Synthesis of benzoazole ionic liquids and evaluation of their antimicrobial activity

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Abstract

A new class of chemicals composed not of molecules but ions, an organic cation and an organic or inorganic anion, has recently attracted attention. When these new kind of salts are in the liquid state below 100 °C, they are named ionic liquids (ILs). In this work, the synthesis of ionic liquids obtained from a 2-mercaptobenzimidazole, 2-mercaptobenzoxazole or 2-mercaptobenzothiazole anion and 1-alkyl-3-methylimidazolium or choline cation are described. The antimicrobial activity against several Gram-positive and Gram-negative bacteria and a yeast was also evaluated.

Keywords: Ionic liquids, benzoazole, choline, antimicrobial activity

Resumo:

Uma nova classe de compostos químicos constituídos não por moléculas mas por íons, um catião orgânico e um anião orgânico ou inorgânico, tem atraído recentemente muita atenção. Quando estes novos tipos de sais estão no estado líquido, abaixo de 100 °C, são chamados líquidos iónicos (LI). Neste trabalho, é descrita a síntese de líquidos iónicos obtidos a partir de 2-mercaptopbenzimidazol, 2-mercaptopbenzoxazol ou 2-mercaptopbenzotiazol e o catião 1-alquil-3-metilimidazolium ou o catião colina. A atividade antimicrobiana destes LI sobre várias bactérias Gram-positivas, Gram-negativas e uma levedura foi avaliada.

Palavras-chave: Líquidos iónicos, benzoazol, colina, actividade antimicrobiana
**Introduction**

Ionic Liquids (ILs) are a new class of recently developed chemicals with singular characteristics that arise from their specific structure, and consequently have attracted attention from chemists. They are liquids composed not of molecules but ions, an organic cation and an organic or inorganic anion. As they are composed of charged units, they have low vapour pressures and are considered not volatile. Usually ILs are viscous liquids highly prized for their ability as solvents of not only organic but also inorganic substances. They can be miscible, immiscible or partially miscible with water, depending on the cation or the anion, and they can be tailored to have the appropriate solvent properties by introducing changes in the chemical structure of the cation or the anion (1, 2).

ILs have been the object of intense investigation towards uncountable chemical and biochemical applications (3). The antimicrobial activity of the first ionic liquids prepared has already been described, as well as their ecotoxicity (4a, b).

In this work, is described the synthesis of ionic liquids obtained from a 2-mercaptobenzimidazole, 2-mercaptobenzoxazole or 2-mercaptobenzothiazole anion and 1-alkyl-3-methylimidazolium or choline cation. The antimicrobial activity of these liquids against several Gram-positive and Gram-negative bacteria and a yeast was also evaluated.

**Materials and Methods**

**Materials and Equipment**

Reagents and solvents of analytical purity were provided by Sigma-Aldrich. 

\[ ^1H \text{NMR spectra were acquired in a Bruker Advance 400 apparatus at 400 MHz, using } D_2O, \text{ CDCl}_3 \text{ or DMSO-d6 as solvents. The chemical shifts are reported in parts per million (ppm, } \delta \text{), using the appropriate signal for residual solvent protons as reference. The growth of microorganisms was measured with an Absorbance Microplate Reader set to 620 nm (Thermo Scientific Multiskan FC).} \]

**Synthesis of ionic liquids**

Synthesis of 1-alkyl-3-methylimidazolium bromide: [Cnmm]Br

Halogenated ionic liquids, 1-ethyl-3-methylimidazolium bromide [C2mim][Br],

**Introdução**

Os Líquidos Iónicos (LI) são uma nova classe de compostos químicos, recentemente desenvolvida, que possui características singulares resultantes da sua estrutura específica e que, por esse motivo, têm sido objeto de muita atenção. Estes novos materiais são líquidos constituídos não por moléculas, mas por iões: um catião orgânico e um anião que pode ser orgânico ou inorgânico. Uma vez que são constituídos por unidades carregadas, têm baixas pressões de vapor e são considerados não voláteis. Normalmente, os LI são líquidos viscosos muito apreciados pela sua capacidade como solventes, não só de substâncias inorgânicas, mas também de substâncias orgânicas. Podem ser miscíveis, imiscíveis ou parcialmente miscíveis com água, dependendo do catião e/ou anião e podem ser modificados para ter as propriedades solventes adequadas, introduzindo alterações na estrutura química do catião e/ou do anião (1, 2).

