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Bile Acid Amphiphiles with Tunable Head Groups as Highly Selective Antitubercular Agents

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Abstract: Tuberculosis faces major challenges for its cure due to a) long treatment period, b) emergence of drug resistance bacteria, and c) poor patient compliance. Disrupting the membrane integrity of mycobacteria as a therapeutic strategy has not been explored well as rigid, waxy, and hydrophobic nature of mycobacterial lipids does not allow binding and penetration of charged amtimicrobial amphiphiles and peptides. Here, we present a new concept that fine-tuning of charged head group modulates the specificity of amphiphiles against bacterial membranes. We show that hard-charged amphiphiles interact with mycobacterial trehalose dimycolates and penetrate through rigid mycobacterial membranes. In contrast, soft-charged amphiphiles specifically inhibit growth of both *E. coli* and *S. aureus via* electrostatic interactions. These subtle variations between interactions of amphiphiles and bacterial membranes could be explored further for design of more specific and potent antimycobacterial agents.

Introduction:

Tuberculosis (TB) remains a global health burden with ~8.6 million individuals infected with *M. tuberculosis* (*Mtb*) and accounting for ~2.0 millions deaths in 2012.[1] Major drawback of current TB regimen targeting essential mycobacterial pathways is 6-month long duration and poor compliance among patients. This situation has further worsened due to emergence of various MDR/XDR TB strains, HIV-TB co-infection, and BCG vaccine failure to impart protection against pulmonary TB.[2] The advent of computational methods along with whole cell and HTS based assays have led to identification of various scaffolds that are currently in clinical trails. The newly identified scaffolds should have i) improved activity against dormant bacteria, ii) novel mechanism of action, iii) specificity against mycobacteria, iv) good pharmacokinetics/pharmacodynamics properties, and v) compatibility with current TB and retroviral therapies.[3]

Antimicrobial peptides (AMPs) induce non-receptor mediated disruptions in the target membranes of microorganisms by virtue of their amphiphilic nature with discrete cationic charge.[4] Amphiphiles mimicking these AMPs with variable cationic charge groups on hydrophobic moieties have been explored for their antimicrobial activities.[5,6] These amphiphiles exert their antimicrobial activity through electrostatic interactions with lipid components of bacterial membranes.[7,8] These membrane disruptive specific amphiphiles have the ability to a) shorten duration of treatment, and b) eradicate drug resistant bacteria.

Mycobacterial non-polar lipids present in outer membrane of mycobacteria may not interact with polar charged amphiphiles/peptides, and hence do not allow their insertion across rigid hydrophobic mycolic lipids. Hence, disrupting the integrity of mycobacterial membranes has not been extensively explored to combat TB.[9] Therefore, the present study was conducted to address: i) lack of specificity of AMPs and amphiphiles against microorganisms, ii) exploring the

disruption of membrane integrity as a mechanism to combat TB, and iii) to better understand interactions between amphiphiles and mycobacterial membranes.

Bile acids are inherently facial amphiphilic in nature due to stereochemical orientation of hydroxyl groups.[10] Savage *et al* have synthesized cholic acid derived cationic antimicrobials that possessed higher affinity for lipid A of bacterial membranes as compared to polymyxin B.[11] In this manuscript, we propose that fine-tuning of charged head groups on bile acid amphiphiles modulates their specificity against mycobacteria. We unraveled that hard-charged amphiphiles specifically kills mycobacteria as hydrophobic, rigid, waxy outer membranes of mycobacteria can allow penetration of these hydrophobic amphiphiles (Fig. 1). Contrastingly, soft-charged amphiphiles specifically kills gram-positive/gram-negative bacteria through electrostatic interactions with polar bacterial lipids.

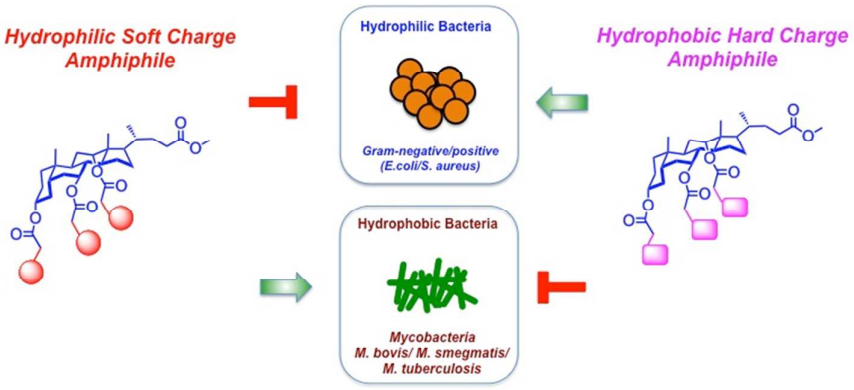

Fig. 1. Schematic presentation of the study showing the selectivity of hard-charged amphiphiles for mycobacteria, and soft-charged amphiphiles for gram-positive and gram-negative bacteria.

