Electronic Supplementary Material (ESI) for RSC Advances.

Synthetic Ionophores as Non-Resistant Antibiotic Adjuvants

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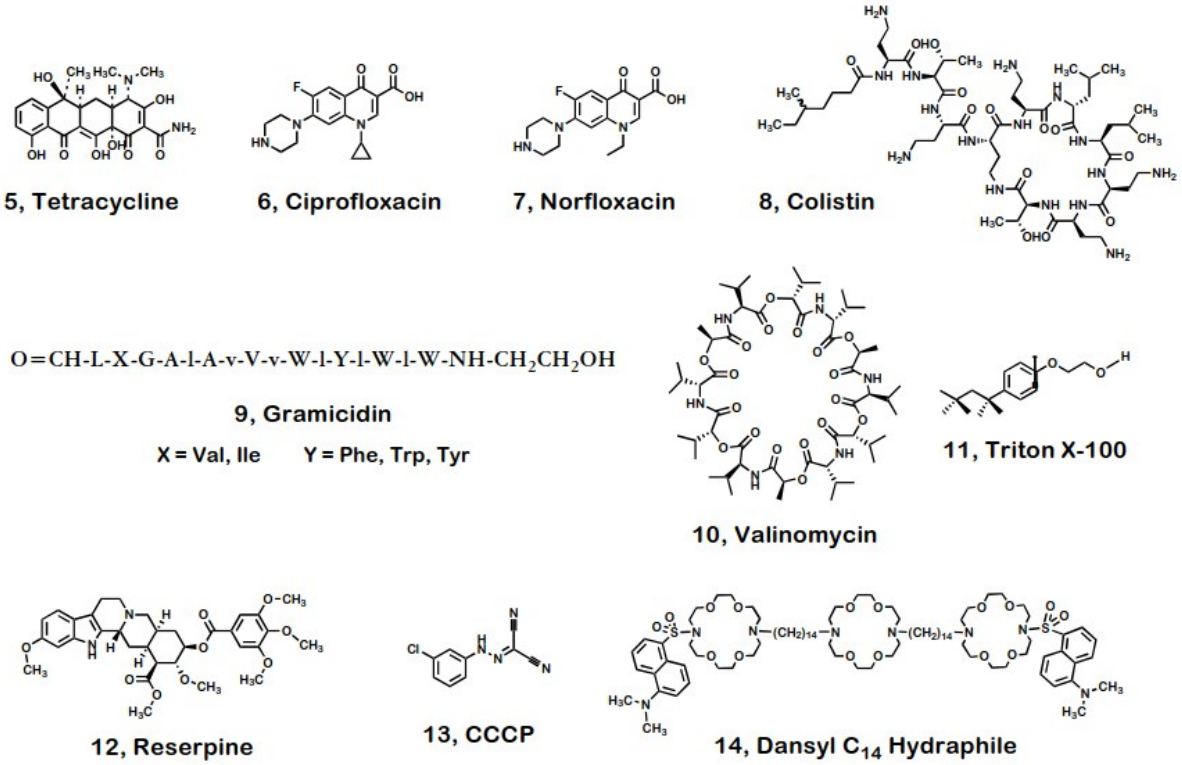

Figure S1. Structures of antibiotics and controls used for the study. The antibiotics studied were tetracycline (**5**), ciprofloxacin (**6**), norfloxacin (**7**) and colistin (**8**). The channel-former gramicidin-D (**9**), the carrier valinomycin (**10**), and the detergent triton X-100 (**11**) served as controls for ion transport. Reserpine (**12**) and CCCP (**13**) are known efflux pump inhibitors. Dansyl C_{14} hydraphile (**14**) was synthesized to study localization of hydraphiles in bacteria.

Table S1: MIC of benzyl C_8-C_{14} hydraphiles (**1** - **4**) against efflux pump expressing tet^R *E. coli*, *K. pneumoniae* (ATCC BAA 2146™), and *S. aureus* 1199B.

