BIOnic Liquids: Imidazolium-based Ionic Liquids with Antimicrobial Activity

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We have synthesized twelve new ionic liquids composed of an imidazolium-based cation in combination with an anion that shows antiobiotic or analgesic activity. These "BIOnic Liquids" have been tested towards their antibiotic activity in a standardized microbiological assay. A surprizingly large number of compounds shows high activity towards a set of bacteria which cannot be explained as simple cumulative effects. The general concept opens up completely new possibilities for the future development of pharmaceutically active compounds.

Key words: Ionic Liquids, Active Pharmaceutical Ingredients (API), Imidazolium Salts, Antibiotics, Antimicrobials, Analgesics

Introduction

Task-specific ionic liquids (TSILs) [1] are second-generation ionic liquids (ILs) that are able to perform a certain task in addition to being "just the solvent". Since Davis' very first publication on the topic in which he described an IL containing an amine functional group to be able to reversibly chemisorb CO_2 [2], numerous examples of TSILs have appeared in the literature.

Ionic liquids with biological activity which are, strictly speaking, nothing but TSILs have been termed "the third generation of ILs" [3]. It is already well-known that certain ILs, although frequently called "green", can be quite toxic [4, 5]. Imidazolium salts in particular have been studied to a larger extent. It was found that the longer the (unbranched) alkyl chain that is attached to the imidazolium ring, the more toxic the salt [6]. Fortunately, this toxicity depends on the trophic level of the living entity: the higher the species in the food chain, the less harmful the salt becomes [5]. Consequently, these salts are much more toxic for bacteria than for human beings, which makes them, by definition, antibiotic substances.

Quite a few ionic liquid antibiotics and antimicrobials have already been described in the literature; comprehensive reviews on the topic have appeared in 2005 [4] and in 2010 [5].

The effect of alkyl chain length on the antimicrobial activity of imidazolium salts has been studied by Jungnickel *et al.* [6], as mentioned already earlier. The groups of Borowiecki [7, 8] and Yin [9] have focussed their studies on ionic liquids bearing a hydroxy group in the cation. Their results were unsatisfactory; the OH group seemed to have little effect on the antimicrobial activity and the influence of the alkyl chain length remained dominant. The same result has been found by the group of Holzgrabe [10]. They tried to pin down the type of interactions responsible for the antimicrobial potential of certain quaternary ammonium salts.

Gathergood and co-workers have recently studied amino acid-functionalized imidazolium salts on their activity towards *MRSA* (methicillin-resistant *Staphylococcus aureus*) [11]. Some of the salts were sufficiently active and additionally showed a reasonable level of biodegradibility.

The combination of the $[C_{16}mim]$ cation with β-lactam antibiotics has been studied by Warner *et al.* [12]. They reported that in more than 90 % of their cases the combination outperformed the commercially available antibiotic.

Closely related to our topic, Rogers and co-workers reported the application of pharmaceutically active ionic liquids that were immobilized onto mesoporous silica [13]. These solids were very robust and could potentially be used as efficient "device for drug delivery and *in vitro* release".

Results and Discussion

Many antibiotics and analgesics currently on the market are available as (sodium) salts. Therefore, the active compounds already exist in anionic form. Since the imidazolium cation is (a) an established part of many common ionic liquids and (b) already well-known for its antibiotic activity (*cf.* introduction) we decided to integrate imidazolium cations into biologically active ionic liquids. As the anions we chose chloramphenicol, fosfomycin and two sulfonamides, and in addition two common analgesics. In the case of the cations we decided for imidazolium with three side chains of different length (butyl, octyl and hexadecyl), since it is already well-known that the length of the side chain defines for the antimicrobial activity of the cation [6]. The compounds are depicted in Fig. 1. A combination of these resulted in twelve new ionic liquids (Table 2).

Table 1. Bacterial reference strains used for the determination of the minimal inhibitory concentration (MIC) of our BIOnic liquids.