Results and Discussion:

Design and Synthesis of Amphiphiles: We engineered twenty bile acid derived amphiphiles with different charged head groups using four bile acids, lithocholic acid (LCA),

chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), and cholic acid (CA) (Fig. 2). Polarity of attached head groups varied from soft-charged ammonium (AMM) to hard-charged trimethyl ammonium (TMA), *N*-methyl piperidine (PIP), pyridine (PYR), and dimethylamino pyridine (DMAP). Amphiphiles were synthesized by chloroacetylation of corresponding bile acid methyl ester followed by quaternization with respective tertiary amines (Scheme 1, ESI), and characterized by ^{1}H-NMR, ^{13}C-NMR and HRMS (ESI).

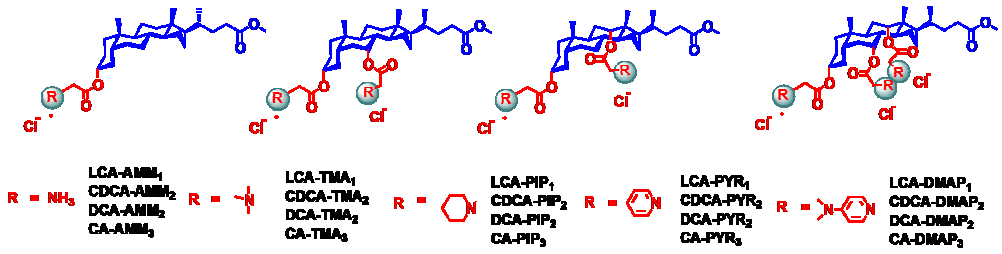

Fig. 2. Molecular structures of lithocholic acid (**LCA)**, chenodeoxycholic acid (**CDCA)**, deoxycholic acid (**DCA)** and cholic acid (**CA)** based amphiphiles with different charged head groups synthesized and studied

Antibacterial activities against different microorganisms: We then evaluated activities of amphiphiles against mycobacterial species, *M. smegmatis (Msm), M. tuberculosis* $H_{37}Rv$ (*Mtb*) and *M. bovis* BCG (Table 1). From structure-activity studies, we concluded that a) multiple-charged amphiphiles are more potent as compared to single-charged amphiphiles; b) among multiple-charged amphiphiles, hard-charged amphiphiles are more potent as compared to soft-charged amphiphiles; c) in general DMAP derived amphiphiles (CDCA-$DMAP_2$, DCA-$DMAP_2$, and CA-$DMAP_3$) were most potent against *Mtb* inhibiting mycobacterial growth in range of 0.78-6.25 μM; d) CA derived amphiphiles followed the order of DMAP > PYR > PIP > TMA > AMM in terms of their anti-tubercular activity; e) ammonium head group derived soft-charged amphiphiles possess no activity against mycobacteria.

Table 1: Antimycobacterial activities, hemolytic activities and therapeutic index of amphiphiles.

Amphiphile	[a]MIC_{99} (μM)			[b]MHC_{50} (□M)	[c]TI	Amphiphile	[a]MIC_{99} (μM)			[b]MHC_{50} (mM)	[c]TI
	BCG	mc^2155	$H_{37}Rv$				BCG	mc^2155	$H_{37}Rv$		
LCA-AMM_1	>50	>50	>50	>1	<20	DCA-AMM_2	25	> 50	> 50	>1	<20
LCA-TMA_1	50	6.25	50	>1	>20	DCA-TMA_2	25	12.5	> 50	>1	<20
LCA-PIP_1	50	6.25	50	>1	>20	DCA-PIP_2	3.12	0.78	6.25	>1	>160
LCA-PYR_1	>50	25	> 50	>1	<20	DCA-PYR_2	6.25	1.56	12.5	>1	>80
LCA-$DMAP_1$	50	12.5	50	>1	>20	DCA-$DMAP_2$	2.34	0.78	4.68	>1	>213
CDCA-AMM_2	37.5	>50	> 50	>1	<20	CA-AMM_3	18.75	> 50	> 50	>1	<20
CDCA-TMA_2	4.68	3.12	12.5	>1	>80	CA-TMA_3	6.25	12.5	50	>1	>20
CDCA-PIP_2	6.25	1.56	12.5	>1	>80	CA-PIP_3	6.25	6.25	25	>1	>40
CDCA-PYR_2	9.375	3.12	25	>1	>40	CA-PYR_3	6.25	3.12	37.5	>1	>26
CDCA-$DMAP_2$	3.12	0.78	3.12	>1	>320	CA-$DMAP_3$	3.12	1.56	6.25	>1	>160
INH	0.78	> 50	0.78	-[d]	-[d]	Levofloxacin	0.39	0.58	0.78	-[d]	-[d]

