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LETTER

Enhanced antimicrobial activities of 1-alkyl-3-methyl imidazolium ionic liquids based on silver or copper containing anions†

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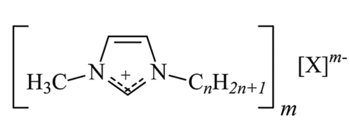
We have developed a series of 1-alkyl-3-methylimidazolium tetrachlorocuprate(II) and dibromoargentate(I) ionic liquids with enhanced antimicrobial activity when compared with 1-alkyl-3-methylimidazolium chloride ionic liquids. These new ionic liquids proved to be effective against a range of pathogenic bacteria and fungi.

Ionic liquids are a novel class of low temperature molten salts, comprised of discrete cations and anions.[1,2] Recently, the antimicrobial activity of a series of imidazolium, pyridinium and quaternary ammonium-derived ionic liquids has been described.[3–9] The toxicity profile of ionic liquids will be an important consideration in the replacement of volatile organic compounds in the chemical industry and in the design of bespoke ionic liquids whose characteristics are tuned to a particular functional niche. We have recently demonstrated that the antibiofilm activity of a series of 1-alkyl-3-methylimidazolium chloride ionic liquids,[10] was in keeping with the findings of several authors who report that antimicrobial activity is dependent on the alkyl chain length of the cationic species.[4–9] In recent years, the emergence of multi-drug resistant pathogens has ignited interest in alternative approaches to antimicrobial chemotherapy, beyond standard antibiotic and biocidal agents. The activity of heavy metals against microbial biofilms has been extensively studied.[11,12] Furthermore, Harrison *et al.* have recently reported synergistic bactericidal and antibiofilm activities of copper(II) and quaternary ammonium cations against *Pseudomonas aeruginosa*.[13] The use of silver(I) in the management of topical wounds, such as burns and ulcers has been documented since the 18th century.[14] Since then, silver has been used in proprietary formulations for the treatment of burns,[15,16] incorporated in topical dressings for wound management[17] and employed as a coating for medical devices, such as urological catheters for the prevention of catheter associated urinary tract infections (CAUTI).[18] The antimicrobial efficacy of silver(I) and copper(II) compounds against methicillin resistant *Staphylococcus aureus* has also recently been demonstrated.[19,20]

In this communication, we present our results on the antimicrobial activities of a series of 1-alkyl-3-methylimidazolium ionic liquids, bearing either $[AgX_2]^-$ or $[CuX_4]^{2-}$ anions against a range of clinically significant pathogens including MRSA and MRSE (methicillin resistant *Staphylococcus epidermidis*). In general, our data demonstrate that incorporation anions containing silver(I) and copper(II) species improves the antimicrobial activity of 1-alkyl-3-methylimidazolium ionic liquids, especially against Gram negative bacteria and fungi. Therefore, combining ionic liquid cations (imidazolium, pyridinium or pyrrolidium) with anions, such as metals with inherent antimicrobial activity, designer antimicrobial ionic liquids may be synthesised whereby anion and cation exert an overall additive or synergistic effect with respect to microbiological toxicity.

In general, 1-alkyl-3-methylimidazolium dibromoargentate(I) ionic liquids exhibit the greatest degree of enhanced antimicrobial activity (reduction in average MIC and MBC values) (Fig. 2 and Table 1) against all strains tested when compared



n = 8, 10, 12, 14, 16, or 18
m = 1, X = AgY_2
m = 2, X = CuY_4
Y = Cl or Br

Fig. 1 General structures of 1-alkyl-3-methylimidazolium dihaloargentate(I), $[C_n\text{mim}][AgY_2]$[21] and 1-alkyl-3-methylimidazolium tetrahalocuprate(II) $[C_n\text{mim}]_2[CuY_4]$.[22]

