

MONONUCLEAR LINEAR-SHAPED ASSEMBLIES OF N,N'-DIETHYLBENZIMIDAZOLYDINE BASED IONIC LIQUID COORDINATED TO AG(I)- AND CU(I)-NHC AND THEIR ANTIBACTERIAL AND ANTIOXIDANT APPLICATIONS

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ABSTRACT

A benzimidazolydine based ionic liquids containing ethyl substituents (L1) were prepared and further converted to ionic silver and copper complex were evaluated for antibacterial and antioxidant properties as evidenced by elemental analysis, FT-IR, (1D and 2D) NMR and EI-MS data followed by investigating the antibacterial and antioxidant activity. The L1, Ag-NHC and Cu-NHC existed and screened for antibacterial activity based on the zone of inhibition, Minimum Inhibitory Concentration determination and stability studies against *Staphylococcus aureus* and *Staphylococcus epidermidis*. The standard drug used was ascorbic acid. Ag-NHC showed maximum inhibitory effects on the radicals [$IC_{50} = 45.3 \mu M$ (DPPH), $47.1 \mu M$ (OH), $49.2 \mu M$ (NO)].

KEYWORDS: NHC, metal complexes, spectral characterization, antibacterial, antioxidant.

1. INTRODUCTION

The coordination chemistry of N-heterocyclic carbenes (NHCs) has established seductively from the time pioneering work (Öfele et al. 1968; Wanzlick et al. 1968), when the isolation and first complexation reviews of stable NHCs (Arduengo III et al. 1991; Arduengo III et al. 1993; Simonovic et al. 2009). The characteristic features of NHCs are strong σ -donor ligands, which can bind steadily to different metal ions with several oxidation states (Braband et al. 2003; Danopoulos et al. 2004). This is certainly responsible for their unexpected stability towards heat, air and moisture and therefore the loss of catalyst is forbidden during the

catalysis (Díez-González et al. 2006). NHCs have been bonded to nearly all transition metals with different synthetic routes, and the complexes have been verified for catalytic and biomedical applications (Glorius et al. 2007; Teyssot et al. 2009). Among numerous metal NHC complexes, Ag-NHC complexes have been synthesized maximum extensively (Lin et al. 2007). In 1993 the initial synthesis of Ag-NHC carbene complex has been widely used for transmetallation reactions where direct synthesis using other metal ions remained difficult or impossible (Garrison et al. 2005). Ag–NHC complexes are easily prepared by three different reaction procedures: (i) azolium salts with silver bases such as Ag_2O , Ag_2CO_3 , and AgOAc ; (ii) free NHC silver salts; and (iii) azolium salts with silver salts under basic phase transfer conditions (Lin et al. 2007; Lee et al. 2002).

Heterocyclic compounds such as benzimidazole, imidazole, triazole, benzodiazepine and indazole are significant classes of heterocyclic compounds revealing a wide spectrum of pharmacological properties (Gök et al. 2014; Ha et al. 2010). Benzimidazoles are observed as a promising class of bioactive heterocyclic compounds, which have involved substantial interest in medicinal chemistry since their various types of biological activities including antiviral, antioxidant, antimicrobial, anti-inflammatory, anticancer, antihypertensive and anticoagulant activities (Tuncbilek et al. 2009; Song et al. 2005). Specifically, most of the compounds were derived from benzimidazole-based NHC ligands have shown hopeful antimicrobial activity (Seenaiah et al. 2014). In previous studies, many groups of researchers have synthesized and determined the antimicrobial activity against microorganisms of a broad range of Ag–NHC complexes (Hindi et al. 2009; Maillard et al. 2013; Yıgıt et al. 2012). Ag^+ is non-toxic to humans in a low concentration, hence a compound that releases Ag^+ to the environment in a fixed rate make an effective antimicrobial agent (Haque et al. 2015).

In the current work, we report that *N,N'*-diethylbenzimidazolydine based ionic liquids and their silver and copper complexes in ionic form were characterized by IR, EI-MS, NMR and further evaluated for antibacterial study against gram positive bacteria followed by MIC determination. Furthermore, stability studies were determined *S. aureus* and *S. epidermidis* up to three weeks. We further evaluated the antioxidant activity of the synthesized compounds.

2. Experimental section

All handlings were carried out under a room temperature. The chemicals and solvents used were of AR grade, were obtained from sigma Aldrich. CHN microanalyses were carried out using a Euro Vector. Infrared spectra were recorded on Agilent Cary 630 FT-IR spectrometer in the range of 4000–400 cm^{-1} using KBr pellets. Raman spectra were recorded on a Bruker RFS FT-R spectrometer in the range of 4000–50 cm^{-1} using KBr pellets. ^1H and ^{13}C spectra were recorded in $\text{DMSO}-d_6$ on a Bruker AVANCE III 400, Bruker AVANCE I 400 and Bruker AVANCE II 200 spectrometer. Chemical shifts (δ) are expressed in ppm downfield from tetramethylsilane (TMS) using the residual protonated solvent as an internal standard. All coupling constants are expressed in hertz. Electrospray ionization mass spectra were recorded using a Waters UPLC-TQD Mass spectrometer.