ATCC no.	Name
29213	*Staphylococcus aureus*
43300	Methicillin-resistant *Staphylococcus aureus* (MRSA)
29212	*Enterococcus faecalis*
25922	*Escherichia coli*
27853	*Pseudomonas aeruginosa*

The twelve BIOnic liquids have subsequently been tested for their antibacterial activity in a standardized microbiological assay by determining the minimal inhibitory concentration (MIC) against five microorganisms (Table 1). For comparison we have also tested the bromide salts of the ionic liquid precursors ("the

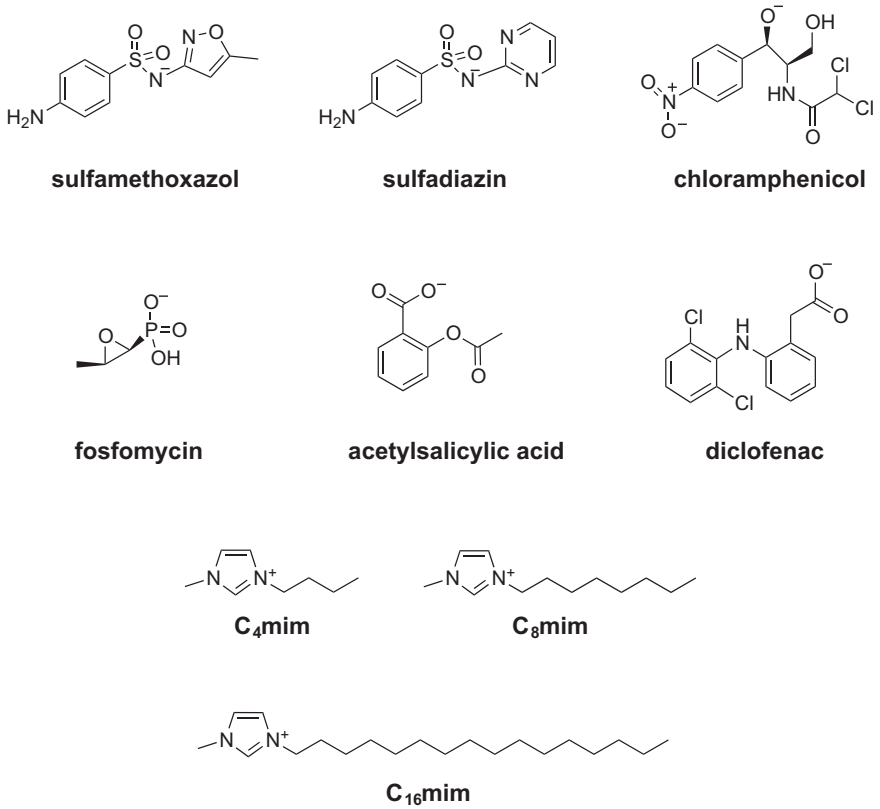

Fig. 1. Pharmacologically active anions and cations used in this study for the synthesis of ionic liquids.

Compound	T_g (°C)	*S. aureus*	MRSA	*E. faecalis*	*E. coli*	*P. aeruginosa*
[C_4mim]Br	76.0	–	–	–	–	–
[C_8mim]Br	−38.1	256	1024	128	512	2048
[C_{16}mim]Br	−19.0	0.125	1	0.125	32	128
Na chloramphenicol	n. d.[a]	8	16	4	4	256
Na sulfadiazin	n. d.	–	512	4	64	1024
Na sulfamethoxazol	n. d.	–	–	4	64	1024
Na_2 fosfomycin	n. d.	4	16	32	2	4
[C_4mim]chloramphenicol	22.9	n. d.	n. d.	n. d.	n. d.	n. d.
[C_4mim]sulfadiazin	−26.0	–	–	16	64	–
[C_4mim]sulfamethoxazol	−30.3	–	–	4	32	2048
[C_4mim]fosfomycin	−49.8	16	16	64	16	16
[C_4mim]diclofenac	n. d.	512	512	512	–	–
[C_8mim]sulfadiazin	3.6	256	2048	8	64	2048
[C_8mim]sulfamethoxazol	−17.3	512	2048	8	32	2048
[C_8mim]fosfomycin	−44.7	n. d.	n. d.	n. d.	n. d.	n. d.
[C_8mim]acetylacetic acid	−51.5	n. d.	n. d.	n. d.	n. d.	n. d.
[C_{16}mim]sulfadiazin	10.5	< 0.125	1	< 0.125	8	128
[C_{16}mim]sulfamethoxazol	−27.0	0.5	2	0.125	8	128
[C_{16}mim]fosfomycin	30.6	< 0.125	1	< 0.125	8	32

[a] n. d.: not determined

Table 2. Minimal inhibitory concentration (MIC) of the ionic liquid precursors (*i. e.* "cations"), of the neat antibiotics (*i. e.* "anions") and of our BIOnic liquids in $\mu g\,mL^{-1}$. A dash (–) denotes no activity at the highest concentration tested ($2048\,\mu g\,mL^{-1}$).

cations") and the neat antimicrobials ("the anions"). The results are shown in Table 2.

