# Imidazolium and Pyridinium Ionic Liquids from Mandelic Acid Derivatives: Synthesis and Bacteria and Algae Toxicity Evaluation

**ARTICLE** *in* ACS SUSTAINABLE CHEMISTRY & ENGINEERING · FEBRUARY 2013

Impact Factor: 4.64 · DOI: 10.1021/sc3001299



**9 AUTHORS**, INCLUDING:

Sónia P M Ventura
University of Aveiro
**58** PUBLICATIONS **1,121** CITATIONS
SEE PROFILE

Fernando Gonçalves
University of Aveiro
**237** PUBLICATIONS **3,182** CITATIONS
SEE PROFILE

Brid Quilty
Dublin City University
**43** PUBLICATIONS **678** CITATIONS
SEE PROFILE

Joao A. P. Coutinho
University of Aveiro
**483** PUBLICATIONS **12,313** CITATIONS
SEE PROFILE

ACS Sustainable Chemistry & Engineering

Research Article

pubs.acs.org/journal/ascecg

# Imidazolium and Pyridinium Ionic Liquids from Mandelic Acid Derivatives: Synthesis and Bacteria and Algae Toxicity Evaluation

Sónia P. M. Ventura,[†,∥] Monika Gurbisz,[‡,§,∥] Mukund Ghavre,[‡,§,∥] Fábio M. M. Ferreira,[†] Fernando Gonçalves,[⊥] Ian Beadham,[‡] Brid Quilty,[○] João A. P. Coutinho,[†] and Nicholas Gathergood*[,‡,§]

[†]CICECO, Departamento de Química, Universidade de Aveiro, 3810-193 Aveiro, Portugal

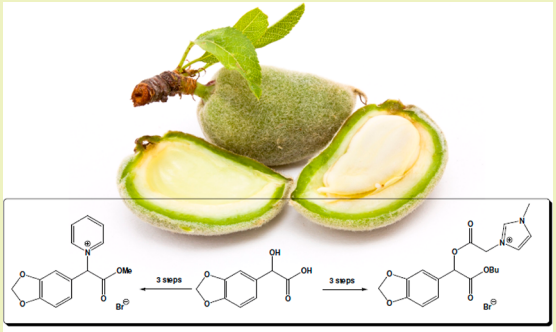
[‡]School of Chemical Sciences and National Institute of Cellular Biotechnology and [○]School of Biotechnology and National Institute of Cellular Biotechnology, Dublin City University, Glasnevin, Dublin 9, Ireland

[§]SFI SRC in Solar Energy Conversion, UCD School of Chemical & Bioprocess Engineering, Engineering & Materials Science Centre, UCD Dublin, Belfield, Dublin 4, Ireland

[⊥]CESAM, Departamento de Biologia, Universidade de Aveiro, 3810-193 Aveiro, Portugal

Supporting Information

**ABSTRACT:** A new class of low bacterial and algal toxicity imidazolium and pyridinium halide ionic liquids (ILs), produced by a short synthesis from substituted mandelic acid derivatives is disclosed. Melting points for most of the ILs were above or close to 100 °C; however, one imidazolium example has a glass transition temperature below room temperature (RT; −3.3 °C). The series of 8 ILs enables an investigation of toxicity on modifying the heterocycle, aromatic ring substitution, ester group, and proximity of cation to aromatic ring present within mandelic acid constituent. Two pyridinium salts, methyl 2-(3,4-methylenedioxyphenyl)-2-pyridinium acetate, bromide salt and methyl 2-(3,4-methylenedioxyphenyl)-2-(2-pyridiniumacetoxy)acetate, bromide salt have low toxicity to all bacteria strains (including *Vibrio fischeri*), and freshwater green algae (*C. Vulgaris* and *P. subcapitata*) screened. All eight pyridinium and imidazolium ILs have low toxicity to Gram-positive (*B. subtilis*) and Gram-negative (*E. coli*, *P. fluorescens*, *P. putida* (CP1), and *P. putgida* (KT 2440)) bacteria strains, although a significant range in $IC_{50}$ values was obtained. Mandelate derived ILs have $EC_{50}$ (*C. Vulgaris* and *P. subcapitata*) values $10^3$−$10^7$ higher (less toxic) than other C14−C18 ionic liquids previously reported.




Low bacterial and algal toxicity ILs prepared from mandelic acids

**KEYWORDS:** *Ionic liquids, Synthesis, Bacterial toxicity, Freshwater green algal toxicity, Mandelic acid*

## INTRODUCTION

Ionic liquids (ILs) are solvents consisting solely of ions, with a generally restricted definition to salts melting below 100 °C.[1] These low-melting salts are often described as "designer solvents" because ionic liquid (IL) scaffolds can be tailored to exhibit diverse physical and chemical properties. ILs may either be inert, acting only as solvents, or can be designed to actively participate in chemical reactions. These ionic compounds have enjoyed a period of categorization as "green solvents",[2] because of their very low vapor pressure and in many cases, lower flammability, compared with common nonchlorinated solvents. However, as ILs have evolved, it has become clear that old generalizations about their "greenness", namely very low vapor pressure[3] and nonflammability,[4] must be discarded and as a result, the IL properties should be assessed on a case-by-case basis. The same principle holds true for IL toxicity and biodegradability properties, and while the generalization has been made that more lipophilic ILs (which disrupt cell membranes) tend to have a higher toxicity toward a range of diverse organisms (bacteria, algae, cladocerans, fish, among others), the toxicity of each new IL must be individually tested to comply with REACH (Registration, Evaluation, Authorization and Restriction of CHemical substances). Even if an IL can be established to have low toxicity in testing[5] at a variety of levels of biological organization,[6] another important factor to be considered is the ease with which the IL can biodegrade to harmless byproducts if an accidental release into the environment were to occur.[7]

Since its infancy 10 years ago,[8] the field of biodegradable ILs has now progressed to a point where several examples have been reported[7] that can be classified as readily biodegradable (at least 60% of the substance is biodegraded within 28 days).[9] While the properties of ILs must be assessed on a case-by-case basis, improved biodegradability can be designed into ILs by including an anion which is expected to be biodegradable, such as octylsulfate[10,11] or dioctylsodium sulfosuccinate "docu-

Received: October 31, 2012
Revised: February 9, 2013
Published: February 27, 2013

sate".[12] Early examples from Gathergood and Scammells[13] using ester-modified 3-methylimidazolium cations were shown to have biodegradability results higher than 67%, which were provided by the presence of the octylsulfate as the anion. However, more recent biodegradation studies have avoided the use of the 3-methylimidazolium cation, which has the disadvantage of breaking down to 1-methylimidazole, a fragment that requires more than 28 days to biodegrade in the presence of an activated sludge.[14] Alternatively, biodegradable cations can be used as a replacement for the common imidazolium, such as the ammonium or pyridinium cores. Even if an IL is proven to biodegrade readily within 28 days, its toxicity must still be taken into account because of the tendency for lipophilic ILs to disrupt cell membranes.[15] While a low toxicity (i.e., low antibacterial, antifungal, and antialgal activity) does not ensure a compound will biodegrade; a compound which is a potent biocide is a far more challenging chemical entity to pass a biodegradation test. Thus, toxicity data is an important factor in determining which ILs to prepare and submit for further biodegradation analysis.

The biological effects of ILs have been examined at different trophic levels,[16−18] ranging from simple organisms named decomposers,[19−23] to more complex biological systems such as the primary producers (algae and plants),[24−27] primary consumers (invertebrates),[16,28−30] and secondary consumers (fish).[29,31,32] The IL toxicity has been found to vary widely between organisms, species, and trophic levels.[17,33] Such tests have provided an important assessment of the toxicity of ILs required by REACH registration—although this varies depending on the quantity (tonnage) of IL—which has been mandatory in Europe since 2007.

The short generation times of bacteria make them an ideal starting point for IL toxicity estimations and structure activity relationship investigations. Numerous toxicological studies across a range of different bacteria have proven that IL toxicity is raised when the lipophilicity of the IL is increased.[34−43] Hence, toxicity toward bacteria is frequently encountered when the alkyl chain attached to ILs exceeds a length of four carbon atoms. Moreover, while it has been proven that both the cation[23,44−47] and anion[34] play an important role in toxicity toward bacteria, the effect of the cation is generally more significant.

Among the inhibition assays used to assess the environmental risk of a compound in aquatic media, the bioluminescence assay using the Gram-negative bacterium, *Vibrio fischeri* is the most popular, due to its rapid, cost-effective, and sensitive response.[48] In this microscale bioassay, the $EC_{50}$ of a chemical compound is accurately measured by the determination of the concentration of a compound at which 50% inhibition of light emission from a specific strain of the bacterium occurs. Several studies into the toxicity of ILs toward *Vibrio fischeri*[5,18,22,23,27,33,49−53] have been presented, where the effect of the anion,[20,22,27] cation,[19] alkyl chain length,[20,23,27,50] and alkyl chain type[50,54] were the most assessed IL features. Despite early assertions that the anion would not have a significant effect on the toxicity of this marine bacterium,[22] a recent comparison between the toxicity of the anions hexafluorophosphate $[PF_6]^-$ and bis(trifluoromethylsulfonyl)-imide $[NTf_2]^-$ clearly demonstrated that the latter exhibits higher effects in the toxicity parameter and that this condition is independent of the cation.[52] In terms of the alkyl chain length, it is a generally agreed upon concordance[5,18] that, when increasing the number of carbons in the substituent chains of an IL, an increase in toxicity is originated.[20,22,23,55,56] However, studies concerning the effect of different cation cores on aquatic toxicity have been less extensive than for the anion. These aquatic experiments have concentrated on the popular imidazolium and pyridinium[17,19,23,52] cations to the detriment of other cations encountered ILs, including pyrrolidinium, piperidinium, ammonium, phosphonium, and guanidinium ions, which remain to be thoroughly investigated.[17,19,46,56−58] Recent studies with morpholinium[59,60] and DABCO[60] ILs were reported.

