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Research Article

Synthesis of macrocyclization cyclophanes and their metal complexes, characterization and antimicrobial activity

Murat TÜRKYILMAZ[*,1], **Murat DÖNMEZ**[1], **Murat ATEŞ**[2]

[1]*Department of Chemistry, Trakya University Faculty of Sciences, Edirne, Türkiye*
[2]*Department of Chemistry, Tekirdağ Namık Kemal University Faculty of Arts and Sciences, Tekirdağ, Türkiye*

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ABSTRACT

Due to their chemical properties, cyclophane-type compounds constitute an interesting organic chemistry class. In the structure of all cyclic compounds, macrocyclization is the most critical issue for high-efficiency synthesis. Especially with a small cyclophane structure, the experimental steps are more complicated than with a prominent cyclophane structure. In this manuscript, three different material groups were applied to synthesize silver cyclophane compounds for smart drug properties. In the first material group, 5,6-dimetil-1H-benzo[d] imidazole (1) and 2,6-bis(chloromethyl)pyridine (2) were reacted to form 5,6-dimethyl-1-((6-((5,6-dimethyl-1H-benzo[d]imidazole-1-il)methyl)pyridine-2-il)methyl)-1H-benzo[d] imidazole compound (3). In the second material group, ethyl 2-bromoacetate (4) reacted to different nitrogen atoms of the cyclophane compound to form a symmetric carbene compound, which is water-soluble (5). In the third material group, the silver (I) and palladium (II) metal complexes were synthesized due to the reaction with silver(I) oxide (6) and palladium (II) chloride (7). Antimicrobial activities of the carbene compounds and silver and palladium complexes (5, 6, and 7) were investigated against bacteria and fungal in more detail. Silver (I) complex (6) shows an antimicrobial agent when mixed with microorganisms, such as Gram-positive, Gram-negative, and fungal, but this property has not been observed in the palladium (II)-carbene complex (7).

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1. INTRODUCTION

Cyclophanes are heterocyclic compounds that combine a benzene ring and a chain that can form a bridge between two non-adjacent positions of the aromatic ring. More complex derivatives, including many aromatic units and bridged compounds, have been synthesized in literature [1, 2]. Cyclophanes are the most well-known and studied compounds inside organic compounds [3, 4]. Different methods were used to form more stable and well-designed compounds in the ring closure step.

Cyclophane compounds have attracted the attention of chemists in recent years and have led to the develop-

***Corresponding author.**
*E-mail address: mturkyilmaz@trakya.edu.tr

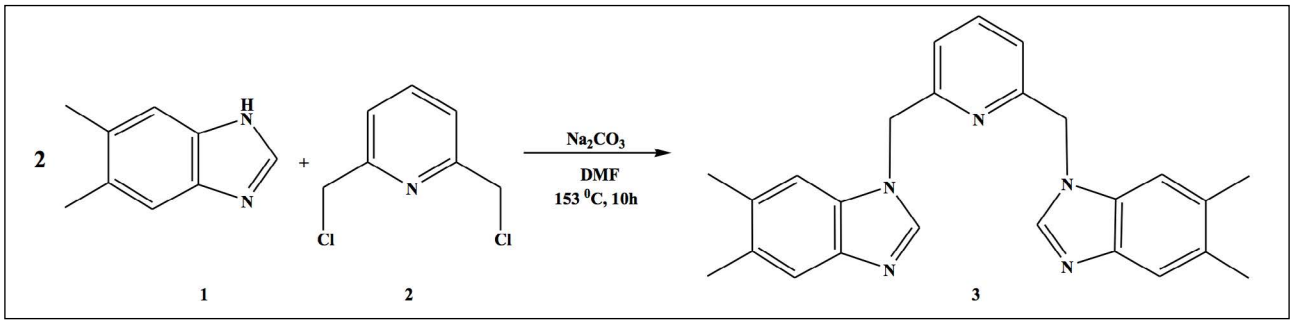

Figure 1. Synthesis ways of macrocycocyclic compounds.