2.1 Synthesis of N,N'-diethylbenzimidazolydine (L1)

L1 were prepared by refluxing 1-ethylbenzimidazole and ethyl bromide (1:1 mmol equivalents) in 1,4-dioxane for 32 h. The reaction was cooled to room temperature. The resultant solid product was washed three times with ethyl acetate and filtered to get white amorphous powder. White powder; yield: 78%; mp 237-239°C. FT-IR (KBr, ν_{max} , cm^{-1}): 3435 ($\text{C}_{\text{aliph}}-\text{N}_{\text{benzimi}}$); 3063 (arom C-H); 2968 (aliph C-H), 1458, 1409, 1364, 1301, 1272, 1245 ($\text{C}_{\text{arom}}-\text{N}_{\text{benzimi}}$); 1447 (aliph CH_2); 1425 (aliph CH_3). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 9.91 (s, 1H, NCHN), 7.65 (s, 1H, Ar-H6), 7.63 (s, 1H, Ar-H5), 7.39 (s, 1H, Ar-H4), 7.37 (s, 1H, Ar-H3), 4.77 (q, $J = 7.3$ Hz, 4H, Al-H8), 1.52 (t, $J = 7.2$ Hz, 6H, Al-H9). ^{13}C NMR (400 MHz, $\text{DMSO}-d_6$): 137.2 (NCHN), 132.1 (Ar-C7), 131.8 (Ar-C2), 126.7 (Ar-C5), 126.3 (Ar-C4), 121.2 (Ar-C6), 119.9 (Ar-C3), 49.1 (Al-C8), 17.3 (Al-C9). Anal. Calcd for $\text{C}_{11}\text{H}_{15}\text{BrN}_2$: C, 51.78; H, 5.93; N, 10.98. Found: C, 51.62; H, 5.88; N, 10.76.

2.2 Synthesis of Ionic Ag-NHC

Compound L1 and Ag_2O (1:0.5 mmol equivalent) were stirred in acetonitrile for 24 h under the exclusion of light and filtered over celite. The solvent was removed under vacuum to get sticky solid which was then dissolved in n-hexane and ethyl acetate was added and milky suspension stirred to get dark brown color powder. The residual solvent was removed under vacuum. Dark brown powder; yield: 82 %; mp >300 °C. FT-IR (KBr, ν_{max} , cm^{-1}): 3413 ($\text{C}_{\text{aliph}}-\text{N}_{\text{benzimi}}$); 3052 (arom C-H); 2925 (aliph C-H), 1461, 1363, 1300, 1238 ($\text{C}_{\text{arom}}-\text{N}_{\text{benzimi}}$); 1449 (aliph CH_2); 1410 (aliph CH_3); 476 (Ag- C_{NHC}). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 7.78 (s, 1H, Ar-H3, H6), 7.74 (s, 1H, Ar-H4, H5), 4.48 (q, $J = 7.3$ Hz, 8H, Al-H8), 1.60 (t, $J = 7.2$

Hz, 12H, Al-H9). ^{13}C NMR (100 MHz, DMSO- d_6): 178.8 (Ag-C1), 142.2 (Ar-C2, C7), 120.5 (Ar-C3, C6), 118.9 (Ar-C4, C5), 46.7 (Al-C8), 17.1 (Al-C9). Anal. Calcd for $\text{C}_{22}\text{H}_{30}\text{AgBrN}_4$: C, 49.09; H, 5.62; N, 10.41. Found: C, 48.84; H, 5.81; N, 10.35.

2.3 Synthesis of Ionic Cu-NHC

A sample of Ag-NHC and CuI (1:1) was dissolved in acetonitrile under a room temperature. The reaction mixture was stirred for 16 h at 50°C. The resulting product was filtered slowly through a celite pad. The filtrate was washed in diethyl ether resulted in the precipitation of pale yellow solid and the solid was dried under vacuum. Pale yellow powder; yield: 82 %; mp >300°C. FT-IR (KBr, ν_{max} , cm^{-1}): 3443 ($\text{C}_{\text{aliph}}\text{-N}_{\text{benzimi}}$); 3050 (arom C-H); 2914 (aliph C-H), 1469, 1352, 1277, 1241 ($\text{C}_{\text{arom}}\text{-N}_{\text{benzimi}}$); 1449 (aliph CH_2); 1410 (aliph CH_3); 469 (Cu- C_{NHC}). ^1H NMR (400 MHz, DMSO- d_6): δ 7.71 (s, 1H, Ar-H3, H6), 7.63 (s, 1H, Ar-H4, H5), 4.47 (q, $J = 7.1$ Hz, 8H, Al-H8), 1.59 (t, $J = 7.1$ Hz, 12H, Al-H9). ^{13}C NMR (50 MHz, DMSO- d_6): 174.8 (Cu-C1), 137.9 (Ar-C2, C7), 125.8 (Ar-C3, C6), 120.4 (Ar-C4, C5), 45.7 (Al-C8), 16.3 (Al-C9). Anal. Calcd for $\text{C}_{22}\text{H}_{30}\text{CuBrN}_4$: C, 48.85; H, 5.59; N, 10.36. Found: C, 48.61; H, 5.53; N, 10.27.

2.4 Biological screening

2.4.1 Antibacterial evaluation

The media Mueller Hinton agar was prepared for antibacterial activity. The concentration of the given samples (10 mg/disc) was applied on the sterile discs. Inhibition of bacterial growth by tested compounds (L1, Ag-NHC and Cu-NHC) was assayed by the agar well diffusion method. The human pathogenic *S. aureus*, *S. epidermidis*, *E. coli* and *P. aeruginosa* bacterial cultures were clinically isolated and used for antibacterial evaluation. Penicillin was used as an antibacterial agent for the positive control and the solvent (DMSO) as negative control. The plates were incubated overnight at 37°C for 24 h and the zone of inhibitions were measured in mm (Xu et al. 2015).