In the context of the ecotoxicological risk assessment of ILs to aquatic environments, algae represent another large and diverse group of eukaryotic organisms. As algae are primary producers of organic matter required by animals in freshwater food chains, their ecology is crucial in providing the energy for sustaining other higher trophic levels. The ubiquity of algae makes these organisms ideal for toxicological studies, and in common with bacteria, they have a short life cycle given a quick response to environmental changes.[61,62] Studies about the acute effects of different ILs[27,29−31,63−67] have been described, being mainly concentrated on green algae species, namely *Oocystis submarina*,[68] *Pseudokirchneriella subcapitata*,[29−31,33,52,63,64,66] *Chlorella vulgaris*,[25,33] *Scenedesmus vacuolatus*,[67] *Scenedesmus quadricauda*, and *Chlamydomonas reinhardtii*.[65] Toxicity data for the different algae exhibit considerable heterogeneity,[5,18,53] which usually stems from the use of dissimilar methods. For example, the use of different techniques for the measurement of cell density, e.g., electrical conductance, fluorometry, or optical density, may give rise to heterogeneous results. Another factor which may lead to heterogeneous results is the exposure time, which has a marked effect on the dose response behavior of *Pseudokirchneriella subcapitata* (*P. subcapitata*), especially in the case of marginally lipophilic ILs (for example the 1-butyl-3-methylimidazolium bromide $[C_4mim]Br$), for which toxicity only emerges over time. As with bacteria, the trend of increasing toxicity with increasing alkyl chain length was observed for algae,[63,66] and it was concluded that the increasing alkyl chain length may lead to interaction with and disruption of biological membranes.[67] Additionally, Cho and co-workers[66] used *P. subcapitata* to study the effect of different IL head groups and anions on growth rate and photosynthetic activity. The results revealed that the toxic effects of ILs on algal growth were more significant than on photosynthesis. In terms of the effect of the IL anion, the growth of freshwater algae was impacted to varying extents by the different anions, with higher toxicity recorded for lipophilic fluorinated anions, such as $[PF_6]^-$ and $[NTf_2]^-$.[52] Finally, despite the reduced number of studies using the cationic IL core with IL feature investigated, the results identify the pyridinium ion as one of the most toxic cations, while the pyrrolidinium ion is among the most benign ionic structures toward the algae species[29,31] In the search for biodegradable and nontoxic ILs, some different structures were developed, such as the oxygenated ILs.[69] These are a promising class of alternative biodegradable solvents with the potential to act as task-specific ionic liquids (TSILs)[70] by metal coordination. The main objective of the present study is to investigate the toxicity of diverse oxygen-functionalized aromatic ILs (belonging to the pyridinium and imidazolium families), toward different freshwater algae species and bacteria.

## RESULTS AND DISCUSSION

**Synthesis of the ILs.** A new class of 1-methylimidazolium and pyridinium ILs derivatives of 3,4-methylenedioxy- and 3,4-

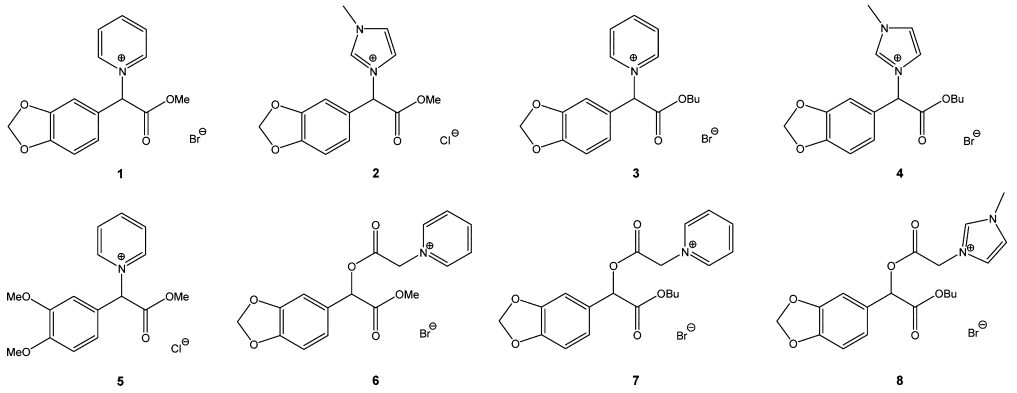


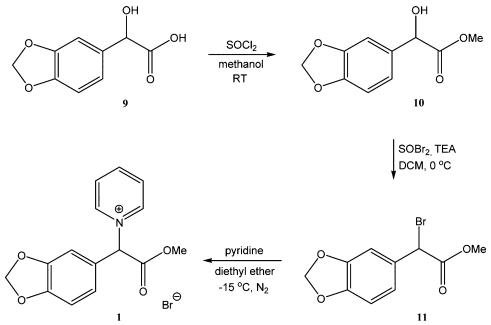
**Figure 1.** Structures of the ILs synthesized.

dimethoxy-mandelic acid were synthesized to serve as "green solvents"[2,5,7,12,71] and "green" catalysts[72] (Figure 1). Following Boethling's "Rules of Thumb",[73] those ILs were designed to be nontoxic, biodegradable, and applicable in catalytic reactions with high selectivities and good catalyst recycling. Consideration of the 12 Principles of Green Chemistry[74] meant that short, efficient, and high atom economy[75] synthetic routes were a priority.

Two different groups of ILs were prepared, both with halide counteranions. The first consists of ionic compounds where a nitrogen atom of a heterocyclic moiety (methylimidazolium or pyridinium) is directly linked to a benzylic carbon **1**−**5** (Figure 1), and the second group is composed of compounds with an acetoxy spacer between benzylic carbon and the nitrogen of pyridinium or methylimidazolium ring **6**−**8** (Figure 1). Excluding the IL **5**, all the remaining ILs (**1**−**4**, **6**−**8**) were prepared in four distinct steps, where chromatographic purification was carried out in the second last step and, except for diethyl ether washes, no additional purification was required.

ILs **1**−**5** were prepared by the same synthetic strategy: esterification of mandelic acid derivative, halogenation, then substitution. Scheme 1 illustrates this approach for IL **1**.

**Scheme 1. Synthesis of IL 1**



3,4-Methylenedioxy-[76−78] and 3,4-dimethoxy-mandelic[79] acid were prepared according to experimental procedures, starting from the disubstituted benzene and glyoxylic acid. Subsequently, methyl esters **10**[80] and **15**[81] and the butyl ester **12** were prepared via Fisher esterification with thionyl chloride and methanol (Scheme 1) or butanol (see the Supporting Information (SI)) as solvents. Excellent yields of 93% and 95% were obtained for methyl **10** and butyl 3,4-methylenedioxymandelate **12**, respectively, and 69% yield for the methyl 3,4-dimethoxymandelate **15** (Table 1).

For ILs **1**−**5**, the next step performed was the chlorination or bromination of the respective esters **10**, **12**, and **15** at the benzylic position which is described in Scheme 1 for **10**. The reaction was carried out in dichloromethane with triethylamine and thionyl chloride (**14**[82] and **16**) or thionyl bromide (**11**[80] and **17**) and the halogenated intermediates were obtained in good yields 63−76% (Table 1). The alkyl halide structures **11**, **14**, **16**, and **17** were then used to *N*-alkylate pyridine or 1-methylimidazole to form ILs **1**−**5**. (Table 2. Reaction conditions for the preparation of **1** are given in Scheme 1. For the experimental procedure for **1**−**5**, see the SI).

Preparation of ILs **3** and **4** derived from butyl-3,4-methylenedioxymandelate **17** was carried out at room temperature in diethyl ether. Pyridinium salt **3** was recovered as a white precipitate (90% yield), whereas the 1-methylimidazolium salt **4** formed a separate viscous phase under the same reaction conditions (58% yield). Preparation of ILs **1** and **2** derived from the precursors **11** and **14**, respectively, required reflux (36 °C) to ensure completion of the *N*-alkylation step. A white precipitate formed in both cases: 1-methylimidazolium IL **2** was isolated with 97% yield, the pyridinium IL **1** isolated with a lower yield of 79%.

Completion of the reaction to form IL **5** proved to be difficult, even in refluxing diethyl ether; therefore, it was prepared in neat conditions at 55 °C. IL **5** was further purified by washing with hot acetone and isolated as a white powder in 61% yield.

Overall yields for ILs **1**−**5** starting from 3,4-methylenedioxymandelic acid or 3,4-dimethoxymandelic acid are **1** 50%, **2** 59%, **3** 65%, **4** 42%, and **5** 27%.

For ILs with an acetoxy linker **6**−**8**, the next step after preparation of methyl ester **10** and butyl ester **12** was the acetylation of the hydroxyl group in the benzylic position (Scheme 2, **13**).