In a common definition, a compound having antimicrobial activity must show a MIC below $200\,\mu g\,mL^{-1}$ [14]. Table 2 shows that this prerequisite is already met by some of our neat ionic liquid bromide salts. We can clearly see that the choice of cation has a strong effect on the antimicrobial activity of the compounds. For ease of comparison, the activities of the neat antimicrobials have also been determined under standardized testing conditions.

A surprisingly large number of our "BIOnic Liquids" shows a high level of antimicrobial activity (Table 2). Depending on the compound and the microorganism, there is no general trend in activity observable. In many cases, the observed MIC resembles the one of either the corresponding imidazolium bromide or of the neat antibiotic. But there are also cases in which the BIOnic Liquid is more effective than each of the individual components (*e. g.* [C_{16}mim]fosfomycin and [C_{16}mim]sulfadiazin against *S. aureus* or [C_{16}mim]sulfamethoxazol against *E. coli*), which can be attributed to efficient cumulative effects. On the other hand, there are cases in which the BIOnic Liquid is less effective than the key component alone (*e. g.* [C_{16}mim]fosfomycin against *E. coli* and *P. aeruginosa*).

Interestingly, the total (average) efficiency of many BIOnic Liquids is higher than the total efficiency of each of the components. Table 3 exemplarily shows the average MIC for [C_{16}mim]fosfomycin.

Table 3. Exemplary efficiency MIC (average) in $\mu g\,mL^{-1}$ of [C_{16}mim]fosfomycin in comparison to its key components. MIC (average) is the sum of the five MICs against our five bacteria reference strains MIC (total) devided by 5.

Compound	MIC (total)	MIC (average)
[C_{16}mim]Br	161.25	32.25
Na_2 fosfomycin	58	11.6
[C_{16}mim]fosfomycin	41.25	**8.25**

Conclusion

The combination of an antimicrobially active imidazolium cation with an anionic antimicrobial leads to new microbiologically active ionic liquids ("BIOnic Liquids"). The average efficiency of the BIOnic Liquids is generally higher than the combined efficiency of their key components.

This methodology opens up many possibilities for time- and cost-efficient development of new antimicrobial agents. By building upon well-known, well-tested and (possibly) already approved chemicals in a modular building block concept, it is possible to save valuable time for the development and testing of new pharmaceutical drugs. In addition, since the product is potentially liquid, the possibility for topical instead of systemical application might prove valuable. Topical

application of active pharmaceutical ingredients can neglect general toxicity issues much more than systemical application in which the drug is distributed throughout the body.

Experimental Section

Syntheses

[C_4mim]Br, [C_8mim]Br and [C_{16}mim]Br have been prepared according to established literature procedures [15]. The BIOnic Liquids have subsequently been synthesized by transforming the corresponding imidazolium bromide into the hydroxide *via* ion exchange resin. The hydroxide salt was then combined with the antibiotic or analgesic to yield the final product by elimination of one equivalent of water.

General procedure for the synthesis of microbiologically active ionic liquids (BIOnic Liquids)

1 eq. of the corresponding imidazolium bromide was dissolved in distilled water and sent through an ion exchange column (Merck ion exchange resin III). To this unstable hydroxide salt in water the corresponding antibiotic or analgesic was directly added, and the mixture was stirred for 2 h at room temperature. The solvent was evaporated under reduced pressure. The purity (especially halide residues) was checked *via* ion chromatography. Since fosfomycin was only available as sodium salt, the neat compound was also produced *via* ion exchange in water (Dowex 50WX8-100).