Table S1: MIC in µM			
Compounds used	***K. pneumoniae* (ATCC BAA 2146™)**	***E. coli* (tet^R)**	***S. aureus 1199B***
Benzyl C_8 hydraphile (**1**)	200	250	128
Benzyl C_{10} hydraphile (**2**)	56	35	16
Benzyl C_{12} hydraphile (**3**)	35	5	1
Benzyl C_{14} hydraphile (**4**)	10	2	≤1
Tetracycline (**5**)	1000	900	N.D.
Ciprofloxacin (**6**)	700	0.5	N.D.
Norfloxacin (**7**)	N.D.	N.D.	64
Colistin (**8**)	0.25	0.25	N.D.
CCCP (**13**)	N.D.	56	N.D.
Reserpine (**12**)	>128	>128	>128
N.D. = not determined; Colistin is inactive against *S. aureus*			

Figure S2: Combination of benzyl C_8-C_{10} hydraphile (**1-2**) and tetracycline or norfloxacin against tet^R *E. coli*, *K. pneumoniae* and *S. aureus* 1199B.

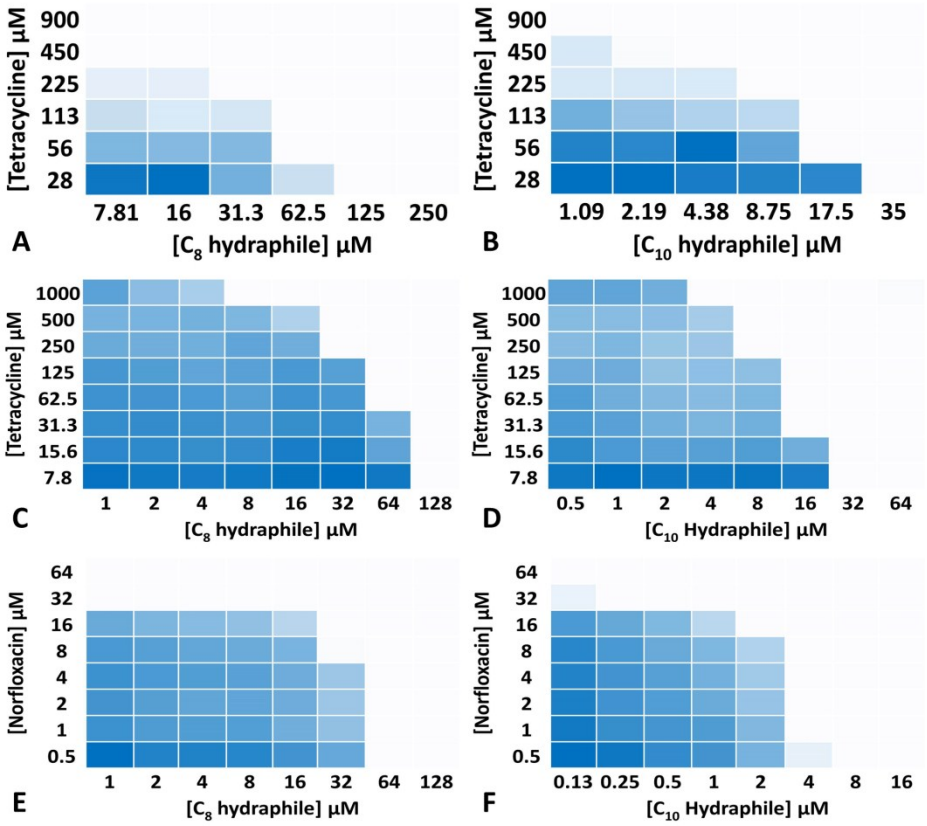

Figure S2. Checkerboard experiments with **1-2** (a-b) with tetracycline against tet^R *E. coli*; (c-d) with tetracycline against *K. pneumoniae* (e-f) with norfloxacin against *S. aureus* 1199B. Color change from blue (0% inhibition) to white (>90% inhibition) indicates increasing growth inhibition.

Figure S3: Tet^R *E. coli* growth curve with benzyl C_8-C_{14} hydraphile (**1**-**4**), tetracycline (**5**) and their combination.