This was carried out with bromoacetyl bromide as the acylating agent and triethylamine or potassium carbonate as the base, yielding intermediates **13** (Scheme 2) and **18** (see the SI) in 80% and 69%, respectively. In both cases, a purification step using column chromatography was necessary. When triethylamine is used, an $\alpha$-bromoester byproduct was formed and, when potassium carbonate was applied, unreacted starting material remained. The last step of the synthesis involves the *N*-alkylation of pyridine or 1-methylimidazole with the halogenated intermediates **13** (Scheme 2) and **18** (see the SI). The reaction was carried out at room temperature, and ILs


dx.doi.org/10.1021/sc3001299 | ACS Sustainable Chem. Eng. 2013, 1, 393−402

**Table 1. Structures and Yield of the Intermediates Formed in the Production of ILs**

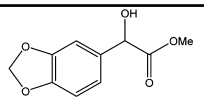
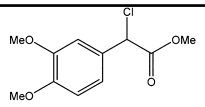
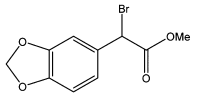
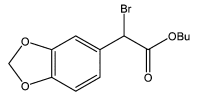
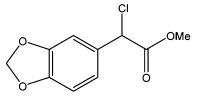
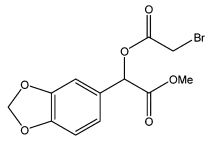
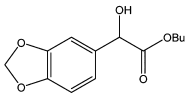
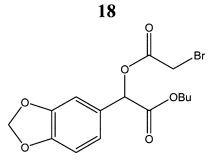
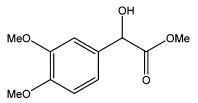
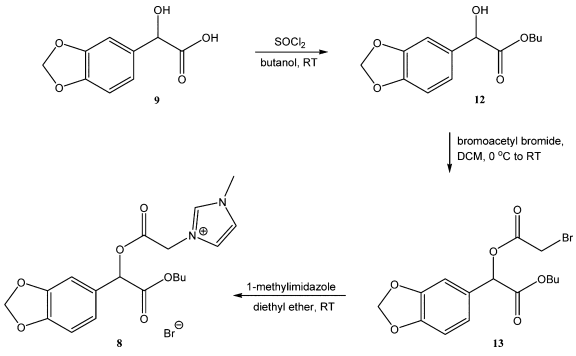
| Intermediate | *Yield (%)* | Intermediate | *Yield (%)* |
|---|---|---|---|
|  **10** | *93* |  **16** | *63* |
|  **11** | *68* |  **17** | *76* |
|  **14** | *65* |  **18** | *69* |
|  **12** | *95* |  **13** | *80* |
|  **15** | *69* | | |

**Table 2. Yield for *N*-Alkylation Step of ILs and Mp or $T_g$**

| IL | yield (%) | Mp (°C) | IL | yield (%) | Mp (°C) |
|---|---|---|---|---|---|
| **1** | 79 | 146−147 | 5 | 61 | 118−119 |
| **2** | 97 | 124−125 | 6 | 90 | 148−149 |
| **3** | 90 | 123−125 | 7 | 50 | 150−152 |
| **4** | 58 | −3.3[a] | 8 | 60 | 94−96 |

[a]Glass transition temperature ($T_g$).

**Scheme 2. Synthesis of IL 8 with Acetoxy Linker**




**7** and **8** precipitated from the diethyl ether solution as white solids, with yields of 50% and 60%, respectively. In the case of IL **6**, heating to reflux of the reaction mixture was required to ensure full conversion, with the final product **6** obtained as a white solid in 90% yield (Table 2). Overall yields for ILs **6**−**8** starting from 3,4-methylenedioxymandelic acid or 3,4-dimethoxymandelic acid are **6** 58%, **7** 38%, and **8** 46%.

Melting points or glass transition temperatures were recorded for the ILs. Due to the interplay of possible bonding interations in ILs **1**−**8** (VdW, ionic, H-bond acceptor, H-bond donor, $\pi-\pi$ stacking), we were interested to determine if a mandelate example which is a liquid at room temperature (RT) could be prepared. IL **4** with a $T_g$ of (−3.3 °C) was identified. While IL **8** has a melting point just below 100 °C, (94−96 °C). The remaining ILs **1**−**3**, **5**−**7** have melting points in the range 118−152 °C, and this would restrict their application as a solvent.

**Toxicity Tests.** The intention of this study is to provide further information about the toxicological impact of the synthesized ILs on two groups of microorganisms. The first are industrial organisms, five bacteria being investigated, and the second is constituted by organisms found in the environment, a marine luminescent bacterium and two freshwater green algae species. (for the experimental method, see the SI). Besides the industrial and environmental issues, this work intends to evaluate the toxic effect of diverse structural variations in ILs (considering the positively charged head groups, the substitution with one or more different side chains, and the corresponding anionic species) using relatively simple, quick, and inexpensive bioassays.

This information may be particularly useful in the design of ILs, considering that it is a goal of many researchers to tune the physicochemical properties of the ILs via the choice of the adequate anionic and cationic components.

**Microtox Assays.** The present study shows low or moderate negative effects of all the ILs synthesized toward the luminescent marine bacteria *Vibrio fischeri*. The results obtained are described by the $EC_{50}$ toxicological parameters


dx.doi.org/10.1021/sc3001299 | ACS Sustainable Chem. Eng. 2013, 1, 393−402

($mg{\cdot}L^{-1}$ and $\mu M$), which describe the IL concentration necessary to inhibit the luminescence of the bacterium by 50%. Those results were determined for two different exposure times, namely 5 and 15 min as reported in Table 3. To discuss the toxicity of these compounds, it is necessary to highlight their complexity, since they present large, bulky, and highly oxygenated structures. All these conditions may promote individual toxic effects, and their conjugated action may have synergetic effects that can be rather complex.

**Table 3. Microtox $EC_{50}$ Results ($mg{\cdot}L^{-1}$ and $\mu M$) for All the ILs Synthesized after 5 and 15 min of Exposure to the Luminescent Marine Bacteria *Vibrio fischeri*, with Respective 95% Confidence Limits (in Brackets)**

| | $EC_{50}$ ($mg{\cdot}L^{-1}$) (lower limit; upper limit) | | $EC_{50}$ ($\mu M$) (lower limit; upper limit) | |
|---|---|---|---|---|
| ionic liquid | 5 min | 15 min | 5 min | 15 min |
| **1** | 438 | 248 | 1240 | 705 |
| | (15; 937) | (117; 511) | (41; 2660) | (332; 1450) |
| **2** | 66 | 71 | 211 | 227 |
| | (2.5; 1730) | (8.0; 622) | (8; 5500) | (26; 2000) |
| **3** | 85 | 85 | 217 | 217 |
| | (20; 372) | (13; 556) | (50; 943) | (33; 1410) |
| **4** | 151 | 87 | 380 | 218 |
| | (136; 166) | (59; 128) | (341; 418) | (148; 321) |
| **5** | 78 | 84 | 240 | 259 |
| | (10; 600) | (60; 92) | (31; 1850) | (185; 284) |
| **6** | 962 | 900 | 2350 | 2200 |
| | (239; 3870) | (37; 2170) | (583; 9440) | (91; 5290) |
| **7** | 21 | 22 | 46 | 48 |
| | (10; 46) | (9.5; 53) | (23; 103) | (21; 118) |
| **8** | 23 | 26 | 51 | 56 |
| | (13; 43) | (18; 37) | (29; 94) | (39; 81) |

The results presented in Table 3 and Figure 1 show that **1**, **4**, and **6** are in agreement with previous studies[56] where the toxicity increases with the exposure time. The toxicity values indicate that these compounds can be divided in two groups, according to the Passino classification[83] based on the $EC_{50}$ values for 15 min: the "practically harmless" (low toxicity to MicroTox assay), constituted by ILs **1** and **6**, and the moderately toxic, composed of the remaining ILs.

The ILs presented in this study diverge in several structural characteristics, such as the cation core, anion, and the size of the cation alkyl side chain. The effect of the elongation of the alkyl chain is here tested on the IL pairs **1**/**3** and **6**/**7** (methyl/butyl), and the results clearly suggest that, as observed for common ILs,[17,20,23,56] the increase in the cation alkyl side chain enhances the toxicity toward the luminescent bacterium. Analyzing the effect of the cation core based on the IL pairs **7**/**8** and **3**/**4** indicates that pyridinium has higher toxic effects when compared with imidazolium. The pair **5**/**1** shows the effect of the cyclic oxygenated structures suggesting that these structures may somewhat enhance the toxicity. However, the influence of the anions (**5**/**1**; chloride/bromide) on the toxicity may also be a contributing factor.

One of the major concerns in the toxicity studies is to provide "benign" IL structures, meaning hydrophobic ILs with lower toxicity. The more hydrophilic ILs are, normally, compounds with lower ability to interact or penetrate into the microorganisms membrane.[39−43,84] For all the ILs synthesized in this study, the parameter solubility in water (Table 4) was measured. The solubility of ILs in water gives us an indication of the hydrophobicity of the ILs. In fact, when the simpler IL $[C_{10}mim]Cl$ was compared with some of the oxygenated-ILs here tested, in terms of toxicity and solubility in water, it is concluded that, our ILs are, in some cases, represented by lower toxicities and similar or, even, higher hydrophobicity. For example, IL **2** has a higher hydrophobicity (0.310 $g{\cdot}mL^{-1}$ solubility in water) and lower toxicity ($EC_{50}$ = 71 $mg{\cdot}L^{-1}$) when compared with the simpler $[C_{10}mim]Cl$ ($EC_{50}$ = 0.152 $mg{\cdot}L^{-1}$ [85] and solubility[86] in water = 0.429 $g{\cdot}mL^{-1}$).