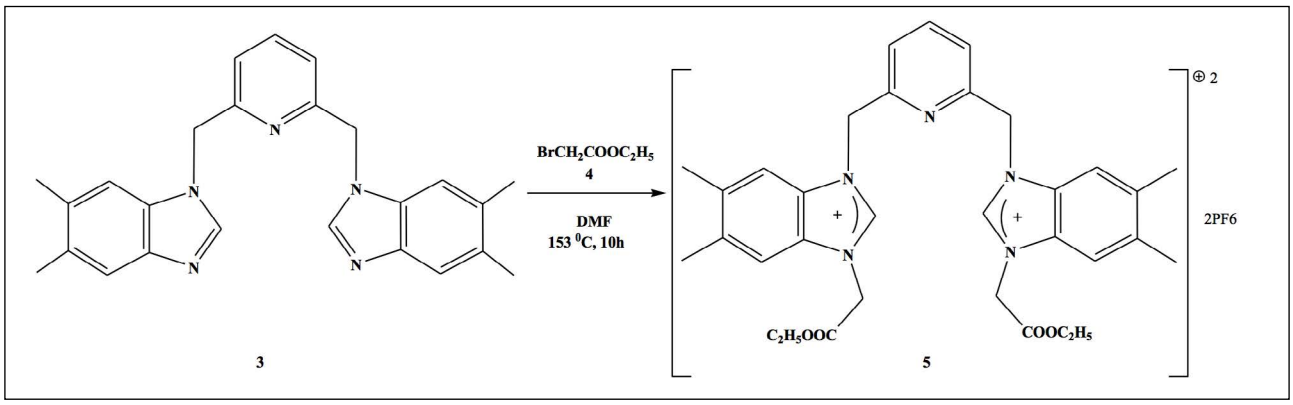

Figure 2. Synthesis methods of carbene compounds.

ment of synthesis methods for chemical reactions [5]. The first cyclophane compound, metacyclophane [6], was synthesized at the end of the 19th century (Fig. 1). The development of cyclophane chemistry began with the scientists Brown and Farthing [7]. The multilayer compounds were obtained as bridged purchased compound 1 (Fig. 2) [8, 9]. Compounds were characterized by different methods to understand bent and contracted benzene rings in the layered structures [10]. In addition, metal complexes were synthesized from 5,6-dimethylbenzimidazolium-linked cyclophane. The critical parameters of these compounds have been obtained as catalytic activities and high stability [11–13].

Chelated palladium (Pd), platinum, and nickel o-cyclophane complexes are highly stable materials. In Heck-type reactions, this stability and other geometric effects contribute significantly to the catalytic activity of o-cyclophane Pd(II) complexes [14]. The synthesis method of 5,6-dimethylbenzimidazolium-linked cyclophanes is similar to the synthesis method of other imidazolium salts [15, 16].

The silver (Ag) metal and silver salt compounds have been used for antimicrobial activity [17]. The synthesis and structural analysis of 5,6-dimethylbenzimidazolium-linked cyclophanes and their metal complexes are investigated in the literature. The antimicrobial activity of silver(I) carbene complex with water-soluble properties on some types of bacteria has been reported [18, 19].

As a result, three different material groups were applied to synthesize silver cyclophane compounds for innovative drug properties. Moreover, this article studied the antimicrobial activities of the carbene compounds and silver and palladium complexes against bacteria and fungi.

2. MATERIALS AND METHODS

2.1. Materials and Measurements

2,6-bis(chloromethyl)pyridine ($C_7H_7Cl_2N$≥99.00%), 5,6-dimetil-1H-benzo[d]imidazole ($C_9H_{10}N_2$≥99%), ethylbromoacetate ($C_4H_7BrO_2$≥98%), deuterium oxide (D_2O≥99.95%), deuterated chloroform ($CDCl_3$≥99.80%), N,N'-dimethylformamide (DMF≥99%), ethanol (C_2H_5OH≥99%), potassium hexafluorophosphate (KPF6≥99%), sodium carbonate (Na_2CO_3≥99%), hexane (C_6H_{14}≥99%), ethyl acetate ($C_4H_8O_2$≥99.50%), silver(I) oxide (Ag_2O≥99%) and palladium(II) chloride ($PdCl_2$≥99%) were purchased in different companies. Gram-negative, Gram-positive bacteria, and fungal strains were used for antimicrobial tests.