2.4.2 MIC determination

MIC test was completed to attain the most effective compounds with the capacity to prevent the growth of pathogenic bacteria at its lowest concentration. Bacterial strains *S. aureus* and *S. epidermidis* were developed instantaneously with vigorous shaking at 37°C. The bacterial growth was determined by identifying the infectious growth after 24 h of incubation at 28°C. The growth rates were dignified by checking the optical density at 600 nm through different time intervals using a UV-vis spectrophotometer (Shimadzu UV-2550). The lowermost

concentration of the antibacterial agent at which has no bacterial growth is detected in the culture suspensions is well-defined as the MIC (Sainis et al. 2016).

2.4.3 Stability experiment

The stability of the tested compounds (L1, Ag-NHC and Cu-NHC) against *S. aureus* and *S. epidermidis* were evaluated for a period 21 days with the working concentrations of 100 µM. 2% of inoculum of the overnight grown *S. aureus* and *S. epidermidis* bacterial cultures were immunized in the compound solution and the stability of long-term was determined by graphical inspection of the bacterial growth monitored by an absorbance reading at 600 nm by UV-vis spectrophotometer (Wang et al. 2016).

2.5 Antioxidant activity

The antioxidant potential of the compounds L1, Ag-NHC and Cu-NHC. The tested compounds (L1, Ag-NHC and Cu-NHC) and ascorbic acid (positive control) were dissolved in DMSO. The tests were carried readily by detecting the variations in absorbance of free radical solution in the occurrence of several concentrations (0.20-30 µM) of tested compounds. In all the cases, ascorbic acid was used as a standard (Kim et al. 2003). For the above three assays, all of the experiments were done in triplicates and the percent inhibition of each compound was determined using the following equation (GÜLÇin et al. 2005).

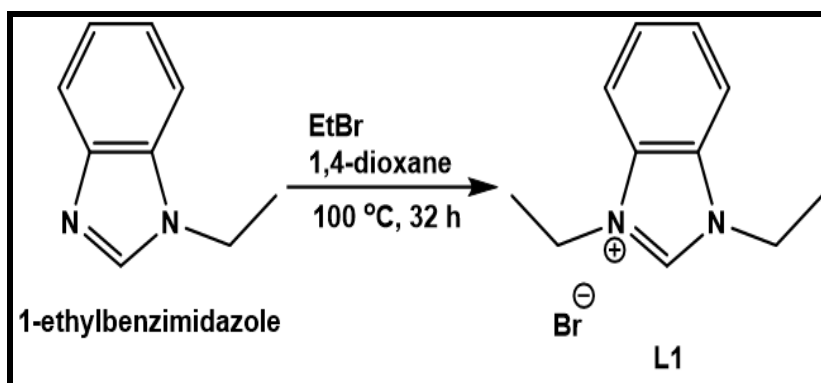
$$\% \text{ scavenging activity} = [1 - A_{\text{sample}} / A_{\text{control}}] \times 100 \dots\dots\dots (1)$$

Where A_{control} = absorbance of free radical solution, A_{sample} = absorbance of free radical solution comprising the tested compound. Moreover, IC_{50} values of each compound was also calculated using the percentage of activity.

3. RESULTS AND DISCUSSION

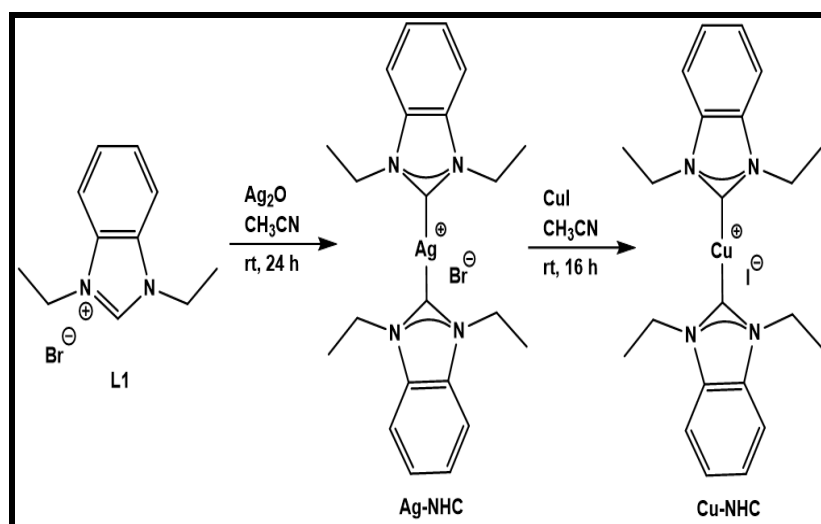
Benzimidazole is a pharmaceutically important heterocyclic moiety where its derivatives exists structural and naturally arising nucleotides which decide them to interrelate with the biopolymers of living systems. It exhibited the greater attention to examine benzimidazolydine salt and respective mononuclear Ag(I)-NHC and Cu(I)-NHC complex contrary to various types of bacteria.

3.1 Synthesis



Scheme 1: Synthesis of N, N'-diethylbenzimidazolydine (L1).

The reaction of one equivalent of *N*-ethylbenzimidazole with ethyl bromide in 1,4-dioxane at 100°C for 32 h afforded *N,N'*-diethylbenzimidazolydine salt with 78% yield. 1,4-dioxane used as a reaction medium for the synthesis of benzimidazolium salts is much suggested because of its appropriate polarity and suitable boiling point (101°C) as shown in Scheme 1. The collection of *N,N'*-diethylbenzimidazolydine salt as halide in pure form becomes difficult.



