ACS Sustainable Chemistry & Engineering

Research Article

pubs.acs.org/journal/ascecg

Synthesis, Toxicity, and Biodegradation of Tunable Aryl Alkyl Ionic Liquids (TAAILs)

Stefan Stolte,*[†,‡] Tobias Schulz,[§] Chul-Woong Cho,[†] Jürgen Arning,[†] and Thomas Strassner[§]

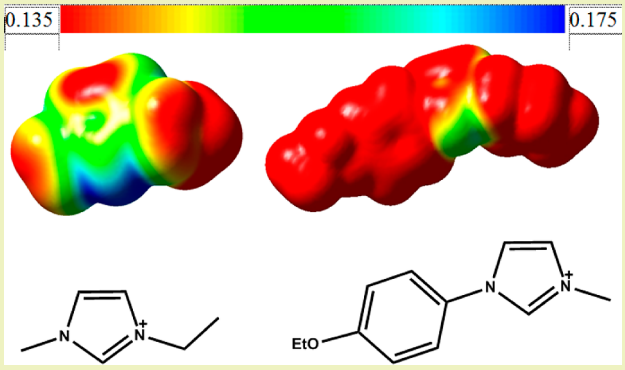
[†]Department of Sustainable Chemistry, University of Bremen, Leobenerstr. UFT, 28359 Bremen, Germany

[‡]Department of Environmental Analysis, University of Gdańsk, ul. Sobieskiego 18/19, 80-952 Gdańsk, Poland

[§]Physical Organic Chemistry, Technical University Dresden, Bergstrasse 66, 01062 Dresden, Germany

Supporting Information

ABSTRACT: TAAILs (Tunable Aryl Alkyl Ionic Liquids) are a new class of ionic liquids with an entirely new cationic substructure and a completely different charge distribution. They have a broad potential for applications, e.g., in metal extraction. In compliance with the basic principles of green chemistry, the optimization of the technological properties of chemicals should be investigated in parallel with the minimization of their (eco)toxicological hazard potential. In this study, we therefore synthesized and assessed the environmental effects of seven congeneric cations with different substitution patterns. In vitro assays with the isolated enzyme acetylcholinesterase and the cytotoxicity assay with IPC-81 cells were used, in conjunction with two in vivo tests using the marine bacteria *Vibrio fischeri* and the limnic green algae *Scenedesmus vacuolatus*, to assess the biological activity of these ionic liquids. Their biodegradability was tested using available procedures. Throughout this manuscript, special emphasis will be placed on the question of whether biological effects observed for TAAILs are hydrophobicity based (as was found for many common ILs) or whether the unique electronic structure of TAAILs changes the toxicity and/or the biodegradability in comparison with "standard" ionic liquid structures.



KEYWORDS: *Green chemistry, Hazard assessment, Ecotoxicity, Biological activity, Biodegradability*

INTRODUCTION

As a substance class defined only by their ionic nature and upper melting point limit of 100 °C, ionic liquids (ILs) have attracted great interest in the chemical community during the past decade.[1] At present, several applications and potential implementations of ILs have been described, e.g., electrodeposition of semiconducting materials and metals,[2] catalytic and stoichiometric reactions,[3] or even wood processing[4] and pharmaceutical applications.[5]

The adaptation of ILs to particular applications has always been the driving force behind their evolution. In most cases, this has led to the development of new anionic species because the physicochemical properties of ILs can be effectively modulated simply by altering the relevant anions.[6] Only recently, however, a new class of ILs with an entirely new cationic substructure has been introduced. These so-called Tunable Aryl Alkyl Ionic Liquids (TAAILs)[7] are distinguished from classical ILs by the substitution pattern of the imidazolium core (Figure 1).

Standard imidazolium ILs carry two alkyl chains (C_{sp3}) allowing no electronic influence of the substituents on the charged aromatic system. The TAAIL concept replaces one alkyl chain with a (substituted) phenyl ring (C_{sp2}), which results in a completely different charge distribution (Figure 1).

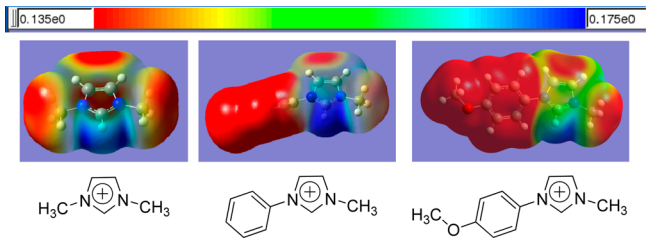


Figure 1. Differences in charge distribution between conventional imidazolium ionic liquids and TAAILs (B3LYP/6-311++G(d,p) level of theory).[8−11] Numbers represent energy per charge in units of Hartree per elemental charge.

While the positive charge of dialkyl cations is located mainly on the alkyl groups, the charge distribution in TAAILs is strongly dependent on the length of the alkyl chain and the substituents at the phenyl ring. Moreover, electronic

Received: November 27, 2012
Revised: February 19, 2013
Published: February 28, 2013

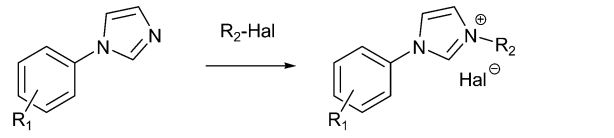
 © 2013 American Chemical Society

communication between the two aromatic systems has been observed.[12] TAAILs exhibit excellent thermal stability and have been proven useful in metal extraction applications.[13]

This new concept allows for the creation of a multifaceted universe of TAAILs with a high potential for tunability. However, besides the fine-tuning of the physicochemical properties necessary for certain applications, environmental and toxicological aspects also have to be considered. In compliance with the basic principles of green chemistry,[14] the optimization of the technological properties of a substance should always be investigated in parallel with the minimization of its (eco)toxicological hazard potential so as to focus on environmentally benign structures already in the early steps of the research and development process. Guided by this approach, the synthesis and a first evaluation of the hazard potential of eight TAAILs is presented in this paper.

During the past few years, the expansion in knowledge about the hazard potential of various ILs generated in different biological test systems[15−17] has shown that some ILs have a low hazard potential and others have a high hazard potential.[18] As expected, the resulting ecotoxicity and biodegradability of ILs depends closely on their molecular structure.

The set of TAAILs under investigation contains seven congeneric cations with different substitution patterns in the R_1 (4-methyl, 4-carboxy, 4-ethoxy, or 2-ethyl) and in the R_2 (propyl, butyl, pentyl, or hexyl) positions combined with halides as counterions (Figure 2).



1 R_1=4-Me
2 R_1=2-Et
3 R_1=4-OEt
4 R_1=4-COOEt

5 R_1=4-Me, R_2=n-Propyl, Hal=Br
6 R_1=4-Me, R_2=n-Butyl, Hal=Br
7 R_1=2-Et, R_2=n-Butyl, Hal=Br
8 R_1=2-Et, R_2=n-Butyl, Hal=I
9 R_1=4-OEt, R_2=n-Butyl, Hal=Br
10 R_1=4-OEt, R_2=n-Pentyl, Hal=Br
11 R_1=4-COOEt, R_2=n-Butyl, Hal=Br
12 R_1=4-COOEt, R_2=n-Hexyl, Hal=Br

Figure 2. TAAILs under investigation (**5**−**12**).