Os LI têm sido objeto de intensa investigação com o fim de se conhecerem as suas potencialidades em inúmeras aplicações químicas e bioquímicas (3). A atividade antimicrobiana dos primeiros líquidos iónicos já foi demonstrada, bem como a sua ecotoxicidade (4a, b).

Neste trabalho, é descrita a síntese de líquidos iónicos obtidos a partir dos anions 2-mercaptop benzimidazol, 2-mercaptopbenzoxazol ou 2-mercaptopbenzothiazol anion and 1-alkyl-3-methylimidazolium ou choline cation. A sua atividade antimicrobiana contra diversas bactérias Gram-positivas e Gram-negativas também foi avaliada.

**Materiais e Métodos**

**Materiais e Equipamento**

Os reagentes e solventes usados, de grau analítico, foram fornecidos pela Sigma-Aldrich. Os espectros de \(^1H\) NMR foram obtidos num aparelho Bruker Avance 400, a 400 MHz, usando \(D_2O\), \(CDCl_3\) ou DMSO-d6 como solventes. Os desvios químicos são referidos em partes por milhão (ppm, \(\delta\)), utilizando como referência o sinal apropriado do protão residual do solvente deuterado. O crescimento microbiano foi medido com um leitor de micro placas ao comprimento de onda de 620 nm (Termo Scientific Multiskan FC).

**Síntese dos Líquidos Iónicos**

Síntese do brometo de 1-alquil-3-metilimidazoló: \([Cnmm]Br\)

Os LI halogenados, brometo de 1-etil-3-metilimidazoló \([C2mim][Br]\), brometo de 1-butil-3-metilimidazoló
1-butyl-3-methylimidazolium bromide [C4 mim][Br] and 1-hexyl-3-methylimidazolium bromide [C6 mim][Br] were prepared according to the procedures described in literature (5, 6) with small modifications: bromoalkane (ethyl, butyl or hexyl) (0.08 mol) was added slowly for 1.5 hours to 1-methylimidazole (6g, 0.073 mol, 5.82 mL). The mixture was stirred for 1 hour for ethyl bromide and 0.5 hour for butyl and hexyl bromide at room temperature (RT) until two phases appeared. Then the reaction mixture was heated, with constant stirring, in a water bath at 70 °C, overnight, for the ethyl derivative, and for 8 hours for the butyl and hexyl derivatives. The mixture was concentrated on a rotatory evaporator, and then washed three times with tetrahydrofuran (THF). Compounds were characterized by 1H NMR and data obtained were in accordance with those reported in the literature (5, 6).

Synthesis of 1-alkyl-3-methylimidazolium hydroxide: [Cnmim][OH]

Synthesis of 1-alkyl-3-methylimidazolium hydroxide was performed as previously described (6) with the following modification: the corresponding 1-alkyl-3-methylimidazolium bromide (0.0731 mol) was dissolved in methanol (MeOH) and then an equimolar amount of potassium hydroxide (KOH) (0.0731 mol, 4.10 g) was added. The mixture was heated for 18 hours, in an oil bath at 60 °C. The resulting potassium bromide (KBr) was then filtered, and the MeOH was evaporated under vacuum. The product was used immediately in the next step without further purification.

Synthesis of 1-alkyl-3-methylimidazolium 2-mercaptopbenzoimidazolide [Cnmim][TBI], 2-mercaptopbenzoxazolide [Cnmim][TBO] and 2-mercaptopbenzothiazolide [Cnmim][TBT] ionic liquids

ILs were prepared by neutralization of the corresponding 1-alkyl-3-methylimidazolium hydroxide. 1-ethyl-3-methylimidazolium hydroxide [C2 mim][OH], 1-butyl-3-methylimidazolium hydroxide [C4 mim][OH] or 1-hexyl-3-methylimidazolium hydroxide [C6 mim][OH] (0.016 mol) were dissolved in MeOH and an equivalent amount (0.016 mol) of the corresponding mercapto compound, 2-mercaptopbenzimidazolide (TBI), 2-mercaptopbenzoxazolide (TBO) or 2-mercaptopbenzothiazolide (TBT), was added slowly. The mixture was stirred overnight at room temperature and was then refluxed 10 hours. Evaporation of MeOH under vacuum afforded the corresponding IL (Table 1).

