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Synthesis, Structural Analysis and Antimicrobial Activities of Novel Water Soluble Ionic Liquids Derived from *N*-Heterocyclic Carbene Salts

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Abstract: Six *N*-heterocyclic Carbene based Ionic Liquids (ILs) have been synthesized by conventional methods. The ILs were spectroscopically characterized by NMR and FT-IR techniques. Their *in vitro* antimicrobial activities were determined towards gram-positive and gram-negative bacterias and yeast strains using minimum inhibition concentration (MIC) assay. The best inhibition performances were obtained with compound **1** due to its more hydrophilic nature compared with the others. It exhibited 1 mg L^{-1} MIC value against to the most bacteria while the others showed 4 mg L^{-1}.

Keywords: ionic liquids, *N*-heterocyclic carbenes, antimicrobial activity, *N*-functional substituents.

INTRODUCTION

The term "Green chemistry" has been accepted as the movement towards environmental friendly chemical processes and products for a recent decade. Researchers in all over the world have attempted considerable efforts to explore novel, environmental-friendly chemicals or materials that they should contribute green chemistry or sustainable chemistry concepts. For achieving this aim, ionic liquids (ILs) are a very best class of novel materials. Ionic liquids are known as a class of compounds, composed of an anion and cation like salts. Their melting point are below 100 °C. ILs have attracted an increasing attention for their unique physical and chemical properties such as low vapor pressure, high polarities, electrical conductivities and non-flammability.[1–3] Since they have multi-faced capabilities, every researcher has handled them from different aspects. In a study polymer material exhibited good solubility in Ils,[4] while in some other studies the researchers tested ILs for their gas solubility.[5] Therefore, they seem to be substituted for volatile organic compounds (VOCs) as different solvents. Besides, ILs and NHCs are used as regenerable reaction media and catalysts as metal-free salts and metal complexes.[6–9] Also, some researchers have investigated them for their use in electrochemical applications too.[10–12] Recent days they have been subjected to physical explorations because of their liquid phase properties such as liquid-liquid equilibrium and solubilities in other liquids,[13–15] and tested NHC derivative ILs in supported membrane studies, extraction[16,17] and in nanochemistry too.[18,19]

N-heterocyclic carbenes (NHCs) are cyclic compounds containing one or mostly two N atoms in the main C ring (Figure 1). Many natural compounds such as caffeine and some amino acids like uraconic acid contain an *N*-heterocyclic part, and NHC salts can be obtained by simple modifications of these natural compounds. (Figure 2).[20–22]

NHCs are mostly used as ligands for complexation reactions with transition metals like Ag(I), Pd(II), Pt(II), *etc*. The transition metal complexes of NHCs have been studied

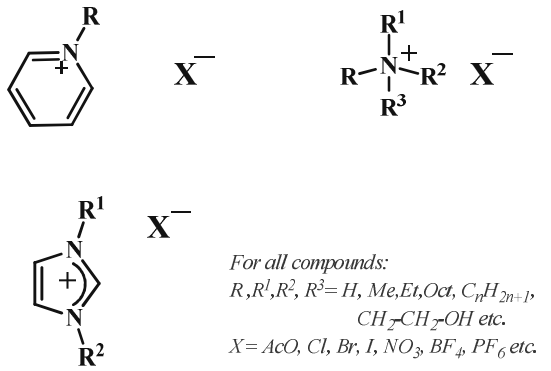


Figure 1. Examples for the types of ILs with N atoms.

and used as catalysts in various types of reactions such as polymerization, cyclization, reduction/oxidation, C–C bond formation/cleavage, hydrosilylation, *etc*.[23] Also, some researches have been conducted to utilize them as antimicrobial agents,[24] as a surface antigen against hepatitis B[25] and even as anticancer drug candidates.[26] The Antimicrobial activities of Ag(I)-NHC complexes have been examined by Young *et. al.* since 2004 to present day.[27–30] Roland *et. al.* also examined a series of Ag(I) NHC complexes for their antimicrobial properties as well.[31] Metal-free NHC compounds and their derivatives were also examined for their biological and medicinal activities by many researchers.[32,33] Antimicrobial activities and other pharmaceutical properties such as toxicity and antiseptic activities of ILs were pioneered by the research groups of Iawa and Gonzalez-Chavez rigorously.[34,35] According to their studies, the primary advantage of the ILs in use is their well adjustable electronic, steric and other properties with changing the substituents and counter anions.