2.2. Instrumentations

In this study, Nuclear magnetic resonance spectroscopy (^{1}H-NMR, ^{13}C-NMR, Varian As 300 mercury), Fourier transform infrared spectroscopy (FT-IR, ATI Unicam 1000), Four-point probe (Qiatek, FFP 4), Thermogravimetric analysis (TGA 400, Perkin Elmer EXSTAR 6300), melt-

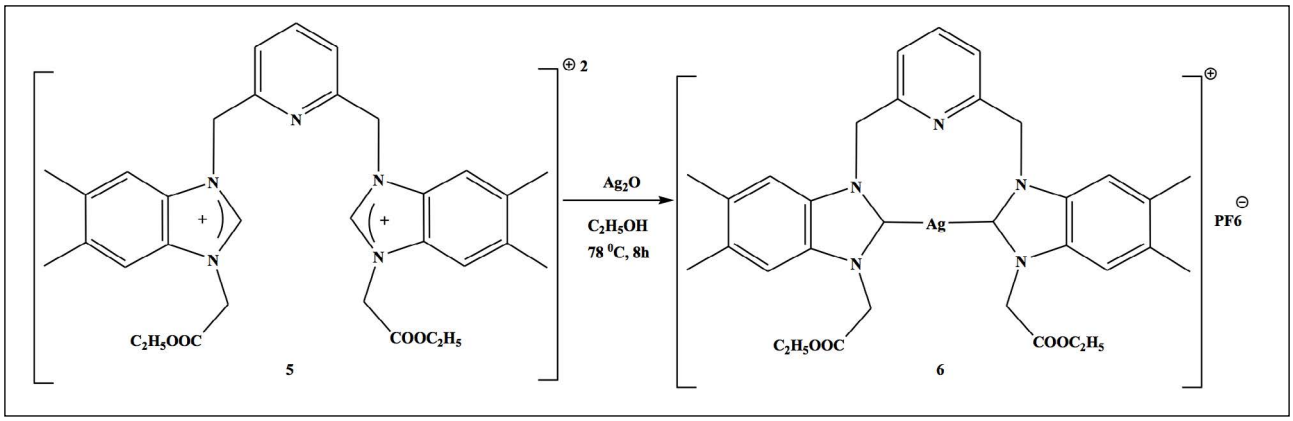

Figure 3. Synthesis ways of silver (I)-NHC complexes.

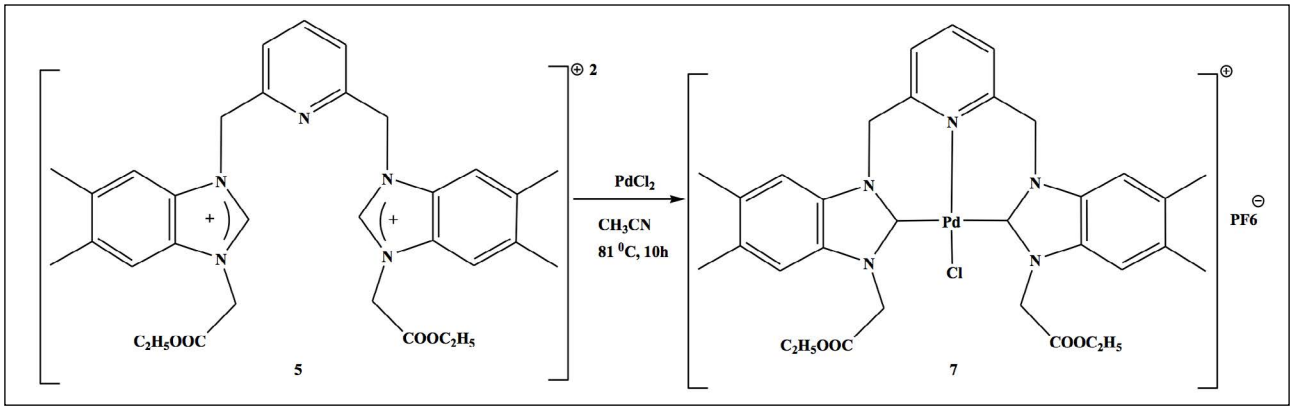

Figure 4. Synthesis ways of palladium(II)-NHC complexes.

ing point analysis (Electrothermal-9200), X-ray diffraction (XRD, Malvern Panalytical Empyrean), elemental analysis (Leco True Spec Micro), and Mass analysis (Shimadzu LC-MS/MS 8040) were used in different steps of the characterization methods.