Synthesis of cholinium 2-mercaptopbenzoimidazolide (ChTBI), 2-mercaptopbenzoxazolide (ChTBO) and 2-mercaptopbenzothiazolide (ChTBT) ionic liquids

Cholinium 2-mercaptopbenzoimidazolide, 2-mercaptopbenzoxazolide and 2-mercaptopbenzothiazolide were prepared according to the procedures described in literature (5, 6) with small modifications: addition was done slowly, during 1.5 h, the bromoalkane (ethyl, butyl or hexyl) (0.08 mol) to 1-methylimidazol (6 g, 0.073 mol, 5.82 mL). The mixture was agitated at temperature ambient, during 1 h in synthesis of the bromo of ethyl and, during 0.5 h, in syntheses of the bromo of butyl and bromo of hexyl, until to appear cimento of two phases. After, it was heated 18 h in a bath at 60 °C, overnight, for ethyl derivative, for 8 h for butyl and hexyl derivatives. The mixture was concentrated on a rotatory evaporator, then it was washed three times with tetrahydrofuran (THF). Compounds were characterized by 1H NMR and data obtained were in accordance with those reported in the literature (5, 6).

Síntese do hidróxido de 1-alkil-3-metilimidazólio: [Cnmim] OH

A síntese do hidróxido de 1-alkil-3-metilimidazólio foi realizada de acordo com o descrito anteriormente (6), com a seguinte modificação: o brometo de 1-alkil-3-metilimidazólio (0,0731 mol) foi dissolvido em metanol (MeOH) e, posteriormente, foi adicionada uma quantidade equimolar de hidróxido de potássio (KOH) (0,0731 mol, 4,10 g). A mistura foi aquecida durante 18 h num banho de óleo a 60 °C. De seguida, o brometo de potássio (KBr) resultante foi filtrado e o MeOH evaporado sob vácuo. O produto foi utilizado imediatamente sem purificação adicional.

Síntese dos LI de 1-alkil-3-metilimidazólio e de 2-mercaptopbenzoimidazolídio [Cnmim][TBI], 2-mercaptopbenzoxazolídio [Cnmim][TBO] e 2-mercaptopbenzothiazolídio [Cnmim][TBT]

Os LI foram preparados por neutralização do correspondente hidróxido do 1-alkil-3-metil imidazólio. O hidróxido de 1-etyl-3-metilimidazol [C2 mim][OH], hidróxido de 1-butil-3-metilimidazol [C4 mim][OH] ou hidróxido de 1-hexil-3-metilimidazol [C6 mim][OH] (0,016 mol) foram dissolvidos em MeOH. A respetiva solução foi adicionada lentamente uma quantidade equivalente (0,016 mol) do composto mercapto correspondente, o 2-mercaptopbenzoimidazol (TBI), o 2-mercaptopbenzoxazol (TBO) ou o 2-mercaptopbenzothiazol (TBT). A mistura reacional foi agitada durante a noite à temperatura ambiente e, em seguida, foi aquecida a refluxo durante 10 h. O líquido iônico correspondente foi obtido após evaporação do MeOH (Tabela 1).
benzoxazolide and 2-mercaptobenzothiazolide compounds were prepared by neutralization of choline hydroxide by 2-mercaptobenzimidazol (TBI), 2-mercaptobenzoxazol (TBO) and 2-mercaptobenzothiazol (TBT). 2-mercaptobenzothiazole, 2-mercaptobenzoxazol and 2-mercaptobenzimidazol were mixed with an equimolar amount of choline (45 % MeOH solution) (0.006 mol) and stirred overnight at room temperature. The MeOH was subsequently evaporated and the oil residue obtained was washed with acetoneitrile and dried under vacuum. IL containing 2-mercaptobenzothiazole was a dense orange oil. ILs containing 2-mercaptobenzimidazol and 2-mercaptobenzoxazol were solids with low melting points: 115 °C – 118 °C and 50 °C – 70 °C, respectively (Table 1). ILs were characterized by 1H NMR (Table 2).