In this study, we have synthesized six novel ILs (compounds **1**–**6**) and examined them for their antimicrobial activity.

MATERIALS AND METHODS

Synthesis and Characterization of *N*-Heterocyclic Carbene Salts

Six novel *N*-heterocyclic carbenes (as of now referred as **1**–**6**) were synthesized by conventional methods described in R&D. All materials and solvents such as imidazole, *N*-methyl imidazole, 2-bromoethaneamine hydrobromide were obtained from Sigma-Aldrich, Merck, and Alfa-Aesar and used without further purification. All synthesizing process were carried out in schlenk type tubes. Reaction progress was monitored by TLC (thin layer chromatography) using aluminum sheets coated with silica gel. Melting points were taken on a Yanagimito micro-melting point apparatus and uncorrected. IR spectra were measured on a Perkin Elmer

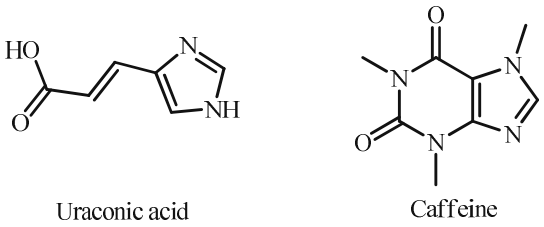


Figure 2. Examples for NHC compounds originated from natural compounds.

Spectrum100 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were taken on spectrometer at VARIAN Infinity Plus 300 and 75 Hz respectively. ^{1}H and ^{13}C shifts were referenced to the internal deuterated solvent. The second peak in the acidic region referred that the "-.HBr" group has not left the molecule during the synthesis process.

Antimicrobial Activity

In vitro antimicrobial activity of the compounds (**1**–**6**) were investigated against microorganisms including three Gram-positive bacteria (*Staphylococcus aureus* ATCC6538*,* vancomycin resistant *Enterococcus faecium* (VREF) DSM 13590*, Bacillus subtilis* ATCC 168, three Gram-negative bacteria (*Escherichia coli* O157:H7 RSKK 234, *Salmonella typhimurium* CCM 5445*, Pseudomonas aeruginosa* ATCC 27853) and three yeast strains *(Candida albicans* ATCC 10231*, Candida krusei* ATCC 6258 and *Candida tropicalis* RSKK 665). The biological methods for cultivation of bacterial strains and determination of antimicrobial activity have been carried out according to CLSI[36] and explained in following lines.

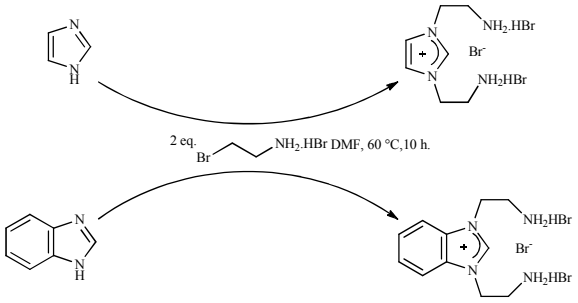
Bacterial strains were cultivated on Mueller-Hinton agar (MHA) and yeasts were grown on Sabouraud Dextrose Agar (SDA). The suspension of the test microorganisms was adjusted according to 0.5 cfu mL^{-1} McFarland turbidimetric standards. The antimicrobial activity of each compound was evaluated by determination of the minimum inhibition concentration (MIC), defined as the lowest concentration of antimicrobial agent that inhibits growth after incubation. The assay was performed on the tested microorganisms by microdilution method according to Clinical and Laboratory Standards (CLSI).[36] To prepare dilution series in the concentration range of 0.004–4 mg mL^{-1}, 20 µL of all dilutions were transferred to the wells filled with MHB medium (180 µL final volume). Microorganism suspension (20 µL) was then added to the wells. MHB medium without compound served as control. Gentamicin sulfate was used as reference antibiotic for bacteria and nystatin for yeasts. The cultures were incubated at 37 °C for 24 h. The growth of the microorganism was determined visually; the first well observed no visible growth was determined as the MIC. All experiments were performed in triplicate.

