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Some 4-dimethylaminopyridinium-based ionic liquids and/or salts. Part I: efficient green ultrasound synthesis, characterization, *in silico* prediction analysis, toxicity and antimicrobial evaluation

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Abstract

An eco-friendly ultrasound-assisted procedure for the preparation of twenty functionalized pyridinium ionic liquids (ILs) **1-20** is described. The characterization of the newly compounds is confirmed by ^{1}H NMR, ^{13}C NMR, ^{11}B NMR, ^{19}F NMR, ^{31}P NMR and mass analysis. All synthesized compounds were screened for some applications, namely, antimicrobial activity and the results are very promising. Preliminary structure activity relationship (SAR) studies have been performed to identify the relation between molecular structure and activity. *In silico* Analysis of ionic liquids and/or salts was carried out based on ADME, Lipinski rule, drug likeness, toxicity profiles and other physico-chemical properties. All compounds were safe in toxicity profile and computed LD50 values were in accepted range (2.63–2.87 mol/kg). *In silico* data has revealed that all ionic liquids and/or salts were in good agreement in term of bioavailability.

Keywords: Green procedure; ionic liquids; ultrasound irradiation; antimicrobial activity; *in silico* Prediction.

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1. Introduction

Ionic liquids (ILs) have recently emerged as an alternative to volatile organic compounds (VOCs) [1-3]. They are synthesized from an anion and an organic cation, such as imidazolium, pyrolidinium or pyridinium and are good solvents that readily dissolve many organic, inorganic and organometallic compounds. They also have many advantageous properties, such as negligible vapor pressure, stability at high temperatures, a low melting point and high ionic conductivity [4].

ILs have been used in organic synthesis [5,6], polymer science [7], as media for the electrodeposition of metals [8,9], in electrochemistry [10-12], supercapacitors [13], solar cells [14]. fuel cells [15] and for their corrosion-resistant behavior [16,17]. ILs have also recently been studied as potent antimicrobial agents [18,19].

Green procedures using ultrasound or microwave and solvent-free conditions are recommended [20,21]. Such conditions require less time and give improved yields [22,23].

Following on from our work on the synthesis of ILs [24-25], we now report an efficient green method for the preparation of novel pyridinium-based ionic liquids using ultrasound.

2. Materials and methods

2.1. Experimental

All new compounds were synthesized and characterized by ^{1}H NMR, ^{13}C NMR, ^{11}B NMR, ^{19}F NMR, ^{31}P NMR. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), ^{11}B NMR (128 MHz), ^{19}F NMR, (376.5 MHz) and ^{31}P NMR (162 MHz), spectra were measured in DMSO or D_2O at room temperature at 400MHz. Chemical shifts (d) were reported in ppm using tetramethylsilane (TMS), as an internal standard. 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 used equal 25 KHz.

2.2. Synthesis

2.2.1. *General procedure for the preparation of pyridinium ionic liquids* **1-5** *using conventional method*

4-(dimethylamino)pyridine (1eq) and an alkyl halide(1.1eq) were added to toluene and stirred at 80 °C for 18 h. The reaction was deemed complete when oil or a solid separated from the initial clear and homogenous mixture. Unreacted starting materials and solvent were removed by extraction or filtration. The pyridinium salt was then washed with ethyl acetate and dried *in vacuo* to remove all VOCs.

2.2.2. General procedure for the synthesis of pyridinium tetrafluoroborates, hexafluorophosphates or trifluoroacetates **6-20** *using conventional method:*

To solution of pyridinium ionic liquids **1-5** (1eq) in dichloromethane was added sodium tetrafluoroborate, potassium hexafluorophosphate or sodium trifluoroacetate (1.2eq). The mixture was stirred at 70°C for 3 h. The solid metal halide was removed by filtration of mixture. The desired ionic liquids were obtained with excellent yields after the evaporation of dichloromethane.

2.2.3. General procedure for the preparation of pyridinium halides **1-5** *under ultrasound irradiation:*

4-(dimethylamino)pyridine (1 eq) and an alkyl halide (1 eq) were placed in a closed vessel and exposed to irradiation for 5 h at RT in a sonicator. The product was then isolated and purified as described in the previous procedure.

2.2.4. General procedure for the preparation of pyridinium tetrafluoroborates, hexafluorophosphates or trifluoroacetates **6-20** *under ultrasound irradiation:*

Pyridinium ionic liquids **1-10** (1 eq) and $NaBF_4$, KPF_6 or CF_3CO_2Na, (1 eq) were placed in a closed vessel and exposed to irradiation for 45 min at RT in a sonicator. The product was then collected as described in the previous procedure.

2.3. Characterization

4-(dimethylamino)-1-ethylpyridinium bromide **1**:

White crystals, Mp 130-132 ºC, ^{1}H NMR ($CDCl_3$, 400 MHz,): δ = 1.36 (t, 3H), 3.08 (s, 6H), 4.06 (t, 2H), 6.78 (d, 2H), 7.94 (d, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz,): δ = 15.2 (CH_3), 39.6 (CH_3), 52.9 (CH_2), 107.6 (CH), 140.9 (CH), 156.2 (C); LCMS (M-Cl) 151.2 found for $C_9H_{15}N_2^+$.

