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Synthesis and Characterization of New Choline-Based Ionic Liquids and Their Antimicrobial Properties

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ABSTRACT

A series of new cholinium-based ionic liquids (ILs) was synthesized by using simple neutralization reaction. The synthesized ILs were choline phenylacetate, choline ricinoleate and choline trichloroacetate. Result obtained from FT-IR, ^{1}H and ^{13}C NMR, mass spectrometry, thermogravimetric and differential scanning calorimetry confirmed the formation of these cholinium-based ILs. These ILs were then tested for their anti-microbial properties towards seven types of bacteria which consist of three Gram-positive bacteria, *Bacillus subtilis* (B29), *Staphylococcus aureus* (S276), *Staphylococcus epidermidis* (S273), two Gram-negative bacteria, *Pseudomonas aeruginosa* (ATCC 15442) and *Escherichia coli* (E266) and two types of yeasts, *Candida albicans* (9002) and *Candida tropicalis* (A3). Interestingly, all of the cholinium ILs possess excellent antimicrobial activity as good as the standard, Streptomycin. The most significant antimicrobial activity was observed in choline phenylacetate against all seven types of bacteria tested in this study.

1. Introduction

A significant attention has been addressed towards finding alternatives to volatile organic compounds (VOCs) previously used as solvents in synthetic organic chemistry in many chemical industries. Halogenated hydrocarbons and many other hazardous materials have caused serious problems to the ecosystem worldwide. Therefore, the use of greener solvent such as ionic liquids (ILs) is important in order to overcome this problem. Normally they are non-volatile, non-flammable and less toxic than conventional organic solvents [1].

(Figure 1) Ionic liquids (ILs) are organic salts that are generally liquid at ambient temperature or melted at temperature below 100°C [2]. Typically, ILs are comprised of heterocyclic organic cations

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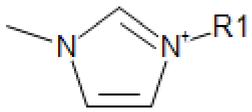
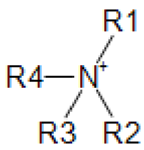
with organic or inorganic anions [3], which provide versatile properties such as low vapour pressure and high polarity, high ionic conductivity, and in-combustibility [4]. ILs are also known as designer solvent as they can be tuned for a particular application or specific physicochemical qualities by merely changing the combination of anion and cation [5]. Ionic liquids are found to be vastly advantageous in many applications such as biocatalysis, organic synthesis, electrochemistry, and synthesis of nanoparticles [4,6,7]. Moreover, most of the ionic liquids demonstrate antimicrobial activity which can make them useful as formulation preservatives or active pharmaceutical ingredients (APIs) [8,9].

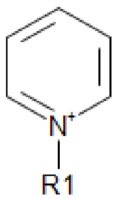
Most commonly described cations:



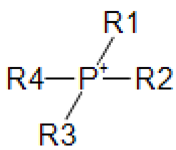
1-alkyl-methyl-imidazolium



Tetraalkyl-ammonium



N-alkyl-pyridinium



Tetraalkyl-phosphonium

($R_{1,2,3,4}$ = alkyl)

Some possible anions:

Water – soluble ⟶ Water – insoluble

$[PF_6]^-$	$[BF_4]^-$	$[CH_3CO_2]^-$, $[CF_3CO_2]^-$
$[(CF_3SO_2O_2)N]^-$	$[CF_3SO_2]^-$	Br^-, Cl^-, I^-, $[NO_3]^-$
$[BR_1R_2R_3R_4]^-$		$[Al_2Cl_7]$

Most commonly described alkyl chain substituents:

Ethyl	Decyl
Butyl	Dodecyl
Hexyl	Tetradecyl
Octly	Hexadecyl

Fig. 1. Some of the most commonly used cation, anion and alkyl chain substituents for ionic liquid synthesis

Despite all unique features of ionic liquids, some of them are completely soluble in water thus highlighting their potential toxicity towards aquatic organisms. Essentially, bacteria and fungi are suitable to be used to examine ILs toxicity due to their short generation time and quick growth compared to other living organisms [10]. Several studies have evidently displayed that a number of ILs have the ability to inhibit bacterial growth [11]. In general, when the lipophilicity of the ILs increases, the antimicrobial activity of the ILs also will increase. The ILs with longer n-alkyl substituent chain displays greater antimicrobial efficacy than those with short chain in all strains tested [12]. Previous studies have concluded that certain pyridinium [13], imidazolium [14] and quaternary ammonium [15] ILs show a trend of increasing toxicity with the elongated chain length of the alkyl substituent [11,16,17]. It has been proposed that some quaternary ammonium compounds can even be applied as disinfectant for medical equipment [18].