[C_4mim]diclofenac

Yield: 77 %. – FT-IR (ATR): $\nu\,(\mathrm{cm}^{-1}) = 621$ (s), 716 (m), 745 (s), 1167 (m), 1362 (m), 1449 (s), 1506 (m), 1558 (s), 1574 (s), 2874 (w), 2959 (m), 3065 (w), 3144 (w). – ^{1}H NMR (300 MHz, D_2O): $\delta = 7.15-7.08$ (s, 3H, 13-H, 15-H, 11-H), 7.01 – 6.98 (d, 2H, 4-H, 5-H), 6.67 – 6.58 (m, 3H, 21-H, 23-H, 25-H), 6.11 (t, 1H, 24-H), 3.85 (t, 2H, 7-H), 3.65 (s, 3H, 6-H), 3.52 (s, 2H, 26-H), 1.52 (m, 2H, 8-H), 1.04 (m, 2H, 9-H), 0.69 (t, 3H, 10-H). – ^{13}C{^{1}H} NMR (75 MHz, D_2O): $\delta = 179.09$ (s, 27-C), 142.38 (s, 22-C), 137.45 (s, 14-C), 130.62 (s, 2-C), 128.90 (s, 21-C, 25-C), 128.50 (s, 23-C, 24-C) 127.04 (s, 16-C), 126.33 (s, 12-C), 123.80 (s, 13-C), 123.27 (s, 15-C), 121.80 (s, 11-C) 121.06 (s, 4-C), 116.13 (s, 5-C), 49.01 (s, 7-C), 42.57 (s, 27-C), 35.45 (s, 6-C), 31.15 (s, 8-C), 18.77 (s, 9-C), 12.72 (s, 10-C).

[C_8mim]acetylacetic acid

Yield: 31 %. – $T_g = -51.5$ °C. – FT-IR (ATR): $\nu\,(\mathrm{cm}^{-1}) = 621$ (w), 664 (w), 704 (w), 756 (s), 854 (w), 918 (w), 1028 (w), 1085 (w), 1194 (m), 1219 (w), 1248 (m), 1368 (br), 1456 (m), 1483 (m), 1570 (m), 1591 (m), 1707 (m), 1749 (w), 2857 (v), 2926 (m), 2955 (w), 3069 (br), 3148 (w). – ^{1}H NMR (300 MHz, [D_6]DMSO): $\delta = 9.21$ (s, 1H, 2-H), 7.90 (d, 1H, 19-H), 7.77 (d, 1H, 20-H), 7.66 (d, 1H, 17-H), 7.54 (d, 1H, 18-H), 7.50 (d, 1H, 5-H), 7.30 (d, 1H, 4-H), 6.63 (m, 2H, 7-H), 4.14 (m, 2H, 8-H), 3.85 (s, 3H, 5-H), 2.23 (s, 3H, 6-H), 1.76 (m, 2H, 9-H), 1.24 (b, 8H, 10-H, 11-H, 12-H, 13-H), 0.85 (t, 3H, 14-H). – ^{13}C{^{1}H} NMR (75 MHz, [D_6]DMSO): $\delta = 169.33$ (s, 22-C), 166.31 (s, 24-C), 163.06 (s, 15-C), 150.01 (s, 16-C), 132.13 (s, 2-C), 131.23 (s, 19-C), 129.89 (s, 17-C), 125.60 (s, 18-C), 123.36 (s, 4-C), 121.99 (s, 20-C), 115.78 (s, 5-C), 48.76 (s, 7-C), 35.72 (s, 18-C), 31.18 (s, 8-C), 29.41 (s, 9-C), 28.50 (s, 10-C), 28.36 (s, 11-C), 25.51 (s, 12-C), 22.08 (s, 13-C), 21.03 (s, 26-C), 13.96 (s, 14-C).

[C_4mim]chloramphenicol

Yield: 31 %. – $T_g = 22.9$ °C. – FT-IR (ATR): $\nu\,(\mathrm{cm}^{-1}) = 623$ (m), 700 (m), 750 (m), 826 (w) 851 (w), 1053 (br), 1167 (m), 1344 (s), 1516 (s), 1647 (m), 2874 (w), 2934 (w), 2959 (w), 3080 (w). – ^{1}H NMR (300 MHz, [D_6]DMSO): $\delta = 9.20$ (s, 1H, 2-H), 8.19 – 8.16 (d, 2H, 11-H/13-H), 7.78 (s, 1H, 4-H), 7.72 (s, 1H, 5-H), 7.61 – 7.58 (d, 2H, 16-H/14-H), 5.79 (s, 1H, 25-H), 4.70 (s, 1H, 27-H), 4.16 (t, 2H, 7-H), 3.85 (s, 3H, 6-H), 3.35 (m, 2H, 28-H), 2.71 (m, 1H, 21-H), 1.76 (m, 2H, 8-H), 1.24 (m, 2H, 9-H), 0.89 (t, 3H, 10-H). – ^{13}C{^{1}H} NMR (75 MHz, [D_6]DMSO): $\delta = 153.10$ (s, 15-C), 146.86 (s, 2-C), 146.52 (s, 11-C), 127.87 127.63 (m, 16-C/22-C, 20-C), 123.91 (s, 4-C), 123.14 (s, 11-C/12-C), 122.56 (s, 5-C), 72.05 (s, 25-C), 63.52 (s, 28-C), 59.12 (s, 21-C), 48.76 (s, 7-C), 36.02 (s, 6-C), 31.66 (s, 8-C), 19.07 (s, 9-C), 13.58 (s, 10-C).

