperature to give a compound of L^3H (478.28 g/mol) as given in Scheme 1. Yield: 1.30 g (90%). m.p. 194 °C. Anal. Calc. for $C_{17}H_{12}N_5O_5S$: C, 42.69; H, 2.53; N, 14.64; S, 6.70%. Found: C, 42.30; H, 2.49; N, 14.52; S, 6.63.

FTIR (ATR, ν_{max}/cm^{-1}): 688, 762, 976, 1081, 1128 (–SO_2 sym), 1245, 1314, 1330 (–SO_2 asym), 1452 (C–O), 1614 (C=N), 3230 (N–H, sym), 3432 (O–H); ^{1}H-NMR (DMSO-d_6, δ, ppm): 13.76 (s, 1H, O–H), 11.65 (s, 1H, N–H), 8.93 (s, 1H, CH=N), 8.45–6.71 (m, 9H, Ar–H). ^{13}C-NMR (DMSO-d_6, δ, ppm): 162.3 (C1), 161.2 (C7), 159.1 (C8), 158.3 (C12), 157.5 (C13), 141.7 (C2), 139.4 (C11), 132.3 (C10), 129.3 (C5), 125.2 (C3), 124.6 (C9), 117.8 (C6), 115.6 (C14), 110.9 (C4).

Preparation of the Cu(II)L3 complex

To a solution of L^3H ligand (0.478 g, 1 mmol) dissolved in 20 mL EtOH, $Cu(CH_3CO_2)_2{\cdot}H_2O$ (0.199 g, 1 mmol) dissolved in 15 mL EtOH was added dropwise. This mixture was reacted at 200 °C for 12 h using the hydrothermal technique. The solid (powder) precipitate was filtered, washed with cold EtOH, and dried in an oven at 80 °C for 24 h to give a compound of CuL^3 (635.89 g/mol) as given in Scheme 1. Yield: 0.43 g (68%). Anal. Calc. for $C_{19}H_{18}BrN_5O_9SCu$: C, 35.89; H, 2.85; N, 11.01; S, 5.04%. Found: C, 35.56; H, 2.80; N, 10.89; S, 5.00.

FTIR (ATR, ν_{max}/cm^{-1}): 1124 (–SO_2 sym), 1328 (–SO_2 asym), 1438 (C–O), 1587 (–C=O, Acetate) 1608 (C=N), 3228 (N–H, sym), 3416 (H_2O).

Antimicrobial activity

In this study, 4 different standard bacterial strains and 1 yeast cell were used. These are Gram-positive *S. aureus* ATCC 25923 and *L. monocytogenes* ATCC 19111, Gram-negative *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853, and yeast *C. albicans* ATCC 10231. The solutions of newly synthesized complexes and ligands were prepared by dissolving them in DMSO at appropriate concentrations. The antimicrobial effects of the prepared complexes on microorganisms were determined by calculating the minimal inhibition concentration (MIC) values according to the broth dilution method. For the broth dilution method, cultures were grown in 5 mL nutrient broth (Merck) at 37 °C for 18 h in shaking at 175 rpm. Bacterial and yeast cells were suspended in 50 mL nutrient broth at a concentration of approximately 10^6 cells/mL by matching with 0.5 McFarland turbidity standards. The nutrient broth is added into 10 different tubes. Then, the complexes are added to the first tubes at appropriate concentrations, and serial dilution is made. In this way, the appropriate MIC values are determined by making half the dilution each time. Then all test tubes are left to grow overnight in an incubator at 37 °C. MIC values were determined after bacterial strains and yeast strains were kept in the incubator for 24 h. The last tube without growth was taken as the MIC (μg/mL), representing the mean of at least three determinations.