On this basis, it is fair-minded to assume that cholinium-based ionic liquids will demonstrate good antimicrobial activity. The aims of this study are therefore to synthesize and characterize a series of novel cholinium ionic liquids and to investigate their antimicrobial activity towards bacteria and yeast.

2. Materials and Methods

2.1 Chemicals

Choline hydroxide (45 wt% aqueous solution) and trichloroacetic acid were purchased from ACROS Organics. Phenylacetic acid was purchased from Merck Chemicals. Nonadecanoic acid was purchased from Fluka. Ricinoleic acid and linoleic acid were purchased from Sigma-Aldrich. The solvents, diethyl ether (99% purity) and chloroform (99% purity) were purchased from Merck. All chemicals and materials are commercially available and of higher analytical grade unless otherwise specified. The chemicals were used without purification, unless otherwise stated.

2.2 Synthesis of Cholinium-based Ionic Liquids

Cholinium-based ionic liquids were synthesized through neutralization of choline hydroxide with three types of carboxylic acids. Our previous works reported on several novel cholinium-based ILs derived from choline hydroxide and five types of carboxylic acids [19]. These ILs were synthesized according to the procedures reported by Taha *et al.,* [9]. They reported the synthesis of choline hydroxide with Good's buffers. Figure 2 shows the reaction mechanism of cholinium-based ILs. The products formed were choline carboxylate and water. Figure 3 shows the structure of three cholinium-based ILs.

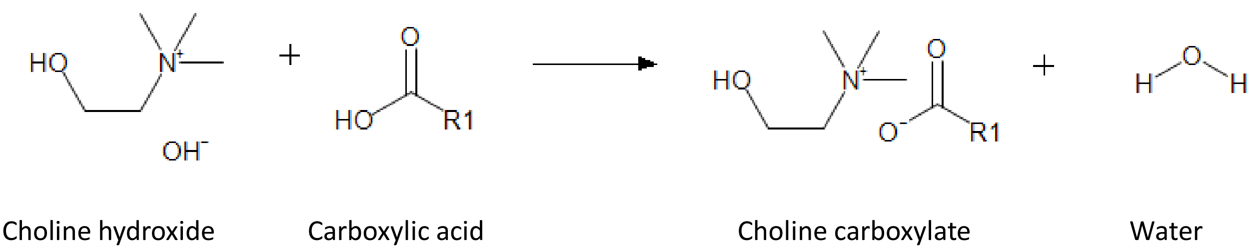


Fig. 2. Reaction mechanism of choline-based ILs

2.2.1 Choline phenylacetate (1a)

Choline phenylacetate was synthesized through neutralization of choline hydroxide with phenylacetic acid. Phenylacetic acid (0.025 mol) was dissolved in 60 mL of distilled water with heat. It was added slowly into 0.025 mol of choline hydroxide. The mixture was stirred continuously for 12 hours at room temperature. The solvent was removed using rotary evaporator, followed by vacuum drying at 70°C for 24 hours. For purification, the sample was washed with toluene for three times. Then, the solvent was removed by vacuum drying at 65°C for 24 hours.

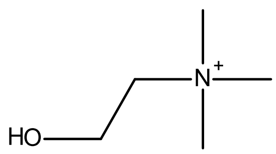
2.2.2 Choline ricinoleate (1b)

Choline ricinoleate was synthesized using method in 1a but replacing phenylacetic acid with ricinoleic acid. For purification, the sample was washed with 2-propanol for three times. Then, the solvent was removed by vacuum drying at 65°C for 24 hours.

2.2.3 Choline trichloroacetate (1c)

Choline trichloroacetate was synthesized using method in 1a but replacing phenylacetic acid with trichloroacetic acid. For purification, the sample was washed with acetone for three times. Then, the solvent was removed by vacuum drying at 65°C for 24 hours. Figure 3 shows the structure of three cholinium-based ILs.