4-(dimethylamino)-1-propylpyridinium bromide **2**:

White crystals, Mp 100-102 ºC, ^{1}H NMR (D_2O, 400 MHz,): δ = 0.79 (t, 3H), 1.75 (sixtet, 2H), 3.08 (s, 6H), 3.99 (t, 2H), 6.77 (d, 2H), 7.91 (d, 2H); ^{13}C NMR (D_2O, 100 MHz,): δ = 9.8 (CH_3), 23.6 (CH_2), 39.4 (CH_3), 59.1 (CH_2), 107.5 (CH), 141.3 (CH), 156.2 (C); LCMS (M-Br) 165.2 found for $C_{10}H_{17}N_2^+$.

1-butyl-4-(dimethylamino)pyridinium bromide **3***:*

White crystals, Mp 190-195 ºC, ^{1}H NMR (D_2O, 400 MHz,): δ = 0.82 (t, 3H), 1.22 (quintet, 2H), 1.72 (sixtet, 2H), 3.10 (s, 6H), 4.04 (t, 2H), 6.78 (d, 2H), 7.93 (d, 2H); ^{13}C NMR (D_2O, 100 MHz,): δ = 12.8 (CH_3), 18.7 (CH_2), 32.1 (CH_2), 39.4 (CH_2), 57.4 (CH_2), 107.5 (CH), 141.4 (CH), 156.2 (C); LCMS (M-Br) 179.2 found for $C_{11}H_{19}N_2^+$.

4-(dimethylamino)-1-pentylpyridinium bromide **4***:*

White crystals, Mp 210-215 ºC, ^{1}H NMR (D_2O, 400 MHz,): δ = 0.74 (t, 3H), 1.18 (m, 4H), 1.73 (sixtet, 2H), 3.09 (s, 6H), 4.02 (t, 2H), 6.77 (d, 2H), 7.91 (d, 2H); ^{13}C NMR (D_2O, 100 MHz,): δ = 13.1 (CH_3), 21.4 (CH_2), 27.4 (CH_2), 29.7 (CH_2), 39.4 (CH_3) 57.6 (CH_2), 107.5 (CH), 141.3 (CH), 156.2 (C); LCMS (M-Br) 193.3 found for $C_{12}H_{21}N_2^+$.

4-(dimethylamino)-1-hexylpyridinium iodide **5***:*

White crystals, Mp 170-172 ºC, ^{1}H NMR (D_2O, 400 MHz,): δ = 0.66 (t, 3H), 1.09 (m, 6H), 1.65 (sixtet, 2H), 3.00 (s, 6H), 3.93 (t, 2H), 6.67 (d, 2H), 7.80 (d, 2H); ^{13}C NMR (D_2O, 100 MHz,): δ = 13.1 (CH_3), 21.7 (CH_2), 24.8 (CH_2), 29.8 (CH_2), 30.3 (CH_2), 39.4 (CH_3) 57.6 (CH_2), 107.4 (CH), 141.3 (CH), 156.2 (C); LCMS (M-I) 207.3 found for $C_{13}H_{23}N_2^+$.

4-(dimethylamino)-1-ethylpyridinium tetrafluoroborate **6**:

Yellow clear crystals, Mp 55-58 ºC, ^{1}H NMR ($CDCl_3$, 400 MHz,): δ = 1.38 (t, 3H), 3.18 (s, 6H), 4.18 (t, 2H), 7.02 (d, 2H), 8.30 (d, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz,): δ = 16.0 (CH_3), 39.6 (CH_3), 52.0 (CH_2), 107.7 (CH), 141.6 (CH), 156.3 (C); ^{19}F NMR ($CDCl_3$, 376.5 MHz): δ = -148.32; ^{11}B NMR ($CDCl_3$, 128 MHz): δ = -1.30; LCMS (M-BF_4) 151.2 found for $C_9H_{15}N_2^+$.

4-(dimethylamino)-1-ethylpyridinium hexafluorophosphate **7**:

White crystals, Mp 70-72 ºC, ^{1}H NMR (DMSO, 400 MHz,): δ = 1.30 (t, 3H), 3.18 (s, 6H), 4.18 (t, 2H), 7.02 (d, 2H), 8.29 (d, 2H); ^{13}C NMR (DMSO, 100 MHz,): δ = 16.0 (CH_3), 39.6 (CH_3), 52.0 (CH_2), 107.7 (CH), 141.6 (CH), 155.8 (C); ^{19}F NMR (DMSO, 376.5 MHz): δ = -71.11; ^{31}P NMR (DMSO, 162 MHz): δ = -144.19 (sep, J = 712.8 Hz); LCMS (M-PF_6) 151.2 found for $C_9H_{15}N_2^+$.

4-(dimethylamino)-1-ethylpyridinium trifluoroacetate **8**:

Waxy, ^{1}H NMR (DMSO, 400 MHz,): δ = 1.37 (t, 3H), 3.18 (s, 6H), 4.20 (t, 2H), 7.03 (d, 2H), 8.34 (d, 2H); ^{13}C NMR (DMSO, 100 MHz,): δ = 16.0 (CH_3), 39.6 (CH_3), 52.0 (CH_2), 107.7 (CH), 140.9 (CH), 155.8 (C); ^{19}F NMR (DMSO, 376.5 MHz): δ = -73.47; LCMS (M-CF_3CO_2) 151.2 found for $C_9H_{15}N_2^+$.