Viability experiments in microorganisms

Microorganisms to be used in this study were incubated for 1 night at 37 °C in a sterile nutrient broth medium. An amount of the microorganism that was left to grow for 1 night was taken and added to another nutrient broth medium. Thus, the number of bacteria was adjusted to 10^5 per mL. At the same time, considering the MIC value determined in the antimicrobial study, the complexes were added to the

medium in amounts determined as MIC, 2×MIC, and 4×MIC. Colony counts were made by taking microorganism samples at 37 °C for 24 h at appropriate times. The obtained colony numbers were converted to logarithmic (log) values, and T_{99} values were calculated. The T_{99} value is expressed as the value corresponding to a 2-log decrease in the number of bacteria over 24 h [23].

■ RESULTS AND DISCUSSION

Synthesis and Characterization of the Ligands and Their Cu(II) Complexes

In this study, sulfa-derived Schiff bases (L^1H-L^3H) were synthesized by reacting equimolar amounts of 4-(2-aminoethyl)benzene sulphonamide, sulfapyridine, and sulfadiazine with 5-bromo-3-nitro salicylaldehyde in EtOH, and followed by Cu(II) complexes of these ligands. All products were purified, and their structures were elucidated with general spectroscopic methods and elemental analysis, and the findings supported each proposed structure. The characteristic IR peaks of the ligands are the peaks of C=N, C–O, O–H, and S=O stretching vibrations. Others are C=C and C=O stretch vibration peaks, aliphatic C–H and N–H, and aromatic C–H stretch vibration peaks. When the IR spectrums of compounds are examined, the O–H stretching vibrations of the phenolic –OH group is broad at the range of 3434–3421 cm^{-1}, and the C=N stretching vibrations of the azomethine group are seen as sharply in the range of 1614–1609 cm^{-1}. These bands support the completion of the formation reaction of compounds L^1H-L^3H. These values agree with those found for similar compounds [14-16].

The most significant changes in the spectra of the Cu^{2+} complexes of L^1H-L^3H ligands were observed in the C=N stretching vibrations of the Schiff base group, the bending vibrations of the phenolic O–H group, the C=N vibrations, and the S=O symmetrical and asymmetrical stretching vibrations. The characteristic C=N (aliphatic) stretching vibration observed in the ligands in the 1614–1609 cm^{-1} range shifted to 1609–1604 cm^{-1} with the formation of metal chelates. This shift indicated that the azomethine group's nitrogen atom was involved in forming the metal-nitrogen (M–N) bond.

In other words, the nitrogen atom gave its unshared electrons to the metal ion and entered into coordination with the metal [24]. In addition, the band observed in the ligands in the range of 1454–1452 cm^{-1} and characteristic of C–O (phenolic) stretching vibration shifted up to 11–14 cm^{-1} in complex structures. This shift supports that deprotonated phenolic oxygen enters coordination with metal ions during complex formation [25]. In addition, the new peak in the range of 1587–1584 cm^{-1} indicates the presence of acetate coordinated with the metal and the presence of metal-coordinated water in the complexes at the peak in the range of 3416–3414 cm^{-1} [26-27]. The most useful infrared spectral bands of the compounds are listed in the experimental section (Fig. S1).

In addition to the IR spectrum data of the complexes, the percentages of C, H, S, and N in the elemental analysis results support the presence of acetate and water molecules that are bound to the structure and support that the predicted structure is in octahedral order [28] (Fig. S1).

The ^{1}H-NMR of the compounds L^1H-L^3H was recorded in DMSO-d_6 at room temperature. It belongs to a singlet –OH proton of one proton observed in the 13.76–13.68 ppm range in the ^{1}H-NMR spectrums taken in DMSO-d_6 solvent of compounds L^1H-L^3H. In addition, the peak was observed as a singlet at a proton intensity range of 9.02–8.93 ppm and also belonged to the azomethine proton in the structure. In addition, the chemical shift observed in the spectrum in the range of 11.68–7.65 ppm belongs to the (–NH) proton(s) attached to the $–SO_2$ group in the structure. The -NH proton(s) of the L1H ligand is lower field than the other ligands because the structure is connected to the linear chain structure instead of the aromatic ring directly. In addition, chemical shifts in the 2.54–2.36 ppm range belong to the methyl groups present in the L^1H ligand. The specified chemical shifts confirm the formation of structures (Fig. S2, S4, S6).