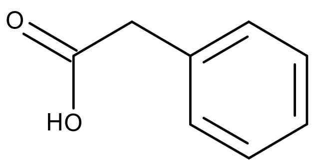
Cation



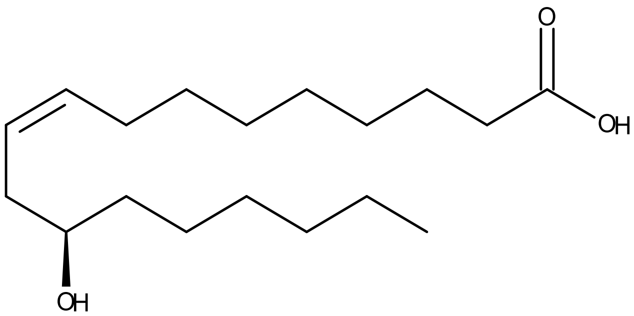
HO^-

Choline hydroxide

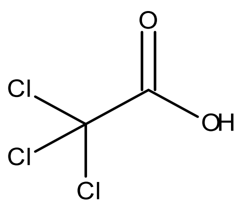
Anion



1a



1b



1c

Fig. 3. Structure of new cholininium-based ILs

2.3 Characterization and Instrumentation

The instruments used for characterization were Shimadzu Europe-GCMS-QP5050A for mass spectrometry, Perkin Elmer-FTIR Spectrometer Spectrum RX1, Varian 500 MHz NMR spectrometer, Differential Scanning Calorimeter (DSC) Mettler Toledo DSC823 and Thermogravimetric Analysis (TGA). Temperature range used for DSC and TGA was between 25-400°C.

2.4 Antimicrobial Activity

Antimicrobial or antifungal test was carried out to determine the effectiveness of choline-based ILs in treating bacteria or fungal infections. The response (sensitivity/resistance) of microbes against antimicrobial compounds varied to one another.

The test was carried out by placing 6mm diameter of paper disc containing IL sample onto a plate which microbes grew on. The microbe culture was standardized to 0.5 Mc Farland Standard which was approximately 10 cells. Streptomycin standard was used for each bacteria. Nystatin standard was used for yeast.

The plates were inverted and incubated at 30-37°C for 18-24-48 hours or until sufficient growth has occurred. After incubation, each plate was examined. The diameters of the zones of complete inhibition (as judged by the unaided eye) were measured, including the diameter of the disc. Zones were measured to the nearest whole millimeter, using sliding caliper or a ruler, which was held on the back of the inverted petri plate. The ILs samples were tested against Bacillus subtilis (B29), Staphylococcus aureus (S276), Staphylococcus epidermidis (S273), Pseudomonas aeruginosa (ATCC 15442) Escherichia coli (E266), Candida albicans (9002) and Candida tropicalis (A3).

3. Results and Discussion

3.1 Properties of Synthesized Ionic Liquids

The structures and characteristics of the synthesized ionic liquids were evaluated using Fourier-transform infrared (FT – IR) spectroscopy, ^{1}H and ^{13}C Nuclear magnetic resonance (NMR) spectroscopy, and mass spectroscopy.

3.1.1 Choline phenylacetate (1a)

Brown liquid (Yield: 74.0 %). FT-IR spectroscopy was used to characterize all the newly synthesized ILs. The absorption peak at around 3381.14 cm^{-1} was a characteristic of –OH stretching frequency of choline hydroxide [10]. The other peak of choline hydroxide was at 1074.09 cm^{-1} which was a characteristic of C–N of aliphatic amines and 1204.75 cm^{-1} for C–O stretching of alcohols. The absorption peaks of phenylacetate were at 1572.09, 3035.94, 2926.53 and 1480.21 cm^{-1}, respectively. ^{1}H NMR (500MHz, CDCl3) δ (ppm): 7.13-7.23 (m, 5H), 3.52 (s, 2H), 2.99 (s, 2H), 2.64 (s, 9H) and 2.59 (s, 2H). ^{13}C NMR (500MHz, $CDCl_3$) δ (ppm): 43.51, 53.87, 53.93, 54.02, 55.86, 67.74, 126.49, 127.32, 129.32, and 176.21. Mass spectrum for choline were at m/z 107 and m/z 58 while for phenylacetate was at m/z 135.