2.3. Antimicrobial Activity

The antimicrobial activity of compounds 3, 5, 6, and 7 was measured in an agar dilution medium. In this experimental setup, the McFarland scale was set to 0.5. Ampicillin was used as a bactericide, and DMSO was used as a stock solution. The antimicrobial activities of these compounds were measured in Tryptic Soy Broth standards (TSB) at 37°C for 24 h. Viability values at 600 nm were measured by comparing 24 and 48-h for the incubation period. The Clinical laboratory standards institute was conducted for antimicrobial tests [20].

2.4. Synthesis Methods of N-Heterocyclic Carbenes

Heterocyclic carbene molecules were obtained with ethyl bromoacetate and cyclophane compound, which is water-soluble azolium salt of a symmetrical structure. Carbene compounds are ionic compounds that dissolve in water. These molecules' chemical and physical properties were comparatively examined in the literature. They are characterized by Four-point probe conductivity, ^{1}H-NMR, ^{13}C-NMR, FT-IR, TGA, melting point measurement, and XRD, as shown in Figure 1, 2.

2.5. Synthesis Ways of Metal Complexes

Silver (I)-NHC (6) and Pd (II)-NHC (7) complexes were obtained by the interaction of Ag_2O and $PdCl_2$ compounds with carbene compound 5, respectively. (Fig. 3, 4).

3. RESULTS AND DISCUSSIONS

3.1. FT-IR Measurements

FT-IR analyses of synthesized compounds 3 and 5 were examined for functional groups in their chemical structures, as given in Table 1.

In the FT-IR analysis of compounds of 3, the -C-H stretching of the CH_2 and CH_3 groups were obtained as 1000 and 838 cm^{-1}. -C-H stretching of the aromatic structure of 5,6-dimetil-1H-benzo[d]imidazole was given as 2944 cm-1. The cyclic ring structure of the imine group (-C=N:) was referred to at 3384 cm^{-1}. Additionally, the carbonyl of the acetate group (-C=O) showed a sharp peak at 1615 cm^{-1} for

Table 1. FT-IR peaks of compounds 3, 5, 6, and 7.

Compound name	υ(CH_3-CH_2) (alkane) (cm^{-1})	υ(C=C) (alkane) (cm^{-1})	υ(CH_2) (alkane) (cm^{-1})	υ(C=O) (carbonyl) (cm^{-1})	υ(NH_2) (amine) (cm^{-1})
3	949	1690	2921	–	3404
	834	1455	2852		
5	1000	1562	2944	1615	3384
	838	1458	2854		
6	1000	1486	2915	1716	3166
	838	1378		1596	2916
7	1000	1378	2885	1736	3155
	838			1599	2855

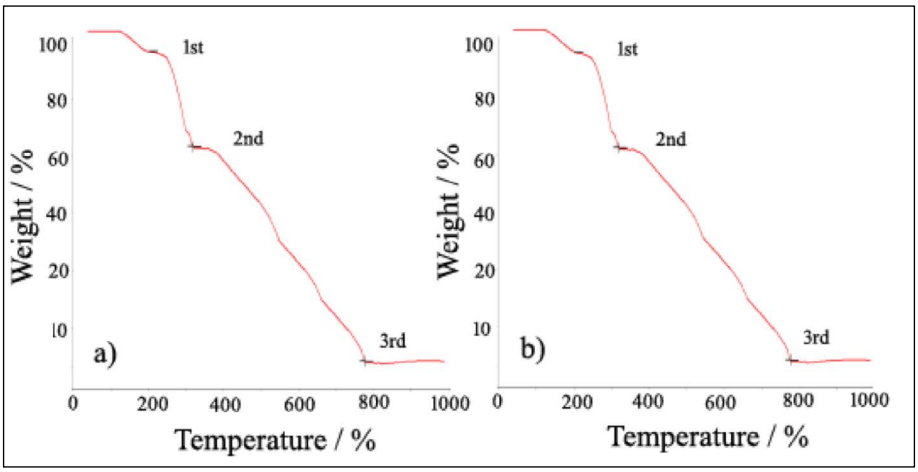

Figure 5. TGA graph compound of **(a)** 6 and **(b)** 7.

the compound of 5. The carbonyl of the acetate group was given at 1716 and 1596 cm^{-1}, and the cyclic ring of the imine group at 3166 and 2916 cm^{-1} for compound 6. In addition, the carbonyl of the acetate group was given at 1736 and 1599 cm^{-1}, and the cyclic ring of the imine group at 3155 and 2855 cm^{-1} for compound 7.