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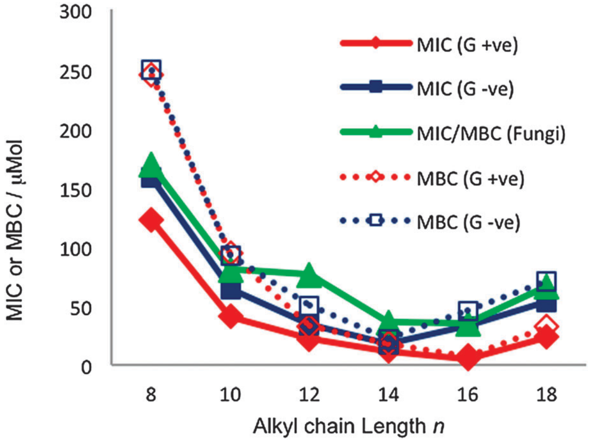

Fig. 2 Mean [$C_n$mim][$AgBr_2$] MIC and MBC values for Gram positive cocci, Gram negative rods and fungi.

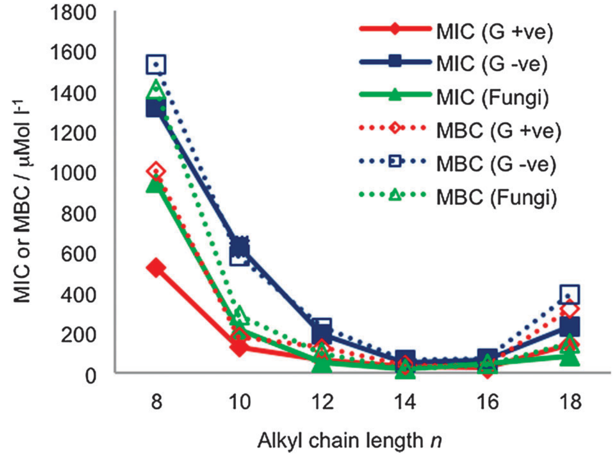

Fig. 3 Mean [$C_n$mim]$_2$[$CuCl_4$] MIC and MBC values for Gram positive cocci, Gram negative rods and fungi.

with data presented here for the corresponding tetrachlorocuprate(II) salts (Fig. 3 and Table 2). For Gram positive organisms tested in this study, there was an improvement in activity with dibromoargentate(I) anions compared with the corresponding 1-alkyl-3-methylimidazolium chlorides.[10] However, enhancement of antimicrobial activity is most pronounced against Gram negative organisms and *C. tropicalis*, for example, the average MIC value for Gram negative organisms tested against [C_{14}mim]$_2$[$CuCl_4$] was almost five fold lower than the corresponding 1-alkyl-3-methylimidazolium chloride.[10]

The most potent compound against Gram positive organisms in this series of ionic liquids was [C_{16}mim][$AgBr_2$], whilst for Gram negative organisms the most toxic compound was [C_{14}mim][$AgBr_2$]. These observations are also in keeping with the data for the [$C_n$mim]$_2$[$CuCl_4$] series of ionic liquids. For the representative fungal strain, the toxicity of [C_{14}mim]$_2$[$CuCl_4$] and [C_{16}mim]$_2$[$CuCl_4$] were essentially equivalent. In general, [$C_n$mim]$_2$[$CuCl_4$] ionic liquids exhibited lower antimicrobial activity than the corresponding [$AgBr_2$]$^-$ salts. [$C_n$mim]$_2$[$CuCl_4$] ionic liquids (with the exception of [C_8mim]$_2$[$CuCl_4$]) were

Table 1 MIC and MBC (μmol l^{-1}) values of 1-alkyl-3-methylimidazolium dibromoargentate(I) ionic liquids ([$C_n$mim][$AgBr_2$])