Scheme 2: Synthesis of complex Ag-NHC and transmetalation of the mononuclear complex Cu-NHC.

Synthesis of mononuclear Ag–NHC complexes using azolium halides as an initial material is of importance, meanwhile using this method pure form of desired complex can be attained with affluence. Scheme 2 displays single simple step for the synthesis, starting from *N*-alkylated benzimidazolydine of mononuclear Ag–NHC complex. Since Ag(I) is a good carbene transfer agent, we tried to transmetalate the Ag(I) ions in Ag-NHC for Cu-NHC complex. A mixture of Ag-NHC and CuI (1:1 mmol equivalents) were stirred in acetonitrile

for 16 h at room temperature led to the formation of Cu-NHC was obtained as a pale yellow solid as a good yield. The compound were soluble in most of the organic solvents and produce stable solutions. The structural and purity of the synthesized compound (L1, Ag-NHC and Cu-NHC) were confirmed on the basis of elemental analysis (CHN analyzer) and spectral studies (IR, ^1H , ^{13}C , DEPT-135, COSY-HSQC and COSY-HMBC NMR, EI-MS).

3.2 IR spectra

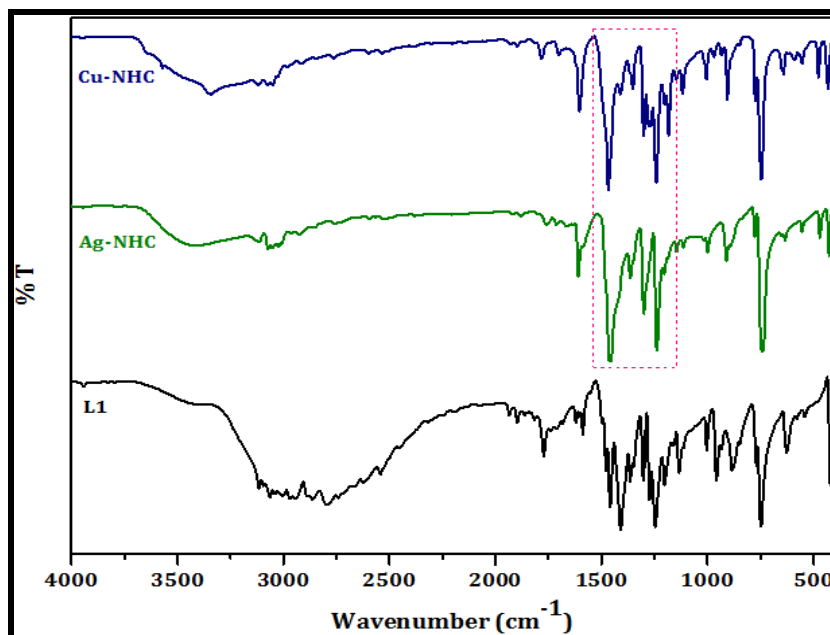


Fig. 1: IR spectra of synthesized compound.

The formation of ligand and metal complexes were represented in Fig. 1. In ligand L1 the aromatic C-H stretching was appeared at 3063 cm^{-1} , respectively. Whereas the aliphatic C-H stretching was observed at 2968 cm^{-1} . The aliphatic CH_2 and CH_3 bending appeared at 1447 and 1425 cm^{-1} confirms the formation of N-alkylated ethyl derivatives in ligand L1. In Ag-NHC and Cu-NHC the aromatic C-H stretching was observed in the range of $3052\text{--}3050\text{ cm}^{-1}$. However the aliphatic C-H stretching was appeared in the range of $2925\text{--}2914\text{ cm}^{-1}$. The aliphatic CH_2 and CH_3 bending was appeared in the range of $1449\text{--}1410\text{ cm}^{-1}$. The Metal-NHC was appeared at 476 cm^{-1} in Ag-NHC and 469 cm^{-1} in Cu-NHC.

3.3 FT-Raman spectra

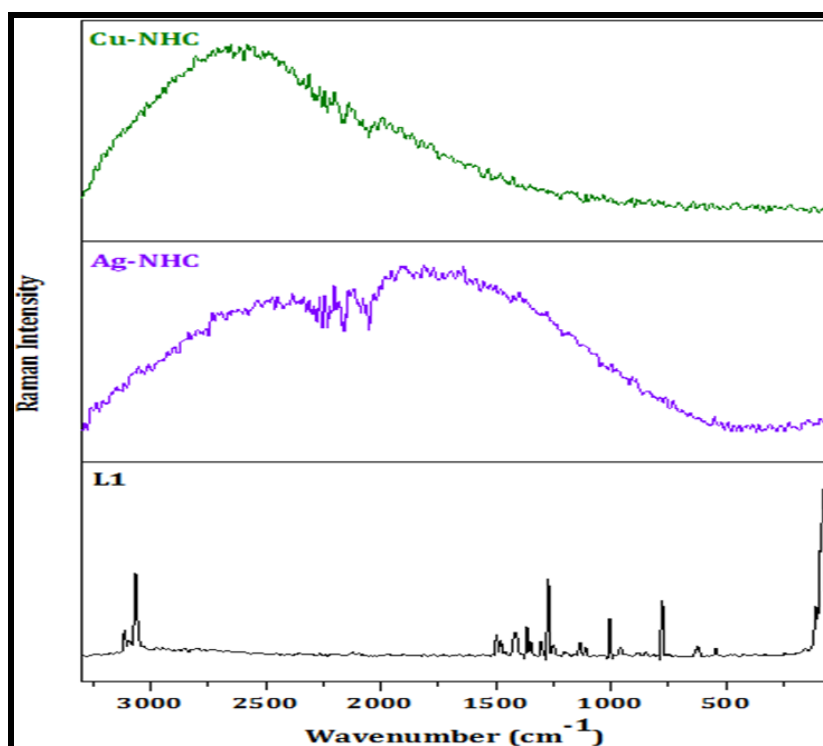


Fig. 2: Raman spectra of synthesized compound.