3.2. Thermal Gravimetric Analysis of Metal Complexes

Thermal gravimetric analysis (TGA) measurements of synthesized silver and palladium-containing metal complexes are presented in Figure 5. Depending on the weight loss of the complexes, organic and inorganic parts were distinguished from each other. This weight loss takes place in three steps. In the 1st step, the moisture of water molecules was removed from the molecules at temperatures between 101 and 399°C. In the 2nd step, there is a weight loss between 400 and 510°C, depending on the carbonization of the metal complexes. In the 3rd step, the carbonization of the organic part in the metal complexes continued at the temperature between 510 and 795°C. In addition, the highest weight loss occurred in the last step (Fig. 5).

3.3. XRD Analysis

XRD measurements of synthesized compounds were obtained in the 2θ=10-100°. Three different peaks were determined for compounds 3 and 5 and four for 6 and 7. For the silver and palladium complexes, three different characteristic peaks correspond to their complexes. Comp. 3; 001; 11.95, 002; 17.71, 003; 75.95, comp. 5; 001; 10.85, 002; 26.59, 003; 46.20, comp. 6; 001; 8.67, 002; 13.19, 003; 13.84, 004; 48.39, comp.7; 001; 8.17, 002; 12.38, 003; 13.34, 004; 49.68, respectively. The compounds were composed of crystalline Ag(I) and Pd(II) metals (Fig. 6). The synthesized carbenes contain C, N, and H as organic elements forming the main skeleton. Hexafluorophosphate, chlorine ions, and silver and palladium metals are bound to this organic part in metal complexes. The XRD peaks obtained in this analysis method consist of the reflection planes of the complexes. The large peak chlorine ion was observed between 8-28°C, which might be bound to the organic structure. In addition, the nano-structured part of the carbenes was also observed in the environment [21].