For a preliminary (eco)toxicity evaluation of these compounds, we investigated the inhibition of isolated acetylcholinesterase (AChE) and applied a 48 h in vitro cytotoxicity assay with IPC-81 cells isolated from the Brown Norway rat to measure general cell viability as a toxicological endpoint. Moreover, two acute in vivo tests with the marine bacteria *Vibrio fischeri* and limnic green algae *Scenedesmus vacuolatus* were performed to assess the ecotoxicity of these ILs. Apart from a low (eco)toxicity, current environmental legislation like the REACh regulation demands the development of nonpersistent chemicals. Therefore, the biodegradability of the TAAILs was also investigated. In this study, we focused on the question if biological effects observed for TAAILs are hydrophobicity based (as it was found for many common ILs) or whether the unique electronic structure of TAAILs changes the toxicity and/or the biodegradability in comparison to common IL structures. A comparative analysis of the ecotoxicity data of TAAILs with the standard ILs 3-ethyl-1-methyl-imidazolium chloride ([C_2MIM] Cl) and 1-methyl-3-octyl-imidazolium chloride ([C_8MIM] Cl) is performed.

Moreover, we want to address the question if already established structure−activity relationships of common ILs can be applied for our aryl alkyl compounds.

The discussion of the results obtained here is guided by a structure−activity relationships approach, which should help to improve the design of inherently safer TAAILs and thereby reduce the risks TAAILs may pose to humans and the environment.

RESULTS AND DISCUSSION

The imidazolium salts are synthesized in two steps. Starting from the substituted anilines, the corresponding 1-aryl-1*H*-imdazoles can be synthesized in good yields. These can be reacted with an 1-alkyl halide in an ACE pressure tube at elevated temperatures to obtain the corresponding 1-aryl-3-alkyl-1*H*-imdazolium halides in almost quantitative yields. A more detailed description of the syntheses and analytical characterization is presented in the Experimental section. The (eco)toxicological test systems used in this study yielded reproducible measurements of the acute toxicity of these ILs, and many data are available for comparison.[19]

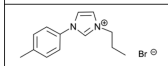
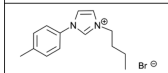
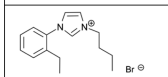
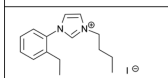
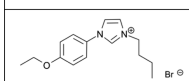
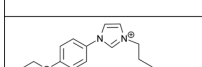
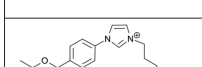
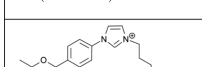
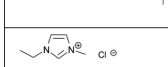
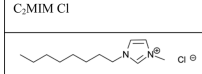
We chose imidazolium-based ILs as reference compounds for lower ([C_2MIM] Cl) (**13**) and higher ([C_8MIM] Cl) (**14**) acute IL toxicity. The toxicities of TAAILs and reference ILs (Table 1) are expressed as the decadic logarithm of half the maximum effective concentration ($\log_{10}IC_{50}$) or inhibitory concentration ($\log_{10}IC_{50}$). It can be assumed that in all tests the observed toxicity/inhibition of these ILs is due solely to the cation because the counterions used (chloride, bromide, and iodide) do not exhibit any intrinsic anion effects in these test systems up to a concentration range of > $\log_{10}$ 3.7 μM (tested as sodium salts).[20−22]

Apart from toxicity data, the cationic hydrophobicity ($\log k_0$) was determined by HPLC according to Ranke et al.[23,24] These values can be used to calculate $\log_{10}IC_{50}$ and $\log_{10}EC_{50}$ without the need for experimentation. The results of enzyme inhibition, cytotoxicity, toxicity prediction, and biodegradation are discussed in the following sections.

Enzyme Inhibition. The enzyme inhibition test with AChE is an important biological marker in (eco)toxicology for evaluating the influence of toxicants on the central nervous system of organisms. The $\log_{10}IC_{50}$ values of TAAILs generally span 2 orders of magnitude, between $\log_{10}$ 0.44 and 2.78 μM. Inhibition was the least in 2-ethylphenyl substituted compounds (**7** and **8**), followed by 4-methylphenyl derivatives (**5** and **6**). The $\log_{10}IC_{50}$ values were similar to or even lower than the inhibition found for [C_2MIM] Cl (**13**). The inhibitory effect of TAAILs substituted with 4-ethoxyphenyl and 4-(ethoxycarbonyl)phenyl (**9**−**12**) was significantly enhanced. The $\log_{10}IC_{50}$ values of (**11**) and (**12**) in particular are more than 1 order of magnitude smaller than that of [C_8MIM] Cl (**14**). These ethoxycarbonyl compounds inhibit the enzyme even more strongly than Aldicarb ($\log_{10}IC_{50}$ 0.69),[22] a known AChE inhibitor, which was used as the positive control in this assay. Arning et al. investigated the AChE inhibition of a large set of ILs and found that a delocalized electron-deficient aromatic system as well as a certain lipophilicity are the key features defining their inhibitory potential.[22] This description applies to TAAILs. Generally, the alkyl chain length in R_2 exhibited a negligible influence when the core structure is kept constant (**5** vs **6**, **9** vs **10**, and **11** vs **12**).

Moreover, the extensively delocalized aromatic system of TAAILs seems to be less important than the substitution

Table 1. Results

	ionic liquid no.	hydrophobicity parameter cation ($log_{10}k_0$)	acetylcholinesterase inhibition		IPC-81		*Vibrio fischeri*		*Scenedesmus vacuolatus*		primary biodegradation	mineralization
			$log_{10}IC_{50}$ [μM] (confidence intervals)		$log_{10}EC_{50}$ [μM] (confidence intervals)						in %	
		exp.	exp.	cal.[c]	exp.	cal.[g]	exp.	cal.[g]	exp.	cal.[g]		
	5	0.990	2.02 (1.98–2.06)	1.73	2.37 (2.35–2.40)	2.98	2.13 (2.10–2.17)	2.74	0.03 (–0.16–0.20)	0.84	0	n.d.
	6	1.309	1.91 (1.85–1.98)	1.57	2.33 (2.28–2.40)	2.62	1.79 (1.75–1.83)	2.13	–1.45 (–2.02 to –0.68)	–0.17	0	0
	7	1.473	2.39 (2.24–2.53)	1.39	2.09 (2.04–2.16)	2.43	1.79 (1.74–1.84)	1.82	0.22 (–0.08–0.56)	–0.70	0	n.d.
	8	1.473	2.78 (2.73–2.83)	1.39	2.02 (1.99–2.04)	2.43	1.79 (1.71–1.87)	1.82	–0.01 (–0.17–0.46)	–0.70	0	n.d.
	9	1.453	1.32 (1.25–1.38)	1.47	1.93 (1.84–2.03)	2.45	1.98 (1.92–2.05)	1.86	–1.37 (–1.62 to –1.13	–0.63	0	n.d.
	10	1.732	1.17 (1.09–1.25)	1.31	1.56 (1.52–1.60)	2.13	1.52 (1.45–1.59)	1.34	n.d.	–1.52	0	n.d.
 IM4-(Ph-4COOEt) Br	11	1.300	0.53 (–0.12–0.65)	1.52	2.67 (2.64–2.70)	2.63	2.43 (2.21–2.63)	2.15	1.02 (0.83–1.12)	–0.15	100	7
	12	1.888	0.44 (–0.10–0.59)	1.20	1.98 (1.96–2.00)	1.96	1.45 (1.27–2.58)	1.05	–0.25 (–0.49–0.01)	–2.02	100	8
 C_2MIM Cl	13	0.22[a]	2.06 (2.02–2.1)[b]	2.54	3.86[e]	3.85	4.33 (4.22– 4.54)[d]	4.16	2.78[d] (2.73–2.84)	3.29	0	n.d.
 C_8MIM Cl	14	1.85[a]	1.6 (1.56–.63)[b]	1.56	2.01 ± 0.05[f]	2.00	1.01 (0.95–1.08)[d]	1.12	–2.67[d] (–3.06 to –2.32)	–1.90	100	41[h]

