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A green ultrasound-assisted synthesis of 4-(dimethylamino)-1-(6-ethoxy-6-oxohexyl)pyridinium bromide: A very promising antibacterial agent

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Abstract

A new ionic liquid, 4-(dimethylamino)-1-(6-ethoxy-6-oxohexyl)pyridinium bromide, was synthesized using a facile and green ultrasound-assisted procedure and its IR, 1H NMR,13C NMR and MS spectroscopic data are presented. The antimicrobial profile of this new ionic liquid was evaluated. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) showed their very promising antimicrobial activity against seven types of human pathogens.

Keywords: 4-(dimethylamino)-1-(6-ethoxy-6-oxohexyl)pyridinium bromide; Ionicliquid; antimicrobial activity

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1. Introduction

Over the last years, Ionic liquids (ILs) have received a great deal of attention as eco-friendly substitutes for volatile organic solvents (VOCs) due to their outstanding properties such as zero vapor pressure, excellent thermal stability, no volatility, no flammability, high ionic conductivity [1,2]. These unique properties of ILs have prompted many researchers to synthesize and investigate their several applications as media for electrodeposition of metals [3], in catalysis and biocatalysis [4,5], as corrosion inhibitors [6,7] and in nuclear industry [8]. In addition, the antimicrobial activity of different classes of ionic liquids against both environmental and clinically important microorganisms have been reported [9,10].

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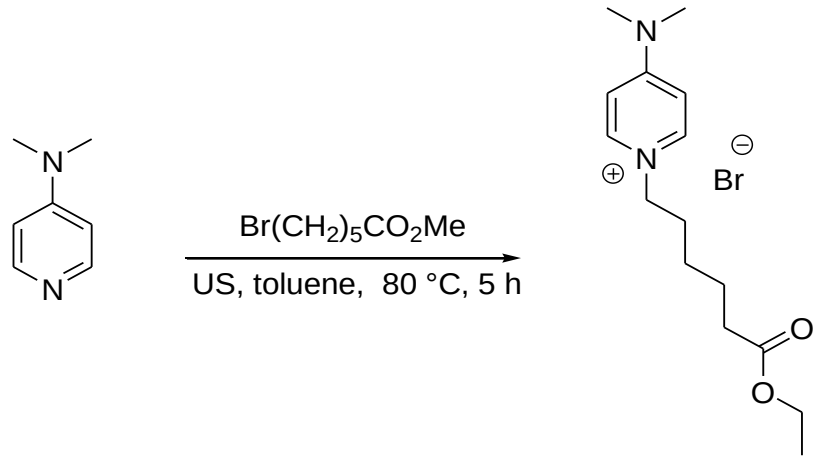
In the present work, and according to our ongoing research interest in ionic liquids synthesis [11-14], a new ionic liquid, 4-(dimethylamino)-1-(6-ethoxy-6-oxohexyl)pyridinium bromide, was synthesized and characterized using ultrasound irradiation as a green technology. Several types of human pathogens were selected to assess the antimicrobial activity of the newly IL.

2. Materials and methods

2.1. Chemistry

The synthesis of 4-(dimethylamino)-1-(6-ethoxy-6-oxohexyl)pyridiniumbromide was easily achieved by the nucleophilic alkylation of 4-(dimethylamino)pyridine with methyl 6-bromohexanoate (scheme 1).



Scheme 1.N-alkylation of 4-(dimethylamino)pyridine under ultrasonic irradiation conditions (US)

The structure of the newly synthesized ionic liquid, 4-(dimethylamino)-1-(6-ethoxy-6-oxohexyl)pyridinium bromide, was confirmed by ^{1}H NMR, ^{13}C NMR, FT-IR, LCMS and elemental analysis.

The ^{1}HNMR spectrum showed a singlet around δ3.11 ppm corresponding to 2CH_3 protons attached to the nitrogen. The characteristic ester protons appeared as triplet at δ at δ 1.12 ppm and quartet at δ 4.04 ppm. The signals at δ6.80 and 7.94 ppm were attributed to the pyridinium protons.

