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A new generation of aprotic yet Brønsted acidic imidazolium salts: effect of ester/amide groups in the C-2, C-4 and C-5 on antimicrobial toxicity and biodegradation†

Rohitkumar G. Gore,[a] Lauren Myles,[b] Marcel Spulak,[c] Ian Beadham,[a] M. Teresa Garcia,[d] Stephen J. Connon*[b] and Nicholas Gathergood*[a]

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Imidazolium Brønsted acidic catalysts substituted with ester/amides have been assessed for both antimicrobial toxicity and biodegradation. Low toxicity to a screen of 20 microbial strains (12 fungi and 8 bacteria) was demonstrated. Imidazolium salts incorporating either ester or amide groups at N-1, C-2, C-4 and C-5 did not pass the readily biodegradable test (ISO 14593). Catalyst selection based on Traffic Signal Light classification of performance, (eco)toxicity, and efficient synthesis is described.

Over the last decade, ionic liquids (ILs) have been extensively investigated as potential replacements for volatile organic compounds (VOCs) for use as (*inter alia*) both tunable reaction media and catalytic solvents.[1] Much of this interest has been focussed on the development of ILs as alternative, 'green' materials; with applications in processes as diverse as ionic compressors, the BASIL™ process and electroplating.[1q] Immense interest in the environmental impact of ILs[2] has led to a plethora of papers dealing with (a) their toxicity[3] (for example antibacterial and antifungal),[4] (b) the importance of biodegradation studies[5] (something only recognised since 2002),[6] and recently (c) bioaccumulation and metabolite identification studies.[7]

The design of a 'green' compound, be it as a solvent,[1] reagent or catalyst[8] should ideally address issues such as low toxicity and ready biodegradability without the generation of toxic, persistent metabolites. *Of equal importance is the functional performance of the environmentally benign material*. The decision to replace a 'toxic' chemical with a 'green' alternative is easier if a performance benefit is also attained. The role of a green chemist (in our view) is to make this decision as easy as possible and to avoid the 'gray area' where environmental protection comes at a performance cost.

By utilising the toxicity and ecotoxicity data available for ILs (in particular those associated with imidazolium and pyridinium salts[3–7]) and the extensive precedent of heterocyclic based reagents and catalysts, we aim to design and prepare catalytically competent green compounds.

The vast majority of ILs screened for biodegradation have failed the test (*e.g.* 'CO_2 Headspace' test (ISO 14593), <60% CO_2 evolution after 28 days).[5] While this does not mean that these ILs are certain to persist in the environment, it does highlight the need to determine structural features that facilitate biodegradation (Fig. 1).[9] Bioaccumulation is also an

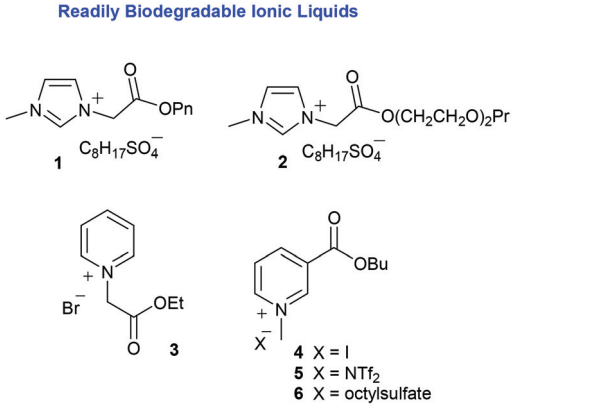
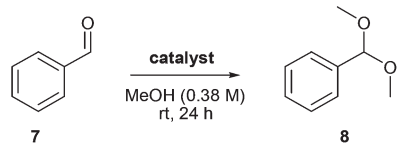


Fig. 1 ILs which 'pass' the CO_2 Headspace test.

[a]*School of Chemical Sciences and National Institute for Cellular Biotechnology, Dublin City University, Glasnevin, Dublin 9, Ireland. E-mail: Nick.Gathergood@dcu.ie; Fax: +353 17005503*
[b]*School of Chemistry, Centre for Synthesis and Chemical Biology, Trinity Biomedical Sciences Institute, University of Dublin, Trinity College, Dublin 2, Ireland. E-mail: connons@tcd.ie; Fax: +353 16712826*
[c]*Department of Inorganic and Organic Chemistry, Charles University, Faculty of Pharmacy, Heyrovského 1203, CZ-500 03 Hradec Králové, Czech Republic*
[d]*Department of Surfactant Technology, IQAC-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain*

†Electronic supplementary information (ESI) available: Determination of Green Chemistry Metrics for Tables 13 and 14. See DOI: 10.1039/c3gc40992a



Scheme 1 Preliminary experiment, 0.1–10 mol% catalyst loadings.[15]

important consideration – caution must be taken when considering primary biodegradation data[5] and recalcitrant breakdown products formed in other biodegradation assays (*i.e.* primary *vs.* readily *vs.* ultimate biodegradation). Work by Doherty[7c] and others[7a,b] has brought attention to this equally important consideration. Gathergood[10] and Scammells[11] have identified functional groups which can both reduce antimicrobial toxicity[10,14] and promote the biodegradation[6,10,11] of ILs.

Our inspiration for this work came from the serendipitous discovery that *N*-alkyl pyridinium ions could catalyse the acetalisation of benzaldehyde in the absence of any discernible acidic species in solution.[12,13] We later demonstrated that this phenomenon was also observed in the case of *N*-alkyl imidazolium ions, which allowed the design of a suite of salts capable of promoting the acetalisation of aldehydes at low catalyst loadings (*e.g.* 5–10 mol%).[14]

Herein we disclose the concurrent assessment of 'green chemistry metrics' including toxicity, ecotoxicity and the application of these results to assist the development of low toxicity and biodegradable ILs. The catalyst optimisation study based on activity and substrate scope is presented in the preceding paper in this journal (Scheme 1).[15]

Experimental

Antifungal activity – experimental method

In vitro antifungal activities of the compounds were evaluated on a panel of four ATCC strains (*Candida albicans* ATCC 44859, *Candida albicans* ATCC 90028, *Candida parapsilosis* ATCC 22019, *Candida krusei* ATCC 6258) and eight clinical isolates of yeasts (*Candida krusei* E28, *Candida tropicalis* 156, *Candida glabrata* 20/I, *Candida lusitaniae* 2446/I, *Trichosporon asahii* 1188) and filamentous fungi (*Aspergillus fumigatus* 231, *Absidia corymbifera* 272, *Trichophyton mentagrophytes* 445) from the collection of fungal strains deposited at the Department of Biological and Medical Sciences, Faculty of Pharmacy, Charles University, Hradec Králové, Czech Republic. Three ATCC strains were used as the quality control strains. All the isolates were maintained on Sabouraud dextrose agar prior to being tested.

Minimum inhibitory concentrations (MICs) were determined by modified CLSI standard of microdilution format of the M27-A3 and M38-A2 documents.[16,17] Dimethyl sulfoxide (100%) served as a diluent for all compounds; the final concentration did not exceed 2%. RPMI 1640 (Sevapharma, Prague) medium supplemented with L-glutamine and buffered with 0.165 M morpholinepropanesulfonic acid (Serva) to pH 7.0 by 10 M NaOH was used as the test medium. The wells of the microdilution tray contained 200 μL of the RPMI 1640 medium with 2-fold serial dilutions of the compounds (2000 to 0.488 μmol L^{-1} for the new compounds) and 10 μL of inoculum suspension. Fungal inoculum in RPMI 1640 was prepared to give a final concentration of $5 \times 10^3 \pm 0.2$ cfu mL^{-1}. The trays were incubated at 35 °C and MICs were read visually after 24 h and 48 h. The MIC values for the dermatophytic strain (*T. mentagrophytes*) were determined after 72 h and 120 h. The MICs were defined as 80% inhibition (IC_{80}) of the growth of control for yeasts and as 50% inhibition (IC_{50}) of the growth of control for filamentous fungi. MICs were determined twice and in duplicate. The deviations from the usually obtained values were no higher than the nearest concentration value up and down the dilution scale.