**Table 4. Water Solubility of the ILs 1−8**

| IL | $M_w$ | solubility of IL in water ($g{\cdot}mL^{-1}$) | IL | $M_w$ | solubility of IL in water ($g{\cdot}mL^{-1}$) |
|---|---|---|---|---|---|
| **1** | 352.2 | 1.30 | **5** | 323.8 | 1.41 |
| **2** | 310.7 | 0.310 | **6** | 410.2 | 0.410 |
| **3** | 394.3 | 0.155 | **7** | 452.3 | 0.013 |
| **4** | 397.3 | 0.070 | **8** | 455.3 | 0.229 |

However, no clear trends linking structural features of ILs **1**−**8**, solubility of IL in water, and toxicity to Microtox assay were found.

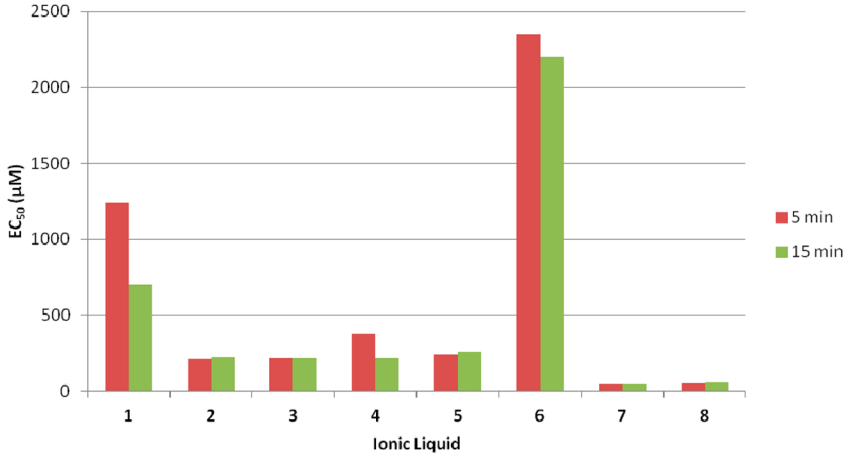



**Figure 2.** Microtox $EC_{50}$ results ($\mu M$) for ILs **1**−**8** after 5 and 15 min of exposure to the luminescent marine bacteria *Vibrio fischeri*.

Table 5. $IC_{50}$ Results for All the ILs Synthesized and Five Different Bacteria Strains

| | $IC_{50}$ (mM) | | | | |
|---|---|---|---|---|---|
| ionic liquid | *E. coli* | *B. subtilis* | *P. fluorescens* | *P. putida* (CP1) | *P. putida* (KT 2440) |
| **1** | 100−200 | 50−100 | 100−200 | 100−200 | 100−200 |
| **2** | 50−100 | 50−100 | 100−200 | 100−200 | 100−200 |
| **3** | 25−50 | 25−50 | 25−50 | 25−50 | 25−50 |
| **4** | 25−50 | 25−50 | 25−50 | 25−50 | 25−50 |
| **5** | >200[a] | >200[a] | >200[a] | >200[a] | 100−200 |
| **6** | 50−100 | 50−100 | 50−100 | 50−100 | 100−200 |
| **7** | 3.13−6.25 | >6.25[a] | >6.25[a] | 3.13−6.25 | 3.13−6.25 |
| **8** | 37.5−75 | 37.5−75 | 75−150 | 75−150 | 75−150 |

[a]Solubility limit. IL $IC_{50}$ value greater than solubility in media.

**Bacteria Toxicity Tests.** Additional antibacterial screening was performed on one Gram-positive bacterium strain (*Bacillus subtilis* (*B. subtilis*; DSMZ 10) and four Gram-negative bacteria (*E. coli* (DSMZ 498), *P. fluoroscens* (DSMZ 50090), *P. putida* (CP1), and *P. putida* (KT 2440) (Table 5)). ILs **1**−**6** were soluble in the Milton media at 0.2 M concentrations, while the least hydrophilic IL **7** has a solubility limit of 6.25 mM and IL **8** has a solubility limit of 150 mM. IL **5** exhibited the lowest toxicity to all five bacteria strains, with an $IC_{50}$ value greater than the solubility limit of 0.2 M for *B. subtilis*, *E. coli*, *P. fluoroscens*, and *P. putida* (CP1) and a 0.1−0.2 M solubility limit for *P. putida* (KT 2440). This methyl ester IL is the only example screened with the dimethoxy substituted aromatic ring compared to the O−$CH_2$−O containing ILs **1**−**4**, **6**−**8**. IL **7** was the most toxic example screened with $IC_{50}$ values of 3.13−6.25 mM for *E. coli*, *P. putida* (CP1), and *P. putida* (KT 2440), which is in close agreement with the results found for this IL against the luminescent bacterium *Vibrio fischeri*. When screened against *B. subtilis* and *P. fluoroscens*, no antibacterial activity was observed for IL **7**, at the maximum solubility limit of 6.25 mM. Of note, changing the pyridinium ring (IL **7**) with the imidazolium group (IL **8**) greatly decreases the antibacterial toxicity (c.f. $IC_{50}$, *P. putida* (CP1) 3.13−6.25 mM (IL **7**) vs 75−150 mM (IL **8**)) ILs **1**−**4**, **6**, and **8** have the same $IC_{50}$ values across the three Gram-negative bacteria strains *P. fluoroscens*, *P. putida* (CP1), and *P. putida* (KT 2440). The increase in ester alkyl group from methyl (IL **1**) to butyl (IL **3**) leads to an increase in toxicity (c.f. $IC_{50}$, *P. putida* (CP1) 100−200 mM (IL **1**) vs 25−50 mM (IL **3**)), while changing from pyridinium (IL **3**) to imidazolium (IL **4**) did not lead to a significant rise in toxicity. The increase in the alkyl chain was also in close agreement with the literature.[5,17,18,38−43] In fact, this behavior is general and practically independent of the microorganisms used and the cations and anions tested.[17] While **1**, **3**, **4**, and **6** have the same $IC_{50}$ value for the other Gram-negative strain *E. coli*, IL **2** and IL **8** were more toxic to this strain compared to the other Gram-negative strains in this study. Comparing the $IC_{50}$ values for the ILs **1**−**4**, **6**, and **8** for the Gram-positive strain (*B. subtilis*) against Gram-negative *P. fluoroscens*, *P. putida* (CP1), and *P. putida* (KT 2440), IL **1**, IL **2**, and IL **8** are more active while ILs **3**, **4**, and IL **6** have the same activity. The increase in ester alkyl group from methyl (IL **1**) to butyl (IL **3**), also leads to an increase in toxicity to (c.f. $IC_{50}$ for *B. subtilis* 50−100 mM (IL **1**) vs 25−50 mM (IL **3**)), however the replacement of pyridinium (IL **3**) by an imidazolium (IL **4**), does not lead to significant rise in toxicity, which is consistent with the trend observed for all bacteria in Table 5.

Finally, when the $IC_{50}$ where analyzed according to the different bacteria morphologies, surprisingly, it is concluded that the different morphologies of Gram-positive and Gram-negative bacteria are not relevant in the $IC_{50}$ results explanation (Table 5). In this context, and analyzing the $IC_{50}$ for the ILs **1**−**6** and **8**, it is observed that the toxic response of the Gram-negative bacterium *E. coli* and the Gram-positive bacterium *B. subtilis* is equal, which is different from previously reported results for common ionic liquids.[87]

Comparing $IC_{50}$ data in (Table 5) with water solubility (Table 4), the effect of the alkyl ester substitutent and *N*-heterocycle are significant. First, the four butyl ester ILs (**3**, **4**, **7**, and **8**) are more toxic and with lower water solubility than the four methyl ester ILs (**1**, **2**, **5**, and **6**). Second, the solubility of the more toxic pyridinium butyl ester ILs **3** and **7** is lower than their less toxic imidazolium analogues (**8** and **4**, respectively). This effect is not so distinguishable with the methyl esters due to the low toxicity of **1**, **2**, **5**, and **6.**

**Freshwater Green Algae Tests.** Aiming to explore in more detail the effects of these oxygenated ILs, their effects toward two freshwater green algae, namely *P. subcapitata* and *Chlorella vulgaris* (*C. vulgaris*), were investigated and the toxicity results presented in Table 6.