The Raman spectra of the ligand and metal-NHCs are represented in Fig. 2. The nonappearance of C=N stretching was clearly unidentified at 1600 cm^{-1} . The band at $1380\text{--}1364\text{ cm}^{-1}$ could be attributed to C-N cm^{-1} stretching vibrations. The strong band lies in the region $3100\text{--}3054\text{ cm}^{-1}$ was assigned to aromatic C=H stretching. The weak band in the range of $1520\text{--}1414\text{ cm}^{-1}$ was assigned to aromatic C=C stretching. The band at $1474\text{--}1463\text{ cm}^{-1}$ could be attributed to CH_3 and CH_2 asymmetric stretching. The weak band at $319\text{--}268\text{ cm}^{-1}$ could be due to aliphatic chains of C-C. From the above given data, the formation of L1, Ag-NHC and Cu-NHC were established. The metal-NHC characteristic peaks at 544 cm^{-1} in Ag-NHC and 539 cm^{-1} in Cu-NHC complexes, and from the above discussion we concluded the development of ligands and metal complexes (Hutchinson et al. 1970).

3.4 NMR spectra

The formation of compound L1 was confirmed by ^1H , ^{13}C , DEPT-135 and COSY (HSQC & HMBC) NMR spectroscopy. The ^1H NMR spectrum of compound L1, the characteristic resonance for the benzimidazolydine H1 proton was appeared at $\delta\ 9.91\text{ ppm}$. There are four characteristic singlet at $\delta\ 7.65, 7.63, 7.39$ and 7.37 ppm corresponding to aromatic proton (H6, H5, H4 and H3) respectively. In addition, it also exhibited the characteristic quartet at δ

4.77 ppm could be attributed to N-alkylation (ethyl) derivative (CH_2). Furthermore the triplet peak at δ 17.3 ppm is corresponding to methyl derivative (CH_3). The ^{13}C NMR spectrum of compound L1 features a signal for the carbon nuclei of the benzimidazolydine NCHN was appeared at δ 137.2 ppm. Also it shows the resonance for CH_2 -C8 and CH_3 -C9 carbon atom observed at δ 49.1 and 17.3 ppm respectively. All the aromatic carbon nuclei appeared in the range δ 131.8-119.9 ppm. From the above discussion we concluded the structure of ligand L1.

The formation of Ag-NHC was completely characterized by ^1H , ^{13}C , DEPT-135 and COSY (HSQC & HMBC) NMR spectroscopy. The characteristic feature of silver complexation is the nonappearance of ^1H signal for benzimidazolydine H1 proton which confirms the positive Ag-NHC formation. The signal responsible for benzimidazolydine NHCN at δ 9.91 ppm was completely absent in Ag-NHC. The silver and copper complexation causes important reform in electron density on benzimidazolydine ring and explains substantial peak shifting of ^1H and ^{13}C signals in silver and copper complexes. In ^{13}C NMR spectrum, the characteristic resonance for Ag-C1 carbon atom appeared at δ 178.8 ppm. The *N,N'*-disubstituted ethyl derivative carbon atom C8 and C9 was observed at δ 46.7 and 17.1 ppm.

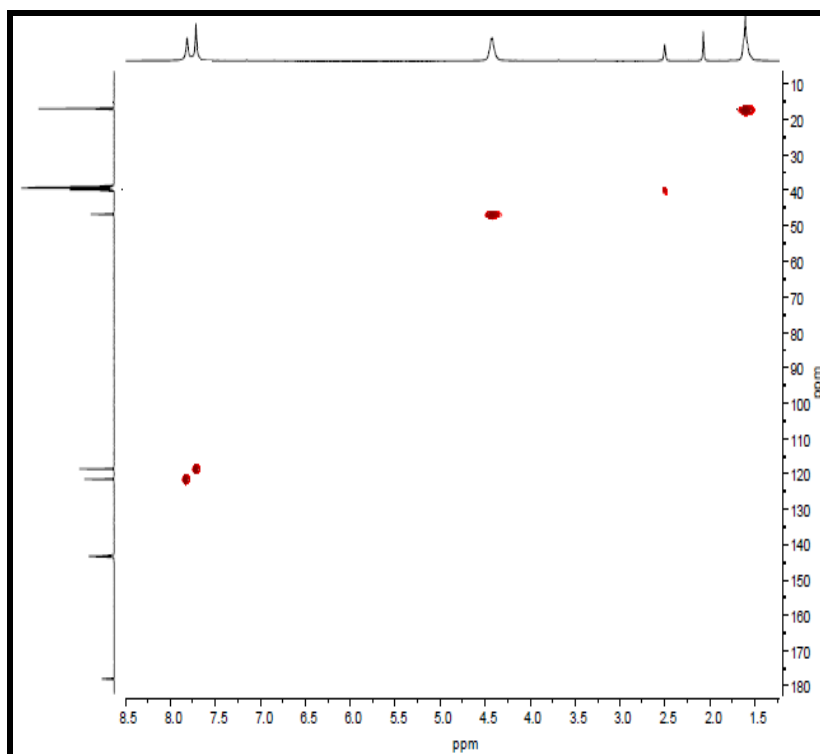


Fig. 4 HC-Heteronuclear Single Quantum Correlation spectra of complex Ag-NHC in $\text{DMSO}-d_6$.