Antimicrobial activity

Microbial strains

The in vitro antimicrobial study was carried out using Gram-positive bacteria (Staphylococcus aureus ATCC 25923, Enterococcus faecalis ATCC 29212, Enterococcus hirae ATCC 10541, Bacillus subtilis ATCC 6633, and Mycobacterium smegmatis ATCC 607), Gram-negative bacteria (Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853 and Klebsiella pneumoniae ATCC 9997) and a yeast (Candida albicans ATCC 10231).

Well diffusion test

The well diffusion assay was used to determine the antimicrobial activity of the compounds (7). Petri dishes containing 20 mL Mueller-Hinton culture medium were inoculated with 0.1 mL of a bacterial cell suspension matching a 0.5 McFarland standard solution. The suspension was uniformly spread using a sterile swab over the surface of the medium. Wells of 5 mm in diameter were made in the agar plates with a sterile glass Pasteur pipette and 50 μL of each compound (1 mg/mL), previously reconstituted by dissolving in dimethyl sulfoxide (DMSO), were added into the well. DMSO was used as a negative control, while vancomycin, norfloxacin, and amphotericin B at 1 mg/mL were used as positive controls for Gram positive and Gram-negative bacteria and the yeast, respectively. The plates were then incubated at 37 °C for 24 hours. The antimicrobial activity was assayed by measuring the diameter of the inhibition zone (in mm) formed around the wells. Each assay was performed in triplicate, at minimum.

Microdilution method

The minimum inhibitory concentrations (MICs) of compounds were determined by means of the two-fold dilution method performed in triplicate, at minimum.

Síntese dos líquidos iónicos de colina e de 2-mercaptobenzimidazolídio (ChTBI), 2-mercaptobenzoxazolídio (ChTBO) e 2-mercaptobenzotiazolídio (ChTBT). Os compostos de colina e de 2-mercaptobenzimidazolídio, 2-mercaptobenzoxazolídio e 2-mercaptobenzotiazolídio foram preparados por neutralização do hidróxido de colina pelo 2-mercaptobenzimidazolídio (TBI), 2-mercaptobenzoxazolídio (TBO) e 2-mercaptobenzotiazolídio (TBT). O composto mercapto correspondente foi adicionado a uma solução a 45 % de colina em metanol (0,006 mol) e a mistura reacional ficou sob agitação de um dia para o outro à temperatura ambiente (TA). Posteriormente, o metanol (MeOH) foi evaporado e o resíduo oleoso foi lavado com acetonitrilo e seco sob vácuo. O líquido iônico com 2-mercaptobenzotiazolídio apresentou-se como um óleo denso e alaranjado. Os LI contendo 2-mercaptobenzimidazolídio e 2-mercaptobenzoxazolídio são sólidos com baixo ponto de fusão: 115 °C – 118 °C e 50 °C – 70 °C, respetivamente (Tabela 1). Os LI foram caracterizados por 1H RMN (Tabela 2).

Actividade antimicrobiana

Estírpes microbianas

A avaliação da actividade antimicrobiana foi realizada in vitro, usando bactérias Gram-positivas (Staphylococcus aureus ATCC 25923, Enterococcus faecalis ATCC 29212, Enterococcus hirae ATCC 10541, Bacillus subtilis ATCC 6633, e Mycobacterium smegmatis ATCC 607), bactérias Gram-negativas (Escherichia coli ATCC 25922, Pseudomonas aeruginosa, ATCC 27853, Klebsiella pneumoniae ATCC 9997) e uma levadura (Candida albicans ATCC 10231).