In ^{13}C NMR spectra, the carbon of the carbonyl group appeared around δ 176.6 ppm. In addition the aromaticcarbons of the pyridinium ring gave the signals between δ 107.5-156.2 ppm.CH_2 and CH_3 groups have been observed at their usual chemical shifts.

The IR spectra showed the principal absorption band at 1730 cm^{-1}indicating the presence of carbonyl group (C=O) in the molecules which confirms the success of the N-alkylation reaction.

2.2. Antimicrobialactivity

Antibacterial activity testing confirmed that 4-(dimethylamino)-1-(6-ethoxy-6-oxohexyl) pyridinium bromide exhibited antibacterial potential with varying degrees. The water soluble IL was screened for its antibacterial activity against a number of clinical bacterial species including; Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Enterobacter, and Acinetobacter baumannii. The antibacterial activity was measured by determination of MIC and MBC values in a range from 0 to 256 µg/mL and evaluated in comparison with two standard antibiotics; erythromycin and vancomycin. IZ, MIC, and MBC values of IL against the test bacterial strains are listed in Table 1.

In general, 4-(dimethylamino)-1-(6-ethoxy-6-oxohexyl) pyridinium bromide exhibited broad spectrum revealed by detected antibacterial activity against all tested Gram-positive and Gram-negative bacteria except for S. epidermidis (MIC values >256 µg/mL). Antibacterial activity measured inhibition zones ranged from 12 to 35 mm. The IL was most effective against Enterobacter sp. and S. aureus with recorded IZ of 35 and 26 mm respectively. The IL possessed relative lower antibacterial activities against the growth of P. aeruginosa and A. baumannii.

Table 1. Antimicrobial activity of 4-(dimethylamino)-1-(6-ethoxy-6-oxohexyl) pyridinium-bromide against seven bacterial strains.

Bacterialstrains	4-(dimethylamino)-1-(6-ethoxy-6-oxohexyl) pyridiniumbromide			Erythromycin			Vancomycin		
	IZ (mm)	MIC (µg/mL)	MBC (µg/mL)	IZ (mm)	MIC (µg/mL)	MBC (µg/mL)	IZ (mm)	MIC (µg/mL)	MBC (µg/mL)
E .coli	15	64	64	7	16	32	16	16	16
Enterobacter sp.	35	16	16	-	-	-	-	-	-
P. aeruginosa	18	64	64	12	16	16	-	-	-
S. aureus	26	16	32	13	8	8	15	16	32
S. epidermidis	-	>256	>256	18	8	8	23	4	4
B. cereus	13	64	128	16	8	8	17	16	32
A. baumannii	12	64	64	7	32	64	12	32	32

The MIC values ranged from 32 to 256 µg/mL, and the MBC values were similar or 2 folds higher than MIC values. MIC values confirmed that 4-(dimethylamino)-1-(6-ethoxy-6-oxohexyl) pyridinium

bromide has potential antibacterial activity against both Enterobacter sp. and S. aureus (MIC, 16 and 32 μg/mL respectively). In addition, 4-(dimethylamino)-1-(6-ethoxy-6-oxohexyl) pyridinium bromide exhibited moderate activities (MIC, 64 μg/mL) against E. coli, P. aeruginosa, and A. baumannii and relatively low activity against B. cereus (MBC, 128 μg/mL). Remarkably, 4-(dimethylamino)-1-(6-ethoxy-6-oxohexyl) pyridinium bromide was more efficient than erythromycin and vancomycin against Enterobacter sp. and P. aeruginosa respectively.

3. Materials and methods

3.1. Experimental

The newly ionic liquid was synthesized and characterized by 1H NMR, 13C NMR, IR, and LCMS. 1H NMR (400 MHz) and 13C NMR (100 MHz) spectra were measured in D2O at room temperature. Chemical shifts (δ) were reported in ppm to a scale calibrated for tetramethylsilane (TMS), which isused as an internal standard. The LCMS spectra were measured with a Micromass, LCT mass spectrometer. IR spectra were recorded in KBr disc on a Schimadzu 8201 PC, FTIR spectrophotometer (□max in cm-1). The ultrasound-assisted reactions were performed using a high intensity ultrasonic processor SUB Aqua 5 Plus-Grant with temperature controller (750 W), microprocessor controlled-2004, the ultrasonic frequency of the cleaning bath usedequal 25 KHz.

