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Synthesis, Characterization and Biological Evaluation of Imidazolium Based Ionic Liquids

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Research Article

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ABSTRACT

Room temperature ionic liquids (RTILs), a class of neoteric solvent have attracted increasing interest over recent years as environmentally benign excellent alternatives to organic solvents in homogeneous and biphasic processes. RTILs are also found to display anti-microbial activities being greatly affected by alkyl chain lengths. In the present investigation, certain imidazolium based ionic liquids were synthesized and the structures were confirmed by elemental and spectral studies such as UV-Vis, FT-IR, ^{1}H-NMR and ^{13}C-NMR and they were screened for their anti - microbial activities against *Vibrio cholera, Staphylo coccus aureus, Micro coccus luteus and Klebsiella aerogenes* at different concentrations. The anti-microbial activity could be summarized as decreasing in the order *Vibrio cholera> Micro coccus luteus Klebsiella aerogenes> Staphylo coccus aureus.* Among the various ionic liquids, 1, 2-Dibutyl-3-methylimidazoliumbromide (BBMIMBr) was found to be more toxic than others. The anti- microbial activity of all the ionic liquids was observed to be greater at the concentration of 48mg.

INTRODUCTION

The pursuit and development of green and environmentally benign technologies is one of the highest priorities for today chemists. Among green synthesis technologies modern synthetic design demands high efficiency in terms of minimization of synthetic steps together with maximization of complexity of molecules[1]. One of the ways to fulfill these goals is the development and use of ionic liquid chemistry which allows a highly efficient synthesis of complex organic molecules starting from simple substrates. Not only they are the efficient catalysts and green solvents, they are also found to have excellent toxic behavior and pronounced biological activities [2]. Therefore the release of ILs into aquatic environments may lead to water pollution because of their high solubility [3]. The properties and toxicities of ILs can be greatly altered by varying the cationic and anionic components. Before the likely industrial release of ILs into the environment, it is necessary to determine their antimicrobial properties. The most excellent part of room temperature molten ionic liquids are salts with bulky nitrogen or phosphorous-bearing cations with alkyl chain substituent and anions such as halides, fluorophosphates, fluoroborates and so on. Over one million simple ionic liquids are hypothetically reachable, with mixtures of two or more ionic liquids production the potential for new reaction media about limitless [4].

The capability to 'tune' the physical, chemical and biological property sets of ionic liquids, by autonomous modification of the properties of the constituent anions and cations, has been the major driving force behind the enormous importance in this rapidly expanding field of chemistry[5]. 'Tuneability' of ionic liquids bring in an unparalleled flexibility in the design of reagents for a particular functional position, these 'designer solvents' are accomplished of providing a range of new reaction media potentially having greater diversity of temperament and application than that of the conventional solvents they are designed to put back [6,7]. Whilst the preponderance of industry in this field has, to date, been heading for towards 'green' applications, biological issues such as constancy,

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biodegradability, recyclability and toxicity have received comparatively slight awareness. However, these issues have paying attention enlarged scrutiny in recent times, and the biological properties of ionic liquids, which in themselves are 'tuneable' have become one of the most argued topics in the ionic liquids arena [4].

Ionic liquids have previously been reported as alternative 'green' solvents for a extensive range of reactions [8] however, in addition to likely concerns about the recyclability of ionic liquids there have also been concerns hoist over the biodegradability or ecological perseverance of ionic liquids [9]. A series of imidazolium compounds were shown to be inadequately biodegradable and it was established that bacteria did not use them as a starting place of carbon under the circumstances of the investigation making them potentially constant polluters [10].

Several ionic liquids are water-soluble and as such could add to pollution of aquatic surroundings. For example, it has been confirmed that imidazolium, pyridinium and pyrrolidinium ionic liquids had LC50 > 100 mg/L against *Danio rero* (Zebra fish) and as such can be look upon as non-lethal [11]. On the other hand, however, the ammonium based ionic liquids had LC50 values extraordinarily lower than that reported for organic solvents and yet proved deadly when zebra fish were exposed to them. Ecotoxicological tests on several ionic liquids have revealed that imidazolium and pyridinium ionic liquids showed noteworthy toxicity towards the freshwater algae *Pseudokirchneriella subcapitata*[12] while imidazolium ionic liquids are noxious to the freshwater crustacean *Daphnia magna* [13] and *Caenorhabditis elegans* [14]. A number of current studies have also demonstrated the latent potentiality of certain ionic liquids to exhibit exceptional antimicrobial activity, thus presenting the thrilling possibility that ionic liquids could have application as biocidal agents in the control of microorganisms in the environment for contamination and infection control. In this present piece of work, an attempt has been made to prepare and characterize certain imidazolium based ionic liquids and they were screened for their antibacterial activity against certain bacteria like *Vibrio cholera, Staphylo coccus aureus, Micro coccus luteus and Klebsiella aerogenes* at different concentrations.