a: MIC_{99} of amphiphiles against three mycobacterial strains; b: MHC_{50} is minimum hemolytic conc. at which 50% hemolysis is observed;
c: Therapeutic index for *M. tuberculosis* $H_{37}Rv$ as ratio of MHC_{50}/MIC_{99} (*M. tuberculosis* $H_{37}Rv$); d: not determined.

To unravel the selectivity of amphiphiles, we determined their activities against gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria (Table 2). MIC_{99} determination studies suggested that a) single-charged LCA amphiphiles showed no growth inhibition even at 512 μM; b) among multiple-charged amphiphiles, soft-charged amphiphiles, (DCA-AMM_2 and CA-AMM_3) inhibited

99% growth at 16-64 μM; c) hard-charged multiple headed amphiphiles are less potet than soft-charged amphiphiles; d) CA derived hard-charged amphiphiles showed no growth inhibition even at 512 μM. Interestingly, soft-charged CA-AMM_3 amphiphile was highly specific in its ability to inhibit growth of *E. coli*/*S. aureus*, whereas, hard-charged CA-$DMAP_3$ amphiphile was highly specific for mycobacterial species. CA-$DMAP_3$ possess MIC_{99} of 1.56-6.25 μM, and selectivity index of ~ 40-320 over *E. coli/S. aureus*. Presence of 50% plasma also maintains its high potency (MIC_{99} = 6.25 μM) against mycobacteria.

Table 2: Antibacterial activities of bile acid amphiphiles against Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria; and toxicities of bile acid amphiphiles against lung epithelial (A549) and macrophage (THP-1) cell lines.

Amphiphile	MIC_{99} (μM)[a]		IC_{50} (μM)[b]		Amphiphile	MIC_{99} (μM)[a]		IC_{50} (μM)[b]	
	E. coli	*S. aureus*	A549	THP-1		*E. coli*	*S. aureus*	A549	THP-1
LCA-AMM_1	> 512	> 512	24.6 ± 8.8	14.0 ± 0.2	**DCA-AMM_2**	16	64	14.7 ± 5.6	13.96 ± 0.3
LCA-TMA_1	> 512	> 512	69 ± 2.5	62 ± 2.6	**DCA-TMA_2**	256	256	157 ± 2.26	105 ±7.3
LCA-PIP_1	> 512	> 512	42 ± 6.5	77 ± 6.0	**DCA-PIP_2**	> 512	128	50 ± 8.1	77 ± 8.3
LCA-PYR_1	> 512	> 512	68 ±1.3	89 ± 5.0	**DCA-PYR_2**	128	> 512	78 ± 5.0	124 ±8.7
LCA-$DMAP_1$	> 512	> 512	112 ±5.9	86 ± 1.5	**DCA-$DMAP_2$**	64	64	50 ±9.6	63 ± 5.4
CDCA-AMM_2	64	128	14.8 ± 9.5	13.7 ± 0.8	**CA-AMM_3**	32	64	75.9 ± 1.3	38.2 ± 5.1
CDCA-TMA_2	256	512	144 ± 7.8	142 ±1.7	**CA-TMA_3**	> 512	> 512	> 200	80 ± 6.02
CDCA-PIP_2	128	256	79 ± 7.1	164 ±1.7	**CA-PIP_3**	> 512	512	> 200	> 200
CDCA-PYR_2	128	256	168 ± 0.1	> 200	**CA-PYR_3**	> 512	> 512	> 200	> 200
CDCA-$DMAP_2$	128	128	154 ± 4.8	137 ± 2.1	**CA-$DMAP_3$**	> 512	256	> 200	> 200
Polymyxin	0.5	1.0	-[c]	-[c]					

a: MIC_{99} of amphiphiles against gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacterial strains; b: IC_{50} is minimum conc. at which 50% cell death is observed against lung epithelial cells (A549) and macrophages (THP-1) ; c: not determined.