4-(dimethylamino)-1-propylpyridinium tetrafluoroborate **9**:

White crystals, Mp 75-77 ºC, ^{1}H NMR (DMSO, 400 MHz,): δ = 0.84 (t, 3H), 1.77 (sixtet, 2H), 3.19 (s, 6H), 4.13 (t, 2H), 7.03 (d, 2H), 8.29 (d, 2H); ^{13}C NMR (DMSO, 100 MHz,): δ = 10.1 (CH_3), 23.6 (CH_2), 39.4 (CH_3), 59.0 (CH_2), 107.5 (CH), 141.9 (CH), 155.8 (C); ^{19}F NMR (DMSO, 376.5 MHz): δ = -148.32; ^{11}B NMR (DMSO, 128 MHz): δ = -1.27; LCMS (M- BF_4) 165.2 found for $C_{10}H_{17}N_2^+$.

4-(dimethylamino)-1-propylpyridinium hexafluorophosphate **10**:

White crystals, Mp 75-77 ºC, ^{1}H NMR (DMSO, 400 MHz,): δ = 0.84 (t, 3H), 1.78 (sixtet, 2H), 3.19 (s, 6H), 4.12 (t, 2H), 7.02 (d, 2H), 8.27 (d, 2H); ^{13}C NMR (DMSO, 100 MHz,): δ = 10.1 (CH_3), 23.6 (CH_2), 39.4 (CH_3), 58.0 (CH_2), 107.5 (CH), 141.9 (CH), 155.8 (C); ^{19}F NMR (DMSO, 376.5 MHz): δ

= -71.10; ^{31}P NMR (DMSO, 162 MHz): δ = -144.21 (sep, *J* = 712.8 Hz); LCMS (M- PF_6) 165.2 found for $C_{10}H_{17}N_2^+$.

4-(dimethylamino)-1-propylpyridinium trifluoroacetate **11**:

Oil, ^{1}H NMR (DMSO, 400 MHz,): δ = 0.81 (t, 3H), 1.74 (sixtet, 2H), 3.18 (s, 6H), 4.17 (t, 2H), 7.03 (d, 2H), 8.37 (d, 2H); ^{13}C NMR (DMSO, 100 MHz,): δ = 10.0 (CH_3), 23.6 (CH_2), 39.4 (CH_3), 57.8 (CH_2), 107.5 (CH), 141.9 (CH), 155.7 (C); ^{19}F NMR (DMSO, 376.5 MHz): δ = -71.10; LCMS (M-CF_3CO_2) 165.2 found for $C_{10}H_{17}N_2^+$.

1-butyl-4-(dimethylamino)pyridinium tetrafluoroborate **12***:*

White crystals, Mp 60-62 ºC, ^{1}H NMR (D_2O, 400 MHz,): δ = 0.88 (t, 3H), 1.22 (quintet, 2H), 1.73 (sixtet, 2H), 3.18 (s, 6H), 4.19 (t, 2H), 7.05 (d, 2H), 8.33 (d, 2H); ^{13}C NMR (D_2O, 100 MHz,): δ = 13.3 (CH_3), 18.6 (CH_2), 32.2 (CH_2), 39.6 (CH_2), 56.3 (CH_2), 107.5 (CH), 141.4 (CH), 156.2 (C); ^{19}F NMR (DMSO, 376.5 MHz): δ = -149.29; ^{11}B NMR (DMSO, 128 MHz): δ = -1.30; LCMS (M-BF_4) 179.2 found for $C_{11}H_{19}N_2^+$.

1-butyl-4-(dimethylamino)pyridinium hexafluorophosphate **13***:*

White crystals, Mp 105-110 ºC, ^{1}H NMR (D_2O, 400 MHz,): δ = 0.90 (t, 3H), 1.23 (quintet, 2H), 1.74 (sixtet, 2H), 3.18 (s, 6H), 4.15 (t, 2H), 7.01 (d, 2H), 8.28 (d, 2H); ^{13}C NMR (D_2O, 100 MHz,): δ = 13.2 (CH_3), 18.6 (CH_2), 32.2 (CH_2), 39.6 (CH_2), 56.4 (CH_2), 107.6 (CH), 141.8 (CH), 155.7 (C); ^{19}F NMR (DMSO, 376.5 MHz): δ = -71.10; ^{31}P NMR (DMSO, 162 MHz): δ = -144.21 (sep, *J* = 712.8 Hz); LCMS (M-PF_6) 179.2 found for $C_{11}H_{19}N_2^+$.

1-butyl-4-(dimethylamino)pyridinium trifluoroacetate **14***:*

Waxy, ^{1}H NMR (D_2O, 400 MHz,): δ = 0.87 (t, 3H), 1.20 (quintet, 2H), 1.71 (sixtet, 2H), 3.16 (s, 6H), 4.12 (t, 2H), 6.98 (d, 2H), 8.25 (d, 2H); ^{13}C NMR (D_2O, 100 MHz,): δ = 12.4 (CH_3), 17.8 (CH_2), 31.4 (CH_2), 38.8 (CH_2), 55.4 (CH_2), 106.8 (CH), 141.1 (CH), 156.8 (C); ^{19}F NMR (DMSO, 376.5 MHz): δ = -74.32; LCMS (M-CF_3CO_2) 179.2 found for $C_{11}H_{19}N_2^+$.