RESULTS AND DISCUSSION

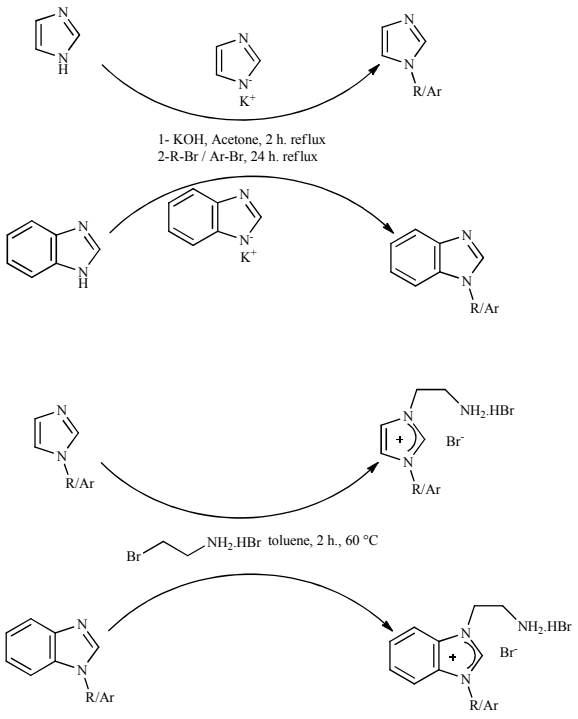
Chemical Structures of ILs

The synthesize reactions of the ILs tested in this study are shown together in the Scheme 3. Six ILs (NHC salts) were prepared and structurally characterized with $–NH_2·HBr$ groups and with $Br^–$ counter anion. The product yields ranged from 68 to 92 as shown in Table 1 which also includes ^1H NMR, ^{13}C NMR, and FT-IR spectroscopic data.

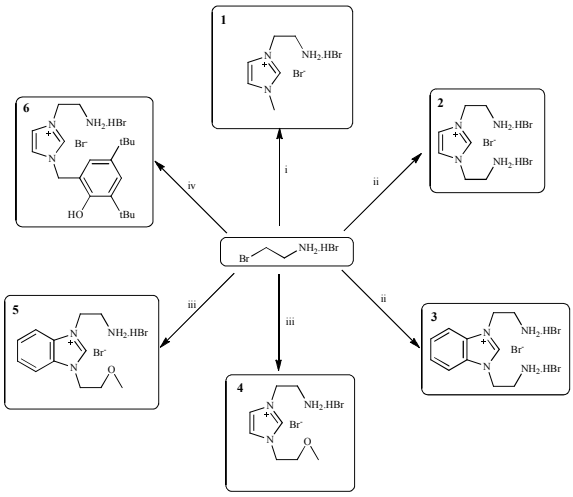
A representative FT-IR spectrum, ^1H NMR and ^{13}C NMR spectra of compound **1** are shown in Figures 3a, 3b and 4, respectively. The FT-IR spectra of these compounds confirm the presence of the $–NH_2.HBr$ groups from the broad peak in 2400–3500 cm^{-1}. The peaks at ~2970s cm^{-1} and 2930s cm^{-1} correspond to methyl and



Scheme 1. One step DMF method for synthesizing symmetrical NHC salts.



Scheme 2. Sequential method for synthesizing dis-symmetrical NHC salts.



Scheme 3. Synthesize routes for the ILs concerned in this study: **i**: *N*-methylimidazole, toluene, 2–3 h, 50–60 °C; **ii**: imidazole or benzimidazole, DMF, 10–12 h,50–60 °C; **iii**: 1-(2-methoxyethyl)-1H-imidazole/benzimidazole, 2–3 h, 50–60 °C; **iv**: 2-((1H-imidazol-1-yl)methyl)-4,6-di-tert-butylphenol, 3 h, 50–60 °C).

