Electronic Supplementary Material (ESI) for RSC Medicinal Chemistry.

Cyrene™ is a Green Alternative to DMSO as a solvent for Antibacterial Drug Discovery against ESKAPE Pathogens

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Supporting Information

I. General Experimental Information

Unless otherwise indicated, all commercially available reagents and solvents were used directly from the supplier without further purification. ^{1}H NMR, ^{13}C NMR and ^{19}F NMR were recorded at ambient temperature in $CDCl_3$ (7.27 ppm). Hexafluorobenzene (-163.0 ppm) was used as an internal standard. Chemical shift values (δ) are expressed as parts per million (ppm) and J values are in Hertz.

II. ^{19}F NMR assay of Levofloxacin

Sample Preparation: To a mixture of levofloxacin (1.0 equiv.) in Cyrene™ (1 mL) or DMSO (1 mL) at rt was added hexafluorobenzene (0.17 equiv.) and the resultant mixture was subjected to ^{19}F NMR analysis.

Table S1: ^{19}F NMR assay of Levofloxacin dissolved in Cyrene™ or DMSO.

Prepared Levofloxacin concentration	**Detected Levofloxacin concentration**	
	Cyrene™ sample	DMSO sample
1.3 mM	1.3 mM	1.3 mM
0.15 M	0.15 M	0.15 M

III. Determination of antibacterial activity

The following bacterial strains were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA): *S. aureus (ATCC 43300), E. faecalis (ATCC 51299), E. coli (ATCC 25922), P. aeruginosa (ATCC 27893), A. baumanii (ATCC 19606), K. pneumoniae (ATCC 700603)*. All antibacterial drugs were purchased from Sigma Aldrich UK.

Bacterial cultures were initiated on LB agar (Fisher, UK, Fisher BioReagents™ BP9724-500) slants, and prior to the assays, the suspensions were prepared in cation-adjusted Mueller Hinton broth (Thermo Scientific™ CM0405B, an Oxoid™ product, and supplemented with CLSI recommended divalent cations[1]) and incubated at 37 °C for 18 h at 100 rpm. Antimicrobial assays were performed by the broth microdilution method, in a 96-well plate format, according to the Clinical and Laboratory Standards Institute guidelines. MIC were defined as the lowest compound concentration at which no bacterial growth was visible ($n \geq 3$)

IV. ROS Mediated Killing

Exponentially growing cultures of the appropriate strains were serially diluted into pre-warmed LB medium to a cell density of 10^5 to 10^6 CFU/mL. Cultures were then treated with 2 x MIC ciprofloxacin for 90 min, followed by immediate plating onto LB agar lacking or containing 2% (vol/vol) DMSO or Cyrene™. After incubation at 37^{o}C overnight, colonies were counted. Shown are the average values from experiments carried out three times. Error bars indicate deviations as standard errors of the mean.

V. References

1. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-third Informational Supplement M100-S23. CLSI, Wayne, PA, USA, 2013.