Antibacterial activity – experimental method

In vitro antibacterial activities[18] of the compounds were evaluated on a panel of three ATCC strains (*Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027) and five clinical isolates (*Staphylococcus aureus* MRSA HK5996/08, *Staphylococcus epidermidis* HK6966/08, *Enterococcus* sp. HK14365/08, *Klebsiella pneumoniae* HK11750/08, *Klebsiella pneumoniae* ESBL HK14368/08) from the collection of fungal strains deposited at the Department of Biological and Medical Sciences, Faculty of Pharmacy, Charles University, Hradec Králové, Czech Republic. The above-mentioned ATCC strains also served as the quality control strains. All the isolates were maintained on Mueller-Hinton agar prior to being tested.

Dimethyl sulfoxide (100%) served as a diluent for all compounds; the final concentration did not exceed 2%. Mueller-Hinton agar (MH, HiMedia, Čadersky-Envitek, Czech Republic) buffered to pH 7.4 (±0.2) was used as the test medium. The wells of the microdilution tray contained 200 μL of the Mueller-Hinton medium with 2-fold serial dilutions of the compounds (2000 to 0.488 μmol L^{-1}) and 10 μL of inoculum suspension. Inoculum in MH medium was prepared to give a final concentration of 0.5 McFarland scale (1.5×10^8 cfu mL^{-1}). The trays were incubated at 37 °C and MICs were read visually after 24 h and 48 h. The MICs were defined as 95% inhibition of the growth of control. MICs were determined twice and in duplicate. The deviations from the usually obtained values were no higher than the nearest concentration value up and down the dilution scale.

Biodegradation method – CO_2 Headspace test

To evaluate the biodegradability of the test salts, the 'CO_2 Headspace' test (ISO 14593) was applied.[19] This method allows the evaluation of the ultimate aerobic biodegradability of an organic compound in aqueous medium at a given concentration of microorganims by analysis of inorganic carbon. The test compound, as the only source of carbon and energy, was added to a buffer-mineral salts medium which had been inoculated with a mixed population of microorganisms

derived from an activated sludge collected from a sewage treatment plant to give a final organic carbon concentration of 20 mg L^{-1}. These solutions were incubated in sealed vessels with a headspace of air, which provided a reservoir of oxygen for aerobic biodegradation. Biodegradation (mineralization to carbon dioxide) was determined by measuring the net increase in total inorganic carbon (TIC) levels over time compared to unamended blanks. Sodium *n*-dodecyl sulfate (SDS) was used as a reference substance. The test ran for 28 days. The extent of biodegradation was expressed as a percentage of the theoretical amount of inorganic carbon (ThID) based on the amount of compound added initially. Assuming 100% mineralization of the test compound, the theoretical amount of inorganic carbon (ThID) in excess of that produced in the blank controls equals the amount of total organic carbon (TOC) added as the test compound to each vessel at the start of the test, that is: ThIC = TOC.

Percentage biodegradation D_t in each case is given by:

$$D_t = \frac{(TIC_t - TIC_b)}{TOC_i} \times 100$$

where TIC_t is the TIC, in milligrams, in test vessel at time *t*; TIC_b is the mean TIC, in milligrams, in blank control vessels at time *t* TOC_i is the TOC, in milligrams, initially added to the test vessel.

Results and discussion

Our previous studies[12–14] included preliminary antifungal and antibacterial screening data for three representative Brønsted acidic ILs (BAILs) **9**, **10** and **14**.[14] Herein we report the complete antimicrobial study for the library of imidazolium salts **9–19** (Fig. 2). In addition, two pyridinium salts **20** and **21** were also screened for catalytic activity in the acetalisation of benzylaldehyde with methanol[12–14] and are included in this toxicity and ecotoxicity assessment.

BAILs (previously reported imidazolium and pyridinium salts by Gathergood and Connon *et al.*)[12–14]

Antimicrobial screening results for **9–21** are shown in Tables 1 and 2. *In vitro* antifungal activities (Table 1) of **9–21** were evaluated on a panel of four ATCC strains (*Candida albicans* ATCC 44859, *Candida albicans* ATCC 90028, *Candida parapsilosis* ATCC 22019, *Candida krusei* ATCC 6258) and eight clinical isolates of yeasts (*Candida krusei* E28, *Candida tropicalis* 156, *Candida glabrata* 20/I, *Candida lusitaniae* 2446/I, *Trichosporon beigelii* 1188) and filamentous fungi (*Aspergillus fumigatus* 231, *Absidia corymbifera* 272, *Trichophyton mentagrophytes* 445). Antifungal activity was not observed for **9–21** at the highest concentration screened (MIC > 2.0 mM).

In vitro antibacterial activities (Table 2) of the compounds **9–21** were evaluated on a panel of three ATCC strains (*Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027) and five clinical isolates (*Staphylococcus aureus* MRSA HK5996/08, *Staphylococcus*

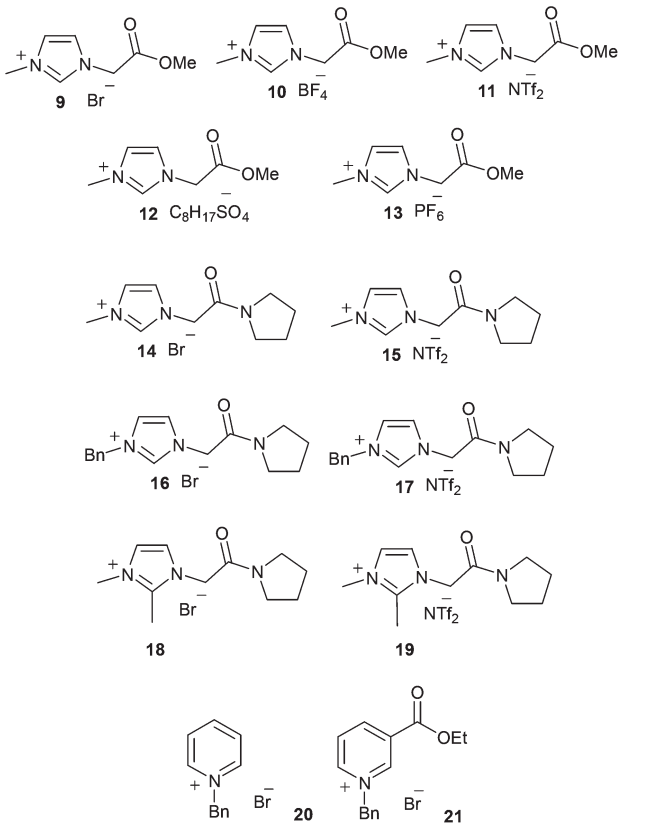


Fig. 2 Previously reported imidazolium and pyridinium salts studied as BAILs.[12–14]

Table 1 Antifungal activity of previously reported BAILs[12–14] (MIC [mM])

Organisms	Time (h)	Esters **9–13**	Me amide **14, 15, 18, 19**	Bn amide **16–17**	PyBr **20, 21**
Candida albicans	24	>2.0	>2.0	>2.0	>2.0
ATCC 44859	48	>2.0	>2.0	>2.0	>2.0
Candida albicans	24	>2.0	>2.0	>2.0	>2.0
ATCC 90028	48	>2.0	>2.0	>2.0	>2.0
Candida parapsilosis	24	>2.0	>2.0	>2.0	>2.0
ATCC 22019	48	>2.0	>2.0	>2.0	>2.0
Candida krusei	24	>2.0	>2.0	>2.0	>2.0
ATCC 6258	48	>2.0	>2.0	>2.0	>2.0
Candida krusei	24	>2.0	>2.0	>2.0	>2.0
E28	48	>2.0	>2.0	>2.0	>2.0
Candida tropicalis	24	>2.0	>2.0	>2.0	>2.0
156	48	>2.0	>2.0	>2.0	>2.0
Candida glabrata	24	>2.0	>2.0	>2.0	>2.0
20/I	48	>2.0	>2.0	>2.0	>2.0
Candida lusitaniae	24	>2.0	>2.0	>2.0	>2.0
2446/I	48	>2.0	>2.0	>2.0	>2.0
Trichosporon beigelii	24	>2.0	>2.0	>2.0	>2.0
1188	48	>2.0	>2.0	>2.0	>2.0
Aspergillus fumigatus	24	>2.0	>2.0	>2.0	>2.0
231	48	>2.0	>2.0	>2.0	>2.0
Absidia corymbifera	24	>2.0	>2.0	>2.0	>2.0
272	48	>2.0	>2.0	>2.0	>2.0
Trichophyton	72	>2.0	>2.0	>2.0	>2.0
mentagrophytes 445	120	>2.0	>2.0	>2.0	>2.0