Teste de difusão em poço

O ensaio de difusão foi utilizado para determinar a actividade antimicrobiana dos compostos (7). As placas de Petri, contendo 20 mL de meio de cultura Mueller-Hinton, foram inoculados com 0,1 mL de uma suspensão bacteriana correspondendo a uma solução padrão de 0,5 McFarland. A suspensão foi uniformemente espalhada utilizando uma zaragatoa estéril sobre a superfície do meio. Poços de 5 mm de diâmetro foram feitos em placas de agar com uma pipeta de Pasteur de vidro estéril e 50 μL de cada composto (1 mg/mL), previamente reconstituídos por dissolução em dimetilsulfóxiido (DMSO), foram adicionados a cada poço. O solvente DMSO foi usado como controlo negativo, enquanto a vancomicina, norfloxacina e anfotericina B, a 1 mg/mL, foram utilizados como controlos positivos para as bactérias Gram-positivas, Gram-negativas e a levedura, respectivamente. As placas foram então incubadas a 37 ºC durante 24 horas. A actividade antimicrobiana foi determinada medindo o diâmetro da zona de inibição (em mm) formada à volta dos poços. Cada ensaio foi efectuado pelo menos em triplicado.

Método de microdiluição
serial broth microdilution assay (7). The compounds, dissolved in DMSO, were diluted with Mueller-Hinton broth medium to concentrations ranging from 500 µg/mL to 0.488 µg/mL. The antimicrobial activity of the solvent was also evaluated. Vancomycin, rifampicin and amphotericin B were used as controls. The MIC values were taken as the lowest concentration of the compound, in µg/mL, that inhibited the growth of the microorganisms following 24 hours of incubation at 37 ºC. Assays were carried out in triplicate for each tested microorganism.

**Results and Discussion**

Eleven ionic liquids were prepared (Figure 1) with a good purity level. Some of these compounds, those obtained from 2-mercaptobenzimidazole (TBI), 2-mercaptobenzoxazole (TBO) and 2-mercaptobenzothiazole (TBT), are here described for the first time. All ionic liquids were synthesized in quantitative yield, with the exception of the 1-alkyl-3-methylimidazolium bromides, which were obtained with a lower yield (62 % - 67 %). Color and physical state at room temperature are described in Table 1.

Characterization of the new compounds was performed by 1H NMR and spectral data are presented in Table 2. The antimicrobial activity of the prepared ionic liquids was investigated by the well diffusion assay. Compounds were tested against five Gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*, *Enterococcus hirae*, *Bacillus subtillis* and *Mycobacterium smegmatis*), three Gram-negative bacteria (*Escherichia

**Resultados e Discussão**

No decorrer deste estudo, foram obtidos onze líquidos iónicos (Figura 1), com bom grau de pureza. Alguns destes compostos, os obtidos a partir de 2-mercaptobenzimidazol (TBI), 2-mercaptobenzoxazol (TBO) e 2-mercaptobenzothiazoal (TBT) são aqui descritos pela primeira vez. Todos os líquidos iónicos foram sintetizados com um rendimento quantitativo, à exceção dos brometos de 1-alkil-3-metilimidazólio que foram obtidos com um rendimento mais baixo, entre 62 % - 67 %. O estado físico à temperatura ambiente dos LI sintetizados, bem como a sua cor, são descritos na Tabela 1.

A caracterização dos novos compostos foi realizada por 1H RMN e os dados espectrais são apresentados na Tabela 2. A atividade antimicrobiana dos líquidos iónicos sintetizados foi investigada através do ensaio de difusão em poço. Este teste foi utilizado como um rastreio para determinar a capacidade destes compostos para inibir
coli, Pseudomonas aeruginosa and Klebsiella pneumoniae), and a yeast (Candida albicans).

Compounds [C4mim][TBO], [C6mim][TBT] and [C2mim][TBI] showed inhibition zones against Gram-positive B. subtillis (8 mm of inhibition zone for compound [C6mim][TBT] and P. aeruginosa (9 mm inhibition zone for both compounds [C4mim][TBO] and [C2mim][TBI]). The remaining imidazolium compounds as well as the choline based ionic liquids showed no inhibition zones, as did the DMSO solvent, against all the bacteria and the yeast tested (Table 3). The described positive results for compounds [C4mim][TBO], [C6mim][TBT] and [C2mim][TBI] were further investigated by the microdilution method. Compounds [C6mim][TBO] and [C2mim][TBI] showed MIC values of 62.5 μg/mL against the Gram-positive B. subtillis and Gram-negative Pseudomonas aeruginosa bacteria, thus revealing modest antibacterial activity. Antimicrobial activity of these compounds is of the same magnitude as the activities of other ILs (8).