MATERIALS AND METHODS

AR grade 1-Methyl imidazole, 1- Bromobutane, Bromoethane, NaH, Acetonitrile, Ether and Trichloroethane were purchased from SD Fine Chemicals Limited and used without further purification. NMR spectra were collected as solutions in deuterochloroform on Bruker spectrometer. IR spectra were obtained on a Varian 800 FTIR as thin films or, for solid samples. All solvents and reagents were used as received and all reactions were run in oven-dried glassware. The homogeneity of the products was checked on TLC plates coated with silica gel-G and visualized by exposure to iodine vapors.

Synthesis of 1-Butyl-3-Methylimidazolium Bromide (BMIMBr)

Equimolar amounts of 1-Methyl imidazole (79.7ml) and 1- Bromobutane (107.4ml) were placed in a two necked round bottom flask. Stirred thoroughly and heated to 70°C for 48h under nitrogen atmosphere. The resulting viscous liquid was cooled to room temperature, washed several times with small portions of ethyl acetate to remove unreacted starting materials and then dried under vacuum at 70 – 80°C to yield a white solid product yield at 85% [2].

FTIR (neat, cm-1): 3439, 3153, 3098, 2961, 2871, 2853, 1634, 1577, 1462, 1381, 1168 cm^{-1};
^{1}H-NMR (400 MHz, $CDCl_3$): δ □0.969 (t,J=7.5 Hz, 3H), 1.424 (sxt, m J=7.5 Hz, 2H), 1.7942 (qnt, m J=7.0 Hz, 2H), 4.121 (s,3H), 4.357 (t, J=7.5 Hz, 2H), 7.751 (t, J=1.5 Hz, 1H), 7.744 (t, J=2.0 Hz, 1H), 9.975 (s,1H); **^{13}C-NMR** (500 MHz, d6-DMSO): δ =□ 1334, 19.25, 31.97, 36.61, 49.57, 122.18, 123.77, 136.64.

Synthesis of 1-Ethyl-3-Methylimidazolium Bromide (EMIMBr)

To a stirred solution of 1-Methylimidazole (41.0 g, 0.5 mol) was added firstly and bromoethane (54.5 g, 0.5 mol) was further added drop wise in a 500 mL three-neck round-bottom flask equipped with a reflux condenser and a magnetic stirrer, and cooled in an ice-bath, because the reaction taking place in it is highly exothermic. Having been vigorously stirred for 5 h, the mixture was refluxed in room temperature until it turned into solid completely. The solid was pounded to pieces and washed four times, each with 50 mL trichloroethane. Then they obtained [Emim] Br (87.9 g) was dried under vacuum at 70° C for 24 h [2].

FT-IR (neat): 3,155, 3,105, 2,927, 2,857, 1,572, 1,460, 1,169, 837, 753, 620 cm^{-1}; **^{1}H-NMR** (400 MHz, $CDCl_3$): δ 9.66 (s, 1H), 7.42 (t, 1H), 7.18 (t, 1H), 4.11 (t, 2H), 3.79 (s, 3H), and 1.27 (t, 3H). **^{13}C-NMR** (75 MHz) δ = 136.24, 123.59, 121.94, 44.91, 36.42, 15.46.

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Synthesis of 1-butyl-2-ethyl-3-methylimidazolium bromide (BEMIM Br)

To a stirred solution of BMIM Br (1.00 g, 4.56 mmol) in acetonitrile (25 mL) was added NaH (60% in mineral oil) (0.22 g, 5.48 mmol). After allowing the mixture to stir for 4 hours, ethyl bromide (2.00 g, 18.3 mmol) was added and the reaction stirred overnight. The solution was filtered to remove any precipitated NaBr and the resulting solution was then evaporated to dryness to afford red/orange oil. The resulting oil was washed with ether (3 x 20 mL) to remove any excess alkyl halide and the residual volatiles were then removed under vacuum. It was characterized with ^{1}H-NMR (acetone-d3) as follows [2].