Table 2 Antibacterial activity of previously reported[12–14] BAILs (MIC [mM])

Organisms	Time (h)	Esters **9–13**	Me amide **14, 15, 18, 19**	Bn amide **16–17**	PyBr **20, 21**
S. aureus ATCC 6538	24	>2.0	>2.0	>2.0	>2.0
	48	>2.0	>2.0	>2.0	>2.0
S. aureus HK 5996/08	24	>2.0	>2.0	>2.0	>2.0
	48	>2.0	>2.0	>2.0	>2.0
S. epidermidis HK 6966/08	24	>2.0	>2.0	>2.0	>2.0
	48	>2.0	>2.0	>2.0	>2.0
Enterococcus sp. HK 14365/08	24	>2.0	>2.0	>2.0	>2.0
	48	>2.0	>2.0	>2.0	>2.0
E. coli ATCC 8739	24	>2.0	>2.0	>2.0	>2.0
	48	>2.0	>2.0	>2.0	>2.0
Klebsiella pneumoniae HK 11750/08	24	>2.0	>2.0	>2.0	>2.0
	48	>2.0	>2.0	>2.0	>2.0
Klebsiella pneumonia ESBL HK 14368/08	24	>2.0	>2.0	>2.0	>2.0
	48	>2.0	>2.0	>2.0	>2.0
Pseudomonas aeruginosa ATCC 9027	24	>2.0	>2.0	>2.0	>2.0
	48	>2.0	>2.0	>2.0	>2.0

epidermidis HK6966/08, *Enterococcus* sp. HK14365/08, *Klebsiella pneumoniae* HK11750/08, *Klebsiella pneumoniae* ESBL HK14368/08). Antimicrobial activity was not observed for **9–21** at the highest concentration screened (MIC > 2.0 mM).

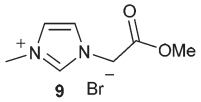
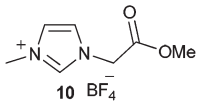
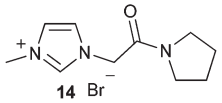
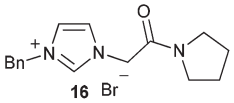
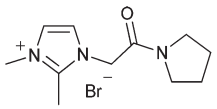
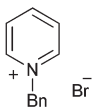
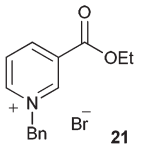
Antimicrobial activity of imidazolium and pyridinium ILs has been reported previously, with increasing toxicity observed across a series of alkyl (C6–C14) substituted methylimidazolium chlorides.[3,20] Gilmore *et al.*[3c] screened 1-hexyl-3-methylimidazolium chloride for antimicrobial activity against a number of pathogens (cocci, rods and fungi) with MIC values of >1.644 mM observed. The data from the screening of **9–21** in Tables 1 and 2 are consistent with Gilmore's results, although it is noted that different strains of organisms are investigated in the two studies. By avoiding the introduction of long (either linear or branched) lipophilic alkyl chains into the salts structure we postulate that undesirable antimicrobial and antifungal toxicity has been limited. No effect on antimicrobial toxicity of the counterion (Br, BF_4, PF_6, NTf_2, octyl sulfate) was observed when comparing the methyl ester series (**9–13**). Salts with either methyl ester (**9–13**) or pyrolidine amide (**14–19**) groups in the side chain showed low toxicity to the strains screened (Tables 1 and 2). Noteworthy is that the more lipophilic imidazolium salts (**16–19**), containing an addition methyl group in the C-2 position (**18–19**), or benzyl group at C-3 (**16–17**) also have low antimicrobial toxicity.

Furthermore, the benzyl substituted pyridinium salts (**20–21**) were not highly toxic to the bacteria and fungi strains screened (upto [2 mM]). This suggests that while the installation of long aliphatic hydrocarbon chains usually leads to ILs with high antimicrobial toxicity,[3] aryl (*e.g.* benzyl) substituents are compatible with our design philosophy to prepare less toxic (as defined as lower antimicrobial toxicity) catalysts.

To evaluate the biodegradability of the test salts, the 'CO_2 Headspace test' (ISO 14593)[19] was implemented (Table 3). This method allows the evaluation of the ultimate aerobic biodegradability of an organic compound in an aqueous medium at a given concentration of microorganisms by analysis of inorganic carbon. The test compound, as the sole source of both carbon and energy, was added at a concentration of 40 mg L^{-1} to a mineral salt medium. These solutions were inoculated with activated sludge collected from an activated sludge treatment plant, washed and aerated prior to use and incubated in sealed vessels with a headspace of air. Biodegradation (mineralisation to carbon dioxide) was determined by measuring the net increase in total organic carbon (TOC) levels over time.

Salts **9**, **10**, **14**, **16**, **18**, **20** and **21** did not pass the CO_2 Headspace test (Table 3; >60% required to pass). Imidazolium salts containing a methyl ester (**9** and **10**) gave low biodegradation, 10 and 14% respectively after 28 days (entries 1–2). No significant effect is observed between the bromide and BF_4 counterion. As both these anions do not contribute to the carbon dioxide evolved on breakdown, the propensity for the cation to biodegrade is determined. Hydrolysis of the methyl ester, and the conversion of a single carbon in **9** and **10** to CO_2 can account for the degree of biodegradation observed. Low to negligible biodegradation was observed, with amide examples **14**, **16** and **18** resistant to breakdown (entries 3–5). The presence of the amide and benzyl group in **16** (entry 4), did not facilitate breakdown under the conditions of the test. This correlates well with both our previous results, and studies from other groups, where amides[6b] and benzyl[7a,11a] substituents did not lead to enhanced biodegradation. Reference innoculum toxicity experiments performed concurrently with the biodegradation tests confirm that salts **9**, **10**, **14**, **16**, **18**, **20** and **21** were non-toxic (not inhibitory) to the innoculum, albeit with poor biodegradation (entries 1–7). As is apparent due the increased stability of the amide *vs.* methyl ester, salts **14**, **16** and **18** are postulated to remain intact during the biodegradation test. All the biodegradation data in Table 3 for the amide and ester-based salts (*i.e.* **9**, **10**, **14**, **16** and **18**) suggest that the imidazolium core is not cleaved during the test (entries 1–5). Analogous trends are observed with pyridinium salts **20** and **21** (entries 6–7). Only 2% biodegradation was observed for benzylpyridinium bromide (**20**), the benzyl moiety not participating in significant breakdown (nor the pyridinium ring). The 25% biodegradation observed for **21** – a loss of approximately two carbon atoms[5b] – can be reasonably attributed to metabolic action on the ethyl ester group. This is consistent with the benzyl moiety resisting breakdown, however one must avoid 'dissecting' an ILs structure into regions and labelling them 'biodegradable and non-biodegradable'. A case in point is the comparison of the readily biodegradable **4** with **21**. For **4** to pass the CO_2 Headspace test (74%), carbon from the pyridinium ring must be released as CO_2. If we assume for both **4** and **21**, hydrolysis of the ester is the first step (100% primary biodegradation), then the two metabolites are closely related (**4**, *N*-methyl *vs.* **21**, *N*-benzyl). However, with **4**, the carboxylic acid/carboxylate metabolite is readily biodegradable, but with **21** no further breakdown is observed. This reaffirms the requisite to obtain biodegradation (and indeed toxicity data) for

Table 3 Biodegradation study (CO_2 Headspace test) of previously reported[12–14] BAILs

Entry	IL	Time 6 days	14 days	21 days	28 days	CL (95%)
1	 **9**	14	14	11	10	9–10[a]
2	 **10**	12	12	13	14	13–15[a]
3	 **14**	1	3	4	3	2–4[a]
4	 **16**	0	0	0	0	0 ± 1.2[b]
5	 **18**	0	1	0	3	0–5[a]
6	 **20**	0	0	0	2	1–4[c]
7	 **21**	8	17	25	25	17–33[c]

[a] SDS reference 28 days 85% (95% CL = 80–91%), confidence limits (CL) were calculated from 5 replicates. [b] SDS reference 28 days 94% (95% CL = 90–98%), confidence limits (CL) were calculated from 4 replicates. [c] SDS reference 28 days 80% (95% CL = 76–84%), confidence limits (CL) were calculated from 5 replicates.

specific chemicals. While predictive tools and models are useful,[21] these need to be supported by experimental data, especially where the chemicals have potential to be used as reagents, catalysts or solvents.