[a]UFT/Merck Ionic Liquids Biological Effects Database. http://www.chem.uft.uni–bremen.de/il–eco/.[19] [b]Arning et al. 2008.[22] [c]According to equation: $logIC_{50} = 2.57 - 0.62 \times log_{10}(k_0)$ published by Arning et al. 2008.[22] [d]Stolte et al. 2007.[24] [e]Stolte et al. 2006.[21] [f]Ranke et al. 2004.[33] [g]According to equations described in the Supporting Information. [h]Docherty et al. 2007.[34] [i]n.d. = not determined.

dx.doi.org/10.1021/sc300146t | ACS Sustainable Chem. Eng. 2013, 1, 410–418

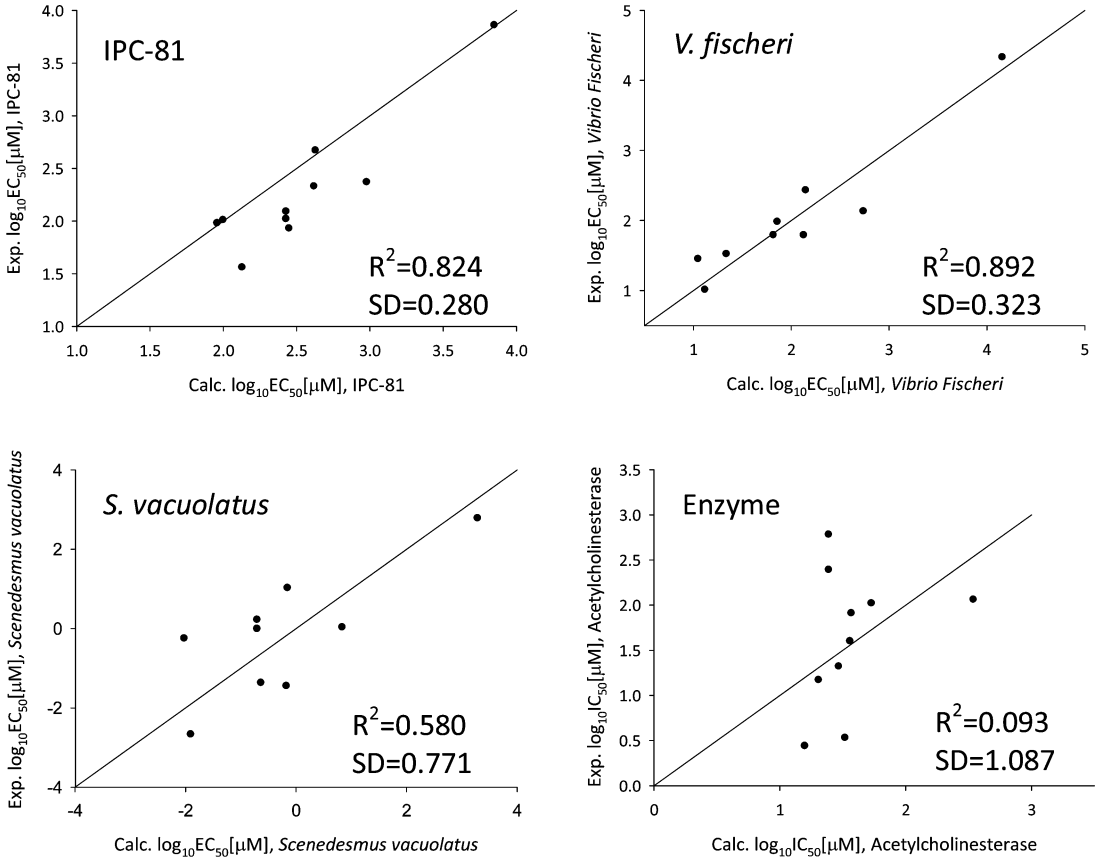


Figure 3. Correlation of the calculated values against experimentally determined $\log_{10}EC_{50}$ values and $\log IC_{50}$ values with a correlation coefficient (R^2), standard deviation (SD) in $\log_{10}$ unit, and 1:1 line.

pattern of the phenyl residue (R_2). Substitution in position 2 reduces inhibition in comparison to the residue in position 4. Such regioselective effects can be explained by the fact that the compounds can bind directly at the active site. This is also supported by the observation that the introduction of ethoxy and ethoxycarbonyl groups causes strong inhibitions because they offer molecular interaction potentials similar to those of the natural substrate acetylcholine. Here, direct binding to the catalytic triad or to the regulatory site—the peripheral anionic site (PAS)—of the enzyme can be assumed.[22] It is known from quaternary ammonium salts that they can interact with both binding sites.[25]

Cytotoxicity. Comparison of the $\log_{10}EC_{50}$ values from the experiments with IPC-81 cells and *Vibrio fischeri* bacteria (Table 1) shows that the values are quite similar for the same TAAIL, with bacteria being consistently slightly more sensitive than IPC-81 cells.

Moreover, the effect concentration lay within a similar range for all tested TAAILs, from $\log_{10}$ 1.56 (**10**) μM to $\log_{10}$ 2.67 μM (**11**). The observed effect concentrations are similar to or even smaller than the values found for [C_8MIM] Cl. Compounds with a longer alkyl group substituted in R_1 but with the same R_2 displayed a greater toxicity (**5** vs **6**, **9** vs **10**, and **11** vs **12**). This observation can most likely be explained by the differences in their hydrophobicities. The interdependence of hydrophobicity and toxicity (also termed baseline toxicity or narcosis) of IL cations in several biological test systems has been widely reported.[15,23] This has led to the rule of thumb that the greater the hydrophobicity of a cation, the greater the observed acute toxic effect. This trend also seems to be valid for TAAILs when the hydrophobicity parameter log k_0 (Table 1; see also the next section) is taken into account. Here, the order of the measured IL toxicity corresponds mainly to the log k_0 of the corresponding cation. The log k_0 values/toxicities are correlated with the number of carbon atoms in the molecule, but they are also influenced (mainly reduced) by the introduction of an oxygen atom—compare, for example, compound (**10**) with compound (**11**).

The TAAILs were also investigated in a growth inhibition test with *Scenedesmus vacuolatus*. These limnic green algae were found to be very sensitive toward hydrophobic ILs,[20,24] an observation also applicable to TAAILs, causing quite strong effects with $\log_{10}EC_{50}$ values around $\log_{10}$ 0 μM. For compounds (**6**) and (**9**), the respective values fell to log −1.45 μM and log −1.37 μM (EC_{50} < 0.05 μM), which correspond to a higher toxicity than expected from hydrophobicity. Moreover, they are even smaller than the values obtained for the photosynthesis-inhibiting herbicide Atrazine.[20]

If we compare the results obtained for TAAILs with those of the reference ILs, we see immediately that the algal toxicities of the TAAILs are distinctly higher than the corresponding values for [C_2MIM] Cl (**13**) and lower than or similar to those of the long-chain [C_8MIM] Cl (**14**).