[C_4mim]sulfadiazin

Yield: 95 %. – $T_g = -25.9$ °C. – FT-IR (ATR): $\nu\,(\mathrm{cm}^{-1}) = 677$ (m), 789 (m), 970 (w), 1001 (m), 1072 (m), 1120 (m), 1167 (w), 1225 (br), 1261 (v), 1408 (s), 1502 (w), 1539 (m), 1577 (m), 1597 (m), 2961 (w), 3102 (w), 3148 (w), 3227 (w), 3339 (w). – ^{1}H NMR (300 MHz, [D_6]DMSO): $\delta = 9.23$ (b, 1H, 2-H), 8.07 – 8.05 (d, 2H, 14-H, 16-H), 7.78 – 7.71 (d, 2H, 4-H, 5-H), 7.45 – 7.42 (d, 2H, 25-H, 21-H), 6.44 – 6.41 (d, 2H, 24-H, 22-H), 6.31 (s, 1H, 15-H), 5.30 (s, 2H, 27-H), 4.18 (t, 2H, 7-H), 3.86 (s, 3H, 6-H), 1.75 (m, 2H, 8-H), 1.24 (m, 2H, 9-H), 0.89 (t, 3H, 10-H). – ^{13}C{^{1}H} NMR (75 MHz, [D_6]DMSO): $\delta = 212.08$ (s, 12-C), 156.91 (s, 14-C, 16-C), 149.63 (s, 23-C), 128.42 (s, 25-C, 21-C), 124.19 (s, 2-C), 123.85 (s, 5-C), 122.26 (s, 4-C), 111.62 (s, 24-C, 22-C), 108.94 (s, 15-C), 48.25 (s, 7-C), 35.46 (s, 6-C), 31.42 (s, 8-C), 18.89 (s, 9-C), 12.93 (s, 10-C).

[C_4mim]sulfamethoxazol

Yield: 99 %. – $T_g = -30.2$ °C. – FT-IR (ATR) $\nu\,(\mathrm{cm}^{-1}) = 673$ (s), 739 (s), 939 (s), 1045 (m), 1090 (s), 1120 (s), 1227 (m), 1267 (w), 1404 (m), 1458 (s), 1597 (m), 2961

(w), 3221 (w), 3333 (w), 3420 (w). – ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 9.20$ (s, 1H, 2-H), 7.77 (s, 1H, 5-H), 7.70 (s, 1H, 4-H), 7.31 – 7.28 (d, 2H, 16-H, 14-H), 6.45 – 6.42 (d, 2H, 11-H, 13-H), 5.72 (s, 1H, 23-H), 5.32 (b, 2H, 17-H), 4.16 (t, 2H, 7-H), 3.85 (s, 3H, 6-H), 2.08 (s, 3H, 27-H), 1.75 (m, 2H, 8-H), 1.26 (m, 2H, 9-H), 0.89 (t, 3H, 10-H). – ^{13}C{^{1}H} NMR (75 MHz, [D$_6$]DMSO): $\delta = 166.00$ (s, 24-C), 164.94 (s, 22-C), 149.63 (s, 12-C), 136.61 (s, 2-C), 134.60 (s, 16-C, 14-C), 123.53 (s, 5-C), 122.19 (s, 4-C), 112.22 (s, 11-C, 13-C), 97.07 (s, 23-C), 48.28 (s, 7-C), 35.54 (s, 6-C), 31.39 (s, 8-C), 18.51 (s, 9-C), 13.30 (s, 27-C), 12.26 (s, 10-C).