Cytotoxicity studies against mammalian cells: Cytotoxicity studies of amphiphiles against mammalian A549 (human lung epithelial cell line) and THP-1 (human monocyte cell line) cells showed that single-charged, LCA amphiphiles are most cytotoxic, and soft-charged amphiphiles are more toxic as compared to hard-charged amphiphiles (Table 2; Fig. S1, S2, ESI). Highly sensitive propidium iodide based cell cycle analysis also showed no change in cell cycle phase of A549 cells on treatment with 100 μM of CA based amphiphiles (Fig. S3, ESI). Hemolytic studies against chicken and sheep blood RBCs showed that all the amphiphiles possessed MHC_{50} values greater than 1 mM (Table 1; Fig. S4, ESI). Therapeutic index (MHC_{50}/MIC_{99}) calculations suggested that hard-charged CA amphiphiles have high therapeutic index over soft-charged amphiphiles, and DMAP derived multiple-charged amphiphiles are most potent with highest therapeutic index.

Mechanism of Action: Above studies concluded that soft-charged CA-AMM_3 selectively inhibits growth of *E. coli*/*S. aureus*, whereas hard-charged CA-$DMAP_3$ is highly selective for mycobacteria. We observed that killing of *E. coli*/*S. aureus* by CA-AMM_3 was dose-dependent and bactericidal in nature whereas no growth inhibition of *E. coli*/*S. aureus* was observed on exposure to CA-$DMAP_3$ (Fig. 3a, Fig. S5, ESI). Incubation of *Msm* with CA-$DMAP_3$ inhibited bacterial growth by 10,000-100,000 fold (Fig. 3b), whereas no such growth inhibition was observed on exposure to CA-AMM_3. Similarly, we observed perturbations in *E.coli*/*S. aureus* membranes only on incubation with CA-AMM_3 (Fig. 3c), whereas CA-$DMAP_3$ specifically inhibited ability of *Msm* to generate proton motive force (Fig. 3d).

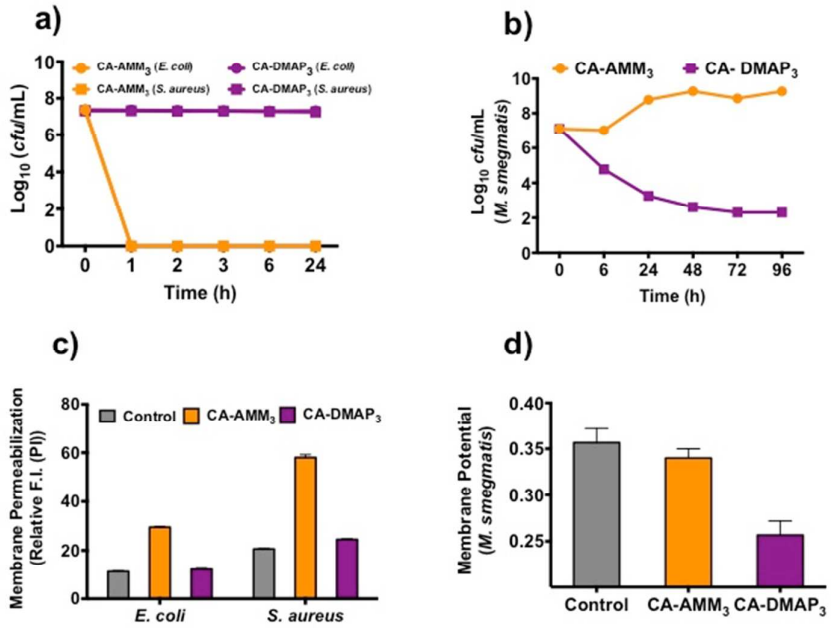

Fig. 3. (a) Time dependent killing of *E. coli* and *S. aureus* suggesting bactericidal effect in the presence of CA-AMM_3. (b) Time dependent killing of *Msm* showing selective bactericidal activity by CA-$DMAP_3$. (c) Membrane permeabilization of *E. coli* and *S. aureus* by propidium iodide showing selective permeabilization of *E. coli* and *S. aureus* by CA-AMM_3. (d) Effect of CA-AMM_3 and CA-$DMAP_3$ on membrane potential of *Msm* suggesting selective activity of CA-$DMAP_3$.

Next, we performed AFM studies to determine both morphological and topological changes in *E. coli* and *M. bovis* BCG on treatment with CA-AMM_3 and CA-$DMAP_3$. Incubation of *E. coli* with CA-AMM_3 induced surface indentations, micelle like structures, and leakage of large amount of cytoplasmic contents (Fig. 4b, Table S1, ESI). Incubation of *M. bovis* BCG with CA-$DMAP_3$ leads to pores and groove formation with rugged surface (Fig. 4f), whereas morphology and topology of CA-$DMAP_3$ treated *E. coli* (Fig. 4c) and CA-AMM_3 treated *M. bovis* BCG (Fig. 4e) was similar to their respective untreated samples (Fig. 4a/d).