Alkyl chain length	8		10		12		14		16		18	
Organism[a]	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. epidermidis (MRSE) ATCC 35984	68.4	137	32.7	65.4	15.7	31.3	15.0	15.0	3.61	3.61	13.9	27.8
S. epidermidis ATCC 12228	68.4	137	32.7	65.4	31.3	31.3	15.0	15.0	3.61	3.61	13.9	27.8
S. aureus NCTC 10788	137	274	32.7	65.4	15.7	31.1	3.76	15.0	7.23	7.23	27.8	27.8
MRSA ATCC 43300	137	274	40.9	81.7	31.3	39.2	7.52	15.0	7.23	14.5	34.8	48.7
E. coli NCTC 8196	137	410	32.7	98.0	31.3	31.3	15.0	30.1	28.9	34.4	27.8	65.9
K. aerogenes NCTC 7427	169	169	79.8	98.0	34.4	68.8	18.0	18.0	34.4	34.4	65.9	65.9
P. mirabilis NCTC 12442	169	169	79.8	79.8	34.4	50.0	18.0	18.0	34.4	68.9	65.9	79.8
P. aeruginosa PA01	186	410	98.0	147	68.8	86.0	30.0	72.2	77.5	96.9	93.7	117
C. tropicalis NCTC 7393	169	337	79.8	98.0	75.8	75.8	36.1	72.2	34.4	68.9	65.9	79.8

[a] *S. epidermidis*, *S. aureus* and MRSA are Gram positive bacteria; *E. coli*, *K. aerogenes*, *P. mirabilis*, and *P. aeruginosa* are Gram negative bacteria and *C. tropicalis* is a fungus.

Table 2 MIC and MBC (μm) values of 1-alkyl-3-methylimidazolium tetrachlorocuprate(II) ionic liquids ([$C_n$mim]$_2$[$CuCl_4$])

Alkyl chain length	8		10		12		14		16		18	
Organism	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. epidermidis (MRSE) ATCC 35984	470	942	102	153	50.9	102	40.0	40.0	22.4	22.4	132	338
S. epidermidis ATCC 12228	470	942	102	153	50.9	102	20.0	40.0	22.4	22.4	132	338
S. aureus NCTC 10788	470	942	102	202	50.9	102	40.0	60.0	22.4	22.4	132	338
MRSA ATCC 43300	470	1177	153	202	102	203	40.0	50.0	33.6	33.6	165	338
E. coli NCTC 8196	942	1412	102	153	50.9	102	40.0	60.0	44.8	44.8	132	338
K. aerogenes NCTC 7427	942	1412	218	218	50.9	102	40.0	40.0	44.8	44.8	338	470
P. mirabilis NCTC 12442	941	11412	218	218	50.9	102	20.0	40.0	44.8	89.6	338	470
P. aeruginosa PA01	1883	>1883	873	1746	407	611	80.0	100	89.6	89.6	169	338
C. tropicalis NCTC 7393	942	1412	218	292	50.9	102	20.0	20.0	44.8	44.8	84.6	151

Table 3 Comparison of the MIC (μmol l^{-1}) of organisms to $[C_{14}mim]Cl$, $[C_{14}quin]Br$, $[C_{14}mim][AgBr_2]$ and $[C_{14}mim]_2[CuCl_4]$. The numbers in brackets are the anion effect ratios, when compared to that of $[C_{14}mim]Cl$

Organism	$[C_{14}mim]Cl$	$[C_{14}quin]Br$	$[C_{14}mim][AgBr_2]$	$[C_{14}mim]_2[CuCl_4]$
MRSA	16	5.8	7.5 (2.1)	40 (0.4)
MRSE	33	5.8	15 (2.2)	40 (0.8)
P. aeruginosa	264	24	30 (8.8)	80 (3.3)
E. coli	33	11.6	15 (2.2)	40 (0.8)
K. aerogenes	33	5.8	15 (2.2)	40 (0.8)
P. mirabilis	530	5.8	18 (29)	40 (14)
C. tropicalis	66	3	36 (1.8)	20 (3.3)