[C$_4$mim]fosfomycin

Yield: 98 %. – $T_g = -49.8$ °C. – FT-IR (ATR): ν (cm^{-1}) = 623 (s), 750 (m), 891 (s), 1038 (s), 1167 (s), 1456 (m), 1558 (m), 2874 (w), 2961 (m), 3096 (w). – ^{1}H NMR (300 MHz, D$_2$O): $\delta = 8.66$ (s, 1H, 2-H), 7.42 (s, 1H, 5-H), 7.38 (s, 1H, 4-H), 4.14 (t, 2H, 7-H), 3.83 (s, 3H, 6-H), 3.50 – 3.31 (b, 1H, 11-H), 2.96 – 2.86 (b, 1H, 13-H), 1.79 (m, 2H, 8-H), 1.44 (m, 2H, 9-H), 1.21 (m, 3H, 14-H), 0.86 (t, 3H, 10-H). – ^{13}C{^{1}H} NMR (75 MHz, D$_2$O): $\delta = 136.43$ (s, 2-C), 73.63 (s, 5-C), 71.60 (s, 4-C), 67.63 (s, 6-C), 54.27 (s, 11-C), 51.50 (s, 13-C), 49.24 (s, 7-C), 35.61 (s, 14-C), 31.25 (s, 8-C), 18.72 (s, 9-C), 13.36 – 12.62 (b, 10-C). – ^{31}P{^{1}H} NMR (121 MHz, D$_2$O): $\delta = 12.20$ (s, 1-P).

[C$_8$mim]sulfadiazin

Yield: 92 %. – $T_g = 3.6$ °C – FT-IR (ATR): ν (cm^{-1}) = 625 (m), 677 (s), 709 (m), 787 (s), 968 (m), 999 (s), 1074 (s), 1121 (s), 1167 (m), 1227 (m), 1410 (s), 1533 (m), 1578 (s), 2855 (w), 2926 (w), 3096 (w), 3219 (w), 3337 (w). – ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 9.28$ (s,1H, 2-H), 8.06 – 8.04 (d, 2H, 18-H, 20-H), 7.77 (s, 1H, 5-H), 7.70 (s, 1H, 4-H), 7.44 – 7.42 (d, 2H, 30-H, 26-H), 6.43 – 6.41 (d, 2H, 29-H, 27-H), 6.30 (t, 1H, 19-H), 5.28 (s, 2H, 31-H), 4.16 (t, 2H, 7-H), 3.86 (s, 3H, 6-H), 1.77 (m, 2H, 8-H), 1.24 (b, 10H, 9-H–13-H), 0.85 (s, 3H, 14-H). – ^{13}C{^{1}H} NMR (75 MHz, [D$_6$]DMSO): $\delta = 157.44$ (s, 20-C, 18-C), 149.97 (s, 28-C), 137.55 (s, 2-C), 137.17 (s, 25-C), 134.59 (s, 30-C, 26-C), 123.97 (s, 5-C), 122.59 (s, 4-C), 112.20 (s, 29-C, 27-C), 109.18 (s, 19-C), 49.04 (s, 7-C), 36.17 (s, 6-C), 31.61 (s, 8-C), 29.88 (s, 9-C), 28.93 (s, 10-C), 28.79 (s, 11-C), 25.95 (s, 12-C), 22.51 (s, 13-C), 14.41 (s, 14-C).

[C$_8$mim]sulfamethoxazol

Yield: 93 %. – $T_g = -17.3$ °C. – FT-IR (ATR): ν (cm^{-1}) = 671 (s), 743 (s), 795 (m), 833 (m), 937 (s), 1042 (m), 1090 (s), 1121 (s), 1225 (s), 1267 (m), 1315 (m), 1398 (m), 1456 (s), 1599 (s), 1649 (m), 2853 (m), 2930 (m), 3117 (w), 3230 (m), 3337 (m), 3397 (m). – ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 9.19$ (s, 1H, 2-H), 7.77 (s, 1H, 5-H), 7.70 (s, 1H, 4-H), 7.32 – 7.29 (d, 2H, 20-H, 18-H), 6.45 – 6.42 (d, 2H, 15-H, 17-H), 5.74 (s, 1H, 27-H), 5.35 (s, 2H, 7-H), 4.15 (t, 2H, 7-H), 3.85 (s, 3H, 6-H), 2.09 (s, 3H, 31-H), 1.76 (m, 2H, 8-H), 1.24 (b, 10H, 9-H–13-H), 0.85 (t, 3H, 14-H). – ^{13}C{^{1}H} NMR (75 MHz, [D$_6$]DMSO): $\delta = 165.54$ (s, 28-C), 149.78 (s, 16-C), 136.60 (s, 2-C), 134.14 (s, 19-C), 127.37 (s, 20-C, 18-C), 123.59 (s, 5-C), 122.25 (s, 4-C), 112.23 (s, 15-C, 17-C), 99.04 (s, 26-C), 96.90 (s, 27-C), 48.75 (s, 7-C),35.73 (s, 6-C), 31.17 (s, 8-C), 29.41 (s, 9-C), 28.48 (s, 10-C), 28.34 (s, 11-C), 25.50 (s, 12-C), 22.06 (s, 13-C), 13.69 (s, 31-C), 12.24 (s, 14-C).