Table 6. $EC_{50}$ Values ($mg{\cdot}L^{-1}$ and $\mu M$) of All the ILs Tested Towards Two Freshwater Microalgae (*P. subcapitata* and *C. vulgaris*)

| | $EC_{50}$ ($mg{\cdot}L^{-1}$) (lower limit; upper limit) | | $EC_{50}$ ($\mu M$) (lower limit; upper limit) | |
|---|---|---|---|---|
| ionic liquid | *P. subcapitata* | *C. vulgaris* | *P. subcapitata* | *C. vulgaris* |
| **1** | 587 | 441 | 1670 | 1250 |
| | (485; 710) | (373; 520) | (1370; 2000) | (1060; 1480) |
| **2** | 125 | 232 | 385 | 746 |
| | (110; 139) | (206; 258) | (353; 449) | (663; 830) |
| **3** | 196 | 344 | 497 | 873 |
| | (155; 237) | (266; 422) | (393; 601) | (675; 1070) |
| **4** | 24.1 | 251 | 61 | 632 |
| | (17.5; 30.7) | (225; 277) | (44; 77) | (566; 698) |
| **5** | 13.2 | 260 | 41 | 803 |
| | (8.6; 17.8) | (218; 301) | (27; 55) | (672; 931) |
| **6** | 281 | 294 | 685 | 717 |
| | (261; 300) | (224; 364) | (637; 733) | (547; 887) |
| **7** | 16.6 | 109 | 37 | 241 |
| | (14.1; 19.7) | (85; 140) | (31; 44) | (188; 310) |
| **8** | 7.1 | 19.9 | 16 | 44 |
| | (6.0; 8.1) | (16.2; 23.6) | (13; 18) | (36; 52) |

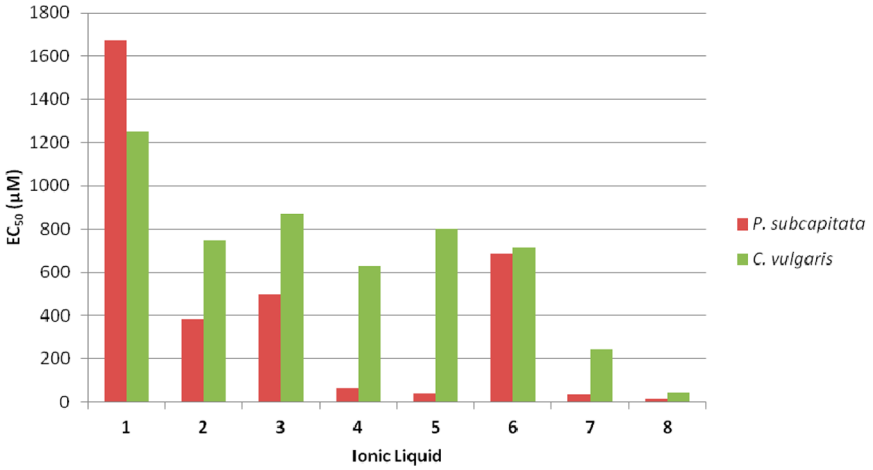



**Figure 3.** $EC_{50}$ values ($\mu$M) of ILs **1**–**8** tested toward two freshwater microalgae (*P. subcapitata* and *C. vulgaris*).

**Table 7. Toxicity of Several IL Halide Salts Towards Freshwater Green Algae**

| name of IL | IL | $M_w$ | algae | $EC_{50}$ (mg·L$^{-1}$) | $EC_{50}$ (mM) | ref |
|---|---|---|---|---|---|---|
| *N*,*N*-dimethyl-*N*-octadecyl-1-octadecanaminium chloride | $[N_{11,18,18}]Cl$ | 586.502 | *P. subcapitata* | 0.46 | $7.84 \times 10^{-4}$ | 88 |
| 1-butyl-1-methylpyrrolidinium chloride | $[C_4mpyrr]Cl$ | 177.715 | *S. vacuolatus* | 431 | 2.43 | 85 |
| 3-decyl-1-methyl-1*H*-imidazolium chloride | $[C_{10}mim]Cl$ | 258.831 | *S. vacuolatus* | $7.05 \times 10^{-5}$ | $2.72 \times 10^{-7}$ | 85 |
| 1-methyl-3-tetradecyl-1*H*-imidazolium chloride | $[C_{14}mim]Cl$ | 314.937 | *S. vacuolatus* | $9.25 \times 10^{-4}$ | $2.94 \times 10^{-6}$ | 85 |
| 3-hexyl-1-methyl-1*H*-imidazolium chloride | $[C_6mim]Cl$ | 202.724 | *S. vacuolatus* | 0.242 | $1.2 \times 10^{-3}$ | 85 |
| 1-butylpyridinium chloride | $[C_4pyr]Cl$ | 171.667 | *S. vacuolatus* | 67.2 | 0.392 | 85 |
| 1-methyl-3-octyl-1*H*-imidazolium chloride | $[C_8mim]Cl$ | 230.777 | *S. vacuolatus* | $4.03 \times 10^{-4}$ | $1.75 \times 10^{-6}$ | 85 |
| 3-butyl-1-methyl-1*H*-imidazolium chloride | $[C_4mim]Cl$ | 174.671 | *P. subcapitata* | 504 | 2.88 | 18 |
| 3-butyl-1-methyl-1*H*-imidazolium bromide | $[C_4mim]Br$ | 219.122 | *P. subcapitata* | 468 | 2.14 | 18 |
| 1-butyl-1-methylpiperidinium bromide | $[C_4mpip]Br$ | 236.192 | *S. vacuolatus* | 450 | 1.90 | 85 |

These two distinct species were used to determine (i) the effect of the ILs here synthesized on a more complex trophic level and (ii) the effect of these ionic structures with respect to different species of the same trophic level. The results reported in Table 6 and Figure 3 show the ILs toxicity for *P. subcapitata* and *C. Vulgaris.*

According to Passino's classification,[83] these ILs are included in different categories, dependent on the algae species investigated. Thus, for the *P. subcapitata*, IL **8** can be classified as slightly toxic (1–10 mg·L$^{-1}$), a moderately toxic label can be attributed to the ILs **4**, **5**, and **7** (10–100 mg·L$^{-1}$), and the remaining IL structures may be considered as low toxicity (100–1000 mg·L$^{-1}$). The *C. vulgaris* is less sensitive to these ionic liquids with the IL **8** categorized as moderately toxic, and the remaining structures exhibit low toxicity. These ILs appear to have an impact on algae different from the ordinary ionic liquids previously described in the literature[33] that we propose is related to the high complexity (c.f. $[C_4mim]Br$) of their chemical structures. However, no clear trends linking structural features of the ILs **1**–**8**, solubility of IL in water, and toxicity to algal assay were found.

Toxicity data[18,85,88] for some simpler common ILs conjugated with the halides Br and Cl, for the algae *P. subcapitata* and *Scenedesmus vacuolatus*, are reported in Table 7 to allow a comparison with the toxicities of the ILs prepared on this work. They show that common ILs, namely $[C_4mim]Br$ and $[C_4mim]Cl$ (*P. subcapitata* 24 h of exposition time) and $[C_4mpip]Br$, $[C_4pyr]Cl$, and $[C_4mpyrr]Cl$ (*Scenedesmus vacuolatus*, 24 h) present slightly higher toxicities than the compounds here studied while some of the ILs reported in literature have significantly higher toxicities, namely *N*,*N*-dimethyl-*N*-octadecyl-1-octadecanaminium chloride $[N_{11,18,18}]Cl$ against *P. subcapitata* (72 h of exposition time) and $[C_6mim]Cl$, $[C_8mim]Cl$, $[C_{10}mim]Cl$, and $[C_{14}mim]Cl$ toward *Scenedesmus vacuolatus* (24 h of exposition time). Moreover, and as explained before, one of the main goals in the toxicity research is to find ILs with lower hydrophobicity and toxicity. Comparing the $EC_{50}$ results of $[C_{10}mim]Cl$ (solubility in water = 0.429 g·mL$^{-1}$) and the IL **6** (solubility in water 0.410 g·mL$^{-1}$), it is clear that, despite their similar hydrophobicity, the toxicity of our IL **6** is significantly lower for both the algae species ($EC_{50}$ = 281 mg·L$^{-1}$ for *P. subcapitata* and $EC_{50}$ = 294 mg·L$^{-1}$ for *C. vulgaris*), when compared with the toxicity of the simpler imidazolium IL ($EC_{50}$ = $7.05 \times 10^{-5}$ mg·L$^{-1}$ for *Scenedesmus vacuolatus*), which means that it is possible to synthesize hydrophobic ILs with considerable lower toxicities, independently of the microorganisms.

A general trend is that IL **1** and **6** have low toxicity to all bacteria strains and freshwater green algae in screen. All ILs have low toxicity to Gram-positive (*B. subtilis*) and Gram-negative (*E. coli*, *P. fluoroscens*, *P. putida* (CP1), and *P. putida* (KT 2440)) bacteria strains, although a significant range in $IC_{50}$ values was obtained. However, both ILs **5** and **7** are moderately toxic to *Vibrio fischeri* and *P. subcapitata*, with low toxicity to *C. vulgaris*. IL **8** was the only compound moderately toxic to *C. vulgaris*, with the others exhibiting low toxicity to this algae. A similar trend was observed for *P. subcapitata*, being IL **8** considered the most toxic albeit slight toxicity, the ILs **4**, **5**, and **7** are noted as moderately toxic, and the ILs **1**, **2**, **3**, and **6** have low toxicity to *P. subcapitata*. On comparison with the lower $M_w$ butyl substituted ionic liquids analogous activities of moderately toxic to low toxicity are found. If one compares


dx.doi.org/10.1021/sc3001299 | *ACS Sustainable Chem. Eng.* 2013, 1, 393–402

ionic liquids of similar $M_w$, due to the high oxygen content of ILs **1**−**8**, the toxicity data would be biased toward our novel ILs. A better guide is to count the carbon content, for **1**−**8**, the range is C14−C20, (*P. subcapitata*, $EC_{50}$ values from 16 to 1670 $\mu$M) c.f. C14−C18 (*S. vacuolatus* $EC_{50}$ $2.72 \times 10^{-4}$ to $1.75 \times 10^{-3}$ $\mu$M). While this is a "back of the envelope" calculation and compares two different algae strains, ILs **1**−**8** $EC_{50}$ values are $10^3$−$10^7$ higher (less toxic).