Estimation of Hydrophobicity and Toxicity. Theoretically, the structural diversity of ILs provides an almost unlimited number of compounds. Because it is impossible to test all of the possible ionic liquid structures for their hazard potentials, there is an urgent need for sound toxicity prediction strategies. Several authors have applied QSAR (Quantitative Structure Activity Relationships) models to data sets of ILs to

determine the general principles governing the toxicity and behavior of these compounds.[26,27]

Ranke et al. used reversed-phase HPLC to estimate the cationic hydrophobicity (log k_0) of ILs.[23] They correlated these values with toxicity data (AChE, rat leukemia cells IPC-81, *Vibrio fischeri* and *Scenedesmus vacuolatus*), as a result of which they were able to demonstrate that the QSAR method could be used to estimate and predict the effect of a given IL structure.[22−24]

The same approach was applied to TAAILs, and effect data were calculated (Table 1) on the basis of log k_0 values using the following QSAR regressions

$$\log_{10} \mathrm{IC}_{50}(\mathrm{AChE})\ [\mu\mathrm{mol\ L}^{-1}] = -0.67 \times \log k_0 + 2.57^{22} \quad (1)$$

$$\log_{10} \mathrm{EC}_{50}(\mathrm{IPC\text{-}81})\ [\mu\mathrm{mol\ L}^{-1}] = 0.794 \times \log k_0 - 3.583 \quad (2)$$

$$\log_{10} \mathrm{EC}_{50}(\textit{Vibrio fischeri})\ [\mu\mathrm{mol\ L}^{-1}] = 1.0887 \times \log k_0 - 3.596 \quad (3)$$

$$\log_{10} \mathrm{EC}_{50}(\textit{Scenedesmus vacuolatus})\ [\mu\mathrm{mol\ L}^{-1}] = 1.7520 \times \log k_0 - 2.7793 \quad (4)$$

Eqs 1 −4 (for details see Supporting Information) allow us to predict the toxicities of TAAILs toward the test systems shown in Table 1 (labeled "cal.") by using log k_0 values. The correlation of the calculated values against experimentally determined $\log_{10}\mathrm{EC}_{50}$ values and $\log_{10}\mathrm{IC}_{50}$ values is given in Figure 3.

The plots show a diverse pattern. The experimental and calculated toxicity data concurred well for *Vibrio fischeri*, but while the TAAILs data points for the IPC-81 test system generally did correlate (R^2 = 0.824), the values tended to be below the 1:1 line (experimental toxicity is higher than the predicted one). For algae, both low correlation of data and strong random deviation from the 1:1 line were found. No meaningful correlation was obtained for the prediction of the inhibitory potential toward AChE.

These results show the applicability and limitation of hydrophobicity-based estimates. The toxicity of the investigated TAAILs toward *Vibrio fischeri* is reliably predictable. Here, the mode of toxic action seems to be based solely on nonspecific interactions with lipid membranes, membrane proteins, and cellular structures in general. The hydrophobicity is a measure of the minimal toxic effect that every chemical can exert on living organisms, but an "excess" toxicity is observed when specific binding, e.g., to enzymes or receptors occurs, which interferes, say, with energy metabolism or signal transduction. The experimentally derived toxicity of IPC-81 is systematically higher (and in the case of the algae, some compounds show a clear deviation) than the calculated (hydrophobicity-based) value; however, this is not clear evidence of "excess" toxicity because for this the deviation would have to be larger than 1 order of magnitude.

In comparison to dialkylimidazolium cations, TAAILs might generally participate in a wider range of molecular interactions, allowing $\pi-\pi$ and cation$-\pi$ interactions that are pivotal in, e.g., substrate−receptor interactions.[28] The strength of such interactions in TAAILs is moderated by ± I and M substituents and their orientations (steric effects). Enzyme inhibition data showed that a minor change in structure could change $\log_{10}\mathrm{IC}_{50}$ values by 1 to 2 orders of magnitude. But even if the inhibition potential of several dialkylimidazolium cations is dependent on hydrophobicity,[22] the correlation cannot be used to estimate such specific binding processes.

Biodegradability. We tested the biodegradability of TAAILs in accordance with OECD guideline 301. For the preliminary biodegradation screening, we used HPLC-DAD to monitor the cation concentration during a test period of 28 days. If no biodegradability occurred, the compounds could not be classified as "readily biodegradable". If HPLC showed that the cation was degraded to a certain extent (primary biodegradation), an additional test using manometic measurements (biochemical oxygen uptake) was performed to track the course of biodegradation. Most of the TAAILs investigated were recalcitrant toward biodegradation, and no primary biodegradation took place with compounds having a different alkyl side chain in R_2 and various substituents (4-methyl, 4-ethoxy or 2-ethyl) in R_1 (Table 1). Primary biodegradation of 4-carboxy substituted compounds (**11** and **12**) was complete within the 28 day test period. The rationale behind the introduction of an ester group into the TAAIL structure was to offer functional groups generally accessible to biodegradation.[29,30] Enzymatic hydrolysis of the ester bond would produce a benzoic acid residue, a potential site of microbial attack, because unsubstituted benzoic acid is readily biodegradable within 5 days (data not shown). Our data suggest that ester−TAAILs were completely converted to other species, but the results of mineralization studies showed that just a small part (7−8%) of the molecule was mineralized. This degradation was potentially due to the ethanol released during enzymatic hydrolysis of the ester bond. However, the degradation pathway was not investigated in this study.

CONCLUSION

An initial evaluation of the hazard potential of seven newly synthesized TAAIL cations is presented. Starting from the substituted anilines, the corresponding 1-aryl-1*H*-imidazoles have been accessible in good yields. A nucleophilic substitution reaction with the 1-alkyl halides leads to the formation of the imidazolium salts.

In comparison with standard dialkylimidazolium ionic liquids, the acute toxicity of TAAILs toward IPC-81, bacteria, and algae lies between those of [C_2MIM] Cl and [C_8MIM] Cl. The observation made with standard ILs, namely, that the greater the hydrophobicity of a cation, the greater the observed acute toxic effect, is not unreservedly applicable to TAAILs. Compared to the dialkylimidazolium cation, TAAILs can participate in a wider range of molecular interactions, allowing $\pi-\pi$ and $\pi-$cation interactions that could permit more specific interactions with biological structures and a different mode of toxic action than the one based on hydrophobicity. This inference is supported by data from enzyme inhibition studies with AChEs. It has been observed that a minor change in structure can change $\log_{10}\mathrm{IC}_{50}$ values by 1 to 2 orders of magnitude. This poses a future challenge for the toxicity assessment of TAAILs, however, because a sound assessment has necessarily to be based on different in vitro and in vivo test systems that respond to different modes of toxic action. Our future aim is the development of TAAILs that contain substructures or functional groups that increase the biodegradability of the compounds. A rapid degradability (short environmental half-life) seems to be an important step within

the benign by design approach of TAAILs, especially when considering the relatively strong effects toward algae.