4-(dimethylamino)-1-pentylpyridinium tetrafluoroborate **15***:*

White crystals, Mp 135-138 ºC, ^{1}H NMR (D_2O, 400 MHz,): δ = 0.76 (t, 3H), 1.20 (m, 4H), 1.75 (sixtet, 2H), 3.11 (s, 6H), 4.04 (t, 2H), 6.79 (d, 2H), 7.93 (d, 2H); ^{13}C NMR (D_2O, 100 MHz,): δ = 12.9 (CH_3), 20.7 (CH_2), 26.7 (CH_2), 29.1 (CH_2), 39.4 (CH_3) 55.7 (CH_2), 106.8 (CH), 141.1 (CH), 154.9 (C); ^{19}F NMR (DMSO, 376.5 MHz): δ = -149.13; ^{11}B NMR (DMSO, 128 MHz): δ = -2.08; LCMS (M-BF_4) 193.3 found for $C_{12}H_{21}N_2^+$.

4-(dimethylamino)-1-pentylpyridinium hexafluorophosphate **16***:*

White crystals, Mp 72-72 ºC, ^{1}H NMR (D_2O, 400 MHz,): δ = 0.77 (t, 3H), 1.22 (m, 4H), 1.78 (sixtet, 2H), 3.14 (s, 6H), 4.04 (t, 2H), 6.81 (d, 2H), 7.96 (d, 2H); ^{13}C NMR (D_2O, 100 MHz,): δ = 16.3 (CH_3),

24.1 (CH_2), 30.1 (CH_2), 32.5 (CH_2), 39.4 (CH_3) 59.2 (CH_2), 110.2 (CH), 144.5 (CH), 158.4 (C); ^{19}F NMR (DMSO, 376.5 MHz): δ = -68.49; ^{31}P NMR (DMSO, 162 MHz): δ = -141.53 (sep, *J* = 712.8 Hz); LCMS (M-PF_6) 193.3 found for $C_{12}H_{21}N_2^+$.

4-(dimethylamino)-1-pentylpyridinium trifluoroacetate **17***:*

White crystals, Mp 72-72 ºC, ^1H NMR (D_2O, 400 MHz,): δ = 0.77 (t, 3H), 1.22 (m, 4H), 1.78 (sixtet, 2H), 3.14 (s, 6H), 4.04 (t, 2H), 6.81 (d, 2H), 7.96 (d, 2H); ^{13}C NMR (D_2O, 100 MHz,): δ = 16.3 (CH_3), 24.1 (CH_2), 30.1 (CH_2), 32.5 (CH_2), 39.4 (CH_3) 59.2 (CH_2), 110.2 (CH), 144.5 (CH), 158.4 (C); ^{19}F NMR (DMSO, 376.5 MHz): δ = -68.49; LCMS (M-CF_3CO_2) 193.3 found for $C_{12}H_{21}N_2^+$.

4-(dimethylamino)-1-hexylpyridinium tetrafluoroborate **18***:*

White crystals, Mp 125-128 ºC, ^1H NMR (D_2O, 400 MHz,): δ = 0.86 (t, 3H), 1.26 (m, 6H), 1.76 (sixtet, 2H), 3.21 (s, 6H), 4.20 (t, 2H), 7.05 (d, 2H), 8.34 (d, 2H); ^{13}C NMR (D_2O, 100 MHz,): δ = 13.7 (CH_3), 21.8 (CH_2), 25.0 (CH_2), 30.2 (CH_2), 30.5 (CH_2), 39.4 (CH_3) 56.5 (CH_2), 107.6 (CH), 141.9 (CH), 155.7 (C); ^{19}F NMR (DMSO, 376.5 MHz): δ = -148.33; ^{11}B NMR (DMSO, 128 MHz): δ = -1.28; LCMS (M-BF_4) 207.3 found for $C_{13}H_{23}N_2^+$.

4-(dimethylamino)-1-hexylpyridinium hexafluorophosphate **19***:*

White crystals, Mp 130-132 ºC, ^1H NMR (D_2O, 400 MHz,): δ = 0.84 (t, 3H), 1.25 (m, 6H), 1.75 (sixtet, 2H), 3.19 (s, 6H), 4.17 (t, 2H), 7.03 (d, 2H), 8.32 (d, 2H); ^{13}C NMR (D_2O, 100 MHz,): δ = 13.7 (CH_3), 21.8 (CH_2), 25.0 (CH_2), 30.2 (CH_2), 30.5 (CH_2), 39.7 (CH_3) 56.6 (CH_2), 107.6 (CH), 141.9 (CH), 155.7 (C); ^{19}F NMR (DMSO, 376.5 MHz): δ = -71.14; ^{31}P NMR (DMSO, 162 MHz): δ = -144.20 (sep, *J* = 712.8 Hz); LCMS (M-PF_6) 207.3 found for $C_{13}H_{23}N_2^+$.

