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Facile Synthesis of Imidazolium Based Ionic Liquids with Organic Anions: Preparation, Characterization, Antimicrobial Activity and Toxicity Studies

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Abstract: A facile synthesis of ionic liquids with organic anions is reported. The synthesis of 1-methylimidazolium lactate (MIM lactate) [**2**], bis-(1-methylimidazolium) succinate (Bis-(MIM) succinate) [**3**] and 1-methylimidazolium stearate (MIM stearate) [**4**] was done through Bronsted acid-base neutralization reaction. Theoretical modeling of optimal geometries using MMFF94 function in Avogadro software showed relatively stable ionic liquid products with Bis-(MIM) Succinate as the most stable. Characterization studies through 1H NMR, LCMS and FTIR spectroscopy were done. Spectroscopic data for [**2**], [**3**], and [**4**] provided evidence for successful synthesis. Antimicrobial activity study through Paper-disc diffusion assay showed only [**4**] exhibiting significant antimicrobial activity against gram negative bacteria, Escherichia coli and Pseudomonas aeruginosa. Toxicity assay studies through Brine shrimp lethality assay showed [**2**] and [**3**] as toxic and [**1**] and [**4**] as weakly toxic. Further studies on the synthesis of more ionic liquids with organic anions, their characterization, bioactivities and other applications are recommended.

Key Words: ionic liquids, imidazolium

1. INTRODUCTION

1.1 Subsection

Ionic liquids are current topics of high interest as it has been shown to have lots of possible beneficial applications as benign solvents, catalysts, electrical conductors, lubricants, electroplating agents, etc. The exciting possibility of the use of ionic liquids as bactericidal agents has not been fully explored. The reported antibacterial ionic liquids were imidazolium halides, where the anions are halogens. We surmise that organic anions are better antimicrobial agents because the lipophilic character of organic anions enables them to penetrate the bacterial cell wall better (Yaganza, E. *et al.*, 2009). Here we report a simple and facile synthesis of ionic liquid with organic anion, their corresponding antimicrobial and toxicity properties.

2. METHODOLOGY

Four imidazolium ionic liquids were synthesized: 1-Ethyl-3-methylimidazolium lactate (EMIM lactate) [**1**], 1-methylimidazolium lactate

(MIM lactate) [2], Bis-(1-methylimidazolium) succinate (Bis- (MIM) succinate) [3] and 1-methylimidazolium stearate (MIM stearate) [4]. The synthesis of EMIM Lactate [1] starts out with the quaternization of 1-methylimidazole with an ethyl group attached to the amine group. Reflux was done with 1-methylimidazole and iodoethane in tetrahydrofuran (THF) to form 1-ethyl-3-methylimidazolium iodide (EMIM I) following the reported protocol (Pogorzelec-Glaser *et al.*, 2006). A Bronsted acid-base reaction was carried out for the synthesis of MIM Lactate [2]. In a reaction vessel, MIM and lactic acid was stirred for 48 hrs under N2 atmosphere. Both reactants were liquid so the use of a solvent was not necessary. The synthesis of bis-(MIM) succinate [3] follows a simple acid-base reaction where succinic acid reacted with 1-methylimidazole in 75ml ethyl acetate, stirred at room temperature under N2 atmosphere for 24 hrs. After the reaction, the solvent was removed under reduced pressure. Similar protocol was done for the synthesis of MIM stearate [4] using 1-methylimidazole and stearic acid.

The ionic liquids that were synthesized were analyzed using Fourier Transform Infrared Spectroscopy, 1H Nuclear Magnetic Resonance. Antimicrobial studies were done using the standard procedure on paper disc assay. The Brine shrimp lethality bioassay for toxicity was based from the studies of Sharma et al. (2013).

3. RESULTS AND DISCUSSION

Prior to the synthesis attempts, the molecular structures of the planned ionic liquids were first simulated since these ionic liquids with organic anions are relatively rare. Its formation was first measured theoretically to ensure ease of preparation. Results show that interaction between the protonated imine and carboxylate would not induce pure ion-ion interactions, but it actually induces the N-H-O hydrogen bonding that is moderate but mostly electrostatic. It can be noted that the theoretical interaction energies were all acquired to be negative, indicating that the imidazolium could form relatively stable ionic liquids with organic anions. Bis- (1-methylimidazolium) succinate [3] was found to be the most stable with interaction energy of -545.246 kJ/mol.

The most common synthetic method of ionic liquids requires a quaternization step. This technique is employed to prepare the necessary EMIM cation. The synthesis of 1-ethyl-3-methylimidazolium lactate [1] was carried out from 1-methylimidazole and ethyl iodide to afford a halide based ionic liquid, 1-ethyl-3-methylimidazolium iodide (EMIM I) quantitatively through the quaternization step. The resulting EMIM I was then reacted with racemic calcium lactate via metathesis. FTIR, NMR and MS data indicate that the 1-ethyl-3-methylimidazolium lactate [1] was not successfully synthesized. The apparent difficulty in the formation of EMIM lactate [1] is already hinted in the theoretical modeling where it produced the least stable (albeit negative value) interaction energy and the longest electrostatic distance. This can be attributed to the apparent weak affinity of the lactate anion with EMIM cation resulting to weak anion exchange between EMIM I and calcium lactate.