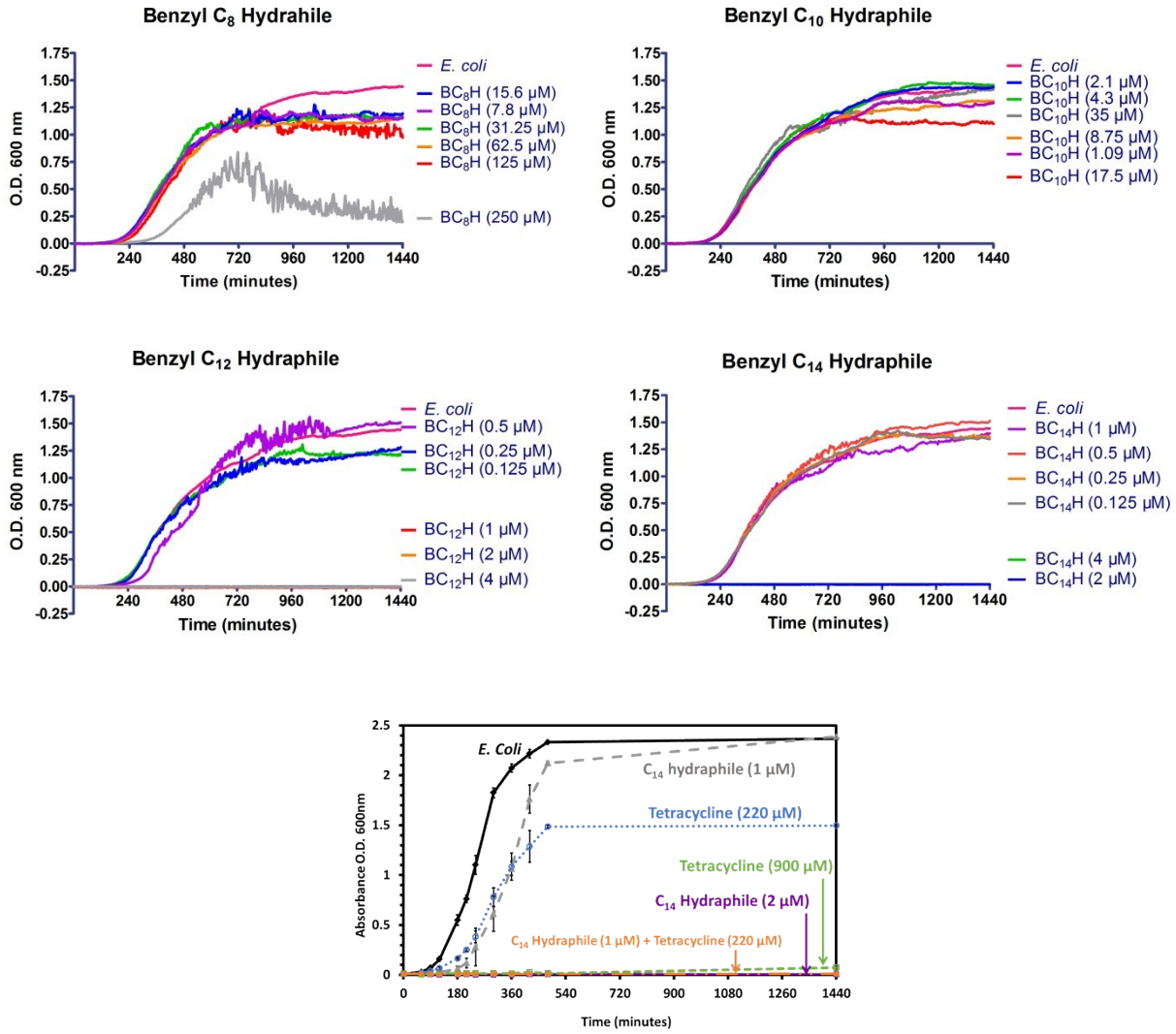

Figure S3. Growth of Tet^R *E. coli* in the presence MIC, and fractional MICs of Benzyl C_8-C_{14} Hydraphile (**1**-**4**) over 24 hours (Four graphs on the top). Readings were taken every 4 minute, average of 2 trials was plotted and error bars were omitted for clarity.

Bottom graph shows the growth of Tet^R *E. coli* in the presence of 1 µM (dashed grey line) and 2 µM (solid purple) benzyl C_{14} hydraphile, 220 µM (dotted blue line) and 900 µM (dashed green line), and combination of 1 µM C_{14} hydraphile and 220 µM tetracycline (orange line). The data represents average optical density (600 nm) from three separate trials. The error bars represent standard deviation in the results.

Table S2: Recovery of tetracycline, ampicillin and ciprofloxacin activity against *Klebsiella pneumoniae* by benzyl C_8-C_{14} hydraphiles.

Table S2: Benzyl C_8-C_{14} hydraphiles recover antibiotic potency against *K. pneumoniae*.