[C$_8$mim]fosfomycin

Yield: 92 %. – $T_g = -44.7$ °C. – FT-IR (ATR): ν (cm^{-1}) = 627 (m), 716 (m), 851 (s), 889 (s), 1083 (s), 1337 (w), 1456 (m), 1558 (m), 2855 (m), 2924 (m), 3144 (w). – ^{1}H NMR (300 MHz, D$_2$O): $\delta = 8.70$ (s, 1H, 6-H) 7.47 – 7.43 (d, 2H, 3-H/4-H), 4.19 (s, 2H, 7-H), 3.89 (s, 3H, 6-H), 3.35 (m, 1H, 15-H), 2.98 – 2.93 (m, 1H, 17-H), 1.87 (m, 2H, 8-H), 1.50 (d, 3H, 18-H), 1.30 – 1.27 (b, m, 9-H–13-H), 0.86 (t, 3H, 14-H). – ^{13}C{^{1}H} NMR (75 MHz, D$_2$O): $\delta = 135.76$ (s, 6-C), 123.43 (s, 4-C), 122.16 (s, 5-C), 54.28 (s, 15-C), 51.89 (s, 17-C), 49.52 (s, 7-C), 35.55 (s, 6-C), 30.91 (s, 8-C), 29.09 (s, 9-C), 28.12 (s, 10-C), 27.92 (s, 11-C), 25.21 (s, 12-C), 21.92 (s, 13-C) 13.33 (s, 14-C, 18-C). – ^{31}P{^{1}H} NMR (121 MHz, D$_2$O): $\delta = 11.99$ (s, 1-P).

[C$_{16}$mim]sulfadiazin

Yield: 93 %. – $T_g = 10.5$ °C. – FT-IR (ATR): ν (cm^{-1}) = 660 (m), 677 (s), 785 (m), 1001 (m), 1072 (s), 1125 (s), 1174 (m), 1225 (m), 1413 (s), 1500 (w), 1578 (m), 1636 (w), 2851 (m), 2920 (m), 3032 (w), 3215 (w), 3325 (w), 3420 (w). – ^{1}H NMR (300 MHz, D$_2$O): $\delta = 9.41$ (s, 1H, 2-H), 8.06 – 8.04 (d, 2H, 38-H/36-H), 7.78 (s, 1H, 4-H), 7.71 (s, 1H, 5-H), 7.46 – 7.43 (d, 2H, 28-H/26-H), 6.44 – 6.42 (d, 2H, 23-H/25-H), 6.33 (t, 1H, 37-H), 5.30 (s, 2H, 29-H) 4.15 (t, 2H, 7-H), 3.87 (s, 3H, 6-H), 1.76 (m, 2H, 8-H), 1.23 (m, 26H, 9-H–21-H), 0.85 (t, 3H, 22-H). – ^{13}C{^{1}H} NMR (75 MHz, D$_2$O): $\delta = 164.27$ (s, 34-C), 156.90 (s, 38-C/36-C), 149.67 (s, 24-C), 136.87 (s, 2-C), 133.75 (s, 27-C), 128.29 (s, 28-C/26-C), 123.56 (s, 4-C), 122.21 (s, 5-C), 111.77 (s, 23-C/25-C), 108.98 (s, 37-C), 48.70 (s, 7-C), 35.68 (s, 6-C), 31.29 (s, 8-C), 29.47 (s, 9-C), 29.05 (m, 10-C–18-C), 28.84 (s, 19-C), 25.50 (s, 20-C), 22.09 (s, 21-C), 13.95 (s, 22-C).

