Synthesis of two novel alanine-based antibacterial and antioxidant chiral ionic liquids

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Abstract

Ionic liquids have drawn a lot of attraction due to their unique properties. In this paper, a simple, inexpensive, easy to handle, and efficient method for the synthesis of 1-(4-((1-carboxyethyl)carbamoyl)benzyl)pyridin-1-ium chloride and 1-(4-(((1-carboxyethyl)carbamothioyl)carbamoyl)benzyl)pyridin-1-ium chloride as two novel chiral ionic liquids based on the *L*-(+)-alanine amino acid is introduced. Their structures were confirmed via ¹H-NMR, ¹³C-NMR and FT-IR spectroscopies. Minimum inhibitory concentration method was used to study their antibacterial properties. Their antioxidant properties were investigated on 2,2-diphenyl-1-picrylhydrazyl as a free radical. Results confirmed these compounds as strong antibacterial and antioxidant ionic liquids, capable of replacing many antibiotic, and potentially, anticancer compounds.

Keywords Chiral ionic liquids · Alanine · Antibacterial · Antioxidant

1 Introduction

Today, the resistance of many microorganism strains against different antibiotics has become a serious health problem in the world. Emergence and spreading of new strains of antibiotic-resistant bacteria not only is of a great threat to the human lives but also creates high costs for the health and care segments of different countries; making the synthesis of new antimicrobial medicines of the highest priority [1–3]. Many antimicrobial compounds such as cationic polymers, peptides, and phenolic compounds have been rigorously investigated by many scientists. Amongst the mentioned groups, ammonium, imidazolium, pyridinium and phosphonium ions-substituted cationic polymers draw a lot of attention to them due to their similar peptide-like structures which result in a highly active antimicrobial property [4–8]. Ionic liquids, by definition, are considered as the salts with a melting point less than 100 °C which are consisted of poorly coordinated

molten cations and anions [9, 10]. These compounds have been known to scientists for more than a century, but due to their unique properties, they have been under strict security protections, and just recently have been made available to the public as proper solvents. Ionic liquids demonstrate a series of unique features. By altering their comprising cations and anions, their physical and chemical properties could change as well, which make them a suitable candidate for applications in molecular modeling, catalysts, bio-materials, etc.[11, 12]. Besides that, as is reported, ionic liquids have a very low vapor pressure which means they can be utilized as a green solvent in replacing the common solvents for the chemical synthesis of many materials [13, 14].

Although many of the currently-studied ionic liquids are not completely biodegradable, studies have proven that under the proper aerobic organisms' degradation, these compounds have minimum impact on the ecosystem and the environment. Investigations on the imidazolium or the

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pyridinium-based ionic liquids have demonstrated that the degradable ester functional group in the sidechain structure of these molecules, tremendously increases the biodegradability of the ionic liquids compared to the case when the ionic liquid molecules consist of simple alkyl chains [15]. Pyridinium-based ionic liquids are consisted of a hydrophilic head and a hydrophobic tale, making them amphiphilic. Many studies have proven the fact that these compounds show behavior similar to the cationic surfactants [16]. Derivatives of long alkyl chain pyridinium- or imidazolium-based ionic liquids which demonstrate substantial surfactant and biological activities, along with the effect of the sidechain ester functional group on the biodegradability of these compounds, have encouraged the researchers to synthesize and study novel antimicrobial ionic liquids [11]. Also, studies on the pyridine-based oxazolidinone derivatives have shown the growth inhibitory effect of these compounds on many antibiotic-resistant bacteria strains which is believed to be due to the increased antibacterial effect of the pyridine [17].

In this study, two novel alanine-based pyridine-containing chiral ionic liquids 1-(4-((1-carboxyethyl)carbamoyl)benzyl)pyridin-1-ium chloride **5** and 1-(4-(((1-carboxyethyl)carbamothioyl)carbamoyl)benzyl)pyridin-1-ium chloride **6** are synthesized in two steps (Scheme 1) with excellent yields. After their structure confirmation, their antioxidant activities are investigated via utilization of 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a free radical. Minimum inhibitory concentration method (MIC) is also used to determine their antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus cereus* bacteria.