Amphiphile used	[Amphiphile] µM	[Antibiotic] µM	Fold enhancement	FIC index
Antibiotic used: Tetracycline				
No amphiphile	-	1000 ± 100	n/a	n/a
Benzyl C_8 hydraphile	2.5	1000 ± 100	1-fold	1
Benzyl C_8 hydraphile	50 (¼ MIC)	250 ± 50	4-fold	0.5
Benzyl C_8 hydraphile	100 (½ MIC)	25 ± 10	40-fold	0.53
Benzyl C_{10} hydraphile	2.5	900 ± 100	1.1-fold	1
Benzyl C_{10} hydraphile	14 (¼ MIC)	300 ± 50	3-fold	0.58
Benzyl C_{10} hydraphile	28 (½ MIC)	125 ± 25	8-fold	0.63
Benzyl C_{12} hydraphile	2.5	500 ± 50	2-fold	0.57
Benzyl C_{12} hydraphile	8.75 (¼ MIC)	300 ± 25	3-fold	0.58
Benzyl C_{12} hydraphile	17.5 (½ MIC)	125 ± 25	8-fold	0.63
Benzyl C_{14} hydraphile	2.5 (¼ MIC)	350 ± 50	3-fold	0.58
Benzyl C_{14} hydraphile	5 (½ [MIC])	62.5 ± 25	16-fold	0.56
Antibiotic used: Ampicillin (beta-lactamase)				
Benzyl C_{14} hydraphile	5 (½ MIC)	> 1000	0-fold	n/a
Antibiotic used: Ciprofloxacin				
No amphiphile	-	700 ± 100	n/a	n/a
Benzyl C_8 hydraphile	100 (½ MIC)	70 ± 20	10-fold	0.6
Benzyl C_{12} hydraphile	8.75 (¼ MIC)	300 ± 25	2-fold	0.67
Benzyl C_{12} hydraphile	17.5 (½ MIC)	175 ± 25	4-fold	0.75
Benzyl C_{14} hydraphile	2.5 (¼ MIC)	400 ± 50	2-fold	0.82
Benzyl C_{14} hydraphile	5 (½ MIC)	250 ± 25	3-fold	0.85

Table S3: Recovery of tetracycline activity against tet^R *E. coli* by gramicidin-D, valinomycin, triton X-100, CCCP, reserpine, and colistin.

Table S3: Combination studies with controls against Tet^R *E. coli*				
Amphiphile used	**[Amphiphile] µM**	**[Tetracycline] µM**	**Fold enhancement**	**FIC index**
No amphiphile	-	900 ± 100	n/a	
CCCP	1	900	1-fold	1
CCCP	21	450	2-fold	0.75
CCCP	42	225	4-fold	0.75
Reserpine	64	450	2-fold	1
Reserpine	128	225	4-fold	1
Colistin	0.03125	225	4-fold	0.52
Colistin	0.0625	56.25	16-fold	0.31
Gramicidin-D	20	900 ± 100	1-fold	1
Valinomycin	20	450 ± 100	2-fold	1
Triton X-100	20	450 ± 100	2-fold	1
Triton X-100	1700 (0.1%)	450 ± 100	2-fold	1
Colistin	0.0625	56.25	16-fold	0.8
Colistin	0.03125	225	4-fold	0.5
Colistin	0.0156	450	2-fold	0.6

Figure S4: Scanning electron micrographs of tet^R *E. coli* treated with benzyl C_{14} hydraphile.