EXPERIMENTAL SECTION

Preparation of Ionic Liquids. The synthesis of compounds **1**,**3**,**4**,**5**, and **12** has been described in the literature.[12]

*1-(2-Ethylphenyl)-1H-imidazole (**2**).* An amount of 38 mL (0.3 mol) of 2-ethylaniline is dissolved in MeOH (50 mL), and aqueous glyoxal (34 mL) is added. The mixture is stirred at room temperature (20 h) until a yellow precipitate forms. The suspension is diluted with MeOH (400 mL), and NH_4Cl (33 g) and formaldehyde solution (37%, 48 mL) are added. Then the reaction mixture is heated for 1 h under reflux. After addition of H_3PO_4 (14 mL, 85%), the solution is heated at reflux for 4 h. The majority of the solvent (ca. 85%) is removed, and ice water and KOH solution are used to adjust the pH value to pH 9. The product is extracted with dichloromethane, the combined organic layers are dried over $MgSO_4$, and the solvent is removed in vacuo. The product is subsequently purified by distillation (33.58 g, 0.195 mmol, 65%), resulting in a low melting solid (mp: 23 °C). ^{1}H NMR (300 MHz, d_6-DMSO) δ = 1.02 (t, J = 7.5 Hz, 3H), 3.60 (q, J = 7.5 Hz, 2H), 7.10 (s, 1H), 7.38 (m, 4H), 7.41 (m, 1H), 7.80 (s, 1H). ^{13}C NMR (125.8 MHz, d_6-DMSO) δ = 14.7, 23.6, 121.2, 126.8, 128.7, 128.9, 129.5, 135.9, 137.8, 139.4. Anal. Calcd for $C_{11}H_{12}N_2$ (172.23): C, 76.71; H, 7,02; N, 16.27. Found: C, 76.15; H, 7.37; N, 16.66.

*1-Butyl-3-(4-methylphenyl)-1H-imidazolium bromide (**6**).* An ACE pressure tube is equipped with 0.025 mol (3.95 g) 1-(4-methylphenyl)-1*H*-imidazole, 3.40 g 1-bromobutane, and 10 mL tetrahydrofurane. The reaction mixture is heated for 20 h at 95 °C, yielding a viscous oil (4.18 g, 14.3 mmol, 57%). ^{1}H NMR (300 MHz, d_6-DMSO) δ = 0.92 (t, J = 7.5 Hz, 3H), 1.32 (m, 2H), 1.89 (m, 2H), 2.38 (s, 3H), 4.31 (t, J = 6.2 Hz, 2H), 7.43 (d, J = 8.5 Hz, 2H), 7.74 (d, J = 8.5 Hz, 2H), 8.16 (s, 1H), 8.39 (s, 1H), 10.12 (s, 1H). ^{13}C NMR (125.8 MHz, d_6-DMSO) δ = 13.3, 18.8, 20.5, 31.1, 48.9, 120.9, 121.4, 123.3, 130.4, 132.3, 135.1, 139.4. Anal. Calcd for $C_{14}H_{19}BrN_2$ (295.22): C, 56.96; H, 6,49; N, 9.49. Found: C, 57.05; H, 6.49; N, 9.56.

*1-Butyl-3-(2-ethylphenyl)-1H-imidazolium bromide (**7**).* An ACE pressure tube is equipped with 0.01 mol (1.72 g) 1-(2-ethylphenyl)-1*H*-imidazole, 1.37 g 1-bromobutane, and 10 mL tetrahydrofurane. The reaction mixture is heated for 20 h at 40 °C, yielding a viscous oil. After precipitating the product from a tetrahydrofurane solution with pentane, it is washed with diethyl ether and dried in vacuo (2.13 g, 6.9 mmol, 69%, mp 65 °C). ^{1}H NMR (300 MHz, d_6-DMSO) δ = 0.94 (t, J = 7.3 Hz, 3H), 1.08 (t, J = 7.5 Hz, 3H), 1.89 (m, 2H), 1.33 (m, 2H), 2.48 (q, J = 7.5 Hz, 2H), 4.31 (t, J = 7 Hz, 2H), 7.48 (m, 1H), 7.56 (m, 3H), 8.11 (m, 2H), 9.64 (s, 1H). ^{13}C NMR (125.8 MHz, d_6-DMSO) δ = 14.3,13.3, 18.7, 23.2, 31.1, 48.9, 122.8, 124.2, 126.9, 127.3, 129.8, 130.9, 133.6, 137.1, 139.2. Anal. Calcd for $C_{16}H_{23}BrN_2O$ (309.24): C, 58.2; H, 6,84; N, 9.06. Found: C, 57.94; H, 6.67; N, 8.90.

*1-Butyl-3-(2-ethylphenyl) −1H-imidazolium iodide (**8**).* An ACE pressure tube is equipped with 0.014 mol (2.41 g) 1-(2-ethylphenyl)-1*H*-imidazole, 2.58 g 1-iodobutane, and 10 mL tetrahydrofurane. The reaction mixture is heated for 20 h at 40 °C, yielding a viscous oil. The crystallized product is washed with tetrahydrofurane and dried in vacuo (1.44 g, 4.1 mmol, 29%, mp 84 °C). ^{1}H NMR (300 MHz, d_6-DMSO) δ = 0.95 (t, J = 7.2 Hz, 3H), 1.08 (t, J = 7 Hz, 3H), 1.33 (m, 2H), 1.89 (m, 2H), 2.48 (m, 2H), 4.28 (t, J = 7 Hz, 2H), 7.50 (m, 4H), 8.08 (m, 2H), 9.56 (s, 1H). ^{13}C NMR (125.8 MHz, d_6-DMSO) δ = 13.3, 14.4, 18.8, 23.1, 31.1, 49.0, 122.8, 124.3, 127.0, 127.3, 129.0, 131.0, 133.6, 137.1, 139.2. Anal. Calcd for $C_{16}H_{23}BrN_2O$ (356.245): C, 50.57; H, 5.94; N, 7.86. Found: C, 50.78; H, 5.98; N, 7.86.

*1-Butyl-3-(4-ethoxyphenyl) −1H-imidazolium bromide (**9**).* An ACE pressure tube is equipped with 0.012 mol (2.26 g) 1-(4-ethoxyphenyl)-1*H*-imidazole, 2.58 g 1-bromobutane, and 10 mL tetrahydrofurane. The reaction mixture is heated for 20 h at 70 °C, yielding a viscous oil, which is crystallized in pentane and dried in vacuo (2.59 g, 7.9 mmol, 66%, mp 90 °C). ^{1}H NMR (300 MHz, d_6-DMSO) δ = 0.94 (t, J = 7.4 Hz, 3H), 1.36 (m, 5H), 1.88 (m, 2H, CH_2), 4.12 (q, J = 7 Hz, 2H), 4.26 (t, J = 7.2 Hz, 2H), 7.18 (d, J = 10 Hz, 2H), 7.72 (d, J = 10 Hz, 2H), 8.05 (s, 1H), 8.27 (s, 1H), 9.81 (s, 1H). ^{13}C NMR (125.8 MHz, d_6-DMSO) δ = 13.3, 14.5, 18.8, 31.1, 48.9, 63.7, 115.5, 121.3, 123.1, 123.4, 127.7, 135.0, 159.2. Anal. Calcd for $C_{16}H_{23}BrN_2O$ (325.244): C, 55.3; H, 6.51; N, 8.61. Found: C, 54.67; H, 6.40; N, 8.58.