FT- IR (neat): 3,150, 3,099, 2,962, 2,872, 1,649, 1,571, 1,168, 1,113 cm^{-1}; **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 7.71 (d, 1H, J = 2.07 Hz), 7.56 (d, 1H, J = 2.07 Hz), 4.13 (t, 2H), 4.05 (s, 3H), 3.19 (q, 2H), 1.94-1.80 (m, 2H), 1.45-1.35 (m, 5H), 0.82-0.62 (m, 3H).

Synthesis of 1, 2 -dibutyl -3-methylimidazolium bromide (BBMIM Br)

To a stirred solution of BMIM Br (1.00 g, 4.56 mmol) in acetonitrile (25 mL) was added NaH (60% in mineral oil) (0.22 g, 5.48 mmol). After allowing the mixture to stir for 4 hours, butyl bromide (2.00 g, 18.3 mmol) was added and the reaction stirred overnight. The solution was filtered to remove any precipitated NaBr and the resulting solution was then evaporated to dryness to afford red/orange oil. The resulting oil was washed with ether (3 x 20 mL) to remove any excess alkyl halide and the residual volatiles were then removed under vacuum. It was characterized with ^{1}H-NMR (acetone-d3) as follows [2].

FT- IR (neat): 3,149, 2,960, 2,869, 1,658, 1,571, 1,168, 1,033 cm^{-1}; **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 7.61 (d, 1H, J = 2.07 Hz), 7.60 (d, 1H, J = 2.04 Hz), 4.35 (t, 2H), 4.03 (s, 3H), 3.25 (t, 2H), 1.95-1.86 (m, 2H), 1.47-1.42 (m, 2H), 1.39-1.34 (m, 4H), 0.98-0.93 (m, 6H); **^{13}C-NMR** (75 MHz) δ = 136.85, 123.78, 121.22, 49.66, 35.90, 32.054, 29.16, 23.45, 22.33, 19.54, 13.51, 13.37.

Synthesis of 2-butyl-1-ethyl-3-methylimidazolium bromide (EBMIMBr)

To a stirred solution of 1-ethyl-3-methylimidazolium bromide (EMIMBr) (2.00 g, 8.37 mmol) in acetonitrile (50 mL) was added NaH (60% in mineral oil) (0.42 g, 10.88 mmol). After allowing the mixture to stir for 4 hours, bromobutane (2.33 g, 25.1 mmol) was added and the reaction was allowed to stir overnight. The solution was filtered to remove any precipitated NaBr and the resulting solution was then evaporated to dryness to afford red/orange oil. The resulting oil was washed with ether (3 x 20 mL) to remove any excess alkyl halide and the residual volatiles were then evaporated under vacuum [2].

FTIR (neat): 3,149, 2,960, 2,869, 1,658, 1,571, 1,168, 1,033 cm^{-1}; **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 7.61 (d, 1H, J = 2.07 Hz), 7.60 (d, 1H, J = 2.04 Hz), 4.35 (t, 2H), 4.03 (s, 3H), 3.25 (t, 2H), 1.95-1.86 (m, 2H), 1.47-1.42 (m, 2H), 1.39-1.34 (m, 4H), 0.98-0.93 (m, 6H); **^{13}C-NMR** (75 MHz) δ = 136.85, 123.78, 121.22, 49.66, 35.90, 32.054, 29.16, 23.45, 22.33, 19.54, 13.51, 13.37.

Synthesis of 1, 2-diethyl-3-methylimidazolium bromide (EEMIMBr)

To a stirred solution of 1-ethyl-3-methylimidazolium bromide (EMIMBr) (2.00 g, 8.37 mmol) in acetonitrile (50 mL) was added NaH (60% in mineral oil) (0.42 g, 10.88 mmol). After allowing the mixture to stir for 4 hours, bromoethane (2.33 g, 25.1 mmol) was added and the reaction was allowed to stir overnight. The solution was filtered to remove any precipitated NaBr and the resulting solution was then evaporated to dryness to afford red/orange oil. The resulting oil was washed with ether (3 x 20 mL) to remove any excess alkyl halide and the residual volatiles were then evaporated under vacuum [2].