The development of Cu-NHC was characterized by ^1H and ^{13}C NMR spectroscopy. In the ^{13}C NMR spectrum, the resonance for the NHCN carbon atoms was observed at δ 174.8 ppm (in DMSO- d_6). All equivalent ^1H and ^{13}C NMR spectral parameters of complex Cu-NHC are slightly related to the corresponding spectral limitations of complexes Ag-NHC. Hence 2D NMR experiments were not performed for Cu-NHC complex. All of the proton and carbon signals were fully assigned by 1D (^1H , ^{13}C and DEPT-135) and 2D NMR measurements (HSQC and HMBC). Heteronuclear single quantum correlation NMR spectra of Ag-NHC complex were represented in Fig. 3.

3.5 Mass spectra

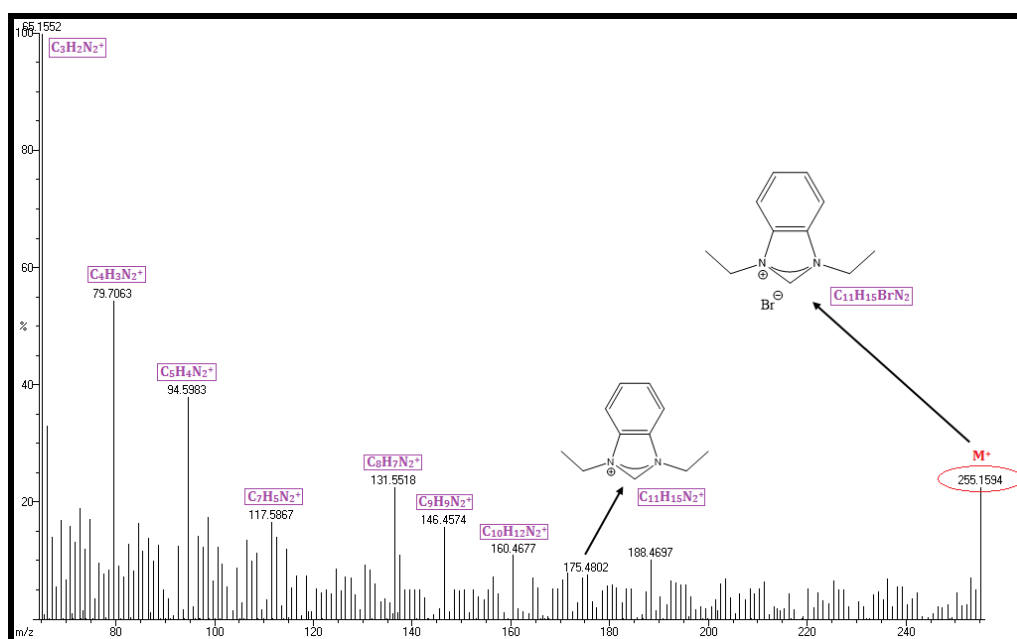


Fig. 5 EI-mass spectra of N,N'-diethylbenzimidazolydine (L1).

The fragmentation pattern of L1 was studied in detail (Fig. 4). L1 had some major fragments such as peak at m/z 255.1594 corresponding to molecular cation ($\text{C}_{11}\text{H}_{15}\text{BrN}_2$), which is in agreement with the calculated mass m/z 255.1590. The peak for $\text{C}_{11}\text{H}_{15}\text{N}_2^+$ appeared at m/z 175.4802 while another cation peak at m/z 160.4677 corresponds to $\text{C}_{10}\text{H}_{12}\text{N}_2^+$. The succeeding fragment formed was of $\text{C}_9\text{H}_9\text{N}_2^+$ at m/z 146.4574 while the peak at m/z 131.5518 corresponds to $\text{C}_8\text{H}_7\text{N}_2^+$. The peak for $\text{C}_7\text{H}_5\text{N}_2^+$ was performed at m/z 117.5867, whereas the following fragment was observed at m/z 94.5983 which could be attributed to $\text{C}_5\text{H}_4\text{N}_2^+$. The subsequent fragment appeared at m/z 79.7063 cation due to $\text{C}_4\text{H}_3\text{N}_2^+$. The resulting fragment performed at m/z 65.1552 corresponds to $\text{C}_3\text{H}_2\text{N}_2^+$. The appearance of corresponding fragments validates the successful formation of L1.

The EI-MS (positive ions) of Ag-NHC featured the most intense peak at 538.2820 m/z (Calcd. For Ag-NHC 538.2822 m/z). The EI-MS (positive ions) confirmed the formation of Cu-NHC by exhibiting an intense peak at 540.9603 m/z (Calcd. For Cu-NHC 540.9605 m/z). In addition, the electrospray ionization mass spectra of complex Ag-NHC and Cu-NHC are given in supporting information (Fig. S12 & S13).