2 Experimental

All of the utilized materials were obtained from Merck Co., Germany and were used without further purification. BrukerTensor-27 was used to collect the FT-IR data; FT-NMR Bruker DRX-500 was exploited to determine the ^1H -NMR at 500 MHz frequency; ^{13}C -NMR was conducted by FT-NMR Bruker DRX at 125 MHz frequency, and specific rotation of the chiral ionic liquids was investigated via Atago AP-300 polarimeter. To further confirm the structural data of the

Scheme 1 Chemical route for the synthesis of the two chiral ionic liquids **5** and **6**

synthesized materials, Electrothermal 9100 Melting Point Apparatus was exploited. The occurrence of the reactions, was performed via aluminum plate and silica gel F245 60 and *n*-hexane/ethyl acetate (2:8) solvent-equipped thin layer chromatography (TLC). All of the experiments were carried under argon gas unless stated otherwise.

2.1 General procedure for the synthesis of (4-(chloromethyl)benzoyl)-L-alanine **3** and ((4-(chloromethyl)benzoyl) carbamothioyl)-L-alanine **4**

For the synthesis of compounds **3** and **4**, a mixture of alanine (1 mmol) and 4-(chloromethyl)benzoyl chloride **1** (1 mmol) or 4-(chloromethyl)benzoyl isothiocyanate **2** (1 mmol) was added to 3 mL acetonitrile as a solvent, under reflux condition at 80 °C for 24 h. Thin layer chromatography (TLC) was used to confirm the completion of the reaction. After the reaction confirmed completed, the mixture was filtered, and the solvent was evaporated under the vacuum condition. The remaining white sediment was recrystallized using an *n*-hexane/ethyl acetate mixture (1:1) and was utilized in further synthesis steps after the structural confirmation.

2.2 General procedure for the synthesis of **5** and **6** chiral ionic liquids

For the synthesis of compounds **5** and **6**, a certain amount of compounds **3** or **4** (1 mmol) was added to pyridine (2 mmol), and the mixture was stirred at 50–60 °C for 2 h. under solventless reaction condition. After the reaction was confirmed completed by the TLC method, 5 mL water was added to the reaction mixture, and shook vigorously for 5 min. Using ethyl acetate as the solvent, the impurities were separated, and the viscous, chiral ionic liquids were isolated with very good yields. Using 0.273 g of each chiral ionic liquids, the equivalent points for compounds **5** and **6** were measured to be 17 and 14.4 mL under 0.05 N sodium hydroxide titration, respectively. This, resulted in the Mw of compounds **5** and **6** equal to 320.77 and 379.86, respectively. Furthermore, the specific rotation of compounds **5** and **6** was studied at various concentrations, and the mean value was observed to be 11.34 and 10.27, respectively.

3 Results and discussion

The structures of the compounds **3** to **6** were confirmed via exploiting the FT-IR, ¹H-NMR and ¹³C-NMR spectroscopy techniques. For instance, the observed peaks of $\delta = 1.41$, 4.42 (CHMe) and 4.68 ppm in the ¹H-NMR data for compound **5**, or the $\delta = 7.39$ –8.49 ppm for the aryl

and pyridine, or the ¹³C-NMR of $\delta = 165.9$ and 174.5 ppm for the carbonyl resonances, all are confirming the proposed structure. All of the spectroscopic data presented here, confirm the proposed structure of their corresponding compounds.

3.1 Spectroscopic data for compound **3**

White powder, isolated yield: 236.8 mg (98%), m.p.: 184–185 °C, C₁₁H₁₂ClNO₃ (M = 241.67); IR (KBr) ($\nu_{\max}/\text{cm}^{-1}$): 3384, 3269–2500 (COOH), 1726, 1640, 1528, 1369, 1237 cm^{-1} ; ¹H NMR (500 MHz, DMSO-*d*₆): 1.37 (3 H, d, ³J 7.3, Me), 4.05 (1 H, quintet, ³J 7.3, CH), 4.79 (2 H, s, CH₂), 7.51 (2 H, d, ³J 8.2, 2 CH), 7.89 (2 H, d, ³J 8.2, 2 CH), 8.67 (1 H, d, ³J 7.1, NH), 12.04 (1 H, br s, COOH) ppm; ¹³C NMR (125 MHz, DMSO-*d*₆): $\delta = 16.7$ (Me), 45.5 (CH₂), 48.1 (CH), 127.8 (2 CH), 128.7 (2 CH), 133.8 (CH), 140.9 (C), 165.7 (C=O), 174.0 (C=O) ppm, Anal. Calcd. for **3** (241.67): C, 54.67; H, 5.01; N, 5.80, Found: C, 54.47; H, 5.66; N, 6.57.