To understand the mechanism of this differential specificity, we next determined activity of these amphiphiles against both *E. coli* and *S. aureus* in the presence of EDTA, a known

destabilizer of lipopolysaccharide (LPS) and proteoglycans.[12] Mechanistic studies showed that presence of EDTA enhances activity of CA-$DMAP_3$ by 64-fold and 16-fold against *E. coli* and *S. aureus,* respectively, whereas only ~ 2-fold increase in activity was observed for CA-AMM_3. These results conclude that presence of LPS and proteoglycans inhibit interactions of CA-$DMAP_3$ with *E. coli*/*S. aureus* bacterial membranes, thereby accounting for lack of its activity against them.

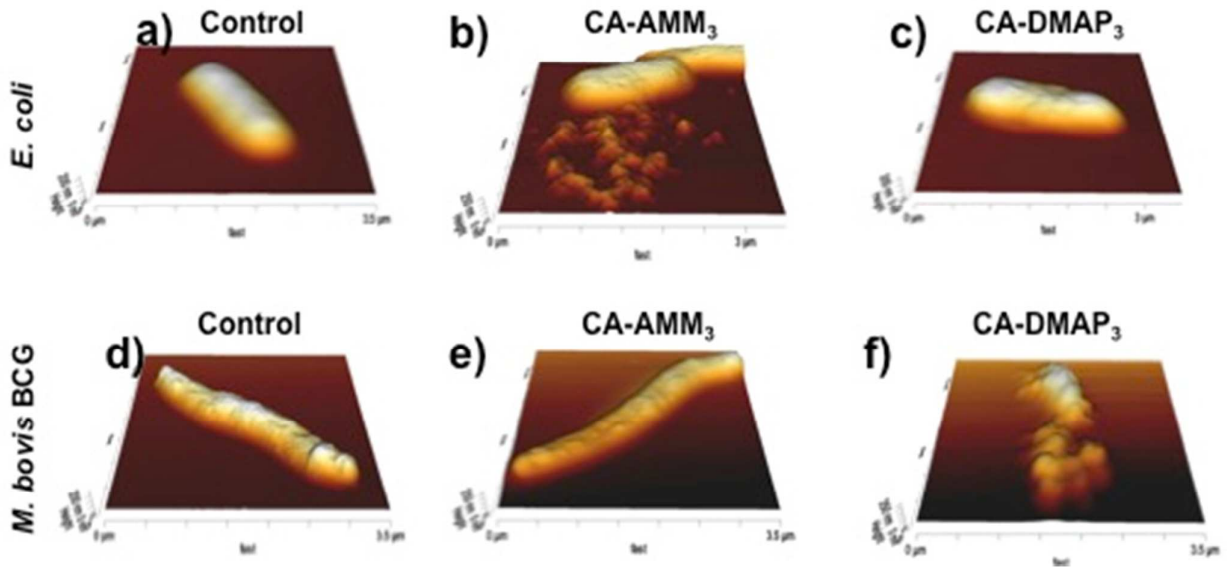

Fig. 4. (a-f) Atomic force micrographs of *E. coli* (a-c) and *BCG* (d-f) on incubation with CA-AMM_3 (b, e) and CA-$DMAP_3$ (c, f) amphiphiles suggesting selective disruptions of *E. coli* membranes by CA-AMM_3 (b) and *BCG* membranes by CA-$DMAP_3$ (f) respectively.

Amphiphile-Membrane interactions: We speculated that presence of hydrophobic mycolic lipids like trehalose dimycolate (TDM),[13] might be responsible for selective killing of mycobacteria by CA-$DMAP_3$. We therefore probed the interactions of CA-AMM_3 and CA-$DMAP_3$ with model bacterial membranes to quantify changes in surface hydration and rigidity of membranes. Using Laurdan probe,[14] we observed that TDM doped mycobacterial model

membranes, PE:PG:TDM (1:1:2) are more dehydrated than *E. coli* modeled membranes, PE:PG:LPS (5:3:1) and PE:PG (1:1) membranes (Fig. 5a-c).