Our previous biodegradation studies have included octyl sulphate as the anion.[5a,b] In the current catalysis/eco(toxicity) study,[14,15] this anion was shown to have a dramatic effect on the performance of the BAIL in the acetalisation reaction of benzylaldehyde with methanol, with only 12% yield using 1 mol% catalyst.[15] As part of this project the decision was made to make only one more octyl sulphate salt (*i.e.* **23**) to confirm the counterion effect on catalyst performance.[15] All the following biodegradation data is derived from catalysts which are halide salts, and BF_4 or NTf_2 variants in cases where these derivatives are more active promoters of the acetalisation reaction[15] and thus warrant compound specific (eco)toxicity screening.

C2-substituted imidazolium salts

We began with the synthesis of a library of imidazolium salts **22–29** incorporating an electron withdrawing group positioned at C-2 (Fig. 3). We also included structures with and without pendant ester/amide functionality in the *N*-alkyl substituent. These materials were evaluated as promoters of the room temperature acetalisation of benzaldehyde in methanol.[15]

Antifungal activity was not observed for **22–29** at the highest concentration screened (MIC > 2.0 mM, Table 4). Antibacterial activity was also not observed for **22–29** at the highest concentration screened (MIC > 2.0 mM, Table 5). Functional

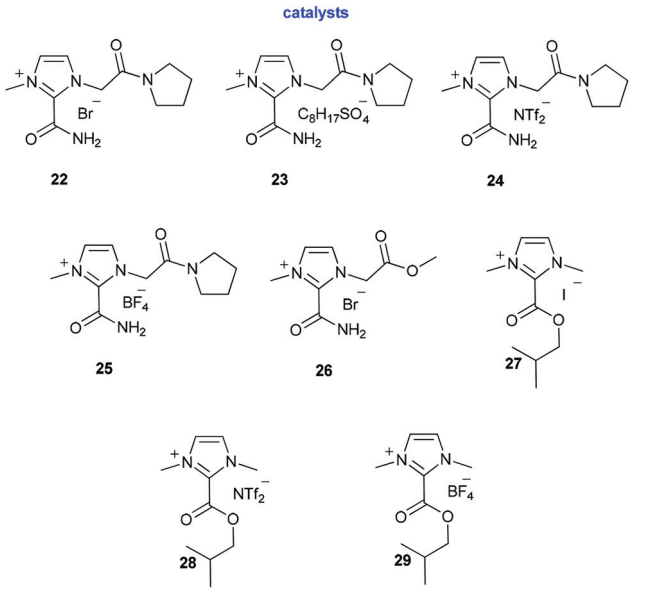


Fig. 3 C-2 substituted imidazolium salts studied as BAILs.

Table 4 Antifungal activity of C-2 substituted imidazolium salts studied as BAILs (MIC [mM])

Organisms	Time (h)	C2-amides **22–26**	C2-esters **27–29**
Candida albicans ATCC 44859	24	>2.0	>2.0
	48	>2.0	>2.0
Candida albicans ATCC 90028	24	>2.0	>2.0
	48	>2.0	>2.0
Candida parapsilosis ATCC 22019	24	>2.0	>2.0
	48	>2.0	>2.0
Candida krusei ATCC 6258	24	>2.0	>2.0
	48	>2.0	>2.0
Candida krusei E28	24	>2.0	>2.0
	48	>2.0	>2.0
Candida tropicalis 156	24	>2.0	>2.0
	48	>2.0	>2.0
Candida glabrata 20/I	24	>2.0	>2.0
	48	>2.0	>2.0
Candida lusitaniae 2446/I	24	>2.0	>2.0
	48	>2.0	>2.0
Trichosporon beigelii 1188	24	>2.0	>2.0
	48	>2.0	>2.0
Aspergillus fumigatus 231	24	>2.0	>2.0
	48	>2.0	>2.0
Absidia corymbifera 272	24	>2.0	>2.0
	48	>2.0	>2.0
Trichophyton mentagrophytes 445	72	>2.0	>2.0
	120	>2.0	>2.0

Table 5 Antibacterial activity of C-2 substituted imidazolium salts studied as BAILs (MIC [mM])

Organisms	Time (h)	C2-amides **22–26**	C2-esters **27–29**
S. aureus	24	>2.0	>2.0
ATCC 6538	48	>2.0	>2.0
S. aureus	24	>2.0	>2.0
HK 5996/08	48	>2.0	>2.0
S. epidermidis	24	>2.0	>2.0
HK 6966/08	48	>2.0	>2.0
Enterococcus sp.	24	>2.0	>2.0
HK 14365/08	48	>2.0	>2.0
E. coli	24	>2.0	>2.0
ATCC 8739	48	>2.0	>2.0
Klebsiella pneumoniae HK	24	>2.0	>2.0
11750/08	48	>2.0	>2.0
Klebsiella pneumonia ESBL	24	>2.0	>2.0
HK 14368/08	48	>2.0	>2.0
Pseudomonas aeruginosa	24	>2.0	>2.0
ATCC 9027	48	>2.0	>2.0

groups tolerated by the fungi and bacteria strains screened include:

• $-CH_2$amide ($-CH_2CONC_4H_8$) at N-1, amide ($-CONH_2$) at C-2, and alkyl (methyl) at C-3; bromide (**22**), octyl sulphate (**23**), NTf_2 (**24**), BF_4 (**25**),

• $-CH_2$ester ($-CH_2CO_2CH_3$), amide ($-CONH_2$) at C-2, and alkyl (methyl) at C-3; bromide (**26**),

• alkyl (methyl) at N-1 and C-3, ester (iso-butyl) at C-2; iodide (**27**), NTf_2 (**28**), BF_4 (**29**).

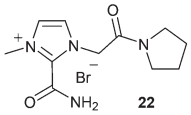
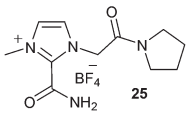
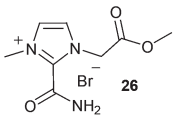
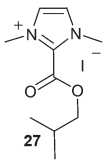
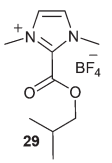
We were pleased that substitution with an iso-butyl ester or primary amide in the C-2 position of the imidazolium salts, for a range of N-1 and C-3 substituted examples, did not increase antimicrobial toxicity.

Based on antimicrobial toxicity alone, all C-2 substituted salts **22–29** are suitable for biodegradation studies. No catalyst was removed from the biodegradation study due to an undesirably high antimicrobial toxicity. As the presence of the octyl sulphate counteranion (*i.e.* **23**) did not confer a clear advantage in catalyst performance *vs.* either its bromide derivative (*i.e.* **22**) or other C-2 substituted imidazolium catalysts,[15] **23** was not included in the biodegradation study. Biodegradation data associated with the bromide **22** enabled a direct comparison of biodegradation data from other salts containing inorganic anions. Halide and BF_4^- bearing salts **22**, **25**, **26**, **27**, and **29**, were selected for biodegradation studies to investigate the effect of ester and amide groups at C-2 (Table 6).

Biodegradation data for **22**, **25**, **26**, **27**, and **29** are shown in Table 6. We were disappointed to find that none of the salts passed the CO_2 Headspace test. Introduction of either a primary amide (**22**, **25** and **26**, entries 1–3) or an iso-butyl ester (*i.e.* **27** and **29**, entries 4 and 5) at C-2 did not lead to significant breakdown of the salt.

Amides **22**, **25** and **26** proved the least biodegradable (12, 14 and 17% respectively, after 28 days, entries 1–3). C-2 esters **27** and **29** gave moderate levels of biodegradation (30 and 35%, respectively, entries 4–5) after 28 days. These values are similar to other CO_2 Headspace data for *n*-alkyl ester

Table 6 Biodegradation (CO_2 Headspace test) C-2 substituted imidazolium salts studied as BAILs

Entry	IL	Time 6 days	14 days	21 days	28 days	CL (95%)
1	 **22**	2	7	8	12	11–13[a]
2	 **25**	7	12	11	14	11–17[b]
3	 **26**	9	13	10	17	9–24[a]
4	 **27**	24	31	32	30	26–34[c]
5	 **29**	26	31	30	35	34–36[a]

[a] SDS reference 28 days 80% (95% CL = 76–84%). [b] SDS reference 28 days 90% (95% CL = 85–95%). [c] SDS reference 28 days 85% (95% CL = 80–91%), confidence limits (CL) were calculated from 5 replicates.

substituted imidazolium bromide ILs (C_3 ester 24%, C_5 ester 41%).[10a] While the C-2 amide example **26** also contains a methyl ester subunit, the increase in biodegradation 17% (*cf.* 12% for **22**) is marginal (entry 3).