*1-(4-Ethoxyphenyl)-3-pentyl-1H-imidazolium bromide (**10**).* An ACE pressure tube is equipped with 0.038 mol (7.09 g) 1-(4-ethoxyphenyl)-1*H*-imidazole, 5.70 g 1-bromopentane, and 10 mL tetrahydrofurane. The reaction mixture is heated for 20 h at 95 °C, yielding a viscous oil. The crude product is crystallized in diethyl ether, washed with ethyl acetate, and dried in vacuo (11.44 g, 0.034 mol, 89%, mp 91 °C) ^{1}H NMR (300 MHz, d_6-DMSO) δ = 0.90 (t, J = 7 Hz, 3H), 1.34 (m, 7H), 1.89 (m, 2H), 4.13 (q, J = 7 Hz, 2H), 4.23 (t, J = 7.4 Hz, 2H), 7.19 (d, J = 9 Hz, 2H), 7.70 (d, J = 9 Hz, 2H), 8.03 (m, 1H), 8.25 (m, 1H), 9.75 (s, 1H). ^{13}C NMR (125.8 MHz, d_6-DMSO) δ = 13.7, 14.5, 21.5, 27.7, 28.8, 49.2, 63.7, 115.5, 121.4, 123.1, 123.4, 127.7, 135.0, 159.2. Anal. Calcd. for $C_{16}H_{23}BrN_2O$ (339.261): C, 56.64; H, 6.83; N, 8.26. Found: C, 55.90; H, 6.92; N, 8.22.

*1-Butyl-3-(4-(Ethoxycarbonyl)phenyl)-1H-imidazolium bromide (**11**).* An ACE pressure tube is equipped with 5.2 mmol (1.12 g) 1-(4-ethylcarbonylphenyl)-1*H*-imidazole, 0.86 g 1-bromobutane, and 10 mL tetrahydrofurane. The reaction mixture is heated for 36 h at 95 °C, yielding a solid product. The crude product is washed with ethyl acetate and tetrahydrofurane and dried in vacuo (0.95 g, 2.7 mmol, 52%, mp 122 °C) ^{1}H NMR (300 MHz, $CDCl_3$) δ = 0.91 (t, J = 7.4 Hz, 3H), 1.33 (t, J = 7.2 Hz, 3H), 1.38 (m, 2H), 1.78 (m, 2H), 4.32 (q, J = 6.9 Hz, 2H), 4.53 (t, J = 7.4 Hz, 2H), 7.73 (s, 1H), 7.96 (d, J = 8.7 Hz, 2H), 7.99 (s, 1H), 8.15 (d, J = 8.7 Hz, 2H), 11.22 (s, 1H). ^{13}C NMR (125.8 MHz, $CDCl_3$) δ = 13.4, 14.2,19.4, 32.1, 50.3, 61.6, 120.6, 121.5, 123.4, 131.8, 131.9, 136.2, 137.4. Anal. Calcd. for $C_{16}H_{23}BrN_2O{\cdot}0.24EtOAc$ (353.254): C, 54.41; H, 6.17; N, 7.48. Found: C, 54.40; H, 6.52; N, 7.48.

Toxicity Testing. *Acetylcholinesterase Inhibition Assay.* The inhibition of AChE was measured using a colorimetric assay based on the reduction of the dye 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB) by the enzymatically formed thiocholine moiety from the AChE substrate acetylthiocholine iodide. The assay is described in detail in Stock et al.[31] Briefly, a dilution series of the test substances in phosphate buffer (0.02 M, pH 8.0) containing max. 1% methanol was prepared directly in the wells of a 96 well microtiter plate. DTNB (2 mM, 0.185 mg mL^{-1} NaHCO3 in phosphate buffer pH 8.0) and the enzyme (0.2 U mL^{-1}, 0.25 mg mL^{-1} bovine serum albumin in phosphate buffer pH 8.0) were added to each well. The reaction was started by the addition of acetylthiocholine iodide (2 mM in phosphate buffer). The final test concentrations were 0.5 mM of DTNB and acetylthiocholine iodide and 0.05 U mL^{-1} AChE. Each plate contained blanks (no enzyme) and controls (no toxicant). Per experiment, each substance was tested with two replicates of nine different concentrations. Two independent experiments with different stock solutions of each substance were performed. Enzyme kinetics were measured at 405 nm at 30 s intervals in a microplate reader (MRX Dynatech) for 5 min. The enzyme activity was expressed as the slope of optical density (in OD min^{-1}) from a linear regression.

Cell Viability Assay with IPC-81 cells. Briefly, promyelocytic rat cells from the IPC-81 cell line are incubated for 4 h in 96 well plates with 2-(4-iodophenyl)-3-(4-nitrophenyl)- 5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt (WST-1) reagent. Each plate contained blanks (no cells) and controls (no toxicant). The cell viability assays were generally carried out for a 1:1 dilution series. Each dose response curve was recorded for nine parallel dilution series on three different 96 well plates. Two independent experiments with different stock solutions of each compound were performed. Positive controls with Carbendazim were checked in regular intervals.

Luminescence Inhibition Assay with Marine Bacteria Vibrio fischeri. This test with the marine bacterium *Vibrio fischeri* was performed according to DIN EN ISO 11348-2.23. The freeze-dried bacteria were purchased from Dr. Lange GmbH (Düsseldorf, Germany). The tests were carried out twice for each substance. First, a range finding was undertaken with two replicates per dilution

series. Those results were validated within a second test using three replicates for each concentration. Within each tes,t at least four controls (2% NaCl solution, phosphate buffered) were used. To exclude pH effects, all selected substances were prepared as phosphate-buffered solutions (0.02 M, pH 7.0, including 2% sodium chloride), and the test solutions were prepared at least 12 h before testing to ensure complete dissolution of the substances. The tests were performed at 15 °C using thermostats (LUMIStherm, Dr. Lange GmbH, Düsseldorf, Germany). The luminescence was measured with a luminometer (LUMIStox 300, Dr. Lange GmbH, Düsseldorf, Germany).

The liquid-dried bacteria was rehydrated according to the test protocol,and then 500 μL aliquots of the bacteria solution were preincubated for 15 min at 15 °C. After measuring the initial luminescence, 500 μL of the diluted samples was added to the prealiquoted bacteria. The bioluminescence was measured again after an incubation time of 30 min. The relative toxicity of the samples was expressed as percentage inhibition compared to the controls. Luminescent bacteria assays were done without any cosolvents.

Reproduction Inhibition Assay with Limnic Green Algae Scenedesmus vacuolatus. For this assay, the unicellular limnic green algae *Scenedesmus vacuolatus* (strain 211-15, SAG (Culture Collection of Algae), Universität Göttingen, Göttingen) was used, and toxicity tests were done using a synchronized culture. The stock culture was grown under photoautotrophical conditions at 28 °C (± 0.5 °C) in an inorganic sterilized medium (pH 6.4) with saturating white light (intensity of 22−33 kilolux) (Lumilux Daylight L 36 W-11 and Lumilux Interna L 36 W-41, Osram, Berlin, Germany). Cells were aerated with 1.5 vol % CO_2 and synchronized by using a 14 h light and 10 h darkness cycle. The stock culture was diluted every day to a cell density of 5×10^5 cells mL^{-1}.