Incubation of CA-AMM_3 induced higher dehydration in PE:PG:LPS and PE:PG membranes (Fig. 5a-c) due to strong electrostatic interactions, thereby accounting for selective activity of CA-AMM_3 against *E. coli*/*S. aureus*. Similarly, minor dehydration of PE:PG:TDM membranes by CA-AMM_3 accounts for poor interactions and inability of CA-AMM_3 to kill mycobacteria. We did not observe any dehydration of PE:PG:TDM membranes upon incubation with CA-$DMAP_3$ (Fig. 5c), thereby suggesting the presence of hydrophobic interactions between CA-$DMAP_3$ and PE:PG:TDM.

Membrane fluidity studies using DPH probe[15] suggested that mycobacterial modeled membranes are more rigid in comparison to other membranes (Fig. 5d-f). Incubation of PE:PG:LPS and PE:PG with CA-AMM_3 does not induce any fluidity suggesting poor interactions of CA-AMM_3 with hydrophobic regions of membranes. Therefore observed dehydration without any alteration in rigidity of PE:PG:LPS and PE:PG confirms carpet-like mechanism for activity of CA-AMM_3 against *E. coli*/*S. aureus*.[16] Contrastingly, presence of hydrophobic, rigid mycolic lipids like TDM prevents interactions of CA-AMM_3 with mycobacterial membranes. Interestingly, CA-$DMAP_3$ increases fluidity of mycobacterial (PE:PG:TDM) membranes as observed in case of alamethicin[17] by virtue of insertion of CA-$DMAP_3$ in hydrophobic membranes. We did not observe this enhanced membrane fluidity on incubation of PE:PG and PE:PG:LPS with CA-$DMAP_3$. These observations suggest that CA-$DMAP_3$ has ability to interact with rigid mycolic lipids and form pore like structures in mycobacterial membranes.[18]

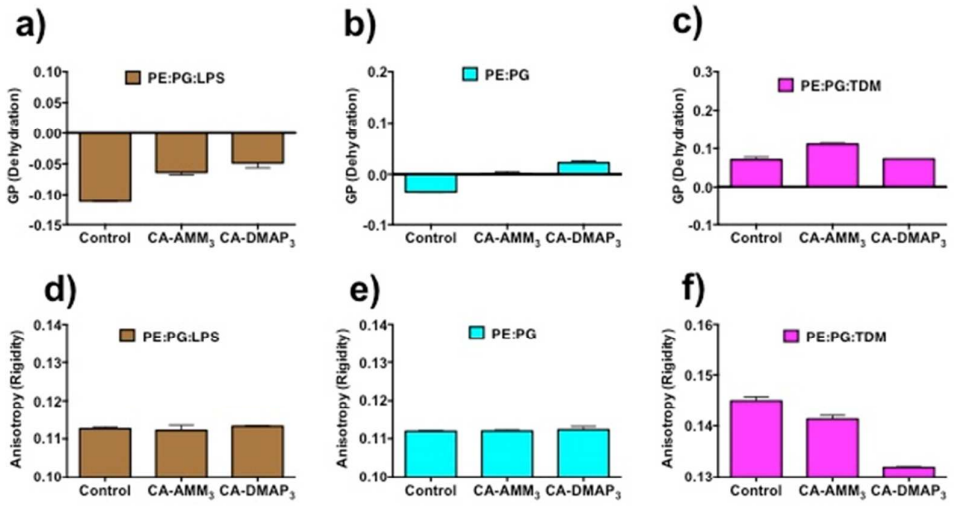

Fig. 5. (a-c) Changes in membrane surface hydration of model membranes on incubation with amphiphiles suggesting dehydrated nature of PE:PG:TDM membranes and maximum dehydration of PE:PG:LPS and PE:PG membranes due to electrostatic interactions. (d-f) Changes in membrane rigidity of model bacterial membranes on incubation with amphiphiles showing high rigidity of PE:PG:TDM membranes and induction of membrane fluidity due to interactions of CA-$DMAP_3$ with modeled PE:PG:TDM membranes.

Conclusions

In summary, we demonstrated that fine-tuning of charged head groups on bile acid scaffold modulates their specificity against bacteria. Soft charged primary amine amphiphiles interact with gram-positive and gram-negative bacterial membranes, whereas hard charged head groups provide effective and selective interactions with hydrophobic mycobacterial membranes. In-depth mechanistic studies revealed that this specificity in mechanism of action for these amphiphiles was due to molecular differences in the cell wall architecture of mycobacterial and gram-positive/ gram-negative bacteria. The present study would help us in understanding the molecular basis of specific amphiphile-membrane interactions and in design of more potent 2nd generation

amphiphiles that are highly specific for a particular bacterial species, which might be useful to combat the problem of drug resistance.