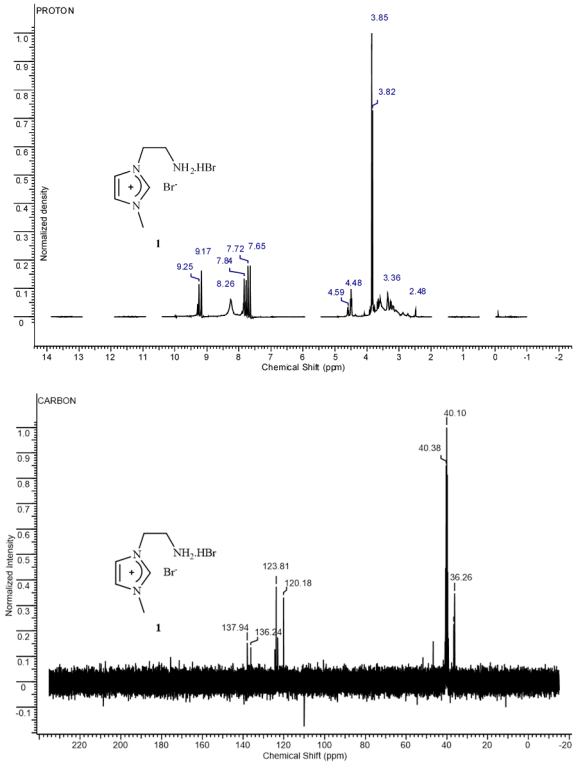


Figure 3. ^1H (a) and ^{13}C (b) NMR spectrum of IL **1**.

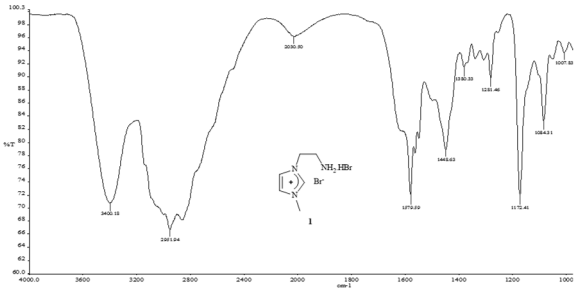


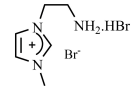
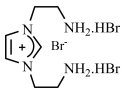
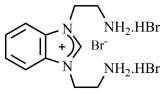
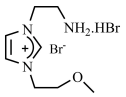
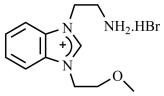
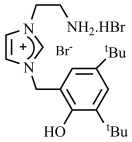
Figure 4. FT-IR spectrum of IL **1**.

methylene C–H bonds. The Amine, methyl and aromatic groups in the structures are consistent with the FT-IR spectra. Also, in the ^{1}H NMR spectra the two peaks in the acidic region show that -H.Br groups in the compounds have not left the structure during the synthesize reactions. The experimental NMR and FT-IR spectra are in good agreement with the expected values and the structures of the synthesized NHC salts.

Synthesis of Dis-Symmetric Compounds 1,4,5,6

To a solution of corresponding mono substituted imidazolium/benzimidazolium mid-product in toluene which contains 0.01 mol of mid-product, 1 eq. (2.05 g or 0.01 mol) of 2-bromoethaneamine hydrobromide was added. The reaction mixture was heated 50-60°C with stirring for 2 or 3 hours. After cooling to room temperature, the solvent was distilled out under vacuum in a gas-vacuum manifold system. The reaction residue was washed with 5 mL of hexane three times and dried in vacuum, resolved in a minimum amount of CH_2Cl_2. The partially purified residue was washed with hexane again and hexane phase was removed by decantation. The residue was dried in a vacuum desiccator.

Table 1. Physical properties of the ILs **1**–**6**.

Compounds	Chemical and Physical Properties												
		^{1}H NMR Shifts				^{13}C NMR Shifts				Solubility in water	Physical situation or melting point	Selected FT-IR Values / cm^{-1}	TLC R_f (acetone : THF 1:1)
Formulae	Mw	C2	Back1	Back2	Tale	C2	Back1	Back2	Tale				
1 	287	9.173	7.724	8.254	4.496	179.312	123.080	124.459	40.096	Good	Liq.	3400.18, 2951.94, 1579.59, 1172.41	0.28
2 	417	9.076	7.800	7.593	3.607	179.082	119.826	134.675	39.832	Good	Liq.	3423.04, 2934.85, 1640, 1387.94, 1099.21	0.6
3 	467	9.659	7.748	7.484	3.637	179.717	127.729	130.840	39.961	Good	Liq.	3312.10, 2763.85, 1650.84, 1386.94, 1097.69	0.55
4 	331	9.208	7.772	7.662	3.652	179.364	120.502	124.297	40.312	Good	Liq.	3349.29, 2942.18, 1655.42, 1388.73, 113.71	0.44
5 	381	9.823	8.257	8.727	3.739	180.126	114.315	116.034	40.677	Good	43–45 °C	3463.33, 3021.00, 1561.18, 1117.11	0.33
6 	491	9.138	7.710	7.966	4.873	179.560	120.031	136.232	40.127	Less than others	67–69 °C	3490, 2952.54, 2868.03, 1575.33, 1361.38, 1003	.078