3.6 Antimicrobial Evaluation

3.6.1 Antibacterial activity

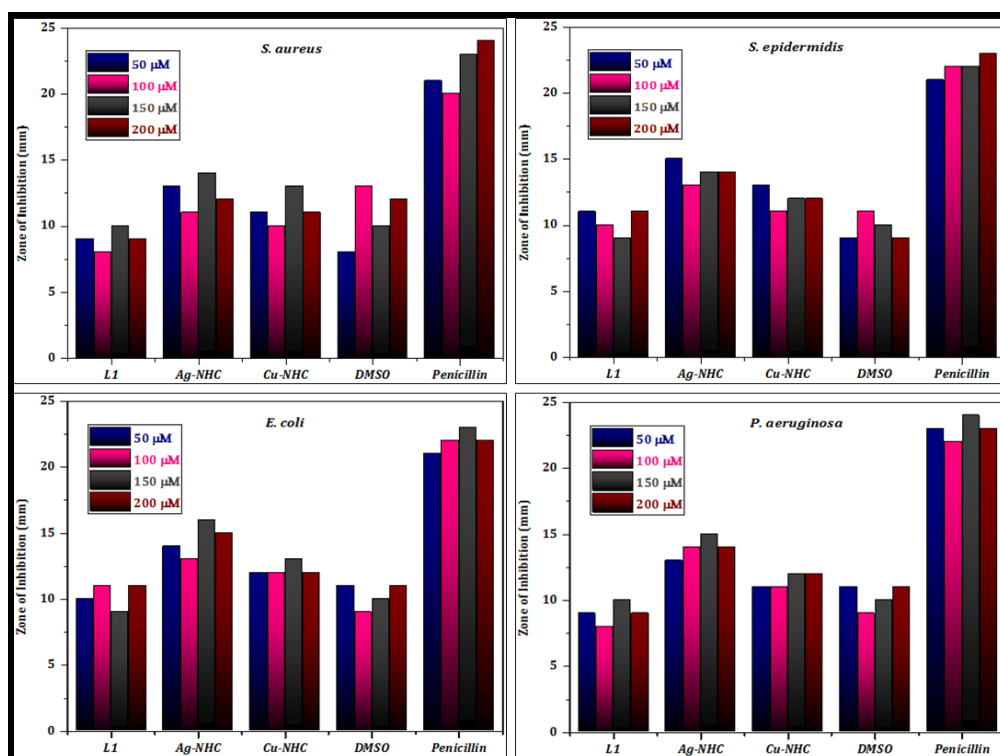


Fig. 5 Antibacterial activity of synthesized compound in the terms of zone of inhibition (mm).

The antibacterial potential of test compounds was investigated against *S. aureus*, *S. epidermidis*, *E. coli* and *P. aeruginosa* in the range of (50-200 μ M) using DMSO (control) and penicillin (reference standard). The activity was measured as a zone of inhibition and ranges from 7 to 15 mm. The lack of substantial increase in a zone of inhibition upon developing the dose of test compound concludes the dose-independent activity (Xu et al. 2015). The zone of inhibition value (mm) were represented in Fig. 5.

3.6.2 MIC determination

Minimum Inhibitory Concentration of an antibacterial agent was made to obtain the most active compounds with the ability to inhibit the growth of pathogenic bacteria at its lowest

concentration. It is normally determined by graphical inspection of bacterial growth for permanent time. OD measurement after 5 d has given perfect data related to visual inspection, as in the case of visual inspection, few sample containers can have analogous growth in the method of turbidity. The maximum potent compound Ag-NHC showed MIC value of 6.25 μM against *S. aureus* and 25 μM against *S. epidermidis*. The moderate active compound Cu-NHC exhibited MIC value of 25 μM against *S. aureus* and 50 μM against *S. epidermidis*. The least active compound L1 displayed 100 μM against *S. aureus* and 50 μM against *S. epidermidis*. The ligand L1 able to interrupt cell membrane due to the presence of side chain and exhibited antibacterial activity. The MIC values of tested compounds (L1, Ag-NHC and Cu-NHC) were represented in Table 1.

Table 1: Minimum Inhibitory Concentration results of tested compounds.

Tested Compounds	L1	Ag-NHC	Cu-NHC
<i>S. aureus</i>	100	6.25	25
<i>S. epidermidis</i>	50	25	50
Note: The MIC values are expressed in μM			

A prominent statement can be made that Ag-NHC have greater MIC in gram-positive pathogens which can execute bacteria through entering cells over loss of essential enzymes that can drag Ag^+ ion on the surface cell wall or damage of cell wall integrity and permeability by elimination of an electron from these cellular mechanisms and thus making gram-positive bacteria unaffected to silver (Sainis et al. 2016).

3.6.3 Stability studies

The stability of silver and copper complexes with respect to time has been calculated by inoculating 100 μM solution of each compound through a bacterial culture in liquid medium. The growth was experimentally visualized on 7, 14 and 21 days. No bacterial growth was observed in the culture of *S. aureus* and *S. epidermidis* up to 7 days in L1, Ag-NHC and Cu-NHC but after 14 days L1 showed turbidity whereas other test compound were still active. After 21 days Ag-NHC showed an absence of bacterial growth but Cu-NHC were observed evidently. Similarly, with *S. epidermidis*, all tested compound were active up to 7 days, whereas L1 and Cu-NHC lost their activity. Only Ag-NHC retained its activity when continued up to 21 days as observed visibly. Ag-NHC can be claimed amongst all test compounds studied as the most active compound. Table 2 represents the study of tested compounds (L1, Ag-NHC and Cu-NHC) for bacterial growth inhibition.

Table 2: Stability studies of tested compounds for bacterial growth inhibition.