The most useful ^{1}H-NMR spectrums bands of the compounds are listed in the experimental section. The observed peaks agree with the structure and literature [29-30]. The chemical shifts of all carbons in the ^{13}C-

NMR spectra of the ligands are given in the experimental part. The resonance observed in the range of 161.2–160.2 ppm belongs to the carbon of the azomethine group (C7). Peaks of other aromatic ring carbons were observed in the range of 162.6–110.9 ppm. In addition, the resonances observed in the range of 44.5–41.7 ppm in the spectrum of the L^1H ligand belong to the carbons (C9 and C8) (Fig. S3, S5, S7). The observed peaks are consistent with the structure and literature [29-30].

Antimicrobial Activity

In this study, the antimicrobial properties of newly synthesized complexes using the microdilution method were investigated. *S. aureus* and *L. monocytogenes* as Gram-positive, *E. coli* and *P. aeruginosa* as Gram-negative, and *C. albicans* as yeast were used as standard strains. In these experiments based on the determination of MIC values, stock solutions of newly synthesized complexes were prepared by dissolving them in DMSO. Therefore, firstly, the MIC value of DMSO for control purposes was calculated and compared with the newly synthesized complexes. According to the results obtained, it was observed that the MIC value of DMSO was > 4000 µg/mL. In other words, it was determined that DMSO did not have a significant antimicrobial effect. When the antimicrobial effects of ligands (L^1H, L^2H, and L^3H) were examined, it was determined that the MIC values ranged between 93.75 and 3000 µg/mL. As a matter of fact, the antimicrobial value of the ligand coded L^1H was found to be 1500, 375, 375, 3000, and 93.75 µg/mL for *E. coli*, *P. aeruginosa*, *S. aureus*, *L. monocytogenes,* and *C. albicans*, respectively. MIC values for another ligand, L^2H, were found to be 1500 µg/mL for *E. coli*, 750 µg/mL for *P. aeruginosa* and *C. albicans*, 93.75 µg/mL for *S. aureus* and 3000 µg/mL for *L. monocytogenes*. When the antimicrobial results of the L^3H ligand are examined, it can be stated that these values are 375 µg/mL in *E. coli*, 187.5 µg/mL in *P. aeruginosa*, 93.75 µg/mL in *S. aureus*, 375 µg/mL in *L. monocytogenes* and 93.75 µg/mL in *C. albicans* (Table 1). In addition, antimicrobial activities of new complexes obtained from ligands were investigated, and MIC values were observed to vary between 11.7 and 3000 µg/mL. As a matter of fact, the MIC values that the newly synthesized L^1-Cu complex is effective on microorganisms were determined as 750 µg/mL for *E. coli*, 93.75 µg/mL for *P. aeruginosa*, 23.4 µg/mL for *S. aureus* and 46.8 µg/mL for *C. albicans*. In addition, these values for L^2-Cu were found to be 750, 93.75, 23.4, 750, and 187.5 µg/mL in *E. coli*, *P. aeruginosa*, *S. aureus*, *L. monocytogenes,* and *C. albicans*, respectively. In the continuation of the study, the MIC values of the new complex coded as L^3-Cu were determined to be 187.5 and 23.4 µg/mL for *E. coli* and *P. aeruginosa* and 11.7 and 375 µg/mL for Gram-positive *S. aureus* and *L. monocytogenes*. Another result is that the MIC value for *C. albicans*, which is yeast, is 187.5 µg/mL.