4-(dimethylamino)-1-hexylpyridinium trifluoroacetate **20***:*

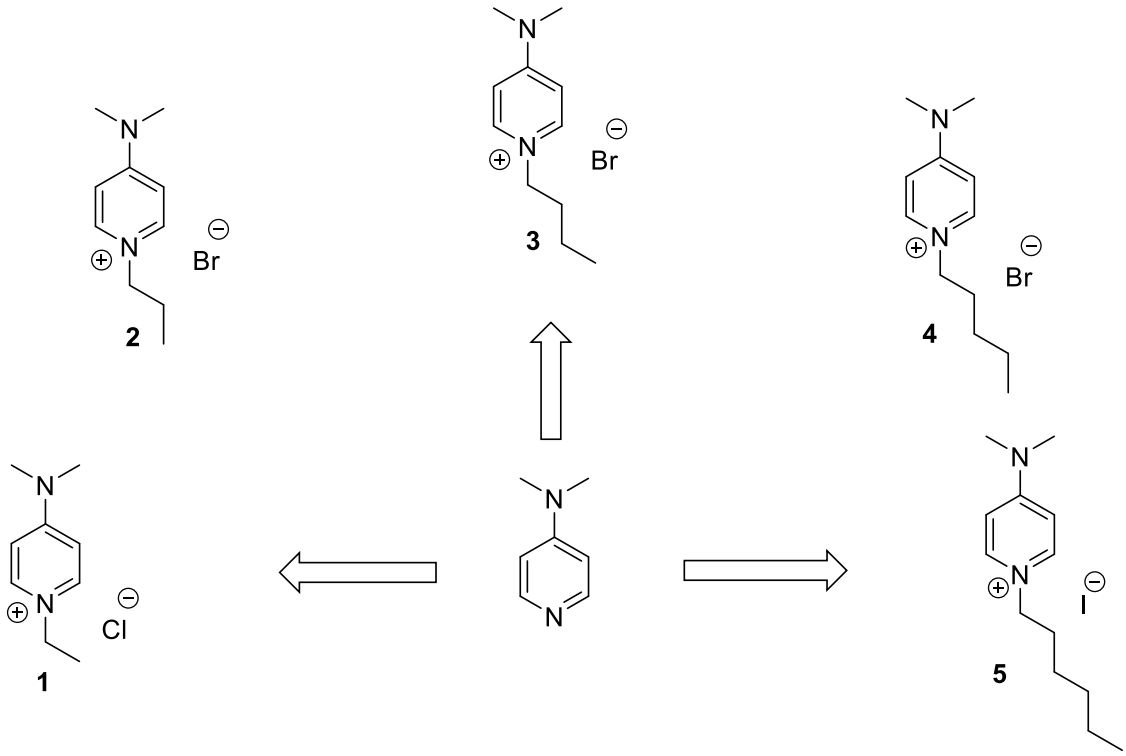
Yellow clear crystals, Mp 150-153 ºC, ^1H NMR (D_2O, 400 MHz,): δ = 0.85 (t, 3H), 1.26 (m, 6H), 1.76 (sixtet, 2H), 3.19 (s, 6H), 4.17 (t, 2H), 7.04 (d, 2H), 8.32 (d, 2H); ^{13}C NMR (D_2O, 100 MHz,): δ = 13.7 (CH_3), 21.8 (CH_2), 25.0 (CH_2), 30.2 (CH_2), 30.5 (CH_2), 39.7 (CH_3) 56.6 (CH_2), 107.6 (CH), 141.9 (CH), 155.7 (C); ^{19}F NMR (DMSO, 376.5 MHz): δ = -73.47; LCMS (M-CF_3CO_2) 207.3 found for $C_{13}H_{23}N_2^+$.

3. Results and discussion

3.1. Chemistry

In continuation to our previous work dealing with the developing of novel functionalized ionic liquids [25-27], in this work we aimed to synthesize a new variety of pyridinium-based ionic liquids under both conventional and ultrasound irradiation methods. To the best of our knowledge, only some compounds has been previously reported by conventional methods [28,29], their preparation under ultrasound irradiation has never been disclosed. Initially, pyridinium-based derivatives (**1–5**) were then

prepared with the conventional method, through the N-alkylation of 4-(dimethylamino)pyridine with the appropriate alkyl bromide in toluene at 80 °C for 18 h (Scheme 1).



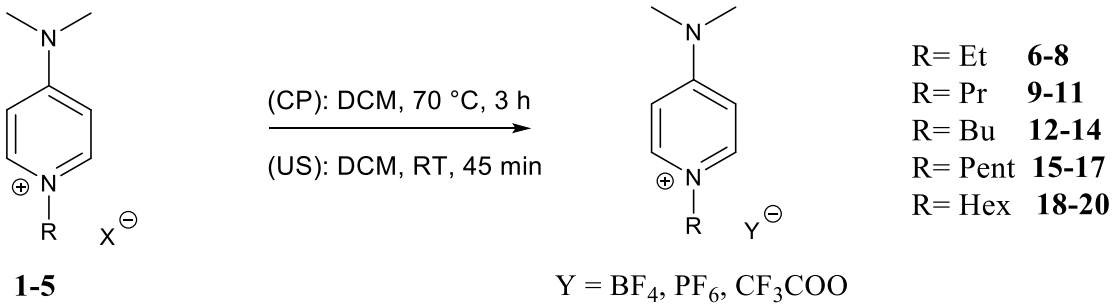
Scheme 1. N-alkylation of 4-(dimethylamino)pyridine under conventional preparation (CP) and ultrasonic irradiation conditions (US). CP: toluene, 80 °C, 18 h; US: toluene, RT, 5 h.

On the other hand, the ultrasound–assisted preparation of pyridinium-based ionic liquids **1-5**, already synthesized by conventional methods, was explored further with the objective of shortening the reaction time. The structures of all the synthesized ILs and/or salts were obtained with good yields (Table 1) and confirmed by ^{1}H NMR, ^{13}C NMR, and FT-IR.

Table 1 Different entries, reaction conditions and reaction yields for the synthesis of pyridinium-based ionic liquids **1-5** *using* conventional preparation (CP) and under ultrasound irradiation (US).