## CONCLUSIONS

A new class of low bacterial and algae toxicity imidazolium and pyridinium halide ionic liquids, produced by a short synthesis from substituted mandelic acid derivatives was here disclosed. The series of ionic liquids prepared enabled an investigation of modifying the heterocycle, aromatic ring substitution, ester group, and proximity of cation to aromatic ring from mandelate starting material and their impact on the compounds toxicity. IL **4** with a $T_g$ of (−3.3 °C) was identified, while the remaining ILs **1**−**3**, **5**−**8** have melting points in the range 94−152 °C. A general trend is that IL **1** and **6** have low toxicity to all bacteria strains and freshwater green algae in screen. All ILs have low toxicity to Gram-positive (*B. subtilis*) and Gram-negative (*E. coli*, *P. fluoroscens*, *P. putida* (CP1), and *P. putida* (KT 2440)) bacteria strains, although a significant range in $IC_{50}$ values was obtained.

Comparing this $IC_{50}$ data in with water solubility, the effect of the alkyl ester substitutent and *N*-heterocycle are significant. First, the four butyl ester ILs (**3**, **4**, **7**, and **8**) are more toxic and with lower water solubility than the four methyl ester ILs (**1**, **2**, **5**, and **6**). Second, the solubility of the more toxic pyridinium butyl ester ILs **3** and **7** is lower than their less toxic imidazolium analogues (**8** and **4**, respectively). This effect is not so distinguishable with the methyl esters due to the low toxicity of **1**, **2**, **5**, and **6.** However, no clear trends linking structural features of the ILs **1**−**8**, solubility of IL in water, and toxicity to either MicroTox or algal assay were found.

Roles of ILs are wide ranging, from solvent to a tailored additive or catalyst[2,5,7,12,53,71] They have found applications in areas including organic, physical, and analytical chemistry, biology, and biomass processing.[53] The search for low toxicity examples will lead to a wider choice of "safer" ionic liquids which can be selected for study in the above applications. Specific applications of ILs **1**−**8** including biomass dissolution studies and electrolytes for dye sensitized solar cells will be published in due course. Overall yields for ILs, starting from 3,4-methylenedioxy- or 3,4-dimethoxy-mandelic acid, were between 27 and 65%. In addition while high atom economy reactions were used where possible, factors including the use of toxic solvents and reagents, solvent use in workup, and waste treatment are issues for many chemists. The challenge to reduce our impact on the environment is always present.

As the design of task specific ionic liquids continues to grow as a research area, the underlying concern is as one modifies the structure of the cation, whether imidazolium, pyridinium, or others, the $M_w$ increases, the carbon scaffold is extended, and the toxicity changes. Conventional wisdom dictates that we must avoid long linear alkyl chains (≥C8), and we propose here instead the use of mandelic acid esters as starting materials. A comparison of the toxicity to algae data of ILs **1**−**8** to other C14−C18 ionic liquids, $EC_{50}$ values $10^3$−$10^7$ higher (less toxic) were found for these mandelate derived ILs **1**−**8**.

## ASSOCIATED CONTENT

### Supporting Information

Experimental procedures for the synthesis of ILs and their intermediates and characterization data (NMR, IR, mp, $T_g$) as well as toxicity and ecotoxicity screening methods. This material is available free of charge via the Internet at http://pubs.acs.org.

## AUTHOR INFORMATION

### Corresponding Author

*Fax: +353 1 700 5503. Tel.: +353 1 700 7860. E-mail address: nick.gathergood@dcu.ie.

### Author Contributions

‖S.P.M.V., Mo.G., and Mu.G. all contributed equally to this work.

### Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

N.G. is thankful for the support of Enterprise Ireland TD-2007-0328 for funding the work of I.B., Mo.G., and Mu.G. This material is also based upon works (N.G. and Mu.G.) supported by Science Foundation Ireland as part of the Solar Energy Conversion Strategic Research Cluster, Grant No. [07/SRC/B1160] which the authors acknowledge. This work was partly funded by Fundação para a Ciência e a Tecnologia (FCT) through the project PTDC/AAC-AMB/119172/2010 and the project Pest-C/CTM/LA0011/2011. The authors also are thankful for the financial support from FCT for the Postdoctoral grant SFRH/BDP/79263/2011 of S.P.M.V.

## REFERENCES

(1) Johnson, K. E. What's an Ionic Liquid? *Interface* **2007**, *16*, 38−41.
(2) Earle, M. J.; Seddon, K. R. Ionic liquids. Green solvents for the future. *Pure Appl. Chem.* **2000**, *72*, 1391−1398.
(3) Armstrong, J. P.; Hurst, C.; Jones, R. G.; Licence, P.; Lovelock, K. R. J.; Satterley, C. J.; Villar-Garcia, I. J. Vapourisation of ionic liquids. *Phys. Chem. Chem. Phys.* **2007**, *9*, 982−990.
(4) Diallo, A. O.; Len, C.; Morgan, A. B.; Marlair, G. Revisiting physico-chemical hazards of ionic liquids. *Sep. Purif. Technol.* **2012**, *97*, 228−234.
(5) Pham, T. P. T.; Cho, C.-W.; Yun, Y.-S. Environmental fate and toxicity of ionic liquids: A review. *Water Res.* **2010**, *44*, 352−372.
(6) Samorì, C.; Malferrari, D.; Valbonesi, P.; Montecavalli, A.; Moretti, F.; Galletti, P.; Sartor, G.; Tagliavini, E.; Fabbri, E.; Pasteris, A. Introduction of oxygenated side chain into imidazolium ionic liquids: Evaluation of the effects at different biological organization levels. *Ecotox. Environ. Saf* **2010**, *73*, 1456−1464.
(7) Coleman, D.; Gathergood, N. Biodegradation studies of ionic liquids. *Chem. Soc. Rev.* **2010**, *39*, 600−637.
(8) Gathergood, N.; Scammells, P. Design and Preparation of Room-Temperature Ionic Liquids Containing Biodegradable Side Chains. *Aust. J. Chem.* **2002**, *55*, 557−560.
(9) OECD. *OECD guideline for testing of chemicals - ready biodegradability*; adopted by council July 1992.
(10) Dávila, M. J.; Aparício, S.; Alcalde, R.; Garcia, B.; Leal, J. M. On the properties of 1-butyl-3-methylimidazolium octylsulfate ionic liquid. *Green Chem.* **2007**, *9*, 221−232.
(11) Wasserscheid, P.; van Hal, R.; Bösmann, A. 1-*n*-Butyl-3-methylimidazolium ([bmim]) octylsulfate—an even 'greener' ionic liquid. *Green Chem.* **2002**, *4*, 400−404.
(12) Davis, J. H., Jr; Fox, P. A. From curiosities to commodities: ionic liquids begin the transition. *Chem. Commun.* **2003**, 1209−1212.

