Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

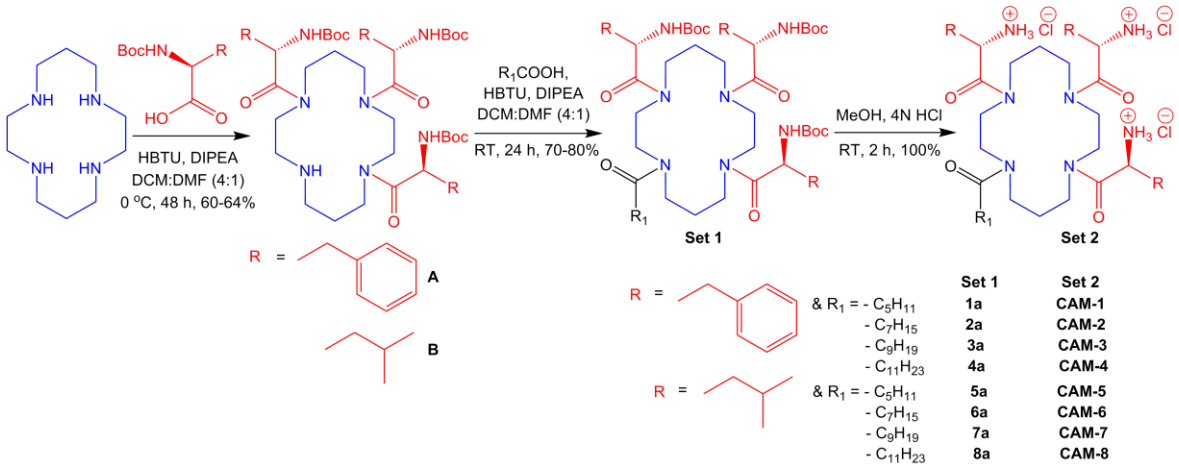
Cyclam-based antibacterial molecule eradicates Gram-negative superbugs with potent efficacy against human corneal infection

Mohini Mohan Konai,[a] Iqbal Pakrudheen,[a] Swagatam Barman,[a] Natalia Sharma,[b] Khatija Tabbasum,[b] Prashant Garg[b] and Jayanta Haldar*[a]

[a]*Antimicrobial Research Laboratory, New Chemistry Unit and School of Advanced Materials, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur, Bengaluru 560064, Karnataka, India.*

[b] *L V Prasad Eye Institute, L V Prasad Marg, Banjara Hills, Hyderabad 500034, Telangana, India.*

Details of Synthesis Protocols and Characterization



Scheme S1: Synthesis of cyclam-based antibacterial molecules, **CAM 1-8**.

General protocol for synthesizing A and B: At first 3 equivalents (~2.5 mmol) of Boc-protected amino acid (*N*-Boc-Phe-OH or *N*-Boc-Leu-OH) was dissolved in dry DCM: anhydrous DMF (4:1) at 0 ºC. Then, 6 equivalents of DIPEA were added to it followed by 3 equivalents of HBTU and the reaction mixture was allowed to stir for 10-15 min. After that, 1 equivalent of cyclam was added drop wise to the reaction mixture after dissolving it in dry DCM. The RB containing reaction mixture was then brought to RT and allowed to stir for 48 h. At the end, the reaction solvent was removed by using rotary evaporator and the crude residue was diluted in ethyl acetate. The ethyl acetate layer was then washed by using 1N HCl (3 times) and saturated Na_2CO_3 solution (3 times). Finally, the ethyl acetate layer was collected through anhydrous Na_2SO_4 and column chromatography was performed on silica gel (60-120 mesh) to obtain pure compounds with 60-64 % yield.

A: Yield-64%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 7.347-7.108 (m, cy(-NH-CH_2-CH_2-CH_2-N(CO-CH(NHBoc)-CH_2-*PhH*)-CH_2-CH_2-N(CO-CH(NHBoc)-CH_2-*PhH*)-CH_2-CH_2-CH_2-N(CO-CH(NHBoc)-CH_2-*PhH*)-CH_2-CH_2-), 15H), 5.398-4.476 (m, cy(-NH-CH_2-CH_2-CH_2-N(CO-*CH*(*NH*Boc)-CH_2-Ph)-CH_2-CH_2-N(CO-*CH*(*NH*Boc)-CH_2-Ph)-CH_2-CH_2-CH_2-N(CO-