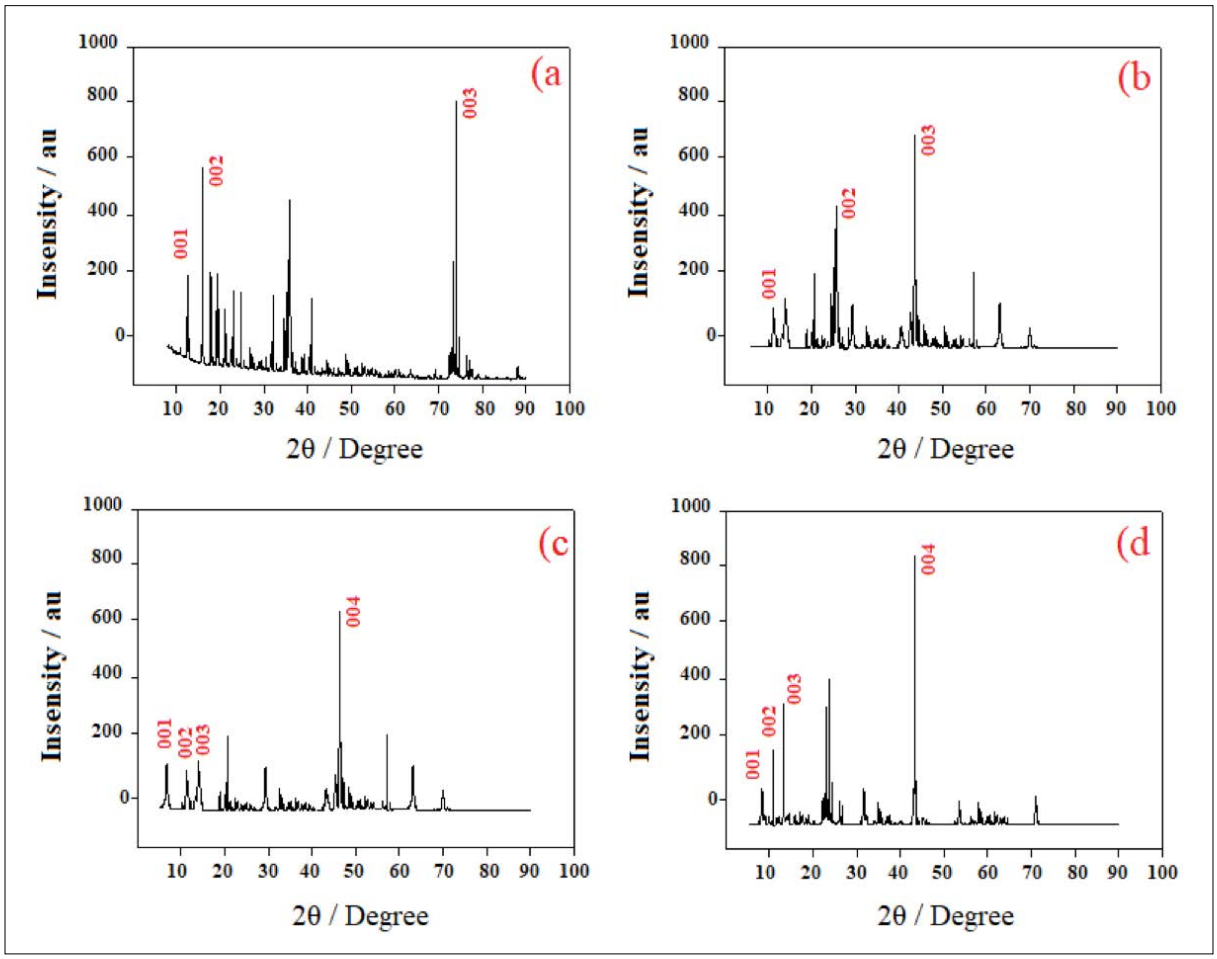

Figure 6. XRD peaks of comp. a) 3, b) 5, c) 6, and d) 7.

3.4. 1H-NMR and 13C-NMR Spectra

In the ^{1}H-NMR (300 MHz, D_2O) spectrum of compound (3) has signals at δ (ppm): 8.21 (s, 1H, *J*=11.7 Hz, for carbene), 7.45 (d, 2H), 7.21 (m, *J*=7.7 Hz, 2H), 6.99 (m, *J*=9.4 Hz, 2H), 5.66 (s, *J*=8.1 Hz, 4H), 2.22 (s, *J*=10.7 Hz, 12H). In the ^{13}C-NMR (75.5 MHz, D_2O) spectrum of compound (3) has signals at δ (ppm): 137.8 (C=C), 112.9 (C=C, for aromatic), 120.9 (C=C), 59.0 (NCH_2), 19.8 (CH_3), 152.5 (C=C), 130.1 (C=C, for aromatic), 128.0 (C=C, for aromatic).

In the ^{1}H-NMR (300 MHz, D_2O) spectrum of compound (5) has signals at δ (ppm): 9.28 (s, 1H, *J*=9.4 Hz, for carbene), 7.13 (d, 2H), 7.50 (t, *J*=7.4 Hz, 2H), 6.70 (d, *J*=9.5 Hz, 2H), 5.50 (m, *J*=6.8 Hz, 6H), 3.91 (s, 4H), 4.43 (t, 4H). 2.30 (s, *J*=10.8 Hz, 12H). In the ^{13}C-NMR (75.5 MHz, D_2O) spectrum of compound (5) has signals at δ (ppm): 147.20 (NCN, for carbene), 112.93 (C=C, for aromatic), 137.87 (C=C), 120.91 (C=C), 59.03 (NCH_2), 19.87 (CH_3), 152.52 (C=C), 130.15 (C=C, for aromatic), 128.05 (C=C, for aromatic), 48.93 (CH_2), 57.54 (CH_2).