The CO_2 Headspace biodegradation data outlined in Table 6 is consistent with the hypothesis that introduction of either an ester or amide at the C-2 position of the imidazolium ring does not promote cleavage of ring leading to further breakdown products and CO_2 formation. While it was clear that **22–29** represented a considerable step forward in terms of catalytic activity,[15] we were aware that the designs were not optimal. One particular cause for concern was the location of the electron-withdrawing substituent: while installing this at C-2 allows the maximum amount of both inductive and mesomeric forms of electron withdrawal to be exerted by the amide/ester moiety at the proposed site of nucleophilic attack by methanol, it also introduces a degree of counterproductive steric crowding (*i.e.* **27–29**), which would be expected to limit catalyst performance.[15] As the introduction of either an amide or an ester at C-2 had not lead to breakdown of the imidazolium ring during the biodegradation test, there was no ecotoxicological benefit to the presence of this group demonstrated. No increase in antimicrobial toxicity (Tables 4 and 5) was observed for **22–29** (*cf.* **9–21**, Tables 2 and 3).

C4-substituted imidazolium salts

It therefore seemed prudent to design a library of green catalysts characterised by the location of the electron withdrawing group at a further remove from C-2. The C-4 substituted analogues **30–35** (Fig. 4) were therefore duly prepared and antimicrobial toxicity and biodegradation determined.

Antifungal activity was not observed for **30–35** at the highest concentration screened (MIC > 2.0 mM, Table 7). Antibacterial activity was not observed for **22–29** at the highest concentration screened (MIC > 2.0 mM, Table 8). Functional groups around the N-1 CH_2CO_2Et imidazolium *motif* tolerated by the fungi and bacteria strains screened include:

• $-CH_2$ester ($-CH_2CO_2CH_2CH_3$), at C-4, and alkyl (methyl) at C-3; bromide (**30**), iodide (**31**) and BF_4 (**32**),

• $-CH_2$ester ($-CH_2CO_2CH_2CH_3$), at C-4, and benzyl at C-3; bromide (**33**), and BF_4 (**34**),

• CH_2amide ($-CH_2CONC_4H_8$) at C4, amide ($-CONH_2$) at C-2, and benzyl at C-3; bromide (**35**).

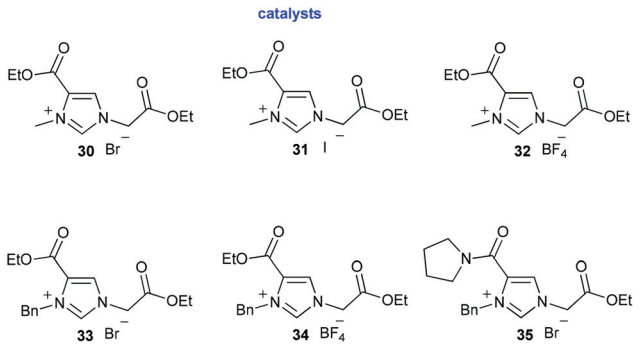


Fig. 4 C-4 substituted imidazolium salts studied as BAILs.

Table 7 Antifungal activity of C-4 substituted imidazolium salts studied as BAILs (MIC [mM])

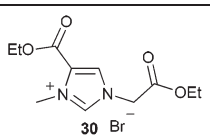
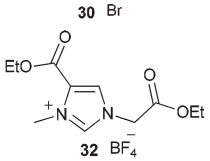
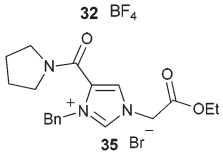
Organisms	Time (h)	C4-esters **30–34**	C4-amide **35**
Candida albicans ATCC 44859	24 48	>2.0 >2.0	>2.0 >2.0
Candida albicans ATCC 90028	24 48	>2.0 >2.0	>2.0 >2.0
Candida parapsilosis ATCC 22019	24 48	>2.0 >2.0	>2.0 >2.0
Candida krusei ATCC 6258	24 48	>2.0 >2.0	>2.0 >2.0
Candida krusei E28	24 48	>2.0 >2.0	>2.0 >2.0
Candida tropicalis 156	24 48	>2.0 >2.0	>2.0 >2.0
Candida glabrata 20/I	24 48	>2.0 >2.0	>2.0 >2.0
Candida lusitaniae 2446/I	24 48	>2.0 >2.0	>2.0 >2.0
Trichosporon beigelii 1188	24 48	>2.0 >2.0	>2.0 >2.0
Aspergillus fumigatus 231	24 48	>2.0 >2.0	>2.0 >2.0
Absidia corymbifera 272	24 48	>2.0 >2.0	>2.0 >2.0
Trichophyton mentagrophytes 445	72 120	>2.0 >2.0	>2.0 >2.0

We were pleased that substitution with either an ester or an amide at the C-4 position of the imidazolium salts, in a range of N-1 and C-3 substituted examples, did not increase antimicrobial toxicity (Tables 7 and 8). Based on antimicrobial toxicity alone, all C-2 substituted salts **30–35** are suitable for biodegradation studies. No salt was removed from the biodegradation study based on undesirable high antimicrobial toxicity. A representative subset (**30**, **32** and **35**) was screened for biodegradation (Table 9). Neither the C-4 ester- (*i.e.* **30** and **32**, entries 1 and 2) or amide- (*i.e.* **35**, entry 3) substituted imidazolium rings underwent breakdown. No toxicity to the innoculum used for the biodegradation assay was found during control experiments.

Table 8 Antibacterial activity of C-4 substituted imidazolium salts studied as BAILs (MIC [mM])

Organisms	Time (h)	C4-esters **30–34**	C4-amide **35**
S. aureus ATCC 6538	24 48	>2.0 >2.0	>2.0 >2.0
S. aureus HK 5996/08	24 48	>2.0 >2.0	>2.0 >2.0
S. epidermidis HK 6966/08	24 48	>2.0 >2.0	>2.0 >2.0
Enterococcus sp. HK 14365/08	24 48	>2.0 >2.0	>2.0 >2.0
E. coli ATCC 8739	24 48	>2.0 >2.0	>2.0 >2.0
Klebsiella pneumoniae HK 11750/08	24 48	>2.0 >2.0	>2.0 >2.0
Klebsiella pneumonia ESBL HK 14368/08	24 48	>2.0 >2.0	>2.0 >2.0
Pseudomonas aeruginosa ATCC 9027	24 48	>2.0 >2.0	>2.0 >2.0

Table 9 Biodegradation (CO_2 Headspace test) of C-4 substituted imidazolium salts studied as BAILs

Entry	IL	Time 6 days	14 days	21 days	28 days	CL (95%)
1	 **30** Br	6	5	8	10	8–11[a]
2	 **32** BF_4	2	4	3	5	4–7[b]
3	 **35** Br	0	0	0	2	0–3[a]

[a] SDS reference 28 days 80% (95% CL = 76–84%). [b] SDS reference 28 days 90% (95% CL = 85–95%). Confidence limits (CL) were calculated from 5 replicates.

C4/C5-substituted imidazolium salts

Pursuant to our search for more active catalysts,[15] 4,5-disubstituted imidazolium structures were proposed. Given the dramatic effect of the introduction of one electron withdrawing group (especially when not located at C-2) on catalyst activity, the final step in our optimisation study involved the design of catalysts characterised by the presence of two such groups at C-4 and C-5 (Fig. 5).

Salts based on the N-1 CH_2CO_2Et-substituted imidazolium ion *motif* (*i.e.* **36–40**) were selected for direct comparison to earlier catalysts activity/toxicity/biodegradation. This series of compounds contained the first examples where hydrophobicity (reduced solubility in media used for antimicrobial screening) was apparent. The solubility limit for **36** and **37** was 0.5 mM, and 1.0 mM for **40**. Salts **38** and **39** were soluble at the maximum concentration limit for the test (2.0 mM).