3.1.2 Choline ricinoleate (1b)

Light brown liquid (Yield: 65.5%). A broad absorption band was found at 3268.95 cm^{-1} which represented –OH group [10]. The other peaks of choline hydroxide at 1079.60 cm^{-1} was characteristic of C–N of aliphatic amines and 1227.93 cm^{-1} for C–O stretching of alcohols. The absorption peaks of ricinoleate were at 2922.47, 2857.48, 1562.91, and 1562.00 cm^{-1}. ^{1}H NMR (500MHz, $CDCl_3$) δ (ppm): 3.91 (s, 2H), 3.48 (d, 2H), 3.30 (s, 1H), 3.17 (s, 9H), 2.14 (d, 1H), 2.07 (t, 2H), 1.98 (t, 1H), 1.49 (d, 12H), 1.43 (s, 4H), 1.32 (d, 8H), 1.17 (s, 2H), 0.76 (t, 3H). ^{13}C NMR (500MHz, $CDCl_3$) δ (ppm): 14.06, 14.11, 28.91, 29.94, 22.55, 22.65, 25.09, 25.33, 25.62, 25.75, 25.79, 26.69, 26.76, 26.83, 38.42, 54.32, 55.91, 60.76, 68.17, 127.91, 128.02, 128.54 and 179.56. Mass spectrum for choline was at m/z 106 and for ricinoleate were at m/z 281 and m/z 254.

3.1.3 Choline trichloroacetate (1c)

Yellow liquid (Yield: 76.5 %) The absorption peak which represented –OH stretching frequency was observed at 3250.67 cm^{-1}. The C–O stretching absorption peaks were assigned at 1632.09 cm^{-1} and 950.92 cm^{-1} for C–H bending. The presence of C–N could be proven by the peak observed at 1075.17 cm^{-1}. The C–Cl band for trichloroacetate was found at 532.97 cm^{-1}. The other absorption peaks for trichloroacetate were found at 1473.72, 1468.51, 1075.17, and 952.12 cm^{-1}. ^{1}H NMR (500MHz, DMSO) δ (ppm): 3.45 (d, 2H), 3.39 (t, 2H), 3.31 (s, 9H), 3.09 (s, 1H). ^{13}C NMR (500MHz, DMSO) δ (ppm): 156.86, 72.63, 57.88, 55.68, 53.58, 53.61, and 53.63. Mass spectrum for choline and trichloroacetate were at m/z 58 and m/z 93, respectively.

3.2 Thermal Analysis

Melting temperature is the temperature when a substance undergoes changes from solid to liquid state. Melting temperature (T_m) of cholinium-based compounds was measured using DSC and the results are tabulated in Table 1. The selected combinations of anions and cations would contribute different characteristic properties of ILs [6]. In this research, all three compounds showed a T_m. However, only one compound fulfills the main properties of ILs as its T_m was measured at below 100 °C. The compound was choline trichloroacetate (1c).

Table 1
Melting temperature (T_m) of choline-based ILs

Cholinium-based ILs	Melting temperature (T_m/°C)
1a*	235.86 (±1.0)
1b*	204.87 (±1.2)
1c	98.27 (±0.3)

* Not considered as ILs due to high melting point (>100 °C)

In comparison, 1a showed the highest T_m value which was 235.86 °C, followed by 204.87 °C and 98.27 ºC for compound 1b and 1c, respectively. Thus, the first two compounds will only be considered as new choline – based salts instead of ionic liquid as the melting temperature is higher than 100 ºC. On the other hand, compound 1c can be considered as new IL due to its low melting point i.e. 98.27 ºC. Besides that, the thermal stability of the compounds was measured by thermogravimetric analysis (TGA). All of the cholinium-based compounds showed decomposition at high temperature ranging from 200 °C to 350 °C.

3.3 Antimicrobial Activity

A number of researches have been conducted to validate the potential of some ILs to exhibit outstanding antimicrobial activity [20]. Pernak *et al.,* have investigated the antimicrobial activity of a series of choline-like quaternary ammonium chloride ILs against a variety of Gram-positive and Gram-negative bacteria. All compounds showed relatively good antimicrobial activity [21]. This study has confirmed that lipophilicity affects the antimicrobial efficacy of the ILs as the increase of the alkyl substituent chain will increase its antimicrobial activity [22]. Similar studies done have also proven that a series of 3-alkoxymethyl-1-methylimidaolium ILs bearing various types of anions displays high antimicrobial activity with longer cationic alkyl chain. The anions show no significant effect on the activity [21]. Furthermore, pyrrolidinium ionic liquids with varying alkyl chain substituents are also known to possess good antimicrobial activity against rods, cocci and fungi [18].