Compound	RX	Yield (%) N-Alkylation (first step)	
		US	CP
1	CH_3CH_2Br	88	77
2	$CH_3(CH_2)_2Br$	87	78
3	$CH_3(CH_2)_3Br$	89	76
4	$CH_3(CH_2)_4Br$	88	78
5	$CH_3(CH_2)_5I$	90	83

The introduction of anions such as tetrafluoroborate, hexafluorophosphate or trifluoroacetate was carried out by an anion exchange reaction (Scheme 2); a high final yield was obtained using this conventional method.



Scheme 2. Anion metathesis using conventional preparation (CP) and ultrasonic irradiation conditions. (CP): MY, dichloromethane, 70 °C, 3 h; US: dichloromethane, RT, 45 min. M = Na, K.

The anion exchange metathesis is easily performed under ultrasonic irradiation. Results in Table 2 showed that very good yields were obtained within very short reaction times, with no effect of the anion types used in this reaction on the yields.

Table 2 Different entries, reaction conditions and reaction yields for the anion metathesis using conventional preparation (CP) and under microwave irradiation (US).

Compound	R	X^-	M^-Y^+	Yield (%) Anion metathesis (second step)	
				US	CP
6			$NaBF_4$	98	95
7	CH_3CH_2	Br^-	KPF_6	96	93
8			NaO_2CF_3	97	92
9			$NaBF_4$	97	93
10	$CH_3(CH_2)_2$	Br^-	KPF_6	97	94
11			NaO_2CF_3	97	92
12			$NaBF_4$	95	95
13	$CH_3(CH_2)_3$	Br^-	KPF_6	98	94
14			NaO_2CF_3	97	93
15			$NaBF_4$	96	94
16	$CH_3(CH_2)_4$	Br^-	KPF_6	97	95
17			NaO_2CF_3	97	92
18			$NaBF_4$	98	93
19	$CH_3(CH_2)_5$	I^-	KPF_6	97	93
20			NaO_2CF_3	96	91

Anion exchange approach is proved without ambiguity by ^{11}B NMR, ^{19}F NMR and ^{31}P NMR since their spectra contained peaks just about δ_B -1 and δ_F -148 ppm for distinctive B or F in BF_4 and peaks just about δ_F -71 and δ_P -144 ppm for distinctive F or P in PF_6.

3.2. Antimicrobial activity

The main goal of the present study is to test the antibacterial activities of some selected newly synthesized ionic liquids. Hence, the water soluble compounds **1-20** were screened for their antibacterial activity against a number of clinical bacterial species including; *Staphylococcus aureus,*

Staphylococcus epidermidis, Escherichia coli, Enterobacter, and *Acinetobacter baumannii.* Antibacterial activity was evaluated in comparison with two standard antibiotics; Vancomycin and Rifampicin. In general, all compound showed antibacterial activities with different spectra. Compounds **3** and **4** were the most potent against *Enterobacter*, while compounds **5** was most potent against *S. aureus*. Compounds **3, 4, 11-17** and **20** were most effective against all bacterial strains tested (Table 3).

Table 3. Antibacterial activity and spectrum of the ILs **1-20**

Test compounds	*S. aureus*		*S. epidermidis*		*E. coli*		*Enterobacter*		*A. baumannii*	
	IZ (mm)	*MIC* (µg/mL)	*IZ (mm)*	*MIC* (µg/mL)	*IZ (mm)*	*MIC* (µg/mL)	*IZ (mm)*	*MIC* (µg/mL)	*IZ (mm)*	*MIC* (µg/mL)
1	-	-	-	-	-	-	3.5	8	1.7	16
2	0.8	64	-	-	1.8	32	4	8	2.6	8
3	2.5	8	1.1	32	2.2	8	4.6	4	3	8
4	2.4	8	2	8	2.2	8	5	4	3	8
5	3.5	4	-	-	0.6	64	3.5	8	0.8	64
6	-	-	-	-	-	-	2.2	32	0.8	32
7	0.7	64	-	-	-	-	2.8	32	1.2	64
8	0.5	128	0.8	64	-	-	2.7	32	1	32
9	0.6	128	-	-	2	16	2.6	16	1.8	32
10	0.8	64	-	-	1.4	32	3.5	8	2.8	8
11	0.9	64	0.5	128	1.8	16	3.2	8	2.9	8
12	1.8	16	0.8	64	1.7	16	3.6	8	2.4	16
13	1.7	16	1.2	32	1.6	16	3.8	8	2	16
14	2	16	1.0	32	1.9	16	4.0	8	2.9	8
15	2.0	16	1.2	32	1.6	32	4.0	8	2.2	16
16	1.9	16	1.4	32	1.8	32	4.1	8	2.6	16
17	2.8	8	1.9	32	2.0	16	4.8	4	2.5	16
18	2.8	8	-	-	-	-	2.7	16	-	-
19	2.9	8	0.7	128	-	-	3.0	8	0.4	128
20	2.7	8	0.6	128	0.5	128	2.9	16	0.6	128
Vancomycin	1.5	16	2.3	8	1.6	16	-	-	1.2	32
Rifampicin	3	4	3.5	4	0.8	32	0.7	32	1.2	16

3.2.1 Antibacterial Spectrum of the compounds 1-20

The antimicrobial activity was initially estimated in terms of inhibition zone (IZ) measurements by agar disc diffusion method. The test was performed by subculturing 24 h fresh bacterial cultures onto the surface of Muller–Hinton agar plates. The antibacterial activity 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 a refrigerator 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. All compounds were tested

against clinical samples of *Staphylococcus aureus*, *Bacillus cereus*, *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* as test strains.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determined by CLSI microdilution-based method [30] were used to evaluate antibacterial potentials. Test compound was dissolved in sterile, distilled water and diluted to a final concentration of 512 µg/mL inMueller-Hinton broth (Becton Dickinson, USA) [31]. 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 [32]. 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 onto nutrient agar plates and tested for colony formation upon subculturing. All experiments were performed in triplicate.