significantly less potent against Gram positive organisms than the corresponding $[C_nmim]Cl$ salts.[10] This apparent antagonism of antimicrobial activity has precedent, since recent studies have demonstrated that copper ions in certain biocide compositions may exert an overall antagonistic effect.[13] Furthermore, Pennanen (2001) indicates that fungi are relatively more susceptible to copper toxicity than bacteria.[23] In contrast, the overall antimicrobial activity was enhanced against Gram negative bacteria and *C. tropicalis*, with $[C_{14}mim]_2[CuCl_4]$ (the most potent copper compound tested). Average MIC values for Gram negative and *C. tropicalis* were reduced three-fold compared with $[C_{14}mim]Cl$.[10] The substitution of the $[AgBr_2]^-$ anion with $[AgCl_2]^-$ in both $[C_{14}mim]^+$ and $[C_{16}mim]^+$ ionic liquids resulted in further enhancement of antibacterial activity against both MRSA and *P. aeruginosa*, whereas no further improvement in antibacterial activity could be achieved by substitution of $[CuCl_4]^{2-}$ with $[CuBr_4]^{2-}$ anions in both $[C_{14}mim]$ and $[C_{16}mim]$ ionic liquids (data not shown).

Finally, the antimicrobial activities of the silver(I) and copper(II) ionic liquids were compared with $[C_{14}mim]Cl$[10] and $[C_{14}quin]Br$[24] (quin = 1-quinolinium) for seven microorganisms (Table 3). As can be seen, the silver(I) anionic ionic liquids appear to be more active than the imidazolium chloride ionic liquids, and can be seen in anion effect ratios. The copper(II) anionic ionic liquids are in generally similar in antimicrobial activity to the corresponding chloride salts, except in the case of *P. aeruginosa* and *P. mirabilis,* and more active than the corresponding chloride salts. However, the recently published 1-tetradecylquinolinium bromide ionic liquids are considerably more potent than all the imidazolium ionic liquids so far tested.[24]

Although the exact mechanism by which antimicrobial ionic liquids exert microbiological toxicity has yet to be fully elucidated, their structural similarity to common biocidal agents such as the quaternary ammonium compounds and dependence on alkyl chain length for toxicity suggest membrane disruption as a likely mechanism of action.[4–10] The mode of action of metal ions, such as copper, is generally *via* their ability to produce reactive oxygen species, thus targeting numerous biochemical pathways within the cell, resulting in oxidation of thiol groups in proteins, DNA damage, lipid peroxidation and the binding-site displacement of similar transition metals.[13,25] In addition, silver ions cause protein denaturation *via* interaction with thiol, amino, imidazole, phosphate and carboxyl groups of membrane proteins and enzymes[26,27] and cause metabolite efflux and inhibition of respiratory pathways in *E. coli*.[28] We confirm that, by utilising anions and cations which are both inherently antimicrobial, enhancement of overall antimicrobial activity may be achieved, thus facilitating the preparation of 'designer' antimicrobial ionic liquids.

Experimental

1-Alkyl-3-methylimidazolium chlorides and bromides were prepared *via* the reaction of 1-methylimidazole with a slight excess of the corresponding 1-haloalkane based on published protocols.[29–31] A series of 1-alkyl-3-methylimidazolium salts of $[AgBr_2]^-$ and $[CuCl_4]^{2-}$ (Fig. 1) were synthesised. These are binary ionic liquids simply made by fusing the imidazolium halide with the metal halide at elevated temperature, as described in the ESI.† For calculation of minimum inhibitory concentrations (MIC) and minimum bactericidal/fungicidal concentrations (MBC or MFC), broth microdilution tests were performed according to NCCLS guidelines,[32] and as described previously.†[10] It should be noted that in the case of silver containing ionic liquids, there was some precipitation of silver halide when in contact with the growth media. With this however, the ionic liquids in question were still more active than compared to the imidazolium halide.

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