Seven strains of bacteria, three Gram-positive, *Bacillus subtilis* (B29), *Staphylococcus aureus* (S276) and *Staphylococcus epidermidis* (S273); two Gram-negative, *Pseudomonas aeruginosa* (ATCC 15422) and *Escherichia coli* (E266) and two types of yeasts, *Candida albicans* (9002) and *Candida tropicalis* (A3) were used to assess the antimicrobial activity of the ILs that have been synthesized. The anti-microbial activities of the synthesized ILs were studied by measuring the inhibition zones on the agar plates. This zone is described as the area on the agar plate where growth of a microbe is prohibited by the test compound [23].

The minimum inhibitory concentration (MIC) of this cholinium-based ILs cannot be evaluated due to low solubility of ILs in nutrient broth. Table 2 shows the inhibition zone diameter of ILs (mm) towards the microorganisms. For the sake of comparison, Streptomycin and Nystatin were tested as positive standards whereas water and DMSO as negative standards.

In this study, cholinium phenylacetate gives excellent inhibition towards all microorganisms, where the inhibition is higher than that of the standard for Gram-positive microorganisms (B29, S276 and S273) and yeasts (9002 and A3), whilst the inhibition is as good as the standard for Gram-negative microorganisms (ATCC 15422 and E266). The excellent properties of cholinium phenylacetate are maybe due to the contributions of phenylacetate group. It has been known that the starting material, phenylacetic acid has been used in commercial production of penicillin [1]. On the other hand, choline ricinoleate was found to have excellent inhibition towards Gram-positive microorganisms S273 and B29. Choline ricinoleate also shows good inhibition towards *Escherichia coli*, however is not very active towards the inhibition of yeasts. On the contrary, choline trichloroacetate has shown excellent inhibition towards both yeasts (9002 and A3) as the inhibition zone diameter is greater than those of the standard.

Table 2
Inhibition zone diameter of choline-based compounds towards bacteria (mm)

Cholinium-based ILs	Inhibition zone diameter of choline-based ILs (mm) towards bacteria						
	B29	*S276*	*S273*	*ATCC 15422*	*E266*	*9002*	*A3*
1a	20	27	25	19	19	30	24
1b	25	12	22	10	21	12	12
1c	15	19	20	9	20	30	24
Std (+ve) (1 mg/ml)	19	20	22	20	23	25	19
H_2O (-ve) (1 mg/ml)	-	-	-	-	-	-	-
DMSO (-ve) (1 mg/ml)	-	-	-	-	-	-	-

4. Conclusions

The main target of this research is to synthesize a series of new cholinium-based ionic liquids (ILs) by neutralization reaction. Three selected carboxylic acids, phenylacetic acid, ricinoleic acid and trichloroacetic acid were added to choline hydroxide to form cholinium-based ILs. The synthesized cholinium-based compounds were cholinium phenylacetate, cholinium ricinoleate and cholinium trichloroacetate.

All the synthesized cholinium-based compounds were characterized using infrared spectroscopy, ^{1}H and ^{13}C NMR spectroscopy, mass spectrometry, thermogravimetric and differential scanning chromatography. Results obtained from all characterization techniques confirmed the formation of these choline-based compounds. One cholinium-based IL was successfully formed which was cholinium trichloroacetate due to low melting temperature below 100°C whereas the other three compounds cannot be classified as ILs but as new choline-based salts.

These cholinium-based compounds were then tested for their antimicrobial properties. Antimicrobial test was carried out towards seven types of bacteria which consist of three Gram-positive bacteria, *Bacillus subtilis* (B29), *Staphylococcus aureus* (S276) and *Staphylococcus epidermidis* (S273); two Gram-negative bacteria, *Pseudomonas aeruginosa* (ATCC 15442) and *Escherichia coli* (E266) and two types of yeasts, *Candida albicans* (9002) and *Candida tropicalis* (A3). All of the cholinium-based compounds show excellent inhibition of microorganisms, with choline phenylacetate showing the most striking inhibition for all the Gram-positive microorganisms, Gram-negative microorganisms and yeasts. These choline-based compounds show promising antimicrobial application. More detailed studies are currently underway.

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