Antifungal activity was not observed for **36–40** at the highest concentrations screened (0.5, 1.0 or 2.0 mM, Table 10). Functional groups around the N-1 CH_2CO_2Et imidazolium motif tolerated by the fungal strains screened include:

- $-CH_2$ester ($-CH_2CO_2CH_3$), at C-4 and C-5, and alkyl (methyl) at C-3; bromide (**36**) and BF_4 (**37**),
- $-CH_2$ester ($-CH_2CO_2CH_3$), at C-4 and C-5, and benzyl at C-3; bromide (**38**), and BF_4 (**39**),
- CH_2amide ($-CH_2CONC_4H_8$) at C4 and C-5, and benzyl at C-3; bromide (**40**).

Antibacterial activity was observed for the first time for this class of compounds, however only to some Gram positive strains (Table 11). ILs **36–40** did not show toxicity to the Gram negative strains (*E. coli*, *Klebsiella pneumoniae*, *Klebsiella pneumoniae-ESBL*, *Pseudomonas aeruginosa*). The Gram positive strain most sensitive to C4/C-5 disubstituted imidazolium salts was *S. epidermidis* (MIC values 0.25 mM, **36**; 1.0 mM **39** and 2.0 **38**). Imidazolium salt **38** was also active against *S. aureus* H 5996/08 (MIC 2.0 mM), and **39** against *S. aureus* CCM 4516/08 (MIC 1.0 mM). ILs **37** and **40** did not exhibit high toxicity (IC_{95}) to all bacteria screened up to the solubility limit (0.5 mM and 1.0 mM, respectively).

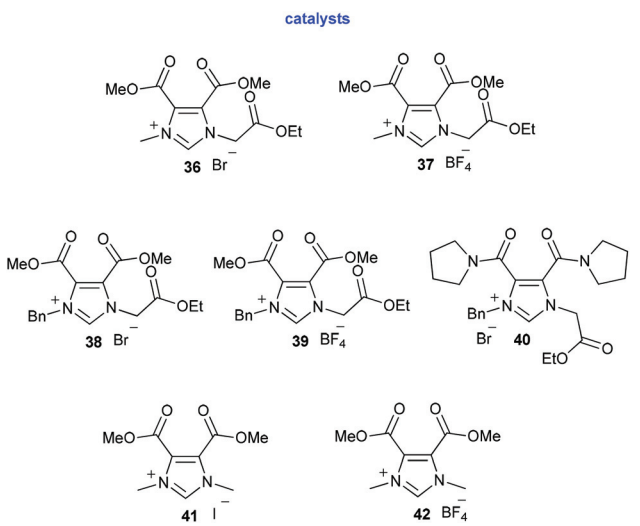


Fig. 5 C-4/C-5 substituted imidazolium salts studied as BAILs.

Biodegradation data of salts **37–40** are shown in Table 12. Results have shown similarity between C-2 substituted and C-4/C-5 disubstituted imidazolium salts. Again neither ester nor amide substitution at C-4 and C-5 on the imidazolium ring of the ILs led to passing the CO_2 Headspace test (entries 1–4). In the case of methyl ester substitution at the C-4 and C-5 positions, salts incorporating halide anions (*i.e.* **38**, entry 1) have exhibited greater degrees of biodegradation (24%), than BF_4 anion derivatives **39** (6%), after 28 days (entry 2). Also ester substitution at C-4/C-5 position did not facilitate breakdown. Salt **37** proved the most amenable to biodegradation (31% after 28 days) of the C-4/C-5 disubstituted imidazolium salts evaluated (entry 3). As expected, the amide substituted salt **40** exhibited negligible biodegradation (*i.e.* 2% after 28 days, entry 4).

A decision was made at this stage of the project to simplify the design of the catalyst. This was based on reducing the lipophilicity (thereby improving water solubility) with the aim of devising low antimicrobial toxicity catalysts. A green chemistry metrics study (*vide supra*) was running concurrently with the catalyst performance study, and the requirement for a short efficient synthesis of the catalysts was now a target. Accordingly, dimethylated catalysts **41** and **42** were prepared (Fig. 5).[15]

Salt **41** exhibited antifungal activity against *Candida albicans* ATCC 44859, *Candida albicans* ATCC 90028, *Candida parapsilosis* ATCC 22019, *Trichosporon beigelii* 1188 and *Aspergillus fumigatus* 231, *Trichophyton mentagrophytes* 445 at a concentration of 0.5 mM (Table 10). Also a MIC value of 1.0 mM was obtained against *Candida krusei* ATCC 6258, *Candida krusei* E28, *Candida tropicalis* 156, *Candida glabrata* 20/I, *Candida lusitaniae* 2446/I. The growth of *Absidia corymbifera* 272 was found to inhibit at the concentration 2.0 mM. Whereas antibacterial activity of **41** was observed at 0.5 mM concentration against *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* MRSA HK5996/08, *Staphylococcus epidermidis* HK6966/08. Also the growth of *Escherichia coli* ATCC 8739, *Enterococcus* sp. HK14365/08, *Klebsiella pneumoniae* HK11750/08, *Klebsiella pneumoniae-ESBL* HK14368/08 was inhibited at the concentration 2.0 mM. Salt **42** did not show any antifungal and antibacterial activity up to 0.5 mM, which was the maximum concentration limit due to the low solubility of the salt in the test (Table 11).

Another finding from the CO_2 Headspace test for 1,3-dimethylimidazolium salts with C-4/C-5 methyl diesters (*i.e.* **41** and **42**) was that structural simplicity (*i.e.* **41**/**42** *vs.* **36–40**) did not assist uptake into biodegradation pathways. Catalysts **41** and **42** have exhibited very poor biodegradation *i.e.* 12% and 3% respectively (Table 12, entries 5 and 6).

Green chemistry metrics

Anastas and Warner proposed the twelve principles of Green Chemistry to assist the design environmental friendly

Table 10 Antifungal activity of C-4/C-5 disubstituted imidazolium salts studied as BAILs (MIC [mM])

Organisms	Time (h)	**36**	**37**	**38**	**39**	**40**	**41**	**42**
Candida albicans	24	>0.5	>0.5	>2	>2	>1	**0.5**	>0.5
ATCC 44859	48	>0.5	>0.5	>2	>2	>1	**0.5**	>0.5
Candida albicans	24	>0.5	>0.5	>2	>2	>1	**0.5**	>0.5
ATCC 90028	48	>0.5	>0.5	>2	>2	>1	**0.5**	>0.5
Candida parapsilosis	24	>0.5	>0.5	>2	>2	>1	**0.5**	>0.5
ATCC 22019	48	>0.5	>0.5	>2	>2	>1	**0.5**	>0.5
Candida krusei	24	>0.5	>0.5	>2	>2	>1	**1**	>0.5
ATCC 6258	48	>0.5	>0.5	>2	>2	>1	**1**	>0.5
Candida krusei	24	>0.5	>0.5	>2	>2	>1	**1**	>0.5
E28	48	>0.5	>0.5	>2	>2	>1	**1**	>0.5
Candida tropicalis	24	>0.5	>0.5	>2	>2	>1	**1**	>0.5
156	48	>0.5	>0.5	>2	>2	>1	**1**	>0.5
Candida glabrata	24	>0.5	>0.5	>2	>2	>1	**1**	>0.5
20/I	48	>0.5	>0.5	>2	>2	>1	**1**	>0.5
Candida lusitaniae	24	>0.5	>0.5	>2	>2	>1	**1**	>0.5
2446/I	48	>0.5	>0.5	>2	>2	>1	**1**	>0.5
Trichosporon beigelii	24	>0.5	>0.5	>2	>2	>1	**0.5**	>0.5
1188	48	>0.5	>0.5	>2	>2	>1	**0.5**	>0.5
Aspergillus fumigatus	24	>0.5	>0.5	>2	>2	>1	**0.5**	>0.5
231	48	>0.5	>0.5	>2	>2	>1	**0.5**	>0.5
Absidia corymbifera	24	>0.5	>0.5	>2	>2	>1	**2**	>0.5
272	48	>0.5	>0.5	>2	>2	>1	>2	>0.5
Trichophyton	72	>0.5	>0.5	>2	>2	>1	**0.5**	>0.5
mentagrophytes 445	120	>0.5	>0.5	>2	>2	>1	**0.5**	>0.5

Table 11 Antibacterial activity of C-4/C-5 disubstituted imidazolium salts studied as BAILs (MIC [mM])