In the ^{1}H-NMR (300 MHz, D_2O) spectrum of compound (6) has signals at δ (ppm): 7.85 (s, 1H), 7.52 (d, *J*=8.2-8.3 Hz, 4H), 7.41 (d, *J*=10.0-10.1 Hz, 2H), 5.52 (t, *J*=8.6-8.7 Hz, 4H), 5.10 (t, *J*=9.2-9.3 Hz, 4H), 3.88 (t, 6H), 2.65 (m, *J*=7.5 Hz, 12H), 1.46 (t, 6H). In the ^{13}C-NMR (75.5 MHz, D_2O) spectrum of compound (6) has signals at δ (ppm): 193.3 (NCN, for carbene), 134.3 (C=C, for aromatic), 112.9 (C=C), 122.0 (C=C), 72.7 (NCH_2), 59.1 (CH_2), 63.2 (OCH_2), 19.8 (CH_3), 172.1 (C=O), 154.9 (C=C), 138.1 (C=C, for aromatic), 130.1 (C=C, for aromatic) ppm.

In the 1H-NMR (300 MHz, D_2O) spectrum of compound (7) has signals at δ (ppm): 8.02 (s, 1H), 7.64 (d, 4H), 7.41 (d, *J*=6.6-6.7 Hz, 2H), 5.52 (t, *J*=9.5 Hz, 4H), 5.10 (t, *J*=7.8 Hz, 4H), 3.88 (t, *J*=9.8-9.9 Hz, 6H), 2.58 (m, *J*=5.4-5.5 Hz, 12H), 1.88 (s, *J*=5.4-5.5 Hz, 6H). ^{13}C-NMR (75.5 MHz, D_2O) spectrum of compound (7) has signals at δ (ppm): 176.7 (NCN, carbene), 134.3 (C=C, aromatic) 112.9 (C=C, aromatic), 122.0 (C=C, aromatic), 72.7 (NCH_2), 59.1 CH_2), 63.2 (OCH_2), 19.8 (CH_3), 13.9 (CH_3), 172.1 (C=O), 154.9 (C=C, aromatic), 138.1 (C=C, aromatic), 130.1 (C=C, aromatic) ppm.

3.5. Four-Point Probe Conductivity Measurements

Solid-state conductivity measurements of compounds 3, 4, 5, 6, and 7 were measured by a Four-point probe instrument. All materials were pressed into pellet forms using the pellet machine. The highest conductivity was obtained as $3.96\times10^{-}$

Table 2. MIC values of comp. (3, 5, 6, and 7) for bacterial resistance in antimicrobial tests

	MIC (µg/mL)				
	Gram-negative bacteria		Gram-positive bacteria		Fungal
Samples	*Escherichia coli*	*Listeria monocytogenes*	*Staphylococcus aureus*	*Bacillus cereus*	*Candida albicans*
3	84.95	86.52	79.95	78.65	81.23
5	72.65	71.32	70.62	72.36	69.38
6	0.01	0.02	0.02	0.02	0.01
7	55.61	54.68	46.38	47.96	41.64

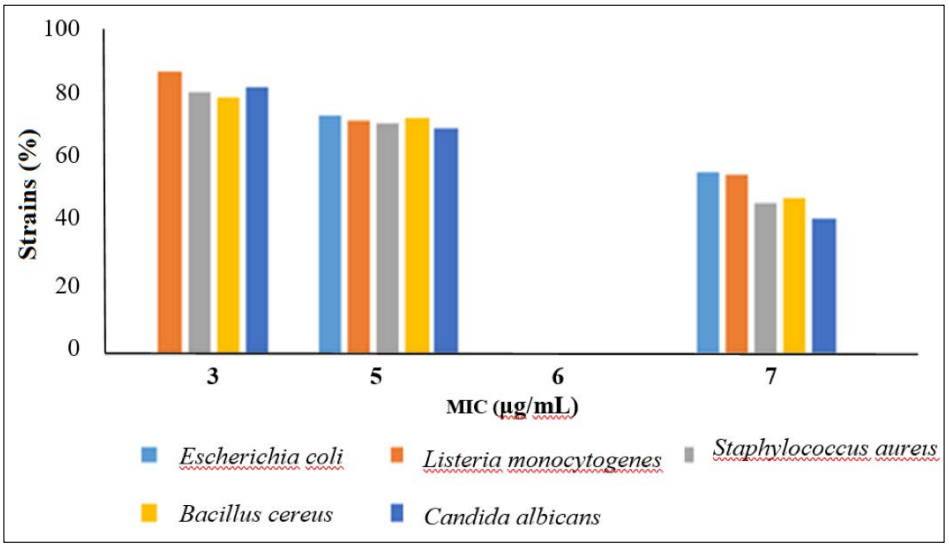

Figure 7. Compounds 3, 5, 6, and 7 were used against bacterial resistance in antimicrobial tests.