4. Pharmacology and toxicity inhibition prediction of ILs and/or salts 1-20

4.1 Pharmacology inhibition prediction

Over the outcome of failure of any drug in clinic stage, ADME-Tox liabilities is necessary to solve these problems for design of a core structure of a novel series of molecules able to inhibit the production of undesirable metabolites. Comparatively to Lipinski's Rule of Five and Oral Bioavailability, in silico physicochemical properties of the synthesized ILs and/or salts as drug candidate 1–20, were calculated and summarized in Table 4 show clearly the very high lipophilicity of most of the tested ILs necessary to cross blood brain barrier (BBB) easily. In addition, all tested ILs displayed TPSA (topological polar surface area) values within acceptable range (3.24–29.54 A°). TPSA is an important descriptor which was shown to correlate well with molecular transport through membranes [33]. Furthermore, the number of rotatable bonds (n-ROTB), molecular weight, hydrogen bond donor/acceptor, were also in satisfactory range [34].

4.2 In silico pharmacokinetic and bioavailability

To qualify the clinical trials, pharmacokinetic and bioavailability studies are required to performed, and the results are listed in Tables 5 and 6. Veber’s rule also play good role in term of the oral bioavailability measurement of drugs. It's important to mention that the oral bioavailability is marked by small molecular weight (less than 500); by the number of rotatable bond (less than 10), by the number of hydrogen bond donors and acceptors (less than 12) and by TPSA values (less than 140).

Table 4. *In silico* prediction of physicochemical calculations

Compd.	MW (g/mol)	Physicochemical properties				Drug likeness					
		TPSA	O/NH	VIOL	VOL	GPC	ICM	KI	NRL	PI	EN
1	231	3.24	0	0	184	-0.84	-0.47	-1.05	-1.01	-1.15	-0.63
2	245	3.24	0	0	201	-0.64	-0.35	-0.93	-0.81	-0.94	-0.50
3	259	3.24	0	0	217	-0.51	-0.27	-0.80	-0.66	-0.78	-0.37
4	273	3.24	0	0	234	-0.39	-0.21	-0.67	-0.52	-0.63	-0.27
5	334	3.24	0	0	257	-0.27	-0.15	-0.55	-0.37	-0.51	-0.20
6	238	3.24	0	0	214	-0.56	-0.29	-0.64	-0.53	-0.46	-0.22
7	296	3.24	0	0	213	-0.23	-0.13	-0.45	-0.39	-0.52	-0.08
8	264	29.54	0	0	225	-0.21	-0.16	-0.43	-0.12	-0.21	-0.09
9	252	3.24	0	0	231	-0.40	-0.21	-0.55	-0.38	-0.31	-0.13
10	310	3.24	0	0	230	-010	-0.07	-0.38	-0.26	-0.38	-0.02
11	278	29.54	0	0	242	-0.11	-0.13	-0.38	-0.03	-0.10	-0.05
12	266	3.24	0	0	247	-0.29	-0.15	-0.45	-0.26	-0.20	-0.04
13	324	3.24	0	0	247	-0.03	-0.05	-0.31	-0.16	-0.27	-0.04
14	292	29.54	0	0	259	-0.05	-0.12	-0.32	-0.04	-0.02	-0.01
15	280	3.24	0	0	264	-0.19	-0.12	-0.36	-0.15	-0.10	0.02
16	338	3.24	0	0	264	0.04	-0.04	-0.24	-0.08	-0.18	0.08
17	306	29.54	0	0	275	0.01	-0.10	-0.27	-0.09	0.04	0.02
18	294	3.24	0	0	281	-0.13	-0.10	-0.28	-0.08	-0.03	-0.05
19	352	3.24	0	0	280	0.08	-0.04	-0.18	-0.03	-0.12	-0.09
20	320	29.54	0	0	292	0.03	-0.10	-0.23	0.12	0.08	0.01
Vancomycin	1449	530	21	3	1207	-3.94	-3.99	-4.00	-4.01	-3.90	-3.95
Rifampicin	823	220	6	3	756	-2.10	-3.27	-3.04	-2.89	-1.61	-2.42

In silico calculation of drug score was in acceptance range which is the combination of drug likeness, Log P, solubility, molecular weight, and toxicity risk within one useful practical value. The tested ILs have a good chance to be a drug candidate if their drug score values are significant. Table 5 clearly shows that all ILs follow the Lipinski rule and Veber's rule in terms of agreement with the in-silico bioavailability. ILs **7, 10, 13, 14, 16, 18** and **19** are rejected by the Ghose rule.