The toxicity tests started with autospores (young algal cells at the beginning of the growth cycle). Algae were exposed to the test substances for one growth cycle (24 h). The endpoint of this assay is inhibition of algal reproduction measured as inhibition of population growth. All cell numbers (stock culture and test) were determined with the Coulter Counter Z2 (Beckmann, Nürnberg, Germany). The tests were performed in sterilized glass tubes (20 mL Pyrex tubes sealed with caps containing a gastight Teflon membrane), algae were stirred over the whole test period of 24 h, and test conditions were the same as for the stock culture except for the CO_2 source. Here, 150 mL of $NaHCO_3$ solution was added to each test tube. The methods for stock culturing and testing are described in detail in ref 32. Laboratory facilities allowed parallel testing of up to 60 tubes. All substances were tested twice. First, a range finding was undertaken (four concentrations, two replicates), and in a second test, the results were verified with eight concentrations per substance in two replicates. The growth inhibition was calculated using the cell counts of the treated samples in relation to the untreated controls (pure medium). For each assay at least six controls were used.

Determination of Hydrophobicity Parameter. The retention factor (log k_0), indicating hydrophobicity of cation can be calculated from the following

$$\log k_0 = (t_r - t_o)/t_o \quad (5)$$

where t_r is the measured retention time and t_o is the system dead time (system hold−up time). We used a Hewlett-Packard system Series 1100 HPLC with a binary pump, online degasser, auto sampler, and UV detector. The column was a Polaris Ether bridged octadecyl column (C18) (Varian, Inc.) with 150 mm length, 3 mm inner diameter, and 3 μm particle size. We performed the measurement at the isocratic condition, which is 60% acetonitrile and 40% buffer solution adjusted by 30 mM ammonium formate (pH of 3) and 0.5 vol % formic acid in 0.5 mL min^{-1} of flow rate. The dead time in the system was measured with Uracil.

Biodegradability Assessment. *Primary Biodegradation.* The primary biodegradation test was conducted according to a modified version of OECD guideline 301. Primary biodegradation of the test compounds was monitored via HPLC-UV for 28 days. The inoculum used was derived from the wastewater treatment plant Bremen−Delmenhorst (Germany). Five grams of sludge flocs were suspended in 1 L mineral medium and preconditioned for 5 days under aerobic conditions. The cell numbers have been determined by a Paddle Tester (Hach Lange, Düsseldorf, Germany). The mineral medium was composed of 8.5 mg L^{-1} KH_2PO_4, 21.75 mg L^{-1} K_2HPO_4, 22.13 mg L^{-1} $Na_2HPO_4 \bullet 2H_2O$, 1.7 mg $L^{-}NH_4Cl$, 36.4 mg L^{-1} $CaCl_2 \bullet 2H_2O$, 22.5 mg L^{-1}, $MgSO_4 \bullet 7H_2O$, and 0.25 mg L^{-1} $FeCl_3$ (pH 7.2). Solutions of the test substances were prepared in a concentration of 200 μM in inoculated test media (100 mL total volume). Blank samples (inoculated media without test substance), abiotic controls (200 μM test substance in inoculated media poisoned with 50 mg L^{-1} $HgCl_2$), and positive controls (inoculated media with 200 μM imidazole) were also prepared. Replicates of test samples, blinds, abiotic, and positive controls were kept in the dark at 20 ± 1 °C. The 100 mL test vessels were closed but not gastight. Losses due to evaporation were determined by weighing and were adjusted by the addition of test media. For every testing day, 500 μL of all samples were taken, centrifuged (RCF 1700, 15 min), and subsequently analyzed via HPLC. The percentage of degradation of each sample was calculated referring to the initial concentration.

The HPLC system used for the determination of primary biodegradation studies was a VWR Hitachi system containing the L-2130 HTA-pump, L-2130 degasser, L-2200 autosampler, L-2300 column oven, L-2450 diode array-detector, and the EZChrom Elite software. The used cation exchange column (125/4 NUCLEOSIL 100-5 SA) with guard column was purchased from Macherey−Nagel (Düren, Germany). The mobile phase was composed of 55% acetonitrile (HPLC grade) and 45% aqueous 25 mM K_2HPO_4/3.9 mM H_3PO_4 buffer. A flow rate of 0.7 mL min^{-1} and a detection wavelength of 212 nm were employed

Ready Biodegradability. The manometric respirometry test was performed according to OECD guideline 301 F. The biological oxygen demand of the substance was determined for 28 days using a BOD-System (OxiTop, thermostatically controlled from WTW GmbH, Weilheim, Germany). The extent of mineralization could be inferred from this test; if 60% biodegradation was exceeded within a certain time frame, the compound was classified as "readily biodegradable".

Acquired from the wastewater treatment plant at Achim (Germany), the inoculum was filtered and aerated before use. A mineral medium containing final concentrations of 85 mg L^{-1} KH_2PO_4, 217.5 mg L^{-1} K_2HPO_4, 221.3 mg L^{-1} $Na_2HPO_4 \bullet 2H_2O$, 17 mg L^{-1} NH_4Cl, 36.4 mg L^{-1} $CaCl_2 \bullet 2H_2O$, 22.5 mg L^{-1} $MgSO_4 \bullet 7H_2O$, and 0.25 mg L^{-1} $FeCl_3$ (pH 7.2) was added to the filtrate. Additionally, 1.16 mg L^{-1} allylthiourea was added in order to inhibit nitrification. The samples, containing inoculated media and ca. 120−150 mg L^{-1} (giving 200 mg ThOD L^{-1}) substance, were prepared, as well as blank samples (inoculated media without test substance) and controls (inoculated media with benzoic acid). In this test, a bacteria number of 10^4 cells L^{-1} was applied (determined by Paddle-Tester; Hach Europe, Düsseldorf). To ensure absorption of the evolved carbon dioxide, the flasks containing vessels with sodium hydroxide were closed with gastight stoppers and stored in the dark at 20 °C.

The oxygen consumption was determined manometrically. Biodegradation of the test substance was calculated by the oxygen uptake for the test substance (corrected by the oxygen demand of the blank samples) with respect to the theoretical oxygen demand (ThOD) of the substance and the amount of substance present in the sample.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Correlation between cationic lipophilicity values and cytotoxicity values ionic liquids toward IPC-81 (Figure S1), *Scenedesmus vacuolatus* (Figure S2), and *Vibrio fischeri* (Figure S3), and relevant equations (eqs S1−S3). Abbreviations (Table S1), measured cationic lipophilicity values, and cytotoxicity values of ionic liquids toward IPC-81 (Table S2), *Scenedesmus vacuolatus* (Table S3), and *Vibrio fischeri* (Table S4). This

material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Fax: 0049 421-218-9863370. Phone: +49 (0)421 218-63370. E-mail: stefan.stolte@uni-bremen.de.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors gratefully thank Ulrike Bottin-Weber, Andrea Böschen, Carla Sernow, and Stephanie Steudte for experimental support and Maria Kaliner for her help finalizing the manuscript. Tobias Schulz acknowledges the financial support from "Fonds der Chemischen Industrie".

REFERENCES

(1) Plechkova, N. V; Seddon, K. R. Applications of ionic liquids in the chemical industry. *Chem. Soc. Rev.* **2008**, *37*, 123−150.

(2) Simka, W.; Puszczyk, D.; Nawrat, G. Electrodeposition of metals from non-aqueous solutions. *Electrochim. Acta* **2009**, *54*, 5307−5319.

(3) Parvulescu, V. I.; Hardacre, C. Catalysis in Ionic Liquids. *Chem. Rev.* **2007**, *107*, 2615−2665.