[C$_{16}$mim]sulfamethoxazol

Yield: 92 %. – $T_g = -27$ °C. – FT-IR (ATR): ν (cm^{-1}) = 623 (m), 669 (s), 740 (m), 833 (m), 937 (m), 1043 (m), 1092 (s), 1123 (s), 1165 (m), 1231 (m), 1269 (m), 1296 (w), 1400 (m), 1458 (s), 1597 (m), 2852 (m), 2922 (m), 3102 (w), 3148 (w), 3219 (w), 3337 (w). – ^{1}H NMR (300 MHz, D$_2$O): $\delta = 9.27$ (s, 1H, 2-H), 7.77 (s, 1H, 4-H), 7.70 (s, 1H, 5-H),

7.32 – 7.29 (d, 2H, 26-H/28-H), 6.45 – 6.42 (d, 2H, 23-H/25-H), 5.73 (s, 1H, 35-H), 5.32 (s, 2H, 29-H), 4.15 (t, 2H, 7-H), 3.85 (s, 3H, 6-H), 2.09 (s, 3H, 39-H), 1.76 (m, 2H, 8-H), 1.23 (b, 26H, 9-H–21-H), 0.85 (t, 3H, 22-H). – $^{13}C\{^1H\}$ NMR (75 MHz, D_2O): δ = 166.46 (s, 34-C), 165.16 (s, 36-C), 149, 65 (s, 24-C), 136.70 (s, 2-C), 134.57 (s, 27-C), 127.28 (s, 28-C/26-C), 123.56 (s, 4-C), 122.23 (s, 5-C), 112.22 (s, 23-C/25-C), 96.95 (s, 35-C), 48.72 (s, 7-C), 35.69 (s, 6-C), 31.29 (s, 8-C), 29.45 (s, 9-C), 29.05 (m, 10-C–18-C), 28.40 (s, 19-C), 25.50 (s, 20-C), 22.09 (s, 21-C), 13.94 (s, 39-C), 12.22 (s, 22-C).

[C_{16}mim]fosfomycin

Yield: 93 %. – T_g = 30.6 °C. – FT-IR (ATR): ν (cm^{-1}) = 623 (m), 714 (s), 853 (m), 966 (m), 1030 (s), 1150 (s), 1222 (m), 1472 (m), 2311 (w), 2847 (s), 2913 (s), 3096 (w), 3138 (w). – ^1H NMR (300 MHz, D_2O): δ = 8.91 (s, 1H, 2-H), 7.54 (s, 1H, 4-H), 7.47 (s, 1H, 5-H), 4.21 (m, 2H, 7-H), 3.92 (s, 3H, 6-H), 3.26 (b, 1H, 23-H), 2.90 (d, 1H, 25-H), 1.84 (b, 1H, 29-H), 1.49 (d, 3H, 26-H), 1.25 (m, 26 H, 9-H–20-H), 0.84 (b, 3H, 21-H). – $^{13}C\{^1H\}$ NMR (75 MHz, D_2O): δ = 136.06 (s, 2-C), 123.84 (s, 4-C), 121.95 (s, 5-C), 53.99 (s, 25-C), 51.68 (s, 23-C), 49.37 (s, 7-C), 35.64 (s, 6-C), 31.91 (s, 8-C), 29.85 (s, 9-C), 29.42 (m, 10-C–17-C), 29.11 (s, 18-C), 26.10 (s, 19-C), 22.58 (s, 20-C), 13.79 (s, 26-C), 13.52 (s, 21-C). – $^{31}P\{^1H\}$ NMR (121 MHz, D_2O): δ = 11.91 (s, 1-P).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by broth micro dilution in cation-adjusted Müller-Hinton broth according to Clinical Laboratory Standard Institute (CLSI) guidelines [16]. The inoculum was prepared using a bacterial suspension of the test strain in 0.9 % NaCl at a final inoculum concentration of 1×10^6 cells per mL using a 0.5 McFarland turbidity standard and subsequent dilution. Inoculation of the microtiter plates was done by delivering 100 μL of the bacterial suspension and 100 μL of the test compound into each well of the plate. The concentration ranges of the compounds tested in twofold dilutions were 0.125 – 2048 mg/L. Plates were incubated in ambient air at 35 °C for 16 – 20 h. Plates were observed for the presence or absence of growth. The minimal inhibitory concentration (MIC) was determined visually as the lowest concentration of drug showing no growth or a significant reduction of growth (> 80 %).

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