3.2 Spectroscopic data for compound **4**

Pale yellow crystals, isolated yield: 291.7 mg (97%), m.p.: 174–175 °C, C₁₂H₁₃ClN₂O₃S (M = 300.76); IR (KBr) ($\nu_{\max}/\text{cm}^{-1}$): 3382, 3161–2600 (COOH), 1732, 1677, 1553, 1255, 1170 cm^{-1} ; ¹H NMR (500 MHz, DMSO-*d*₆): 1.67 (3 H, d, ³J 7.5, Me), 4.15 (2 H, s, CH₂), 5.12 (1 H, quintet, ³J 7.5, CH), 7.32 (2 H, t, ³J 8.1, 2 CH), 7.75 (2 H, d, ³J 8.1, 2 CH), 9.29 (1 H, s, NH), 11.15 (1 H, d, ³J 7.6, NH), 12.11 (1 H, br s, COOH) ppm; ¹³C NMR (125 MHz, DMSO-*d*₆): $\delta = 17.4$ (Me), 46.4 (CH₂), 49.6 (CH), 126.9 (2 CH), 127.1 (2 CH), 132.4 (CH), 139.5 (C), 167.5 (C=O), 173.1 (C=O), 180.2 (C=S) ppm, Anal. Calcd. for **4**: C, 47.92; H, 4.36; N, 9.31, Found: C, 50.47; H, 4.81; N, 9.94.

3.3 Spectroscopic data for compound **5**

White powder, isolated yield: 304.7 mg (95%), m.p.: 79–82 °C, $[\alpha] = +11.34$. C₁₆H₁₇ClN₂O₃ (M = 320.77); IR (KBr) ($\nu_{\max}/\text{cm}^{-1}$): 3231, 3155–2600 (COOH), 1731, 1647, 1541, 1211, 1163 cm^{-1} ; ¹H NMR (500 MHz, DMSO-*d*₆): 1.41 (3 H, d, ³J 7.6, Me), 4.42 (1 H, quintet, ³J 7.6, CH), 5.78 (2 H, s, CH₂), 7.43 (2 H, d, ³J 8.2, 2 CH), 7.72 (2 H, d, ³J 8.2, 2 CH), 7.88 (1 H, d, ³J 7.6, NH), 7.99 (2 H, t, ³J 6.8, 2 CH), 8.47 (1 H, t, ³J 6.8, CH), 8.82 (2 H, d, ³J 6.9, 2 CH), 12.16 (1 H, br s, COOH) ppm; ¹³C NMR (125 MHz, DMSO-*d*₆): $\delta = 16.8$ (Me), 48.6 (CH₂), 62.5 (CH), 128.6 (2 CH), 128.7 (2 CH), 128.8 (2 CH), 134.5 (C), 137.6 (C), 145.1 (2 CH), 146.4 (CH), 165.9 (C=O), 174.5 (C=O) ppm, Anal. Calcd. For **5**: C, 59.91; H, 5.34; N, 8.73, Found: C, 59.45; H, 5.87; N, 9.19.

3.4 Spectroscopic data for compound 6

White powder, isolated yield: 357.0 mg (94%), m.p.; 86–87 °C, $[\alpha]_D^{25} = +10.27$. $C_{17}H_{18}ClN_3O_3S$ ($M = 379.8$); IR (KBr) ($\nu_{\max}/\text{cm}^{-1}$): 3357, 3255–2500 (COOH), 1666, 1577, 1491, 1379, 1339, 1167 cm^{-1} ; ^1H NMR (500 MHz, $\text{DMSO}-d_6$): 1.48 (3 H, d, 3J 7.1, Me), 4.86 (1 H, quintet, 3J 7.1, CH), 6.04 (2 H, s, CH_2), 7.67 (2 H, d, 3J 8.2, 2 CH), 7.98 (2 H, d, 3J 8.2, 2 CH), 8.21 (1 H, t, 3J 6.8, 2 CH), 8.66 (1 H, t, 3J 6.7, CH), 9.34 (2 H, d, 3J 6.8, 2 CH), 11.25 (1 H, d, 3J 7.1, NH), 11.57 (1 H, s, NH), 12.88 (1 H, br s, COOH) ppm; ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$): $\delta = 17.3$ (Me), 52.7 (CH_2), 62.7 (CH_2), 126.7–152.7 (C-aromatic and pyridine), 168.5 (C=O), 172.6 (C=O), 179.9 (C=S) ppm, Anal. Calcd. For **6**: C, 53.75; H, 4.78; N, 9.33, Found: C, 53.57; H, 5.16; N, 9.83.