^{6} S/cm for compound 5. The other conductivity results are 5.76×10^{-7} S/cm, 2.17×10^{-6} S/cm, 5.94×10^{-7} S/cm, and 1.31×10^{-6} S/cm for compounds of 3, 4, 6, and 7, respectively. Pd(II)-NHC complex is more electrically conductive than Ag(I) due to the easy electron transfer from valance and conduction bands [22].

3.6. Antimicrobial Activities

The antibacterial activity measurement of four compounds was tested on four different bacteria and one yeast species. The results of the tests are separately given in Table 2. The antimicrobial activities of Ag(I) and Pd(II)-NHC complexes have shown that they have a relatively broad spectrum of antimicrobial activity [23, 24]. The silver complex of comp. 3, 5, 6, and 7 showed inhibitory effects against four different bacteria (*Escherichia coli* O157: H7 (ATCC 25922), *Listeria monocytogenes* (ATCC 19115), *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 11778) and a type of mushroom (*Candida albicans* (ATCC 10231). On the other hand, they had a very low inhibitory effect on different properties of bacterial species and fungal. Compared to the sulfamethoxazole drug, it has much lower antimicrobial activities. Complex six is effective against bacteria and fungal even though the stated absorbance measurements have some statistical flaws [25, 26]. The absorbance values were added for informational purposes even though the other synthetic compounds 3, 5, and 7 did not exhibit the anticipated inhibitory effects on the target bacteria (Fig. 7).

The tested compounds have a MIC range of 5 to 100 µg/mL. Compounds 3 and 5 were found to have similar activities against Gram-positive and Gram-negative bacteria, and fungal (Fig. 7). These results contained useful information for synthesizing NHC compounds with high antimicrobial activity. The efficiencies of synthesized carbenes and complexes of Ag(I)-NHC (6) and Pd(II)-NHC (7) were evaluated against antimicrobial agents [27].

4. CONCLUSIONS

The symmetric structure of the NHC ligands (3, 5) and NHC complexes containing the new pioneer (6, 7) were synthesized in this study. Many characterization techniques

were used, such as FT-IR, ^{1}H-NMR, ^{13}C-NMR, TGA analysis, XRD spectroscopy, and Four-point probe conductivity measurements. As a result of comparing the XRD results of the calculated compounds with the results of a similar cyclophane, the structures were confirmed.

Complexes 6 and 7 have shown transitions of $^{1}A_{1g} \rightarrow {}^{1}A_{2g}$, ^{1}A1g → $^{1}B_{1g}$, and $^{1}A_{1g} \rightarrow {}^{1}E_{g}$, respectively. The absorbance values observed at 285, 315, and 297 nm represent the transition charge transfer from NHCs to the Ag^{+} and Pd^{2+} ions by UV-vis spectrophotometer. According to the results of the electronic spectrum, the synthesized silver complex has a linear geometry coordinated with the ligand of the central Ag^{+} ion. In contrast, the synthesized palladium complex has a square planar geometry coordinated with the ligand of the central Pd^{2+} ion.

In this study, when the Ag(I)-NHC complex (6) is compared with other compounds (3,5,7), they have shown higher antimicrobial activity even at much lower concentrations. According to the findings, compounds containing silver ions have antimicrobial agent properties. Complex six's lowest microbial inhibition concentration (MIC) values were measured as 0.01 µg/ml for Escherichia coli and Candida albicans, respectively. The Ag(I)-NHC complex (6) showed higher antimicrobial activity than the carbene compound and Pd(II)-NHC complex (7).

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ETHICS

There are no ethical issues with the publication of this manuscript.

DATA AVAILABILITY STATEMENT

The authors confirm that the data that supports the findings of this study are available within the article. Raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

FINANCIAL DISCLOSURE

This study received financial support from the Trakya University Research Fund (TUBAP-2014-106).

PEER-REVIEW

Externally peer-reviewed.

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