Hence, a new synthetic route was used, starting from the commercially available 1-methylimidazole in direct reaction with an acid in a Bronsted Acid-Base reaction. Imidazole moiety serves as a proton transfer agent which allows it to be able to act as a weak base. In this reaction lactic acid will cause the protonation of imidazole thus forming a 1-methylimidazolium cation and the deprotonated lactic acid, lactate, will then be an anion. This is an acid-base reaction between a weak acid and a weak base. The spectroscopic evidence acquired from FTIR and 1H NMR confirms the successful synthesis of the ionic liquid 1-methylimidazolium lactate [2]. The integration for the NMR peaks however indicates that the cations and anions are not in strict 1:1 ratio. One hydrogen indicative for the peaks of imidazolium cation has an integration value of around 10 while that for the peaks of lactate anion was shown to be around 4. This suggests that in one MIM Lactate [2] ionic compound, two cations interact for every one anion. The possibility of the N+-H of imidazole to form H-bonding with the OH at carbon 2 of lactate is likely.

This would allow two imidazolium cations, to interact with one lactate ion through electrostatic interaction and through hydrogen bonding.

A similar protocol was then carried out to synthesize with bifunctional anion. This dianion system with 4 carbon bridge is relatively new and an interesting ionic liquid. In this case, one succinic acid molecule could protonate two 1-methylimidazoles. Spectroscopic data indicate the successful synthesis Bis- (MIM) succinate [**3**]. This indicates that an acid-base reaction could be used to produce an ionic liquid with an organic dianion.

The synthesis of MIM stearate [**4**] followed the same Bronsted acid-base reaction where the stearic acid is the proton donor and 1-methylimidazole is the proton acceptor. The FTIR and 1H NMR for MIM stearate [**4**] concludes the successful synthesis through acid-base reaction. This new ionic liquid with a long alkyl chain group adds lipophilic character to the ionic liquid which would provide interesting and novel applications.

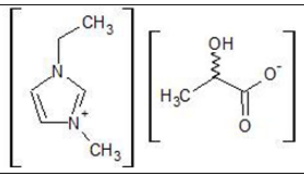
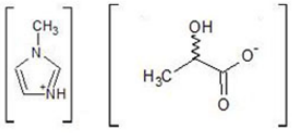
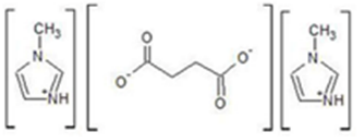
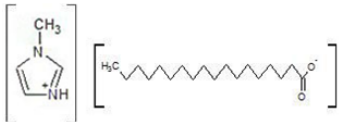
Ionic Liquid	Chemical Structure
EMIM lactate **[1]**	
MIM lactate **[2]**	
Bis- (MIM) succinate) **[3]**	
(MIM stearate) **[4]**	

Fig. 1 Chemical Structures of Ionic Liquid

The synthesized ionic liquids were tested for their antimicrobial activity in terms of inhibition zones (mm) against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The ionic liquids did not really show any significant antimicrobial activity as compared to the positive control, Ceftazidime. However, it could be observed that only MIM stearate [**4**] was effective in inhibiting the growth of gram negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*. This can be attributed to the strongly lipohilic alkyl chain of the MIM stearate [**4**].

The newly synthesized ionic liquids were subjected to the brine shrimp toxicity assay which showed that both EMIM Lactate and MIM Stearate are weakly toxic, MIM Lactate and MIM Succinate are both toxic. Organic anions are reactive to a wide variety of organic compounds in vivo, this may cause its toxicity level to heighten, the more lipophilic an organic compound, the more chance of it becoming more toxic because it will be easier for the compound to cross the plasma membrane. That is why MIM lactate [**2**] and MIM succinate [**3**] are toxic to the brine shrimp. However, MIM stearate [**4**] was found to be weakly toxic. Seeing that it is lipophilic it should have been toxic to the brine shrimp. Kadono et al. (2006) reported on the alteration of toxicities of fatty acid salts, like stearates, which depends on the composition of the water. Detoxification effects were observed for different fatty acids when they were dissolved in river and tap water instead of pure distilled water. As the mineral content, such as calcium or magnesium of the water increases, it was concluded that the toxicity of the fatty acid salt decreases. In this study, the ionic liquids were dissolved in synthetic sea water so as to imitate the natural living conditions of brine shrimp. This may have caused the decrease in toxicity of MIM stearate [**4**].

4. CONCLUSIONS

The synthesis of ionic liquids with organic anions could follow a more facile route. Instead of undergoing quaternization and anion exchange

reactions to produce the ionic liquid, a simpler bronsted acid-base neutralization reaction could be done. Ionic liquids with organic anions could provide an avenue for a new class of ionic liquids with very interesting properties like antimicrobial activity and toxicity. The synthesis of [**2**], [**3**] and [**4**] was successful while that of [**1**] was not. This indicates that the usual quaternization and anion exchange synthesis route for ionic liquid could not work for the synthesis of ionic liquids with organic anions. The more facile acid-base neutralization would be more preferred. The synthesis through protonation by Bronsted acid-base neutralization reaction is much more favored since it is facile, cost-effective, and high yield. Furthermore, MIM stearate [**4**] was found to be the ionic liquid with an organic anion which was active against gram negative bacteria and was also weakly toxic.

5. ACKNOWLEDGMENTS

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