Choline based ionic liquids, [Ch][TBO], [Ch][TBT] and [Ch][TBI] as well as the imidazolium based ionic liquids, [C4mim][Br], [C4mim][TBT] and [C6mim][TBO] did not exhibit any antimicrobial activity. In literature (1, 4, 9), it is indicated that three substrutures of an ionic liquid have importance in the evaluation of their toxicity against several organisms, including bacteria, algae, plants and invertebrates: a) a o crescimento de bactérias, quer Gram-positivas, quer Gram-negativas e também de uma levedura. Os compostos foram testados contra cinco bactérias Gram-positivas (Staphylococcus aureus, Enterococcus faecalis, Enterococcus hirae, Bacillus subtilis and Mycobacterium smegmatis), três bactérias Gram-negativas (Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae), and a levedura (Candida albicans). Os compostos [C4mim][TBO], [C6mim][TBT] and [C2mim][TBI] revelaram zonas de inibição contra a bactéria Gram-positiva B. subtillis (8 mm de zona de inibição para o composto [C6mim][TBT]) e P. aeruginosa (9 mm de zona de inibição para ambos os compostos [C4mim][TBO] e [C2mim][TBI]). Os restantes compostos de imidazolium, bem como os compostos de colina não indicaram zonas de inibição (tal como o solvente DMSO) contra todas as bactérias Gram-positivas, Gram-negativas e a levedura testadas (Tabela 3). Os resultados positivos descritos anteriormente para os compostos [C4mim][TBO], [C6mim][TBT] e [C2mim][TBI] foram posteriormente estudados pelo método de microdiluição. Os compostos [C6mim][TBO] e [C2mim][TBI] revelaram valores de CMI de 62,5 μg/mL contra as bactérias Gram-positiva B. subtillis e Gram-negativa Pseudomonas aeruginosa, indicando assim uma atividade antibacteriana moderada. A atividade antimicrobiana destes compostos é da mesma ordem de grandeza das atividades de outros LI descritos na literatura (8).

Os líquidos iónicos contendo o catião colina [Ch][TBO], [Ch][TBT] e [Ch][TBI] não apresentaram qualquer atividade antimicrobiana. Da mesma forma, os líquidos iónicos contendo o catião alquil-metilimidazolídio, [C4mim]Br, [C4mim][TBT]

| Ionic Liquid/ Líquido Iônico | Physical state at RT/ Estado Físico à TA | Color/ Cor |
|-------------------------------|----------------------------------------|------------|
| [C2mim][Br]                  | Solid/Sólido                           | White/Branco |
| [C4mim][Br]                  | Solid/Sólido                           | Light yellow/Amarelo claro |
| [C6mim][Br]                  | Liquid/Líquido                         | Light yellow/Amarelo claro |
| [C2mim][TBI]                 | Solid/Sólido                           | White/Branco |
| [C4mim][TBO]                 | Liquid/Líquido                         | Light Brown/Castanho claro |
| [C4mim][TBT]                 | Solid/Sólido                           | Yellow/Amarelo |
| [C6mim][TBO]                 | Liquid/Líquido                         | Brown/Castanho |
| [C6mim][TBT]                 | Liquid/Líquido                         | Brown/Castanho |
| [Ch][TBI]                    | Solid/Sólido                           | Beige/Bege |
| [Ch][TBO]                    | Solid/Sólido                           | Light Brown/Castanho claro |
| [Ch][TBT]                    | Liquid/Líquido                         | Light Orange/Laranja claro |
positive portion designated as head-group, b) the substituents present in that head-group, and c) the anion.