Effects of Complexes on the Life of Bacteria and Time-Killing Activities

The effects of L^1-Cu and L^2-Cu, which have the most antimicrobial effects, on the survival of Gram-positive *S. aureus* and Gram-negative *P. aeruginosa* was investigated in this study, which was carried out considering the data obtained from the MIC study. In the

Table 1. Antimicrobial effects of complexes on microorganisms (µg/mL)

	Gram-negative (-) bacteria		Gram-positive (+) bacteria		Yeast
	E. coli ATCC 25922	*P. aeruginosa* ATCC 27853	*S. aureus* ATCC 25923	*L. monocytogenes* ATCC 19111	*C. albicans* ATCC 10231
L^1H	1500.00	375.00	375.00	3000.00	93.75
L^2H	1500.00	750.00	187.50	3000.00	750.00
L^3H	375.00	187.50	93.75	375.00	93.75
L^1-Cu	750.00	93.75	23.40	3000.00	46.80
L^2-Cu	750.00	93.75	23.40	750.00	187.50
L^3-Cu	187.50	23.40	11.70	375.00	1500.00
DMSO	> 4000.00	> 4000.00	> 4000.00	> 4000.00	> 4000.00

study conducted for 24 h, the complexes were added to the medium containing the bacteria in MIC, 2×MIC, and 4×MIC concentrations, and the results were evaluated according to the colony count. Table 2 shows the effects of the complexes added to the nutrient broth medium on the colony-forming abilities of the microorganisms. Bacteria samples taken from the liquid medium at 0, 12, and 24 h were inoculated into the solid medium, kept in the incubator for 24 h, and the former colonies were counted and recorded.

The results obtained in the second stage of the study were converted to logarithmic values and recorded by calculating T_{99} values (Table 3). Therefore, as a result of this study, how long the newly synthesized complexes kill the microorganism *in vitro* will be determined precisely. Both *P. aeruginosa* and *S. aureus* control samples showed a very high increase in colony numbers after 24 h. As a matter of fact, while the number of colonies at the 0 h was 45×10^4 in the *P. aeruginosa* control sample, it was determined that this number was 550×10^{10} at the end of 24 h. In addition, at the end of 24 h (L^1-Cu addition) in the samples to which MIC, 2×MIC, and 4×MIC were added, the number of bacteria was found to be 6300, 240, and 39, respectively. In the samples with L^2-Cu added, it was determined that the bacterial numbers were 5800 with the addition of MIC, 180 with the addition of 2×MIC and 105 with the addition of 4×MIC. Adding L^3-Cu (MIC, 2×MIC, 4×MIC) to the broth containing *P. aeruginosa* also revealed that the bacterial counts were 4570, 15, and 0.

When the survival test results of Gram-positive *S. aureus* were examined, it was seen that the colony numbers in the control samples were 26×10^4 at 0 h and 1524×10^{11} at the end of 24 h. Although the number of bacteria in the samples added to the medium at MIC concentration was 26×10^4 at 0 h, this number was found

Table 2. Bacterial cell count (colony forming unit-cfu/mL)

Complexes	Microorganisms		0 h ($\times10^4$ cfu/mL)	12 h (cfu/mL)	24 h (cfu/mL)
L^1-Cu	*P. aeruginosa* ATCC 27853	Control	45	67×10^5	550×10^{10}
		MIC	45	88×10^3	63×10^2
		2×MIC	45	47×10^2	24×10^1
		4×MIC	45	250×10^1	39
	S. aureus ATCC 25923	Control	26	224×10^5	1524×10^{11}
		MIC	26	158×10^3	282×10^1
		2×MIC	26	104×10^2	198
		4×MIC	26	458×10^1	8
L^2-Cu	*P. aeruginosa* ATCC 27853	Control	45	67×10^5	550×10^{10}
		MIC	45	350×10^3	58×10^2
		2×MIC	45	145×10^2	180
		4×MIC	45	452×10^1	105
	S. aureus ATCC 25923	Control	26	224×10^5	1524×10^{11}
		MIC	26	205×10^3	76×10^2
		2×MIC	26	276×10^2	64
		4×MIC	26	327×10^1	2
L^3-Cu	*P. aeruginosa* ATCC 27853	Control	45	67×10^5	550×10^{10}
		MIC	45	557×10^3	296×10^1
		2×MIC	45	653×10^1	45
		4×MIC	45	250×10^1	5
	S. aureus ATCC 25923	Control	26	224×10^5	1524×10^{11}
		MIC	26	463×10^2	457×10^1
		2×MIC	26	48×10^2	15
		4×MIC	26	59×10^1	0