Organism	Time (h)	**36**	**37**	**38**	**39**	**40**	**41**	**42**
S. aureus	24	>0.5	>0.5	>2	**2**	>1	**0.5**	>0.5
ATCC 6538	48	>0.5	>0.5	>2	**2**	>1	**0.5**	>0.5
S. aureus	24	>0.5	>0.5	**2**	>2	>1	**0.5**	>0.5
HK 5996/08	48	>0.5	>0.5	**2**	>2	>1	**0.5**	>0.5
S. epidermidis	24	**0.25**	>0.5	**2**	**1**	>1	**0.5**	>0.5
HK 6966/08	48	**0.25**	>0.5	**2**	**1**	>1	**0.5**	>0.5
Enterococcus sp.	24	>0.5	>0.5	>2	>2	>1	**1**	>0.5
HK 14365/08	48	>0.5	>0.5	>2	>2	>1	**2**	>0.5
E. coli	24	>0.5	>0.5	>2	>2	>1	**1**	>0.5
ATCC 8739	48	>0.5	>0.5	>2	>2	>1	**2**	>0.5
Klebsiella pneumoniae	24	>0.5	>0.5	>2	>2	>1	**2**	>0.5
HK 11750/08	48	>0.5	>0.5	>2	>2	>1	**2**	>0.5
Klebsiella pneumonia ESBL	24	>0.5	>0.5	>2	>2	>1	**2**	>0.5
HK 14368/08	48	>0.5	>0.5	>2	>2	>1	**2**	>0.5
Pseudomonas aeruginosa	24	>0.5	>0.5	>2	>2	>1	>2	>0.5
ATCC 9027	48	>0.5	>0.5	>2	>2	>1	>2	>0.5

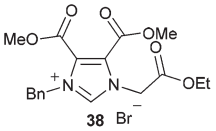
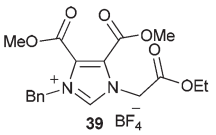
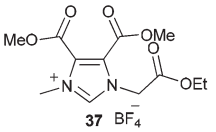
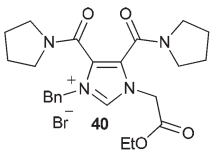
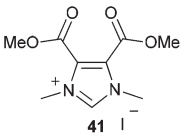
synthesis of product and processes.[22] Every reaction generates waste in the process, the most common contributors are solvents, unwanted side products and excess reagents. In order to evaluate the 'greenness' of any process, 'Green Chemistry Metrics'[23] such as Atom Economy (AE),[24] Mass Intensity (MI), Solvent Intensity (SI), Sheldon Environmental impact factor (E-factor), GSK Reaction Mass Efficiency (GSK RME), Andraos Reaction Mass Efficiency (Andraos RME), Stoichiometric Factor (SF), Material Recovery Parameter (MRP), and Carbon Efficiency (CE) be determined.[23]

These metrics can guide chemists towards the 'greener' technologies and has been already adopted in industry.[25,26] From the outset we expected *ca.* 30–50 novel imidazolium ion-based catalyst/salts would be prepared as part of this study. Multi-step synthetic routes are employed, so in excess of 100 reactions would be performed. With this in mind a limited Green Chemistry Metrics[27] study was performed with the aim that inefficient, waste intensive, reactions and routes could be avoided, and efficient routes developed for the lead catalysts. Metrics studied are shown in Table 13 including: Andraos RME, AE, 1/SF and MRP for each synthetic step in the preparation of the BAILs **9–42**[15] and number of steps (NS) (see ESI† for details).

As the project progressed, factors which were highlighted as cause for concern were

- excess reagents
- solvent use for chromatography
- solvent use for extraction of product and
- number of steps (NS) in the synthesis.

Table 12 Biodegradation study: C-4/C-5 disubstituted imidazolium salts studied as BAILs

Entry	IL	Time 6 days	14 days	21 days	28 days	CL (95%)
1	 **38** Br^-	12	18	22	24	21–26[a]
2	 **39** BF_4^-	1	2	3	6	3–8[a]
3	 **37** BF_4^-	15	21	30	31	30–31[a]
4	 **40**	0	0	1	2	0–4[a]
5	 **41** I^-	3	9	12	12	11–13[b]
6	 **42** BF_4^-	0	4	5	3	2.5–3.5[b]

[a] SDS reference 28 days 90% (95% CL = 85–95%). Confidence limits (CL) were calculated from 5 replicates. Confidence limits (CL) were calculated from 5 replicates. [b] SDS reference 28 days 94% (95% CL = 90–98%). Confidence limits (CL) were calculated from 4 replicates.

By studying these metrics during the catalysts/toxicity/biodegradation study, we were able to design a final class of catalyst **41** and **42**, which can be prepared by a short and efficient synthesis. We recommend future studies focus on further reductions of solvent use in the synthesis of BAILs.

Overview

In Table 14 an overview of all the catalyst's performance data, green synthesis, number of steps, antimicrobial toxicity and biodegradation is shown. As the design and development of more active catalysts progressed (from **9** to **42**), we can see that significant enhances in performance was found for the 4,5-disubstituted imidazolium salts **36–42**. As more complex Brønsted acidic catalysts were investigated, the synthetic routes increased in the number of steps, and the efficiency of the process (as determined by metrics in Table 13), decreased. This was addressed with compounds **41** and **42**, where short, efficient synthesis was prioritised at the design stage. Average Atom Economy (AAE) for the synthesis of Brønsted acidic catalysts is classified in Table 14. Where catalysts have the same rating, the more detailed green chemistry metric analysis in Table 13 is included. Antifungal toxicity was low for all generations of Brønsted acidic catalysts prepared, with the sole exception of **41**; which exhibits MIC values in the range 0.5–2 mM. Antibacterial toxicity was low for Brønsted acidic catalysts **9–35**, however, with the 4,5 disubstituted salts this was now a cause for concern with some examples. The biodegradation data shows that our efforts to prepare an imidazolium salt which is readily biodegradable and passes the CO_2 Headspace test were unsuccessful.

Key for Traffic Signal Light[28–31] **Classification in** Table 14

Catalyst Activity (**Cat. Act.**)
- Green: ≥90% yield with 0.1 mol% catalyst loading
- Amber: ≥90% yield with 1 mol% catalyst loading
- Red: <90% yield with 1 mol% catalyst loading

Catalysts Synthesis (**Cat. Syn.**, Number of steps, NS)
- Green: <3
- Amber: 3
- Red: >3

Catalysts Synthesis (**Cat. Syn.**, Average Atom Economy, AAE)
- Green: 1–0.85
- Amber: 0.85–0.70
- Red: <0.70

Antibacterial Toxicity (**Tox. Bac.**)
- Green: >2 mM all strains, or up to solubility limit
- Amber: MIC 0.25–2.0 mM
- Red: MIC <0.25 mM

Antifungal Toxicity (**Tox. Fung.**)
- Green: >2 mM all strains, or up to solubility limit
- Amber: MIC 0.25–2.0 mM
- Red: MIC <0.25 mM

Biodegradation (**Biodeg.**)
- Green: 60+% Readily Biodegradable
- Amber: 20–59%
- Red: 0–19%

Conclusions

There are very few examples of readily biodegradable ILs known which contain inorganic anions. Most examples which pass with the Closed Bottle Test or CO_2 Headspace test are due to organic anions which contribute favourably to the overall evolution of CO_2. Pyridinium salts substituted with an ester group are readily biodegradable. A concern with the biodegradation of imidazolium salts is the difficulty in breaking down the heterocyclic ring present in the compound or metabolites. We postulated that substitution of the imidazolium ring with EWG (*e.g.* esters and amides) would facilitate reaction at the aromatic ring, leading to additional degradation pathways,

Table 13 Green Chemistry Metrics[27]