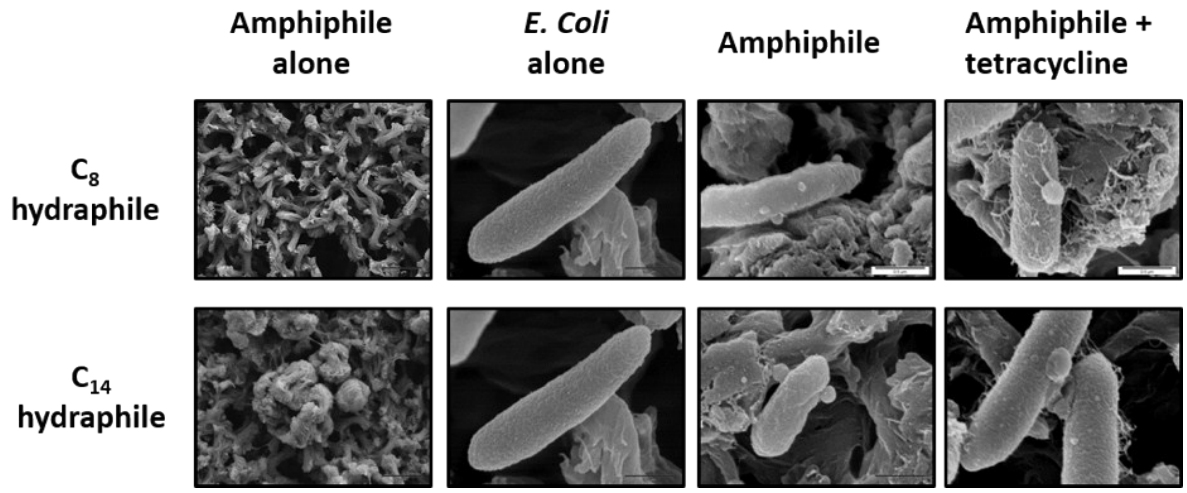

Figure S4. Scanning electron microscopy images of tet^R *E. coli* treated with benzyl C_8 hydraphile (top panel, scale =0.5 μm) and benzyl C_{14} hydraphile (bottom panel, scale =0.5 μm). The column labeled 'amphiphiles alone' shows the nylon membrane (top, scale = 2 μm) and benzyl C_{14} hydraphile aggregate (bottom, scale = 2 μm).

Figure S5: Localization of dansyl labeled C_{14} hydraphile in human embryonic kidney (HEK-293) cells.

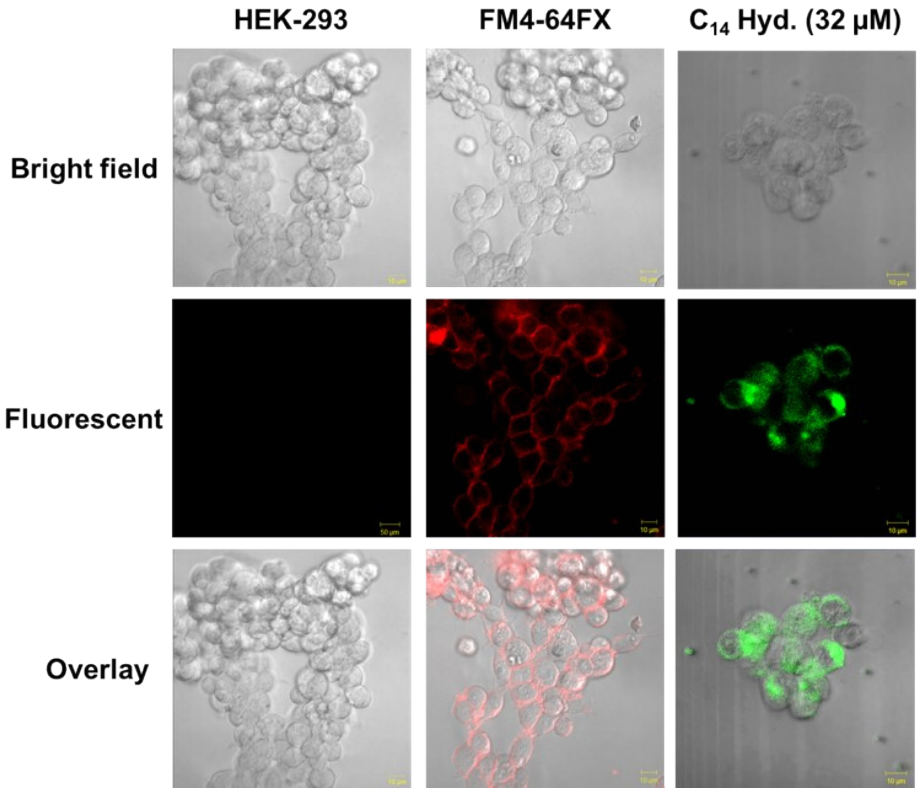

Figure S5. Localization of FM4-64FX (red), and dansyl labeled C_{14} hydraphile (green) in mammalian cells. The magnification is 400-X and the scale is 10 µm.

Figure S6: Change in membrane permeability of tet^R *E. coli* and *S. aureus* 1199B in the presence of compounds 1-4, triton X-100 and colistin.