Synthesis of Symmetric Compounds 2,3

To a solution of imidazole/benzimidazole in DMF, which contains 0.01 mol of starting material, 2 eq. (4.1 g or 0.02 mol) of 2-bromoethaneamine hydrobromide was added after stirring for 20–30 min. The reaction mixture was heated 50–60 °C with stirring for 10 or 12 hours. After cooling them to room temperature, the products (compounds **2** and **3**) were exposed to the same purification procedure with dis-symmetric compounds.

Antimicrobial Activities of ILs

In vitro antimicrobial activity of the compounds (**1–6**) were tested against microorganisms including three gram-positive, three gram-negative bacteria and three yeast strains The biological methods for cultivation of bacterial strains and determination of antimicrobial activity have been carried out according to CLSI.[34] The growth of the microorganism was determined visually; the first well observed no visible growth was determined as the MIC. All experiments were performed in triplicate. All results have been given in Table 2.

CONCLUSIONS

The antimicrobial activities of six imidazolium derivative compounds were determined on six different bacterias and three yeasts. MIC values determined for ILs are given in Table 1. We observed that approximately all of compounds have a small antimicrobial activity against gram-negative bacteria, whereas, MIC values of all samples against *S. aureus* (4 mg L^{-1}) were promising. All of the compounds were shown better antimicrobial activity against the yeasts compared to the bacterial strains. Among them compound **1** showed a significant antimicrobial activity against to both bacterias and yeasts compared to other compounds (**2–6**). Also, compound **3** was effective towards only gram-negative bacteria, *P. aeruginosa* and yeast *C. tropicalis* in relatively lower concentrations. Compound **6** exhibited considerable activity with all yeasts at lower concentrations. Any considerable antimicrobial activities were not observed for compound **2**, **4**, **5** and **6** at the highest concentration (4,0 mg L^{-1}). As an interesting outcome, the compound **1** exhibited better MIC values at a wide range of concentration (1–4 mg L^{-1}) despite its simplicity.

In summary, six different water soluble NHC salts were synthesized in high yields and structurally analyzed. Also, their antimicrobial activities were examined. All synthesized compounds showed antimicrobial activities while the compound **1** is still the best among the others. We believe that this result might safely be attached to the fact that the compound **1** is the smallest one. The smaller molecules can penetrate into the cell through the membrane and damage the organelles. As a matter of fact, our results can be looked down according to MIC values. However, it should be considered that an antimicrobial is not only used as medicine but also, it is quite useful for daily use such as surface disinfection and in agricultural practices. Also, these substances can be further investigated for drug delivery systems, and they will give synergetic profits in use.

Table 2. MIC values (mg L^{-1}) for ILs.

Microorganism	MIC / mg L^{-1}							
	Compounds						Gentamicin	Nystatin
	1	**2**	**3**	**4**	**5**	**6**		
Gram positive bacteria								
Staphylococcus aureus	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	0.008	n.t.[(a)]
Enterococcus faecium	2	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	0.008	n.t.[(a)]
Bacillus subtilis	2	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	0.0005	n.t.[(a)]
Gram negative bacteria								
Escherichia coli	4	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	0.001	n.t.[(a)]
Salmonella typhimurium	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	0.002	n.t.[(a)]
Pseudomonas aeruginosa	4	> 4.0	2	> 4.0	> 4.0	> 4.0	0.0005	n.t.[(a)]
Yeasts								
Candida albicans	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	2	n.t.[(a)]	0.006
Candida krusei	1	4	> 4.0	4	2	2	n.t.[(a)]	0.003
Candida tropicalis	1	1	4	2	2	0.5	n.t.[(a)]	0.006

[(a)] n.t. – not tested.

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Appendix A. Supplementary Material.

Supplementary data also can be found in the supplementary material file named "suppkunduracioglu2014.docx" and "suppkunduracioglu2014.pdf." Also, NMR and FT-IR source files and pictures of spectra can be found in "suppkunduracioglu2014.rar" file too.

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