Table 3 shows that a small inhibition in the growth of the Gram-positive bacteria was obtained with [C6mim][TBO], [C4mim][TBO] and [C2mim][TBI], and these were also slightly active against P. aeruginosa. It is interesting to note that regarding the bacteria tested, changing the head group from an alkyl imidazolium to choline removed the antibacterial effect, which could indicate that the head group was the responsible for this activity. However, the lack of activity of [C4mim]Br and [C4mim][TBT] does not support this hypothesis. It is also stated (9, 10) that the length of the alkyl chain attached to the head group influences the toxicity of the ionic liquid - the longer the chain, the more active the ionic liquid. It was thus expected that [C6mim][TBO] would be more active than the corresponding IL, [C4mim][TBO], which contains a smaller alkyl chain linked to the same head group, but this was not observed. In a previous work (6), it was also found that toxicity against bacteria does not follow the same rules previously found in organisms with a higher degree of organization. Different susceptibility of Gram-positive and Gram-negative bacteria to the same IL is likely due to the different compositions of the cell walls and [C6mim][TBO], also not being active against the microorganisms tested.

In the literature (1, 4, 9), it is referred that three sub-structures of a liquid ionic have importance for the evaluation of the corresponding bacteria, algae, plants, and invertebrates: a) the positive portion, or cation, designated as the head-group, b) the substituents present in the head-group, and c) the anion. A Table 3 shows that a small inhibition in the growth of the Gram-positive bacteria was obtained with [C6mim][TBT], [C4mim][TBO] and [C2mim][TBI], and these were also slightly active against P. aeruginosa. It is interesting to note that regarding the bacteria tested, changing the head group from an alkyl imidazolium to choline removed the antibacterial effect, which could indicate that the head group was the responsible for this activity. However, the lack of activity of [C4mim]Br and [C4mim][TBT] does not support this hypothesis. It is also stated (9, 10) that the length of the alkyl chain attached to the head group influences the toxicity of the ionic liquid - the longer the chain, the more active the ionic liquid. It was thus expected that [C6mim][TBO] would be more active than the corresponding IL, [C4mim][TBO], which contains a smaller alkyl chain linked to the same head group, but this was not observed. In a previous work (6), it was also found that toxicity against bacteria does not follow the same rules previously found in organisms with a higher degree of organization. Different susceptibility of Gram-positive and Gram-negative bacteria to the same IL is likely due to the different compositions of the cell walls and [C6mim][TBO], also not being active against the microorganisms tested.

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Conclusion

In conclusion, a new approach for the synthesis of benzoazole ionic liquids has been developed. The resulting compounds have been tested for antibacterial and antifungal activities. The present series of compounds exhibited moderate antimicrobial activity against Gram-positive and Gram-negative bacteria, which opens the avenue to use these compounds as chemotherapeutic agents. Further studies on the synthesis of a library of ionic liquids and evaluation of their antimicrobial activity must be performed, as the antimicrobial activity of this huge class of chemicals is difficult to predict and is an enormous challenge to chemists and microbiologists.

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Conflict of Interest

The authors declare that there is no financial or personal relationship that can be understood as representing a potential conflict of interest.

Table 3/ Tabela 3: Antibacterial activity of ILs

| Compound Composto | Inhibition zone/Zona de inibição(mm) (C=1mg/mL) | MIC/CMI (µg/mL) |
|--------------------|-----------------------------------------------|-----------------|
|                    | B. subtilis | P. aeruginosa | B. subtilis | P. aeruginosa |
| [C4mim][TBO]       | -*         | 9             | nt         | >125         |
| [C6mim][TBT]       | 8          | -*            | 62,5       | nt           |
| [C2mim][TBI]       | -*         | 9             | nt         | 62,5         |
| TBT                | 9          | 8             | 250        | 62,5         |
| TBI                | -*         | 8             | nt         | 62,5         |
| TBO                | -*         | 7             | nt         | 62,5         |
| Positive control** | 27         | 35            | <7,82      | <7,82        |
| DMSO               | -*         | -*            | 125        | 125          |

* no inhibition; **Gram + bacteria: Vancomycin, Gram - : Norfloxacyn; nt, not tested

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Conflito de interesses

Os autores declaram que não há nenhuma relação financeira ou pessoal, que possa ser entendida como representando um potencial conflito de interesses.
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