FT- IR (neat): 2982.28, 2928.24, 2177.28, 1652.65, 1533.07, 1458.25, 1386.16, 1232.26, 1168.73, 1130.68 cm-1; **^{1}H-NMR** (400 MHz, CDCl3): δ 7.757 (d, 1H), 7.639 (d, 1H), 4.429 (q, 3H), 4.332 (q, 3H), 4.114 (s, 3H), 1.614 – 1.536 (m 6H)

Antimicrobial Activity

Well Diffusion Method

Antibacterial activity of the ionic liquids was tested by using well diffusion method [15]. The prepared culture plates were inoculated with different bacteria by using plate method. Wells were made on the agar surface with 6mm cork borer. The ionic liquids were poured into the well using sterile syringe. The plates were incubated at 37±2°C for 24 hr for bacterial activity. The plates were observed for the zone formation around the wells. The zone of inhibition was calculated by measuring the diameter of the inhibition zone

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around the well (in mm) including the well diameter. The readings were taken in three different fixed directions in all three replicates and the average values were tabulated.

RESULTS AND DISCUSSION

In continuation of our studies on the synthesis and biological activities of certain imidazolium based ionic liquids, we herein report the synthesis of ionic liquids such as 1-Butyl-3-Methylimidazolium Bromide (BMIMBr), 1-Ethyl-3-Methylimidazolium Bromide (EMIMBr), 1-butyl-2-ethyl-3-methylimidazolium bromide (BEMIMBr), 1, 2 -dibutyl -3-methylimidazolium bromide (BBMIM Br), 2-butyl-1-ethyl-3-methylimidazolium bromide (EBMIMBr) and 1, 2-diethyl-3-methylimidazolium bromide (EEMIMBr). Though these ionic liquids are readily available in the market, synthesis was carried out to minimize expenses as these ionic liquids are costly and to optimize the reaction conditions. Under our optimal conditions synthesis of all ionic liquids gave excellent yield and the products were identified and purified HPLC methods and characterized by FT-IR, ^{1}H-NMR and ^{13}C-NMR studies.

Antimicrobial Activity

Even though ionic liquids reduce the costs and environmental migration of air pollution, release of ILs into water systems may lead to aquatic pollution owing to their high solubilities [18]. Therefore, it becomes a bounden duty of a chemist to check the toxicity and biological activities of ILs before they are let out into our atmosphere. Bacteria have short generation times and serve as ideal starting points for ILs – structure – activity relationship experiments. In this paper, we present an examination of the toxicity of the aforementioned ILs to a range of bacteria. Specifically, we focused on the toxicity and antimicrobial activity of six ILs with increasing alkyl chain substituents. The antibacterial study was conducted using the famous Well diffusion method. The inhibition zone of the micro-organisms due to the treatment of different synthesized compounds is mentioned in Tables 1.

Table 1: Inhibition zone of different Imidazolium ionic liquids against bacterial pathogens

S.No	Name of the organisms	Conc.	Compounds					
			BMIMBr	BEMIMBr	BBMIMBr	EMIMBr	EBMIMBr	EEMIMBr
1	*Staphylococcus aureus*	Control	–	–	–	–	–	–
		10µL	10	10	10	10	12	12
		20 µL	18	13	16	15	18	15.5
		30 µL	20.5	17.5	17.5	20	19.5	18.5
		40 µL	22	19	20.5	21.5	21	19.5
2	*Vibrio cholerae*	Control	–	–	–	–	–	–
		10µL	12	10	20	11	17.5	12.5
		20 µL	19	11	22	14	19.5	19.5
		30 µL	21	14.5	22.5	17.5	22.1	22
		40 µL	22.5	16	23.3	20	22.5	23.5
3	*Micrococcus luteus*	Control	–	–	–	–	–	–
		10µL	10	14	16	13	14.5	13
		20 µL	14	16	19.5	15	20	19
		30 µL	17.5	18.5	22	19	22	19.5
		40 µL	19	21.5	23	21	23.5	21.5
4	*Klebsiella pneumoniae*	Control	–	–	–	–	–	–
		10µL	11	13	10	13.5	10	11
		20 µL	14.5	18.5	15	17	13	14.5
		30 µL	17	19	22	18	16	15