Tested Compounds	<i>S. aureus</i>			<i>S. epidermidis</i>		
	7	14	21	7	14	21
L1	-	+	+	-	+	+
Ag-NHC	-	-	-	-	-	-
Cu-NHC	-	-	+	-	+	+

Note: The negative sign (-) indicates growth not observed and the positive sign (+) indicates growth observed

The precursor benzimidazolydine salts had no activity in contradiction of the similar strains of bacteria, even at high concentrations associated to the relative complexes. This tendency emphasizes the incidence of aromatic moiety to show activity for a longer time. This results the significance of having aromatic or steric substitutions for providing additional stability to Ag-NHC and to extent the Ag^+ release over a continuous time lesser activity for extensive time (Wang et al. 2016).

3.7 Antioxidant studies

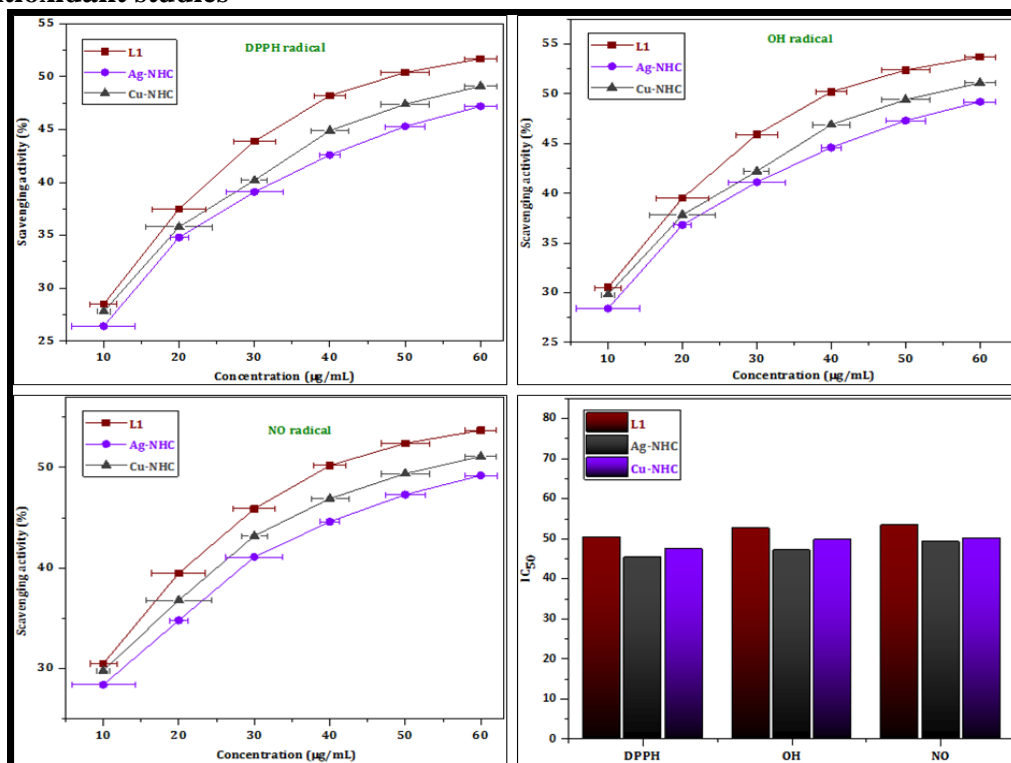


Fig. 6: Radical scavenging potential of the tested compounds (L1, Ag-NHC and Cu-NHC) at different concentrations. Bar diagram illustrates the IC_{50} values for the inhibitory properties of the compounds on DPPH, OH and NO radical.

The radical scavenging potency of the newly synthesized compounds (L1, Ag-NHC and Cu-NHC), by their ability to control the DPPH, OH and NO radical. The antioxidant activity of

the tested compounds also have the selected free radicals. It is noticeable that the scavenging ability of the compounds was dose dependent and destruction ratio was better with increasing sample concentration. The calculated IC₅₀ values of compounds L1, Ag-NHC and Cu-NHC were found to be 50.4, 45.3, 47.4 μ M for DPPH radical, 52.6, 47.1, 49.7 μ M for OH radical and 53.4, 49.2, 50.1 μ M for NO radical, respectively. Moreover, the compounds exhibited selective scavenging ability in the direction of DPPH, OH radical than NO radical. This tendency is equivalent to antioxidant action of various compounds reported by various researchers. In ligand L1 the existence of acidic proton was appeared at C1 position due to the appearance of acidic proton ligand shows higher % inhibition. The proton is removed by silver coordination in respective Ag-NHC complexes, which may reduce further the activity. (Cole et al. 1974) labelled that pure benzimidazole and their alkyl derivatives have no uninterrupted antioxidant whereas (Babizhayev et al. 1998) relating structure-activity connection of imidazole derivatives, revealed that imidazole ring is answerable for free radical scavenging properties. Still our properties are comparable to Cole's conclusions.

CONCLUSIONS

The newly synthesized benzimidazolydine based ionic liquids containing ethyl substituent and their ionic silver and copper complexes were obtained as a fine powder and stable towards moisture. The mononuclear silver complex undergoes transmetalation with CuI to give homo-mononuclear copper complex. The symmetrically substituted silver complex had shown MIC value of 6.25 μ M in *S. aureus* but ineffective to show the similar value in *S. epidermidis* which arises due to the resistance for silver. This resulted in maximum MIC in gram-positive bacteria. However, the antioxidant results showed that the compounds exhibit significant scavenging action against DPPH and OH rather than NO radical. Moreover, Ag-NHC compound has the greatest radical scavenging capability and probably it can be considered additional for medicinal applications.

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