3.2. Synthesis

4-(dimethylamino)pyridine (1eq) and an alkyl halide (1.1eq) were placed in a closed vessel and exposed to irradiation for 5 h at 70 °C in a sonicator. The reaction was deemed complete when a solid separated from the initial clear and homogenous mixture. Unreacted starting materials and solvent were removed by filtration. The pyridinium salt was then washed with ethylacetate and dried in vacuo to remove all VOCs.

3.3. Characterization

4-(dimethylamino)-1-(6-ethoxy-6-oxohexyl)pyridiniumbromide:

White crystals, Mp 102-105 ºC, 1H NMR (D2O, 400 MHz,): δ = 1.12 (t, 3H), 1.21 (t, 2H), 1.52 (quintet, 2H), 1.77 (sixtet, 2H), 2.26 (quintet, 2H), 3.11 (s, 6H), 4.04 (m, 4H), 6.80 (d, 2H), 7.94 (d, 2H). 13C NMR (D2O, 100 MHz,): δ = 13.4 (CH3), 23.7 (CH2), 24.6 (CH2), 29.6 (CH2), 33.7 (CH2), 39.6 (CH3), 57.3 (CH2), 61.5 (CH2), 107.5 (CH), 141.3 (CH), 156.2 (C), 176.6 (C=O). IR (KBr) □max 3131 (C-H Ar), 1728 (C=O), 1602-1471 (C=C), 1164 (C-N), 1081 (C-O) cm-1. LCMS (M-Br) 265.3 found for C15H25N2O2+. Anal. Calcd. For C15H25BrN2O2 (345.27): C: 52.18; H: 7.30; N: 8.11; Found: C: 52.02; H: 7.19; N: 8.24.

3.4. Determination of IZ, MIC and MBC

The antimicrobial potential was estimated in terms of inhibition zone (IZ) measurements by agar disc diffusion method. The test was performed by transferring 24h fresh bacterial cultures onto the surface of Muller–Hinton agar plates to yield confluent growth. The antibacterial potential was assayed using filter paper discs (6 mm i.d.) loaded with 10 µg of tested compound. Loaded discs were transferred onto the centre of inoculated Petri-plates which were then maintained for 2 h in are frigerator at 4°C to allow for the diffusion of the bioactive compound. The diameter of the inhibition zone was measured (in mm) after 24 h incubation at 37°C. Sterile distilled water was used as a control. Clinical samples of Escherichia coli, Enterobactersp, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, and Acinetobacter baumannii were used as test strains.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of test compound were determined by CLSI microdilution-based method [15]. Test compound was dissolved in sterile, distilled water and diluted to a final concentration of 512 µg/mL in Mueller-Hinton broth (Becton Dickinson, USA) [16]. Two-fold serial dilutions were prepared in a 96-well microtiter plate. Bacterial suspension containing approximately 1 × 108 CFU/mL were prepared from 24 h agar plates with Mueller Hinton broth. Aliquots of 100 µL of each bacterial suspension was mixed with 100 µL serially diluted tested compound in microtiter plate [17]. Uninoculated wells were prepared as control samples. Plates were incubated at 37°C for 24 h. The MIC was defined as the lowest concentration of test compound producing no visible growth. The MBC was determined by transfer of aliquots from wells containing no growth on to nutrient agar plates and tested for colony formation upon subculturing. All experiments were performed in triplicate.

Conclusion

A new eco-friendly ionic liquid, 4-(dimethylamino)-1-(6-ethoxy-6-oxohexyl)pyridinium bromide ,was prepared by using ultrasound irradiation. The newly synthesized IL displayed a very promising antimicrobial activity.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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