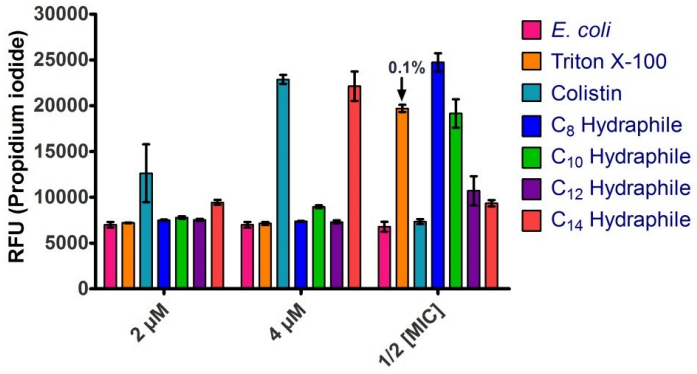

Figure S6a. Permeability of propidium iodide to *E. coli* cell membranes mediated by compounds **1**-**4**, triton X-100 and colistin. The X-axis represents the concentration of the compounds used whereas the y-axis represents the relative fluorescence unit (RFU). Error bars represent standard deviation in three trials.

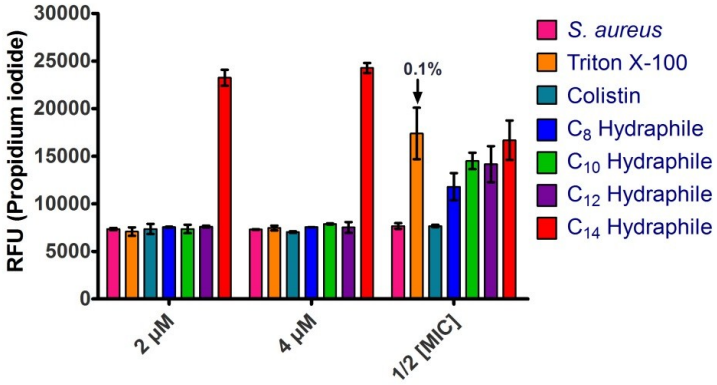

Figure S6b. Permeability of propidium iodide to *S. aureus* cell membranes mediated by compounds **1**-**4**, triton X-100 and colistin. The X-axis represents the concentration of the compounds used whereas the y-axis represents the relative fluorescence unit (RFU). Error bars represent standard deviation in three trials.

Figure S7. Confocal images showing permeability of PI and FDA in tet^R *E. coli* and HEK-293 cells mediated by compounds **1**-**4** and triton X-100.

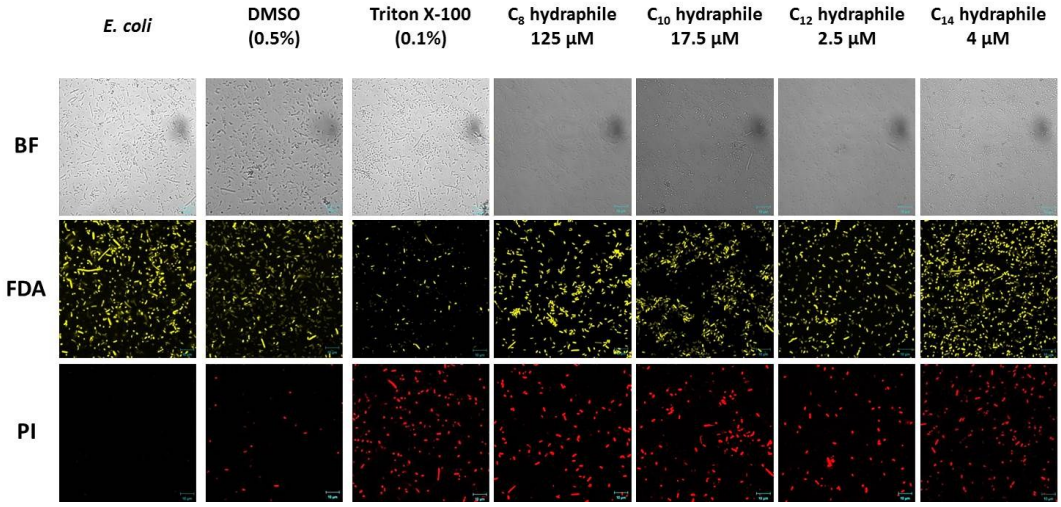

Figure S7a. Benzyl C_8-C_{14} hydraphiles ($^1/_2$ [MIC]) mediated permeability of propidium iodide and fluorescein diacetate in tet^R *E. coli*. *E. coli* alone, DMSO (0.5% v/v) and triton X-100 (0.1% v/v) were used as controls.