(13) Gathergood, N.; Scammells, P. J.; Garcia, M. T. Biodegradable ionic liquids: Part III. The first readily biodegradable ionic liquids. *Green Chem.* **2006**, *8*, 156−160.
(14) Philipp, B.; Hoff, M.; Germa, F.; Schink, B.; Beimborn, D.; Mersch-Sundermann, V. Biochemical Interpretation of Quantitative Structure−Activity Relationships (QSAR) for Biodegradation of N-Heterocycles: A Complementary Approach to Predict Biodegradability. *Environ. Sci. Technol.* **2007**, *41*, 1390−1398.
(15) Ranke, J.; Muller, A.; Bottin-Weber, U.; Stock, F.; Stolte, S.; Arning, J.; Stormann, R.; Jastorff, B. Lipophilicity parameters for ionic liquid cations and their correlation to in vitro cytotoxicity. *Ecotoxicol. Environ. Saf.* **2007**, *67*, 430−438.
(16) Kulacki, K. J.; Chaloner, D. T.; Costello, D. M.; Docherty, K. M.; Larson, J. H.; Bernot, R. J.; Brueseke, M. A.; Kulpa, C. F., Jr; Lamberti, G. A. Aquatic toxicity and biodegradation of ionic liquids - A synthesis. *Chim. Oggi/CHEM. TODAY Green Chem.* **2007**, *25* (Supplement), 32−36.
(17) Matzke, M.; Arning, J.; Ranke, J.; Jastorff, B.; Stolte, S. *Handbook of Green Chemistry*; Wiley-VCH Verlag GmbH & Co. KGaA: New York, 2010.
(18) Ranke, J.; Stolte, S.; Stormann, R.; Arning, J.; Jastorff, B. Design of Sustainable Chemical ProductsThe Example of Ionic Liquids. *Chem. Rev.* **2007**, *107*, 2183−2206.
(19) Couling, D. J.; Bernot, R. J.; Docherty, K. M.; Dixon, J. K.; Maginn, E. J. Assessing the factors responsible for ionic liquid toxicity to aquatic organisms via quantitative structure−property relationship modeling. *Green Chem.* **2006**, *8*, 82−90.
(20) Garcia, M. T.; Gathergood, N.; Scammells, P. J. Biodegradable ionic liquids: Part II. Effect of the anion and toxicology. *Green Chem.* **2005**, *7*, 9−14.
(21) Stepnowski, P.; Skladanowski, A. C.; Ludwiczak, A.; Laczynska, E. Evaluating the cytotoxicity of ionic liquids using human cell line *HeLa*. *Hum. Exp. Toxicol.* **2004**, *23*, 513−517.
(22) Ranke, J.; Molter, K.; Stock, F.; Bottin-Weber, U.; Poczobutt, J.; Hoffmann, J.; Ondruschka, B.; Filser, J.; Jastorff, B. Biological effects of imidazolium ionic liquids with varying chain lengths in acute Vibrio fischeri and WST-1 cell viability assays. *Ecotoxicol. Environ. Saf.* **2004**, *58*, 396−404.
(23) Docherty, K. M.; Kulpa, C. F. J. Toxicity and antimicrobial activity of imidazolium and pyridinium ionic liquids. *Green Chem.* **2005**, *7*, 185−189.
(24) Latala, A.; Nedzi, M.; Stepnowski, P. Toxicity of imidazolium and pyridinium based ionic liquids towards algae. Bacillaria paxillifer (a microphytobenthic diatom) and Geitlerinema amphibium (a microphytobenthic blue green alga). *Green Chem.* **2009**, *11*, 1371−1376.
(25) Latala, A.; Nedzi, M.; Stepnowski, P. Toxicity of imidazolium and pyridinium based ionic liquids towards algae. Chlorella vulgaris, Oocystis submarina (green algae) and Cyclotella meneghiniana, Skeletonema marinoi diatoms. *Green Chem.* **2009**, *11*, 580−588.
(26) Latala, A.; Nedzi, M.; Stepnowski, P. Acute toxicity assessment of perfluorinated carboxylic acids towards the Baltic microalgae. *Environ. Toxicol. Pharmacol.* **2009**, *28*, 167−171.
(27) Matzke, M.; Stolte, S.; Thiele, K.; Juffernholz, T.; Arning, J.; Ranke, J.; Welz-Biermann, U.; Jastorff, B. The influence of anion species on the toxicity of 1-alkyl-3-methylimidazolium ionic liquids observed in an (eco)toxicological test battery. *Green Chem.* **2007**, *9*, 1198−1207.
(28) Bernot, R. J.; Brueseke, M. A.; Evans-White, M. A.; Lamberti, G. A. Acute and chronic toxicity of imidazolium-based ionic liquids on Daphnia magna. *Environ. Toxicol. Chem.* **2005**, *24*, 87−92.
(29) Pretti, C.; Chiappe, C.; Baldetti, I.; Brunini, S.; Monni, G.; Intorre, L. Acute toxicity of ionic liquids for three freshwater organisms: Pseudokirchneriella subcapitata, Daphnia magna and Danio rerio. *Ecotoxicol. Environ. Saf.* **2009**, *72*, 1170−1176.
(30) Wells, A. S.; Coombe, V. T. On the Freshwater Ecotoxicity and Biodegradation Properties of Some Common Ionic Liquids. *Org. Proc. Res. Dev* **2006**, *10*, 794−798.
(31) Wang, S. H.; Huang, P. P.; Li, X. Y.; Wang, C. Y.; Zhang, W. H.; Wang, J. J. Embryonic and Developmental Toxicity of the Ionic Liquid 1-Methyl-3-octylimidazolium bromide on Goldfish. *Environ. Toxicol.* **2010**, *25* (3), 243−250.
(32) Pretti, C.; Chiappe, C.; Pieraccini, D.; Gregori, M.; Abramo, F.; Monni, G.; Intorre, L. Acute toxicity of ionic liquids to the zebrafish (Danio rerio). *Green Chem.* **2006**, *8*, 238−240.
(33) Ventura, S. P. M.; Gonçalves, A. M. M.; Gonçalves, F.; Coutinho, J. A. P. Assessing the toxicity on $[C_3mim][Tf_2N]$ to aquatic organisms of different trophic levels. *Aquat. Toxicol.* **2010**, *96*, 290−297.
(34) Cieniecka-Roslonkiewicz, A.; Pernak, J.; Kubis-Feder, J.; Ramani, A.; Robertson, A. J.; Seddon, K. R. Synthesis, anti-microbial activities and anti-electrostatic properties of phosphonium-based ionic liquids. *Green Chem.* **2005**, *7*, 855−862.
(35) Pernak, J.; Chwala, P. Synthesis and anti-microbial activities of choline-like quaternary ammonium chlorides. *Eur. J. Med. Chem.* **2003**, *38*, 1035−1042.
(36) Pernak, J.; Goc, I.; Mirska, I. Anti-microbial activities of protic ionic liquids with lactate anion. *Green Chem.* **2004**, *6*, 323−329.
(37) Pernak, J.; Rogoza, J.; Mirska, I. Synthesis and antimicrobial activities of new pyridinium and benzimidazolium chlorides. *Eur. J. Med. Chem.* **2001**, *36*, 313−320.
(38) Pernak, J.; Sobaszkiewicz, K.; Mirska, I. Anti-microbial activities of ionic liquids. *Green Chem.* **2003**, *5*, 52−56.
(39) Martinez, M. F. R.; Kelessidou, N.; Law, Z.; Gardiner, J.; Stephens, G. Effect of solvents on obligately anaerobic bacteria. *Anaerobe* **2008**, *14*, 55−60.
(40) Cornmell, R. J.; Winder, C. L.; Schuler, S.; Goodacre, R.; Stephens, G. Using a biphasic ionic liquid/water reaction system to improve oxygenase-catalysed biotransformation with whole cells. *Green Chem.* **2008**, *10*, 685−691.
(41) Rebros, M.; Gunaratne, H. Q. N.; Ferguson, J.; Seddon, K. R.; Stephens, G. A high throughput screen to test the biocompatibility of water-miscible ionic liquids. *Green Chem.* **2009**, *11*, 402−408.
(42) Wood, N.; Stephens, G. Accelerating the discovery of biocompatible ionic liquids. *Phys. Chem. Chem. Phys.* **2010**, *12*, 1670−1674.
(43) Wood, N.; Ferguson, J. L.; Gunaratne, H. Q. N.; Seddon, K. R.; Goodacre, R.; Stephens, G. M. Screening ionic liquids for use in biotransformations with whole microbial cells. *Green Chem.* **2011**, *13*, 1843−1851.
(44) Babalola, G. O. Anti-bacterial activity of synthetic *N*-heterocyclic oxidizing compounds. *Lett. Appl. Microb.* **1998**, *26*, 43−46.
(45) Kelman, D.; Kashman, Y.; Rosenberg, E.; Ilan, M.; Ifrach, I.; Loya, Y. Antimicrobial activity of the reef sponge Amphimedon viridis from the Red Sea: evidence for selective toxicity. *Aquat. Microb. Ecol.* **2001**, *24*, 9−16.
(46) Li, G.; Shen, J.; Zhu, Y. Study of pyridinium-type functional polymers. II. Antibacterial activity of soluble pyridinium-type polymers. *J. Appl. Polym. Sci.* **1998**, *67*, 1761−1768.
(47) Ventura, S. P. M.; Goncalves, A. M. M.; Sintra, T.; Pereira, J. L.; Goncalves, F.; Coutinho, J. A. P. Designing ionic liquids: the chemical structure role in the toxicity. *Ecotoxicology* **2013**, *22*, 1−12.
(48) Johnson, B. T. Microtox Acute Toxicity Test. *Small-scale Freshwater Toxicity Investigations*; Springer: The Netherlands, 2005; Vol. *1*, pp 69−105.
(49) Luis, P.; Garea, A.; Irabien, A. Quantitative structure−activity relationships (QSARs) to estimate ionic liquids ecotoxicity $EC_{50}$ (Vibrio fischeri). *J. Mol. Liq.* **2010**, *152*, 28−33.
(50) Samori, C.; Pasteris, A.; Galletti, P.; Tagliavini, E. Acute toxicity of oxygenated and nonoxygenated imidazolium-based ionic liquids to Daphnia magna and Vibrio fischeri. *Environ. Toxicol. Chem.* **2007**, *26*, 2379−2382.
(51) Ventura, S. P. M.; Gardas, R. L.; Gonçalves, F.; Coutinho, J. A. P. Ecotoxicological risk profile of ionic liquids: octanol-water distribution coefficients and toxicological data. *J. Chem. Technol. Biotechnol.* **2011**, *86*, 957−963.
(52) Ventura, S. P. M.; Gonçalves, A. M. M.; Gonçalves, F.; Ventura, S. P. M.; Gonçalves, A. M. M.; Coutinho, J. A. P. Fragrance material review on a-methylcinnamic alcohol. *Toxicol. Lett.* **2011**, *205*, S124.