IL	NS	ARME	AE	1/SF	MRP	Yield (1 = 100%)
9	1	0.062	1.000	0.935	0.069	0.960
10	2	0.048	0.702	1.000	0.072	0.956
		0.062	1.000	0.935	0.069	0.960
11	2	0.527	0.834	0.901	1.000	0.702
		0.062	1.000	0.935	0.069	0.960
12	2	0.080	0.780	1.000	0.167	0.616
		0.062	1.000	0.935	0.069	0.960
13	2	0.057	0.716	0.810	0.104	0.948
		0.062	1.000	0.935	0.069	0.960
14	2	0.026	1.000	1.000	0.033	0.809
		0.049	0.506	1.000	0.251	0.389
15	3	0.584	0.845	1.000	1.000	0.691
		0.026	1.000	1.000	0.033	0.809
		0.049	0.506	1.000	0.251	0.389
16	2	0.011	1.000	1.000	0.018	0.628
		0.049	0.506	1.000	0.251	0.389
17	3	0.745	0.863	1.000	1.000	0.863
		0.011	1.000	1.000	0.018	0.628
		0.049	0.506	1.000	0.251	0.389
18	2	0.042	1.000	0.967	0.053	0.817
		0.049	0.506	1.000	0.251	0.389
19	3	0.643	0.849	1.000	1.000	0.758
		0.042	1.000	0.967	0.053	0.817
		0.049	0.506	1.000	0.251	0.389
22	3	0.007	1.000	1.000	0.016	0.464
		0.497	0.661	1.176	0.969	0.659
		0.035	0.528	0.630	0.198	0.523
23	4	0.023	0.813	1.000	0.061	0.461
		0.007	1.000	1.000	0.016	0.464
		0.497	0.661	1.176	0.969	0.659
		0.035	0.528	0.630	0.198	0.523
24	4	0.633	0.856	1.000	1.000	0.739
		0.007	1.000	1.000	0.016	0.464
		0.497	0.661	1.176	0.969	0.659
		0.035	0.528	0.630	0.198	0.523
25	4	0.015	0.759	1.000	0.032	0.623
		0.007	1.000	1.000	0.016	0.464
		0.497	0.661	1.176	0.969	0.659
		0.035	0.528	0.630	0.198	0.523
26	3	0.005	1.000	0.999	0.030	0.170
		0.497	0.661	1.176	0.969	0.659
		0.035	0.528	0.630	0.198	0.523
27	2	0.015	1.000	0.432	0.055	0.621
		0.090	0.570	0.621	0.467	0.545
28	3	0.668	0.781	1.000	1.000	0.856
		0.015	1.000	0.432	0.055	0.621
		0.090	0.570	0.621	0.467	0.545
29	2	0.014	0.860	1.000	0.017	0.969
		0.090	0.570	0.621	0.467	0.545
30	3	0.016	1.000	0.611	0.029	0.888
		0.001	0.547	0.503	0.001	0.659
		0.007	0.531	0.115	0.219	0.496
32	3	0.108	0.877	0.981	0.139	0.906
		0.001	0.508	0.770	0.001	0.665
		0.007	0.531	0.115	0.219	0.496
33	3	0.011	1.000	0.431	0.038	0.661
		0.001	0.508	0.770	0.001	0.665
		0.007	0.531	0.115	0.219	0.496
34	4	0.009	0.797	0.851	0.041	0.957
		0.011	1.000	0.431	0.038	0.661
		0.001	0.508	0.770	0.001	0.665
		0.007	0.531	0.115	0.219	0.496
36	3	0.025	1.000	0.690	0.041	0.886
		0.014	0.608	0.732	0.037	0.846
		0.017	0.523	1.000	0.042	0.773
37	4	0.162	0.890	1.000	0.198	0.924
		0.036	0.552	0.746	0.107	0.814
		0.014	0.608	0.732	0.037	0.846
		0.017	0.523	1.000	0.042	0.773

Table 13 *(Contd.)*

IL	NS	ARME	AE	1/SF	MRP	Yield (1 = 100%)
38	4	0.047	1.000	0.721	0.087	0.739
		0.036	0.552	0.746	0.107	0.814
		0.014	0.608	0.732	0.037	0.846
		0.017	0.523	1.000	0.042	0.773
39	5	0.015	0.813	0.961	0.020	0.981
		0.047	1.000	0.721	0.087	0.739
		0.036	0.552	0.746	0.107	0.814
		0.014	0.608	0.732	0.037	0.846
		0.017	0.523	1.000	0.042	0.773
40	4	0.031	1.000	0.504	0.085	0.717
		0.029	0.617	0.531	0.088	1.000
		0.001	0.804	0.582	0.003	0.475
		0.017	0.523	1.000	0.042	0.773
41	3	0.184	1.000	0.998	0.215	0.858
		0.014	0.608	0.732	0.037	0.846
		0.017	0.523	1.000	0.042	0.773
42	3	0.167	0.867	0.891	0.238	0.909
		0.014	0.608	0.732	0.037	0.846
		0.017	0.523	1.000	0.042	0.773

NS = number of steps, AE = atom economy, ARME = Andraos reaction mass efficiency, SF = stoichiometric factor, MRP = material recovery parameter. Values determined using Green Metrics Lab Book.[27]

Table 14 Proposed 'Traffic Light' system applied to salts **9–42**

Salt	Cat. Act.	Cat. Syn. (NS)	Cat. Syn. (AAE)	Tox. Bac.	Tox. Fung.	Biodeg.
9	R	G	G	G	G	R
10	R	G	G	G	G	R
11	R	G	G	G	G	*R*
12	R	G	G	G	G	*A*[a]
13	R	G	G	G	G	*R*
14	R	G	A	G	G	R
15	R	A	A	G	G	*R*
16	R	G	A	G	G	R
17	R	A	A	G	G	*R*
18	R	G	A	G	G	R
19	R	A	A	G	G	*R*
20	nr[b]	A	A	G	G	R
21	A	A	A	G	G	A
22	R	A	A	G	G	R
23	R	R	A	G	G	*R*
24	R	R	A	G	G	*A*[a]
25	R	R	A	G	G	R
26	R	A	A	G	G	R
27	R	G	A	G	G	A
28	R	A	A	G	G	*A*
29	A	G	A	G	G	A
30	A	A	R	G	G	R
31	A	A	*R*	G	G	*R*
32	A	A	R	G	G	R
33	A	A	R	G	G	*R*
34	A	A	A	G	G	*R*
35	A	A	*R*	G	G	R
36	A	A	A	A	G	*A*
37	G	R	R	G	G	A
38	A	R	R	A	G	A
39	G	R	R	A	G	R
40	A	R	A	G	G	R
41	A	A	A	A	A	R
42	G	A	R	G	G	R

[a] ($OctSO_4$ salt). [b] nr = no reaction at 20 mol% loading. Entries in italics are estimated. NS = number of synthetic steps, AAE = Average Atom Economy.

and increased biodegradation. One potential problem was toxicity to the biodegradation innoculum, so a preliminary antibacterial and antifungal toxicity screen was performed. The interest in this class of imidazolium ion-based BAILs came from results using pyridinium salts, where the addition of electron withdrawing groups (*e.g.* esters) to the aromatic ring, significantly increased their catalytic activity in acetalisation reactions. Thus, a possible outcome from this work is that the highest catalytically active Brønsted acidic salt would be the most biodegradable of the series. Unfortunately this was not the case. While the catalytic activity is greatly enhanced on addition of the ester and amide groups,[15] all the salts studied exhibited poor biodegradation. CO_2 evolution can be attributed to alcohol oxidation after hydrolysis of ester groups, with no result supporting imidazolium breakdown. Antibacterial and antifungal toxicity was low for all salts, except a few C4/C5 examples.

Optimisation of Brønsted acidic catalysts based on toxicity and biodegradation data (and catalyst performance) was a goal. As the project progressed, we were pleased that substitution of the imidazolium ring did not lead to high antimicrobial toxicity, with only moderate toxicity determined for several C-4/C-5-substituted imidazolium salts. All salts screened for biodegradation were non-toxic to the innoculum as confirmed by reference experiments. Our biodegradation results demonstrated that there was no preferred position for the electron withdrawing group (all positions gave poor results), thus C-4/C-5-substituted catalysts were selected. Concurrent assessment of the green chemistry metrics for the synthesis of the Brønsted acid catalysts was completed. This was an integral part of the design process, as from the outset it was apparent that as the complexity of the catalyst targets increased, the synthetic route was likely to be less efficient (*e.g.* number of steps, overall yield). By determining the green chemistry metrics of all salts, we were able to assess which salts were prepared by the least efficient routes, identify problems (*e.g.* solvent use) and design improved synthetic routes (see the previous paper in this issue). The Traffic Light System was applied to the green chemistry metrics evaluated, including number of synthetic steps to prepare catalyst, synthesis efficiency, antibacterial and antifungal toxicity, and biodegradation. This facilitates comparison of the Brønsted acidic catalysts against a wide range of metrics. Salts **38–40** were classified RED for catalyst synthesis and catalyst preparation, with **39** GREEN for catalyst activity. The optimal Brønsted acidic catalysts prepared were **41** and **42**, with the latter GREEN for catalyst activity and AMBER for catalyst synthesis and catalyst preparation.

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