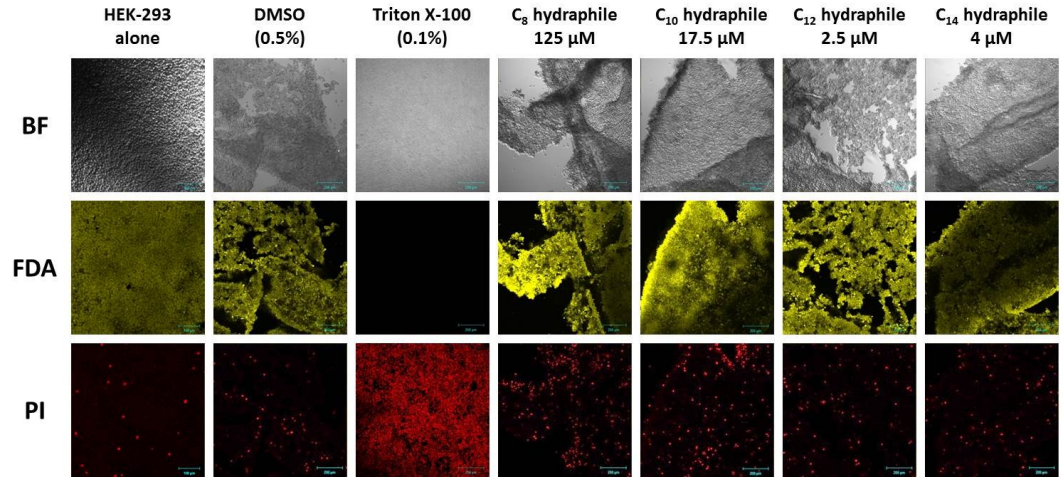

Figure S7b. Benzyl C_8-C_{14} hydraphiles ($^1/_2$ [MIC]) mediated permeability of propidium iodide and fluorescein diacetate in HEK-293 cells. HEK-293 alone, DMSO (0.5%) and triton X-100 (0.1%) were used as controls.

Figure S8: Release of potassium ions from tet^R *E. coli* in the presence of benzyl C_8-C_{14} hydraphiles and Gramicidin.

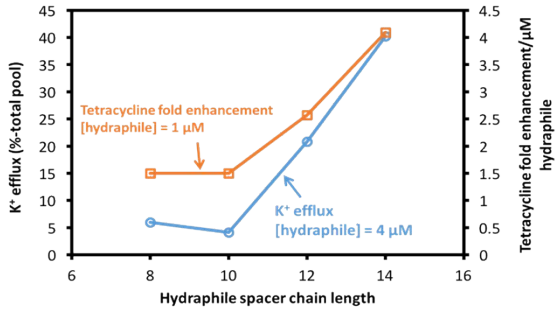

Figure S8(a). Comparison of benzyl C_8-C_{14} hydraphiles to transport potassium ions (open circles) and recovery of tetracycline activity (open squares) against tet^R *E. coli*. Potassium release is expressed in percent of total potassium content of tet^R E. coli released (y-axis, left) and tetracycline recovery in fold enhancement (y-axis, right). The x-axis shows the number of carbon atoms in the spacer chains of hydraphiles.