(4) Fort, D. A.; Remsing, R. C.; Swatloski, R. P.; Moyna, P.; Moyna, G.; Rogers, R. D. Can ionic liquids dissolve wood? Processing and analysis of lignocellulosic materials with 1-n-butyl-3-methylimidazolium chloride. *Green Chem.* **2007**, *9*, 63−69.

(5) Stoimenovski, J.; MacFarlane, D. R.; Bica, K.; Rogers, R. D. Crystalline vs. ionic liquid salt forms of active pharmaceutical ingredients: A position paper. *Pharm. Res.* **2010**, *27*, 521−526.

(6) Yao, C.; Pitner, W. R.; Anderson, J. L. Ionic liquids containing the tris(pentafluoroethyl)trifluorophosphate anion: A new class of highly selective and ultra hydrophobic solvents for the extraction of polycyclic aromatic hydrocarbons using single drop microextraction. *Anal. Chem.* **2009**, *81*, 5054−5063.

(7) Ahrens, S.; Peritz, A.; Strassner, T. Tunable aryl alkyl ionic liquids (TAAILs): The next generation of ionic liquids. *Angew. Chem., Int. Ed.* **2009**, *48*, 7908−7910.

(8) Frisch, M. J.; Pople, J. A.; Binkley, J. S. Self-consistent molecular orbital methods 25. Supplementary functions for Gaussian basis sets. *J. Chem. Phys.* **1984**, *80*, 3265−3269.

(9) Gaussian 03 Literature Citation. http://www.gaussian.com/g_misc/g03/citation_g03.htm (accessed October 29, 2012).

(10) Frisch, M.; Trucks, G.; Schlegel, H.; Scuseria, G.; Robb, M.; Cheeseman, J.; Montgomery, J.; Vreven, T.; Kudin, K.; Burant, J.; Millam, J.; Iyengar, S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J.; Hratchian, H.; Cross, J.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R.; Yazyev, O.; Austin, A.; Cammi, R.; Pomelli, C.; Ochterski, J.; Ayala, P.; Morokuma, K.; Voth, G.; Salvador, P.; Dannenberg, J.; Zakrzewski, V.; Dapprich, S.; Daniels, A.; Strain, M.; Farkas, O.; Malick, D.; Rabuck, A.; Raghavachari, K.; Foresman, J.; Ortiz, J.; Cui, Q.; Baboul, A.; Clifford, S.; Cioslowski, J.; Stefanov, B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R.; Fox, D.; Keith, T.; Laham, A.; Peng, C.; Nanayakkara, A.; Challacombe, M.; Gill, P.; Johnson, B.; Chen, W.; Wong, M.; Gonzalez, C.; Pople, J. Gaussian 03, Revision C.02, 2003.

(11) Dennington, R.; Keith, T.; Millam, J. GaussView, Version 5, 2009.

(12) Schulz, T.; Ahrens, S.; Meyer, D.; Allolio, C.; Peritz, A.; Strassner, T. Electronic effects of para-substitution on the melting points of TAAILs. *Chem.–Asian J.* **2011**, *6*, 863−867.

(13) Strassner, T.; Schulz, T.; Bernhard, G.; Raff, J.; Lehmann, F. Patent PCT/EP2012/064940, 2011.

(14) Anastas, P. T.; Warner, J. C. *Green Chemistry: Theory and Practice*; Oxford University Press: New York, 2000

(15) Bernot, R. J.; Brueseke, M.; Evans-White, M.; Lamberti, G. Acute and chronic toxicity of imidazolium-based ionic liquids on *Daphnia magna*. *Environ. Toxicol. Chem.* **2005**, *24*, 87−92.

(16) Steudte, S.; Stepnowski, P.; Cho, C.-W.; Thöming, J.; Stolte, S. (Eco)toxicity of fluoro-organic and cyano-based ionic liquid anions. *Chem. Commun.* **2012**, 10−12.

(17) Latała, 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*. *Aquat. Toxicol.* **2005**, *73*, 91−98.

(18) Ranke, J.; Stolte, S.; Störmann, R.; Arning, J.; Jastorff, B. Design of sustainable chemical products–the example of ionic liquids. *Chem. Rev.* **2007**, *107*, 2183−2206.

(19) UFT/Merck Ionic Liquids Biological Effects Database. Center for Environmental Research and Sustainable Technology. http://www.il-eco.uft.uni-bremen.de/ (accessed March 4, 2013).

(20) 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.

(21) Stolte, S.; Arning, J.; Bottin-Weber, U.; Matzke, M.; Stock, F.; Thiele, K.; Uerdingen, M.; Welz-Biermann, U.; Jastorff, B.; Ranke, J. Anion effects on the cytotoxicity of ionic liquids. *Green Chem.* **2006**, *8*, 621−629.

(22) Arning, J.; Stolte, S.; Böschen, A.; Stock, F.; Pitner, W.-R.; Welz-Biermann, U.; Jastorff, B.; Ranke, J. Qualitative and quantitative structure activity relationships for the inhibitory effects of cationic head groups, functionalised side chains and anions of ionic liquids on acetylcholinesterase. *Green Chem.* **2008**, *10*, 47−58.

(23) Ranke, J.; Müller, A.; Bottin-Weber, U.; Stock, F.; Stolte, S.; Arning, J.; Störmann, R.; Jastorff, B. Lipophilicity parameters for ionic liquid cations and their correlation to in vitro cytotoxicity. *Ecotoxicol. Environ. Saf.* **2007**, *67*, 430−438.

(24) Stolte, S.; Matzke, M.; Arning, J.; Böschen, 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.

(25) Leonetti, F.; Catto, M.; Nicolotti, O.; Pisani, L.; Cappa, A.; Stefanachi, A.; Carotti, A. Homo- and hetero-bivalent edrophonium-like ammonium salts as highly potent, dual binding site AChE inhibitors. *Bioorg. Med. Chem.* **2008**, *16*, 7450−7456.

(26) García-Lorenzo, A.; Tojo, E.; Tojo, J.; Teijeira, M.; Rodríguez-Berrocal, F. J.; González, M. P.; Martínez-Zorzano, V. S. Cytotoxicity of selected imidazolium-derived ionic liquids in the human Caco-2 cell line. Sub-structural toxicological interpretation through a QSAR study. *Green Chem.* **2008**, *10*, 508−516.

(27) 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.

(28) Zacharias, N.; Dougherty, D. A. Cation-pi interactions in ligand recognition and catalysis. *Trends Pharmacol. Sci.* **2002**, *23*, 281−7.

(29) Boethling, R. S.; Sommer, E.; DiFiore, D. Designing small molecules for biodegradability. *Chem. Rev.* **2007**, *107*, 2207−2227.

(30) 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.

(31) Stock, F.; Hoffmann, J.; Ranke, J.; Störmann, R.; Ondruschka, B.; Jastorff, B. Effects of ionic liquids on the acetylcholinesterase − A structure-activity relationship consideration. *Green Chem.* **2004**, *6*, 286−290.

(32) Backhaus, T.; Scholze, M.; Grimme, L. The single substance and mixture toxicity of quinolones to the bioluminescent bacterium Vibrio fischeri. *Aquat. Toxicol.* **2000**, *49*, 49−61.

(33) Ranke, J.; Mölter, 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.
(34) Docherty, K. M.; Dixon, J. K.; Kulpa, C. F. Biodegradability of imidazolium and pyridinium ionic liquids by an activated sludge microbial community. *Biodegradation* **2007**, *18*, 481−493.