3.5 Study of antibacterial activity of compounds 5 and 6

Minimum inhibitory concentration (MIC) method was used as the chosen method for determining the antibacterial activity of the synthesized compounds **5** and **6**. Based on the CLSI standards [18], to determine the antibacterial activity, the selected bacteria strains were inoculated to the Mueller–Hinton broth which was injected with a series of 100 μL , 0.4–1000 $\mu\text{g}/\text{mL}$ of compounds **5** and **6**. Suspensions of the 18 h old inoculated Mueller–Hinton broth samples were prepared via utilization of normal saline (1:100) and then, after adjusting its turbidity within the McFarland standards, 100 μL of the prepared suspensions were added to the wells using the agar well diffusion method. After incubation, resazurin dye was exploited as the reagent to distinguish between the active and inactive bacteria wells so that the MIC could be determined. The results are presented in Table 1. The reported data suggest a slightly stronger antibacterial activity of compound **6**. Also, it is obvious that although the precursors of compound **5** and **6** (i.e. compounds **3** and **4**) demonstrate antibacterial activities, the final chiral ionic liquids **5** and **6** outrank these precursors in this category, which is believed to be due to the ionic nature of the latter compounds which the precursors lack.

3.6 Study of antioxidant activity of compounds 5 and 6

2,2-diphenyl-1-picrylhydrazyl (DPPH) was utilized as the free radical source to measure the antioxidant activity of compounds **5** and **6**. In this method, by measuring the decreased capacity of the free radical which has a $\lambda_{\max}$ equal to 517 nm, the inhibitory concentration 50% (IC_{50}) can be measured and reported as the required inhibitory concentration which decreases the absorption value in $\lambda_{\max}$ to 50%. When DPPH accepts an electron from the antioxidant compound, its color would change from purple to yellow and will demonstrate a decrease in the adsorption value which has a linear relation to the amount of the utilized antioxidant compound.

Different samples of compounds **5** and **6** with 5 different concentrations (1–5 $\mu\text{g}/\text{mL}$) were prepared and added to a mixture of 187 μL of ethanol and 63 μL of DPPH which was injected into the wells. These samples were put into a PowerWave XS2 plate reader at 517 nm for their absorption (A) to be determined. By using the I% formula: $(A \text{ blank} - A \text{ sample})/A \text{ blank} \times 100$, the IC_{50} was calculated. The butylated hydroxytoluene (BHT) was exploited as a positive control [19]. After graph extrapolation by utilization of the trend-line equation (Fig. 1), the calculated IC_{50} value of each compound was determined. The data are reported in Table 2.

The lower the IC_{50} value, the more active the compound is. As can be seen, the antioxidant activity of compound **6** was higher than compound **5**. This difference which was measured around 1.67 folds, is believed to be due to the presence of =S atoms in the structure of compound **6**. Proportionally, compounds **3** and **4** are not as strong in free radical suppression in comparison to compounds **5** and **6**, which is believed to be caused by their lack of ionic activity. The data suggest compound **6** with a 2.69 mM concentration can inhibit the activity of 50% of DPPH radicals. This value was observed to be 0.25 mM for the BHT as the standard.

Table 1 The antibacterial activity of the synthesized compounds **5** and **6**

Compound	MIC unit	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Bacillus cereus</i>	<i>Pseudomonas aeruginosa</i>
3	mg/mL	27	20	31	29
4	mg/mL	19	11	22	20
5	mg/mL	> 1	> 1	> 1	> 1
6	mg/mL	1	> 1	1	1
Cefixime	$\mu\text{g}/\text{mL}$	32	0.25	1	64

Fig. 1 Comparison between the antioxidant activity of compounds **5** and **6**, based on their inhibition values

Table 2 The antioxidant activity of the synthesized compounds **5** and **6**

Compound	IC ₅₀ (mM)
3	10.87
4	8.43
5	4.50
6	2.69
BHT	0.25

4 Conclusion

In this study, an easy, simple to handle, one-pot two-step synthesis of two novel chiral ionic liquids was presented. The advantages of this process are simple purification of the products, very high isolated yields of reaction (> 95%) and environmentally-friendly. Addition of the *L*-alanine made the synthesized compounds more water soluble. The study of the antibacterial and antioxidant activity of the products **5** and **6** has proved these chiral ionic liquids as strong antibacterial and antioxidant compounds which can potentially be utilized as the future antibiotics against many antibiotic-resistant strains, or be exploited as strong antioxidants against various cancers, or as supplements and medicines in the treatment of similar diseases.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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