Table 3. Survival times of bacteria under the influence of complexes (time-killing activities) (T_{99} values)

Strain	Complexes		0 h (log)	24 h (log)	T_{99} (h)
Gram(-) *P. Aeruginosa* ATCC 27853	L^1-Cu	Control	6.65	13.74	>24.00
		MIC	6.65	4.79	>24.00
		2×MIC	6.65	3.38	15.38
		4×MIC	6.65	2.59	12.50
	L^2-Cu	MIC	6.65	4.94	>24.00
		2×MIC	6.65	3.25	14.20
		4×MIC	6.65	3.02	13.30
	L^3-Cu	MIC	6.65	4.47	22.20
		2×MIC	6.65	2.65	12.50
		4×MIC	6.65	2.39	11.76
Gram(+) *S. Aureus* ATCC 25923	L^1-Cu	Control	6.41	15.18	>24.00
		MIC	6.41	4.26	22.47
		2×MIC	6.41	2.99	14.28
		4×MIC	6.41	1.90	11.10
	L^2-Cu	MIC	6.41	5.24	>24.00
		2×MIC	6.41	2.80	13.30
		4×MIC	6.41	1.30	9.50
	L^3-Cu	MIC	6.41	3.65	17.39
		2×MIC	6.41	2.17	11.30
		4×MIC	6.41	1.00	8.80

to be 2820 for L^1-Cu, 7600 for L^2-Cu, and 4570 for L^3-Cu after 24 h (Table 2). Again, with the addition of L^1-Cu, L^2-Cu, and L^3-Cu in 2×MIC concentration to the medium, the bacterial counts were determined to be 198, 64, and 15, respectively, after 24 h. Finally, the addition of L^1-Cu, L^2-Cu, and L^3-Cu at 4×MIC concentration to the medium caused a decrease in bacterial numbers. The number of bacteria was 8 when L^1-Cu was added to the medium, 2 when L^2-Cu was added, and 0 when L^3-Cu was added to the medium.

Colony numbers obtained in the second part of this study were converted to logarithmic values, and T_{99} values were calculated. It is known that the T_{99} value is considered as the time corresponding to 2 logarithmic drops. The T_{99} value is considered important data in terms of showing us how long the bacteria live in any environment. Table 3 presents logarithmic values and T99 calculations of the effects of newly synthesized complexes on P. aeruginosa and S. aureus. The medium in which no complex was added was considered the control. And it was determined that the control samples continued to live more than 24 h according to the T_{99} value. When L^1-Cu is added to the medium at MIC concentration, it can be stated that *P. aeruginosa* continues to live for more than 24 h. As a result of the addition of the same complex to the medium in 2×MIC concentration, it was observed that the life of the bacteria decreased, and this period was 15.38 h.

On the other hand, it was determined that the bacteria continued to live for 12.5 h by adding complex at 4×MIC concentration to the medium. In addition, with the addition of L^2-Cu and L^3-Cu with MIC value to the medium, it was observed that *P. aeruginosa* lived for more than 24 and 22.2 h. On the other hand, the addition of L^2-Cu to the medium at 2×MIC concentration caused the survival of the bacteria to decrease to 14.2 h, and the addition of L^3-Cu to 12.5 h. In addition, it was determined that the bacteria continued to live for 13.3 h with the addition of L^2-Cu at 4×MIC concentration to the medium in which the bacteria were present and 11.76 h with the addition of L^3-Cu.

Table 3 shows the results of the studies that determined how long the Gram-positive *S. aureus* could survive with the addition of complexes at different concentrations. According to this, it was observed that

the bacteria continued to live for 22.47, > 24, 17.39 h in media with the addition of L^1-Cu, L^2-Cu, and L^3-Cu, respectively, at MIC concentrations. When the complex was added to the medium at 2×MIC concentration, T_{99} values were found to be 14.28, 13.3, and 11.3 h. On the other hand, the addition of the complexes (L^1-Cu, L^2-Cu, and L^3-Cu; 11.1, 9.5, and 8.8 h, respectively) to the medium at 4×MIC concentration resulted in significant reductions in the survival of *S. aureus*.