Table 5 .*In silico* bioavailability prediction

Compound No	*In silico* Bioavailability			
	Lipinski	**Ghose**	**Veber**	**Bioavailability score**
1	Yes	Yes	Yes	0.55
2	Yes	Yes	Yes	0.55
3	Yes	Yes	Yes	0.55
4	Yes	Yes	Yes	0.55
5	Yes	Yes	Yes	0.55
6	Yes	Yes	Yes	0.55
7	Yes	No, 1 Violation	Yes	0.55
8	Yes	Yes	Yes	0.55
9	Yes	Yes	Yes	0.55
10	Yes	No, 1 Violation	Yes	0.55
11	Yes	Yes	Yes	0.55
12	Yes	Yes	Yes	0.55
13	Yes	No, 1 Violation	Yes	0.55
14	Yes	No, 1 Violation	Yes	0.55
15	Yes	Yes	Yes	0.55
16	Yes	No, 1 Violation	Yes	0.55
17	Yes	Yes	Yes	0.55
18	Yes	No, 1 Violation	Yes	0.55
19	Yes	No, 1 Violation	Yes	0.55
20	Yes	Yes	Yes	0.55
Vancomycin	No, 3 Violation	No, 4 Violation	No, 2 Violation	0.17
Rifampicin	No, 3 Violation	No, 3 Violation	No, 1 Violation	0.17

During the development of a new drug for any disease, an assessment of toxicity risk parameters is required to qualify a drug candidate, and low toxicity/ side effects indicate a good therapeutic index for any drug. Table 6 shows that the compounds showed satisfactory gastrointestinal absorptions and BBB permeability or inhibition to cytochrome P450 isomers (CYP1A2 and CYP2D6).

Table 6. *In silico* pharmacokinetics prediction of **1-20**

Compoud No	*In silico* Pharmacokinetics					
	GI absorption	**BBB permeant**	**P-gp**	**CYP1A2 inhibitor**	**CYP2D6 inhibitor**	**Log K_p(skin permeation), cm/s**
1	High	Yes	No	No	No	-6.87
2	High	Yes	No	No	No	-6.59
3	High	Yes	No	No	No	-6.42
4	High	Yes	No	No	No	-6.12
5	High	No	No	No	No	-6.19
6	High	Yes	No	No	No	-6.03
7	Low	No	No	No	No	-5.68
8	High	Yes	No	No	No	-6.90
9	High	Yes	No	No	No	-5.74
10	Low	No	No	No	No	-5.39
11	High	Yes	No	No	No	-6.61
12	High	Yes	No	No	No	-5.57
13	Low	No	No	No	No	-5.22
14	Low	No	No	No	No	-5.39
15	High	Yes	No	No	No	-5.28
16	Low	No	No	No	No	-4.92
17	High	Yes	No	No	No	-6.15
18	High	No	No	No	No	-4.98
19	Low	No	No	No	No	-4.62
20	High	Yes	No	No	No	-5.85
Vancomycin	Low	No	Yes	No	No	-16.99
Rifampicin	Low	No	Yes	No	No	-7.44

GI: Gastro Intestinal; **P-gp**: P-glycoprotein; **BBB**: Blood Brain Barrier; **CYP1A2**: Cytochrome P450 family 1 subfamily A member 2 (PDB: 2HI4); **CYP2D6**: Cytochrome P450 family 2 subfamily D member 6 (PDB: 5TFT)

4.3 Log p and inhibitor toxicity prediction

The hydrophobicity and log P value are related each other because when its value is increasing, the drug will be more hydrophobic. When the drug is more hydrophobic, then the drug will be able to

circulate longer in our body, because it wouldn't be easy to secrete it. The Table 7 shows all synthesized ILs 1–20 showed log P value ranges from 1.13 to 4.47. The mutagenic or carcinogenic properties are detected by the toxicity of the compounds based on the functional group similarity with mutagenic or carcinogenic ones.

Table 7. *In silico* screening of toxicity and log p of ILs **1-20**

Compd.	AMES Toxicity	Carcinogenecity	Rat acute toxicity LD_{50} (mol/kg)	Log*P*
1			2.66	2.13
2			2.66	2.94
3			2.65	3.19
4			2.63	3.69
5			2.64	4.47
6			2.78	1.13
7			2.87	1.23
8			2.87	2.18
9			2.76	1.63
10			2.83	1.74
11			2.83	2.68
12			2.74	2.19
13			2.81	2.30
14			2.83	3.24
15			2.72	2.69
16			2.78	2.80
17			2.80	3.74
18			2.72	3.20
19			2.78	3.31
20			2.79	4.25
Vancomycin			2.58	0.13
Rifampicin			2.68	2.62

:non toxic ; : toxic

The result of toxicity analysis of synthesized ILs and/or salts **1–20** presented in Table 7 indicates that all the synthesized ILs have no AMES toxicity and carcinogenicity. For further investigation of the in vivo antimicrobial activity, computed LD50 doses have been calculated in rat acute toxicity models, and the compounds seem to be relatively safe (2.63–2.87 mol/kg).

Conclusion

Collectively, new environmentally friendly functionalized pyridinium-based ionic liquids and/or salts were prepared by using ultrasound irradiation. Comparison of CP and US methods afforded a lot of advantages and recommendations for the use of the green ultrasound assisted reactions. On the other hand, preliminary evaluation of their antimicrobial properties show very interesting and promising results. These good results were confirmed by *in silico* screening which provide the support to antimicrobial activity. Physico-chemical evaluation showed good druglikeness and interacted with various enzymatic targets. In addition, toxicity analysis has revealed that all compounds were safe for mutagenecity and carcinogenicity and computed LD50 values were in accepted range (2.63–2.87 mol/kg). In addition, all ionic liquids and/or salts have pursued Lipinski rules. *In silico* Bioavailability analysis has demonstrated that all synthesized ionic liquids and/or salts follow Lipinski's rule and Veber rule.

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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