(53) Petkovic, M.; Seddon, K. R.; Rebelo, L. P. N.; Silva Pereira, C. Ionic liquids: a pathway to environmental acceptability. *Chem. Soc. Rev.* **2011**, *40*, 1383−1403.
(54) Samorì, C.; Malferrari, D.; Valbonesi, P.; Montecavalli, A.; Moretti, F.; Galletti, P.; Sartor, G.; Tagliavini, E.; Fabbri, E.; Pasteris, A. Introduction of oxygenated side chain into imidazolium ionic liquids: evaluation of the effects at different biological organization levels. *Ecotoxicol. Environ. Saf.* **2010**, *73*, 1456−1464.
(55) Romero, A.; Santos, A.; Tojo, J.; Rodríguez, A. Toxicity and biodegradability of imidazolium ionic liquids. *J. Hazard. Mater.* **2008**, *151*, 268−273.
(56) Ventura, S. P. M.; Marques, C. S.; Rosatella, A. A.; Afonso, C. A. M.; Gonçalves, F.; Coutinho, J. A. P. Toxicity assessment of various ionic liquid families towards Vibrio fischeri marine bacteria. *Ecotoxicol. Environ. Saf.* **2012**, *76*, 162−168.
(57) Luis, P.; Ortiz, I.; Aldaco, R.; Irabien, A. A novel group contribution method in the development of a QSAR for predicting the toxicity (Vibrio fischeri $EC_{50}$) of ionic liquids. *Ecotoxicol. Environ. Saf.* **2007**, *67*, 423−429.
(58) Stolte, S.; Arning, J.; Bottin-Weber, U.; Muller, A.; Pitner, W.-R.; Welz-Biermann, U.; Jastorff, B.; Ranke, J. Effects of different head groups and functionalised side chains on the cytotoxicity of ionic liquids. *Green Chem.* **2007**, *9*, 760−767.
(59) Pernak, J.; Borucka, N.; Walkiewicz, F.; Markiewicz, B.; Fochtman, P.; Stolte, S.; Steudte, S.; Stepnowski, P. Synthesis, toxicity, biodegradability and physicochemical properties of 4-benzyl-4-methylmorpholinium-based ionic liquids. *Green Chem.* **2011**, *13*, 2901−2910.
(60) Pretti, C.; Renzi, M.; Focardi, S. E.; Giovani, A.; Monni, G.; Melai, B.; Rajamani, S.; Chiappe, C. Acute toxicity and biodegradability of *N*-alkyl-*N*-methylmorpholinium and *N*-alkyl-DABCO based ionic liquids. *Ecotox. Environ. Safe.* **2011**, *74*, 748−753.
(61) Blaise, C. R. In *Ecotoxicology Monitoring*; Richardson, M. L., Ed.; VCH: Weinheim, 1993; pp 83−108.
(62) Lewis, M. A. In *Fundamentals of aquatic toxicology: Effects, environment fate, and risk assessment*, 2nd ed.; Rand, G. M., Ed.; Taylor and Francis: Washington, DC, USA, 1995; pp 135−170.
(63) Cho, C.-W.; Pham, T. P. T.; Jeon, Y.-C.; Vijayaraghavan, K.; Choe, W.-S.; Yun, Y.-S. Toxicity of imidazolium salt with anion bromide to a phytoplankton Selenastrum capricornutum: Effect of alkyl-chain length. *Chemosphere* **2007**, *69*, 1003−1007.
(64) Cho, C.-W.; Pham, T. P. T.; Jeon, Y.-C.; Yun, Y.-S. Influence of anions on the toxic effects of ionic liquids to a phytoplankton Selenastrum capricornutum. *Green Chem.* **2008**, *10*, 67−72.
(65) Kulacki, K. J.; Lamberti, G. A. Toxicity of imidazolium ionic liquids to freshwater algae. *Green Chem.* **2008**, *10*, 104−110.
(66) Pham, T. P. T.; Cho, C.-W.; Min, J.; Yun, Y.-S. Alkyl-chain length effects of imidazolium and pyridinium ionic liquids on photosynthetic response of Pseudokirchneriella subcapitata. *J. Biosci. Bioeng.* **2008**, *105*, 425−428.
(67) Stolte, S.; Matzke, M.; Arning, J.; Boschen, A.; Pitner, W.-R.; Welz-Biermann, U.; Jastorff, B.; Ranke, J. Effects of different head groups and functionalised side chains on the aquatic toxicity of ionic liquids. *Green Chem.* **2007**, *9*, 1170−1179.
(68) Latala, A.; Stepnowski, P.; Nedzi, M.; Mrozik, W. Marine toxicity assessment of imidazolium ionic liquids: Acute effects on the Baltic algae Oocystis submarina and Cyclotella meneghiniana. *Aqua. Toxicol.* **2005**, *73*, 91−98.
(69) Morrissey, S.; Beadham, I.; Gathergood, N. Selective hydrogenation of trans-cinnamaldehyde and hydrogenolysis-free hydrogenation of benzyl cinnamate in imidazolium ILs. *Green Chem.* **2009**, *11*, 466−474.
(70) Hough, W. L.; Smiglak, M.; Rodríguez, H.; Swatloski, R. P.; Spear, S. K.; Daly, D. T.; Pernak, J.; Grisel, J. E.; Carliss, R. D.; Soutullo, M. D.; Davis, J. H.; Rogers, R. D. The third evolution of ionic liquids: active pharmaceutical ingredients. *New J. Chem.* **2007**, *31*, 1429−1436.
(71) Beadham, I.; Gurbisz, M.; Gathergood, N. *Handbook of Green Chemistry: Designing Safer Chemicals*; first ed.; Boethling, R., Voutchkova, A., Eds.; Wiley-VCH Verlag GmbH & Co. KGaA: New York, 2012; Chapter 6, Vol. *9*, pp 137−158.
(72) Beadham, I.; Gurbisz, M.; Gathergood, N. *Handbook of Green Chemistry: Designing Safer Chemicals*, first ed.; Boethling, R., Voutchkova, A., Eds.; Wiley-VCH Verlag GmbH & Co. KGaA: New York, 2012; Chapter 7, Vol. *9*, pp 159−226.
(73) Boethling, R. S.; Sommer, E.; DiFiore, D. Designing Small Molecules for Biodegradability. *Chem. Rev.* **2007**, *107*, 2207−2227.
(74) Anastas, P.; Warner, J. *Green Chemistry: Theory and Practice*; Oxford University Press: New York, 1998.
(75) Trost, B. M. The atom economy–a search for synthetic efficiency. *Science* **1991**, *254*, 1471−1477.
(76) Bjorsvik, H.; Liguori, L.; Minisci, F. High Selectivity in the Oxidation of Mandelic Acid Derivatives and in O-Methylation of Protocatechualdehyde: New Processes for Synthesis of Vanillin, iso-Vanillin, and Heliotropin. *Org. Process Res. Dev.* **2000**, *4*, 534−543.
(77) Ueno, Y.; Oohira, N.; Watabe, M.; Oota, A. *Production of 3,4-methylenedioxymandelic acid*, Japan Patent, JP 08-59650 A, 1996.
(78) Bauer, K.; Molleken, R. *Process for the preparation of 3,4-methylenedioxymandelic acid*. U.S. Patent, US4190583 A4190581, 1980.
(79) Dury, M. *Production of alkoxymandelic acids, e.g. intermediates for alkoxyaromatic aldehydes, comprises reacting alkoxyaromatic compound with glyoxylic acid in presence of strong protonic acid*. France Patent, FR2830861, 2832003, 2003.
(80) Rawson, D. J.; Dack, K. N.; Dickinson, R. P.; James, K. The Design and Synthesis of a Novel Series of Indole Derived Selective $ET_A$ Antagonists. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 125−128.
(81) Aggarwal, V. K.; Thomas, A.; Schade, S. Trans-1,3-dithiane-1,3-dioxide; a chiral acyl anion equivalent. Enantioselective synthesis of α-hydroxy- carboxylic acids, esters, amides and ketones. *Tetrahedron.* **1997**, *53*, 16213−16228.
(82) Slotta, K. H.; Haberland., G. Zur Gewinnung der Homopiperonylsäure. *J. Praktische Chem.* **1934**, *139*, 211−219.
(83) Passino, D. R. M.; Smith, S. B. Acute bioassays and hazard evaluation of representative contaminants detected in great lakes fish. *Environ. Toxicol. Chem.* **1987**, *6*, 901−907. For an alternative assessment model see the GHS (global harmonised classification system) http://www.osha.gov/dsg/hazcom/ghs.html (accessed February 22, 2013).
(84) Ventura, S. P. M.; Gardas, R. L.; Gonçalves, F.; Coutinho, J. A. P. Ecotoxicological risk profile of ionic liquids: octanol-water distribution coefficients and toxicological data. *J. Chem. Technol. Biotechnol.* **2011**, *86*, 957−963.
(85) Centre for Environmental Research and Sustainable Technology (UFT), 2012, http://www.il-eco.uft.uni-bremen.de/ (accessed February 22, 2013).
(86) Domanska, U.; Bogel-Lukasik, E.; Bogel-Lukasik, R. 1-Octanol/water partition coefficients of 1-alkyl-3-methylimidazolium chloride. *Chem.—Eur. J.* **2003**, *9*, 3033−3041.
(87) Ventura, S. P. M.; de Barros, R. L. F.; Soares, C. M. F.; Lima, A. S.; Coutinho, J. A. P. Simple screening method to identify toxic/non-toxic ionic liquids: Agar diffusion test adaptation. *Ecotoxicol. Environ. Saf.* **2012**, *83*, 55−62.
(88) *MSDS*; Sigma Aldrich: St. Louis, MO, 2010.