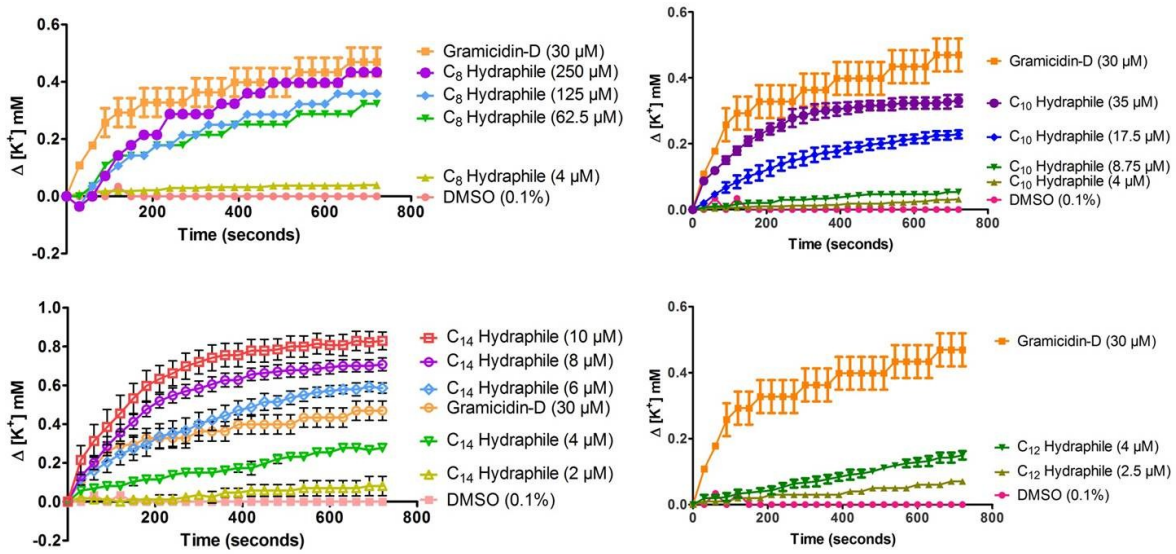

Figure S8 (b-e). Potassium release from *E. coli* over 720 seconds in the presence of various concentrations of benzyl C_8 hydraphile (a), benzyl C_{10} hydraphile (b), benzyl C_{14} hydraphile (c), and benzyl C_{12} hydraphile (d). Error bars represent standard deviation in three trials.

Table S4. Combination studies with hydraphiles and ethidium bromide against *S. aureus* 1199B.

Table S4. Recovery of ethidium bromide activity against *S. aureus* 1199B			
Amphiphile used	**[Amphiphile] μM**	**[EthBr] μM**	**Fold enhancement**
No amphiphile	-	16	n/a
C_8 hydraphile	32 (¼ MIC)	16	1-fold
C_8 hydraphile	64 (½ MIC)	8	2-fold
C_{10} hydraphile	4 (¼ MIC)	16	1-fold
C_{10} hydraphile	8 (½ MIC)	1	16-fold
C_{12} hydraphile	0.25 (¼ MIC)	16	1-fold
C_{12} hydraphile	0.5 (½ MIC)	2	8-fold
C_{14} hydraphile	0.25 (¼ MIC)	4	4-fold
C_{14} hydraphile	0.5 (½ MIC)	0.5	32-fold

Figure S9: Survival of human hepatocytes in the presence of 1-100 µM benzyl C_{14} hydraphile and CCCP.

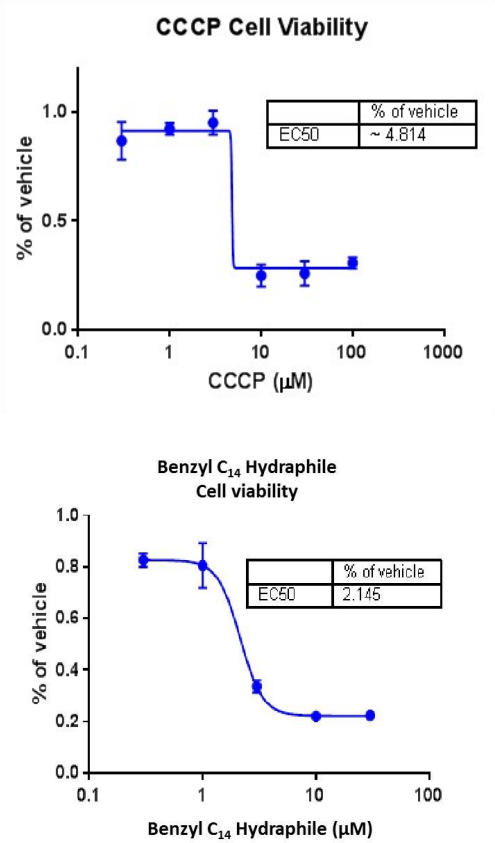

Figure S9. Cytotoxicity of benzyl C_{14} hydraphile and CCCP at 1-100 µM against human hepatocytes. The x-axis represents the concentration of the compound used. The y-axis represents the percent of cells survived as compared to the vehicle (DMSO) in the presence of the respective compounds used. The error bars represent standard deviation in three trials.