Experimental Section

Materials and methods: Bile acids were purchased from Sigma-Aldrich. CCCP, INH, $DiCO_2$ were purchased from Invitrogen corporation. All the synthesized compounds were purified using Combi-flash chromatography using 230-400 mesh size silica gel. ^{1}H NMR and ^{13}C NMR spectra were recorded using Brucker 400 MHz spectrometer. Chemical shifts (δ) are reported in ppm with tetramethylsilane as internal standard. High-resolution mass spectra were measured on AB-SCIEX-5600 mass spectrometer. *E. coli* (MTCC 443) and *S. aureus* (MTCC 737) was purchased from MTCC. A549 cell line was purchased from Sigma Aldrich and THP-1 cells were kind gift from NCCS, Pune. *M. smegmatis* and *M. tuberculosis* $H_{37}Rv$ were kind gift from Dr. Clifton E. Barry, NIH and *M. bovis* BCG was kind gift from Prof. Anil K. Tyagi (Dept. of Biochemistry, UDSC, India).

Antibacterial activity: We determined antibacterial activity of bile acid amphiphiles using a slight modification in method mentioned by Hancock *et al*.[19] The bacterial strains were grown for 6 hrs and diluted to 10^5 *cfu*/mL. 150 µL of this bacterial suspension was added to a 96 well plate containing required concentration of amphiphiles. The plate was then incubated at 37 °C with continuous shaking for 12h, and OD_{600} nm was measured using molecular probes M5 microplate reader. MIC_{99} value was determined by taking the average of triplicate values for each concentration and has been performed in duplicates. Polymyxin was used as positive control in our assays. For experiments with EDTA, both the bacterial cultures were grown in nutrient broth containing 10 mM EDTA and MIC_{99} was determined as described above in the presence of 10 mM EDTA. The antimycobacterial activity of these amphiphiles was determined using inverted plate reader method as described previously.[20] The plates were incubated at 37 °C and MIC_{99} values were read microscopically using an inverted plate reader after 14 days for *M. bovis* BCG,

Mtb, and after 2 days for *Msm*. Each reading was made three independent times. Standard drug such as isoniazid and levofloxacin were used as positive controls in our assays. For killing curves early-log phase cultures were incubated with CA-AMM_3 and CA-$DMAP_3$, at designated time points. Bacterial enumeration was performed by plating 10-fold serial dilutions on MB7H10 plates, and plates were incubated at 37°C.

Cytotoxicity Assay:[21] THP-1 cell line was maintained in RPMI media as per standard protocol and were differentiated in macrophages by overnight addition of 50 ng/mL of PMA (phorbol 12-myristate 13-acetate). For cytotoxicity assay, 5 x 10^3 cells were seeded per well in a 96 well plate. After 24 h, cells were overlayed with medium containing various concentration of amphiphiles. After overlaying macrophages for 48 h, 20 μL of 5 mg/ml MTT {3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide} was added to each well. After incubation for 4 h, cells were lysed by addition of 200 μL of 1:1 mixture of DMSO and MeOH to dissolve the formazen crystals and absorption at 540 nm was measured. Cell viability was calculated using equation [{A_{540} (treated cells) - background}/{A_{540} (untreated cells) - background}] 100.

Cell cycle analysis: Cell cycle analysis against A549 cells for bile acid amphiphiles were performed according to published protocol.[21]

Hemolytic assay:[22] Hemolytic assays were performed using chicken and sheep blood. For RBC isolation, blood was centrifuged at 4000 rpm for 5 min. For hemolytic assays, 195 μL of erythrocyte suspension (5% or 20% RBC) were added per well in a 96-well plate and incubated with facial amphiphiles at desired concentrations. After incubation for 1 h, plate was centrifuged at 1200 x g for 15 min. The supernatant was diluted 1:100 in 1x PBS and absorption at 413 nm was measured. All our assay plates included positive (1% PBST), buffer (1x PBS) and solvent (MeOH) controls. The percentage of hemolysis was determined from $\{(A - A_0)/(A_{total} - A_0)\} \times 100$, where A is the absorbance of the test well, A_0 is the absorbance of the negative controls, and

A_{total} is the absorbance of 100% hemolysis wells at 413 nm. All the experiments were performed at least two times in duplicates

Membrane permeablization studies:[23] *E. coli* and *S. aureus* cells were grown till mid log phase. Cells were harvested by centrifugation, washed with 1x PBS and re-suspended in 1x PBS. These washed *E. coli* and *S. aureus* bacilli suspension were pre-incubated with amphiphiles at a concentration of 80 μM or 160 μM, respectively, followed by addition of 15 μM propidium iodide (PI). The uptake of PI was measured by the increase in fluorescence of PI for 10 min as a measure of membrane permeabilization using excitation wavelength of 535 nm and emission wavelength of 617 nm.

