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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 in μM				
Compounds used	<i>K. pneumoniae</i> (ATCC BAA 2146 TM)	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	<u>≤1</u>	
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 <i>S. aureus</i>				

Table S1: MIC of benzyl C₈-C₁₄ hydraphiles (1 - 4) against efflux pump expressing tet^R *E. coli*, *K. pneumoniae* (ATCC BAA 2146TM), and *S. aureus* 1199B.

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 μ M (dashed grey line) and 2 μ M (solid purple) benzyl C₁₄ hydraphile, 220 μ M (dotted blue line) and 900 μ M (dashed green line), and combination of 1 μ M C₁₄ hydraphile and 220 μ M tetracycline (orange line). The data represents average optical density (600 nm) from three separate trials. The error bars represent standard deviation in the results.

Amphiphile used[Amphiphile] μ M[Antibiotic]Fold enhancementFIC indexNo amphiphile Benzyl C8 hydraphile- 1000 ± 100 n/an/aBenzyl C8 hydraphile2.5 1000 ± 100 1-fold1Benzyl C8 hydraphile50 (¼ MIC) 250 ± 50 4-fold0.5Benzyl C8 hydraphile100 (¼ MIC) 25 ± 10 40-fold0.53Benzyl C8 hydraphile100 (¼ MIC) 25 ± 10 40-fold1Benzyl C8 hydraphile100 (¼ MIC) 25 ± 10 40-fold0.53Benzyl C10 hydraphile2.5 900 ± 100 1.1-fold1Benzyl C10 hydraphile14 (¼ MIC) 300 ± 50 3 -fold0.58Benzyl C10 hydraphile14 (¼ MIC) 300 ± 50 3 -fold0.63Benzyl C12 hydraphile2.5 500 ± 50 2 -fold0.57Benzyl C12 hydraphile 2.5 500 ± 50 2 -fold0.57Benzyl C12 hydraphile 17.5 (¼ MIC) 300 ± 25 3 -fold0.58Benzyl C12 hydraphile 2.5 (¼ MIC) 125 ± 25 8 -fold0.63Benzyl C14 hydraphile 5 (½ MIC) 125 ± 25 6 -fold0.58Benzyl C14 hydraphile 5 (½ MIC) 350 ± 50 3 -fold0.58Benzyl C14 hydraphile 5 (½ [MIC]) 62.5 ± 25 16 -fold0.56Benzyl C14 hydraphile 5 (½ [MIC])> 1000 0 -fold n/a	Table S2: Benzyl C ₈ -C ₁₄ hydraphiles recover antibiotic potency against <i>K</i> .					
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Benzyl C14 hydraphile $5 (\frac{1}{2} [MIC])$ 62.5 ± 25 16 -fold 0.56 Antibiotic used: Ampicillin (beta-lactamase)Benzyl C14 hydraphile $5 (\frac{1}{2} MIC)$ > 1000 0 -fold n/a Antibiotic used: CiprofloxacinNo amphiphile $=$ 700 ± 100 n/a	hydraphile	2.0 (/ 1.1.2.0)		0 1010	0.00	
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Antibiotic used: Amplemin (beta-factalitase)Benzyl C_{14} hydraphile5 (1/2 MIC)> 10000-foldn/aAntibiotic used: CiprofloxacinNo amphiphile $-$ 700 ± 100 n/a	nydraphile Antibiotic used. Ampicillin (bata lastomasa)					
Defizyr C_{14} hydraphile5 (1/2 MIC)> 10000-foldn/aAntibiotic used: CiprofloxacinNo amphiphile700 ± 100n/a	Benzyl C.	Antibiotic useu.	Ampicinii (Deta-	lactamase)		
Antibiotic used: Ciprofloxacin	hydraphile	5 (½ MIC)	> 1000	0-fold	n/a	
No amphiphile $ 700 \pm 100$ n/a n/a	Antibiotic used: Ciprofloyacin					
100 ± 100 $11/a$ $11/a$	No amphiphile		700 ± 100	n/a	n/a	
Benzyl C_8 100 (1/ MIC) 70 + 20 10 feld	Benzyl C_8	100(1/MIC)	70 ± 20	10 6-14	0.6	
hydraphile $100(\frac{1}{2}) 100 \pm 20$ 10-101d 0.6	hydraphile	$100(\frac{7}{2})$ MIC)	70 ± 20	10-1010	0.6	
Benzyl C ₁₂ 8 75 (1/ MIC) 300 ± 25 2 fold 0.67	Benzyl C ₁₂	8 75 (¹ / MIC)	300 ± 25	2 fold	0.67	
hydraphile $8.75(74 \text{ WHC}) = 500 \pm 25$ 2-101d 0.07	hydraphile	0.73(74 WHC)	500 ± 25	2-101u		
Benzyl C_{12} 17.5 (¹ / ₂ MIC) 175 + 25 4-fold 0.75	Benzyl C ₁₂	17.5 (½ MIC)	175 + 25	4-fold	0.75	
hydraphile 17.5 (721110) 175 225 1161 0.75	hydraphile	17.0 (72 mile)	170 - 20	1010		
Benzyl C ₁₄ $2.5 (\frac{1}{4} \text{ MIC})$ 400 ± 50 2-fold 0.82	Benzyl C_{14}	2.5 (¼ MIC)	400 ± 50	2-fold	0.82	
hydraphile	hydraphile				-	
beilzyi C_{14} bydraphile 5 (½ MIC) 250 ± 25 3-fold 0.85	benzyi C_{14}	5 (½ MIC)	250 ± 25	3-fold	0.85	

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

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	[Amphiphile]	[Tetracycline]	Fold	FIC
used	μM	μŇ	enhancement	index
No amphiphile	-	900 ± 100	n/a	
СССР	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 C₈ hydraphile (top panel, scale =0.5 μ m) and benzyl C₁₄ hydraphile (bottom panel, scale =0.5 μ m). The column labeled 'amphiphiles alone' shows the nylon membrane (top, scale = 2 μ m) and benzyl C₁₄ hydraphile aggregate (bottom, scale = 2 μ m).



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 μ m.

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 C₈-C₁₄ 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 C₈-C₁₄ 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. Recovery of ethidium bromide activity against S. aureus 1199B			
Amphiphile	[Amnhinhile] uM	[EthBr]	Fold
used	[Ampmphile] µM	μM	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 (¹ / ₂ 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

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



Figure S9: Survival of human hepatocytes in the presence of 1-100 μ M benzyl C₁₄ hydraphile and CCCP.

Figure S9. Cytotoxicity of benzyl C_{14} hydraphile and CCCP at 1-100 μ M 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.