Among the various ionic liquids examined, the ionic liquid 1,2-Dibutyl-3-methylimidazoliumbromide (BBMIMBr) was found to be more toxic under the chronic test conditions for all the bacteria taken for investigation and prevented their colony formation more potentially than others. The trend of increasing antibacterial activity to *Vibrio cholera, Micro coccus luteus, Klebsiella aerogenes and Staphylo coccus aureus* with increasing hydrophobicity, corresponding to an increasing alky chain of especially the C-1 (and C-2) substituted groups was observed (Table 1) in the 1,2-dibutyl-3-methylimidazolium bromide (BBMIMBr), 1-butyl-2-ethyl-3-methylimidazolium bromide (BEMIMBr), 1-Butyl-3-Methylimidazolium Bromide (BMIMBr) 1-ethyl-2-butyl-3-methylimidazolium bromide (EBMIMBr). Thus, those ionic liquids with longer alkyl chains on the cations were found to exhibit better antibacterial activities than those

with small alky chains. 1-Ethyl-3-Methylimidazolium Bromide (EMIMBr) showed no acute antibacterial effect as it has smaller alkyl groups on C-1 and C-2. This outcome was consistent with previous conclusions drawn by earlier workers on the toxicity of ILs toward fresh snail (*Physa acuta*) and bioluminescent bacteria (*Vibrio fischeri*) and acetyl cholinesterase, respectively [15, 16]. Another worker also found a similar trend of increased marine algal growth inhibition, nonetheless [17]. The increasing order of antibacterial activity is observed as EMIMBr< EBMIMBr < BMIMBr < BEMIMBr < BBMIMBr. The anti-microbial activity could be summarized as decreasing in the order *Vibrio cholera> Micro coccus luteus > Klebsiella aerogenes> Staphylo coccus aureus.* The anti- microbial activity of all the ionic liquids was observed to be greater at the concentration of 48mg. Since the trend of increased growth inhibition with longer alkyl chain length holds good for all bacteria investigated, the antibacterial effect may be related to common cellular structure [18]. Many others have suggested that the mechanism of antibacterial activity for ILs is through membrane disruption because of the ILs structural similarity to detergents, pesticides and antibiotics that attack the lipid structure [19, 20]. Another suggested mechanism of IL antibacterial activity is related to acetyl cholinesterase inhibition in more complex test organisms, such as the electric organ in Electrophorus electricus [21]. In future studies, we will use bacterial model systems to further examine the mechanism of IL antibacterial activity, focus on the mutagenicity of ILs and determine if this mechanism is common to more complex test organisms. We strongly believe that this study will be very useful to study the destiny of many potentially toxic chemicals like ionic liquids in the environment with increasing alkyl chain length and also to ascertain their water solubility and bioaccumulation in organisms [22, 23].

CONCLUSIONS

Since ionic liquids are tunable and fashionable chemicals, they have been used in a extensive variety of applications. Many have been developed for use as solvents in industrial chemistry, and in general their use bestows a number of compensation over using other solvents; greater reaction rates, recyclability of reactants and catalysts and improves product recovery. Having negligible vapour pressure, it has been suggested that ionic liquids will not contribute to air pollution and are thus green alternative to conventional organic solvents which are generally volatile, flammable and toxic. As a result of this, many studies have been conducted on the use of ionic liquids as novel green solvents to replace established solvents for particular reactions. However, toxicity of ionic liquids has been demonstrated by a number of groups, including our own, in a variety of environmental niches, and raises questions over their 'green' credentials. Nonetheless the toxicity of ionic liquids is in itself a property which can be tuned and exploited for other beneficial uses, for example in developing novel antimicrobials. In this present piece of work, an attempt has been made to synthesize and characterize as many as half a dozen imidazolium based ionic liquids (Though they are readily available they were synthesized to optimize the reaction conditions and to reduce the expenses of purchasing these ionic liquids). These ILs were screened for their antibacterial activities against bacteria such as *Vibrio cholera, Staphylo coccus aureus, Micro coccus luteus* and *Klebsiella aerogenes* at different concentrations. This study has demonstrated the effects of alkyl chain length on the antibacterial activities. Although, Ionic liquids are considered attractive chemicals due to their low volatility, the present results show that they exhibit strong antibacterial activity toward almost all bacteria taken for investigation. A longer chain length showed stronger antibacterial activity. The increasing order of antibacterial activity is observed as EMIMBr< EBMIMBr < BMIMBr < BEMIMBr < BBMIMBr. The anti-microbial activity could be summarized as decreasing in the order *Vibrio cholera> Micro coccus luteus > Klebsiella aerogenes> Staphylo coccus aureus.* We have demonstrated the utility of ionic liquids as anti biofilm agents; biofilms are complex, organized communities of bacteria which have been shown to have greater tolerance and resistance to antimicrobials, accounting for the majority of chronic and acute infections as well as the majority of bacterial communities in aquatic environments. In summary, the biological properties of ionic liquids may yet prove their most exciting and the benefits of rationally designed, bespoke ionic liquid-based antimicrobials to human health has yet to be harnessed.

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