Membrane potential studies:[24] *Msm* was grown till OD_{600} nm of 1.0 and bacteria was pre-incubated with amphiphiles or INH or CCCP. Bacterial cells were immediately exposed to 15 μM of $DiCO_2$ (3,3′-diethyloxacarbocyanine iodide) at room temperature. After 30 minutes of labeling, cells were washed twice with 1x PBS and green fluorescence (Ex_{480} nm/Em_{530} nm) and red fluorescence (Ex_{488} nm/Em_{610} nm) was measured in a 96-well plate reader (Biotek synergy Hr). Cells treated with no drug were kept as control for background fluorescence. Membrane potential was calculated as ratio of red fluorescence to green fluorescence using Biotek Synergy Hit and Gene5 software.

Atomic force microscopy studies (Sample preparation and imaging):[25] Poly-L-lysine coated (1.0 mm uniform thickness) microscopic slides were obtained from Polysciences Inc. All the AFM-imaging experiments were done on JPK NanoWizard® AFM head using an AC air mode cantilever. The pyramidal tip cantilever used purchased from ACTA is made of silicon with a spring constant of 40 N/m. All the data was processed using JPKSPM data processing software. For AFM studies bacteria were grown till OD_{600} nm of 1.0, harvested, washed twice with 1x PBS and subsequently exposed to CA-AMM_3 or CA-$DMAP_3$ in a 96-well plate. After incubation for 24 h, samples were applied on poly-L-lysine coated slides and dried using slow stream of nitrogen

gas. Imaging was done using AC air/tapping mode with 15 μm z-scale size. All images were obtained with a scan speed of 0.5 Hz and a resolution of 1024 × 1024 pixels. The height, width, and 3D-toplogical information was acquired and processed with JPKSPM data processing software.

Amphiphile-Membrane interactions: Membrane vesicles with desired lipid ratios were prepared as described previously.[26] Changes in surface hydration of vesicles were studied after incubation of the vesicles with 10 weight percentages of facial amphiphiles at 25^{o}C for 4h. We recorded generalized polarization of Laurdan in a 96-well plate in Molecular Devices M5 instrument. Fluorescence of Laurdan was recorded using excitation wavelength of 350 nm and emission wavelength of 440 nm and 490 nm. Generalized polarization (GP) was calculated using equation GP = (I440 - I490)/(I440 + I490). Similarly, we measured changes in steady state anisotropy of DPH in a 96-well plate using λ_{ex} at 350 nm and λ_{em} of 452 nm after incubation of these vesicles with amphiphiles at 25^{o}C in Molecular devices M5 instrument.

Notes and references

† Electronic Supplementary Information (ESI) available: [Figure S1-S6, Table S1, Synthesis of amphiphiles]. See DOI: 10.1039/b000000x/

AB conceived the idea. AB and RS designed the experiments. MS, PB, AS synthesized the amphiphile molecules. SB performed all the experiments with *E. coli* and *S. aureus*. SB, SK, RS performed experiments with mycobacteria. MS performed the Laurdan and DPH based experiments with model membranes. VS performed AFM experiments. SB, MS, VS, RS, and AB analyzed the data. AB and RS have written the manuscript. AB and RS supervised the overall research.

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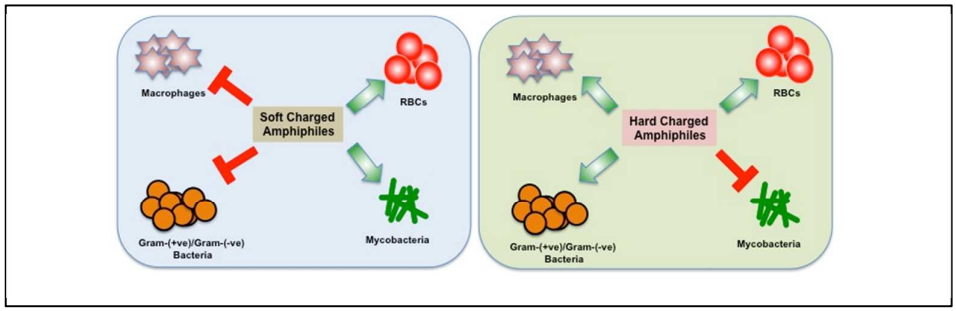
Table of Contents



Hard-charged amphiphiles are highly selective against mycobacteria, whereas soft-charged amphiphiles are active against gram-positive and gram-negative bacteria.