The development of resistance of microorganisms to antibiotics adversely affects the fight against diseases. This necessitated the discovery of new chemicals. Therefore, studies on the synthesis of new complexes to be used for chemotherapeutic purposes have become very important in recent years. Investigations, primarily to obtain new antimicrobials from plants, are continuing rapidly [31-32]. However, the increasing resistance of microorganisms to chemotherapeutics has made it necessary to synthesize new chemicals. Studies on the synthesis of new complexes and the acquisition of new antimicrobials in the laboratory environment have gained importance in recent years, and quite a lot of new studies are being done on this subject [33-39]. It is known that sulfonamides are the oldest and most well-known antimicrobial agents widely used against bacterial and fungal infections. Like sulfonamides, Schiff bases are commonly used in drug discovery.

Even now, sulfonamides are used to treat infections caused by bacteria and other microorganisms. It is thought that the study conducted here is very important in terms of supporting this. As a matter of fact, according to the results obtained, it was determined that ligands and complexes synthesized from ligands had high antimicrobial effects. Moreover, it was determined that the newly synthesized copper complexes showed higher activity than the ligands. It is stated that this effect is due to the atomic structure of copper and the possibility of easy electron transfer. It is known that the increased copper level in the cell creates an oxidative stress effect. Therefore, oxidative stress causes oxidative damage in cells. It is known that increased copper level weakens membrane integrity and causes deterioration of protein functions [40]. It is considered one of the most important factors that cause the disappearance of microorganisms from the environment. This study is important for being a precursor for using the newly synthesized complexes as a new chemotherapeutic for the future. As a result of bacterial survival experiments, it was revealed that the number of both bacteria (*P. aeruginosa*, *S. aureus*) increased during 24 h in control samples. However, the addition of the complex to the medium caused significant reductions in the number of bacteria. In addition, no bacteria were found at the end of 24 h in environments where high concentrations of complexes were added. Moreover, it was determined that the higher the amount of complex added to the medium, the corresponding decrease in the number of bacteria. In this study, it was also determined that Gram-positive bacteria were affected more than Gram-negative bacteria. It is known that this is due to the Gram-positive bacterial wall structure. Therefore, it can be stated that the new chemicals synthesized here are important in terms of shedding light on future studies.

■ CONCLUSION

In this study, syntheses of 4-(2-{(*E*)-[(5-bromo-2-hydroxy-3-nitrophenyl)methylidene]amino}ethyl) benzene-1-sulfonamide L^1H, Cu(II)L^1, 4-{(*E*)-[(5-bromo-2-hydroxy-3-nitrophenyl)methylidene]amino}-*N*-(pyridin-2-yl) benzene-1-sulfonamide L^2H, Cu(II)L^2, 4-{(*E*)-[(5-bromo-(2-hydroxy-3-nitrophenyl)methylidene]amino}-*N*-(pyrimidin-2-yl) benzene-1-sulfonamide L^3H, Cu(II)L^3 were described.

Ligand and copper complexes were found to have significant antimicrobial activities on all microorganisms. However, it was also determined that the newly synthesized complexes had higher antimicrobial activity than the ligands. Moreover, it has been determined that all newly synthesized complexes have more antimicrobial effects than others, especially on *S. aureus* and *P. aeruginosa*. And it has been determined that L^3-Cu has a very high antimicrobial effect on both *S. aureus* and *P. aeruginosa*. It is thought that this study is important in terms of giving us the idea of using newly synthesized complexes in the pharmacological field in the future.

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■ AUTHOR CONTRIBUTIONS

Önder İdil wrote the experimental part on biological activity studies (Antimicrobial and Time killing) and prepared the draft of the article. Hakan Şahal and Erdal Canpolat completed the synthesis and characterization and wrote this part. Mustafa Özkan revised the manuscript. All authors agreed to the final version of this manuscript.

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