C<u>*H*</u>(*N*<u>*H*</u>Boc)-CH_2-Ph)-CH_2-CH_2-), 6H), 3.743-1.955 (m, cy(-*N*<u>*H*</u>-*C*<u>*H*$_2$</u>-CH_2-*C*<u>*H*$_2$</u>-N(CO-CH(NHBoc)-*C*<u>*H*$_2$</u>-Ph)-*C*<u>*H*$_2$</u>-*C*<u>*H*$_2$</u>-N(CO-CH(NHBoc)-*C*<u>*H*$_2$</u>-Ph)-*C*<u>*H*$_2$</u>-CH_2-*C*<u>*H*$_2$</u>-N(CO-CH(NHBoc)-*C*<u>*H*$_2$</u>-Ph)-*C*<u>*H*$_2$</u>-*C*<u>*H*$_2$</u>-), 23H), 1.558-1.335 (m, cy(-NH-CH_2-*C*<u>*H*$_2$</u>-CH_2-N(CO-CH(NH-COO-*C(C*<u>*H*$_3$</u>*)*$_3$)-CH_2-Ph)-CH_2-CH_2-N(CO-CH(NH-COO-*C(C*<u>*H*$_3$</u>*)*$_3$)-CH_2-Ph)-CH_2-*C*<u>*H*$_2$</u>-CH_2-N(CO-CH(NH-COO-*C(C*<u>*H*$_3$</u>*)*$_3$)-CH_2-Ph)-CH_2-CH_2-), 31H); HRMS (m/z): 942.57086 [$(M+H)^+$] (Observed), 942.57045 [$(M+H)^+$] (Calculated).

B: Yield-60%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 5.257-5.086 (m, cy(-NH-CH_2-CH_2-CH_2-N(CO-CH(*N*<u>*H*</u>Boc)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-N(CO-CH(*N*<u>*H*</u>Boc)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-CH_2-N(CO-CH(*N*<u>*H*</u>Boc)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-), 3H), 4.657-4.535 (m, cy(-NH-CH_2-CH_2-CH_2-N(CO-*C*<u>*H*</u>(NHBoc)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-N(CO-*C*<u>*H*</u>(NHBoc)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-CH_2-N(CO-*C*<u>*H*</u>(NHBoc)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-), 3H), 4.073-2.633 (m, cy(-*N*<u>*H*</u>-*C*<u>*H*$_2$</u>-CH_2-*C*<u>*H*$_2$</u>-N(CO-CH(NHBoc)-CH_2-$CH(CH_3)_2$)-*C*<u>*H*$_2$</u>-*C*<u>*H*$_2$</u>-N(CO-CH(NHBoc)-CH_2-$CH(CH_3)_2$)-*C*<u>*H*$_2$</u>-CH_2-*C*<u>*H*$_2$</u>-N(CO-CH(NHBoc)-CH_2-$CH(CH_3)_2$)-*C*<u>*H*$_2$</u>-*C*<u>*H*$_2$</u>-), 17H), 2.285-1.495 (m, cy(-NH-CH_2-*C*<u>*H*$_2$</u>-CH_2-N(CO-CH(NHBoc)-*C*<u>*H*$_2$</u>-*C*<u>*H*</u>$(CH_3)_2$)-CH_2-CH_2-N(CO-CH(NHBoc)-*C*<u>*H*$_2$</u>-*C*<u>*H*</u>$(CH_3)_2$)-CH_2-*C*<u>*H*$_2$</u>-CH_2-N(CO-CH(NHBoc)-*C*<u>*H*$_2$</u>-*C*<u>*H*</u>$(CH_3)_2$)-CH_2-CH_2-), 13H), 1.406 (bs, cy(-NH-CH_2-CH_2-CH_2-N(CO-CH(NH-COO-*C(C*<u>*H*$_3$</u>*)*$_3$)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-N(CO-CH(NH-COO-*C(C*<u>*H*$_3$</u>*)*$_3$)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-CH_2-N(CO-CH(NH-COO-*C(C*<u>*H*$_3$</u>*)*$_3$)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-), 27H), 1.027-0.883 (m, cy(-NH-CH_2-CH_2-CH_2-N(CO-CH(NHBoc)-CH_2-CH*(C*<u>*H*$_3$</u>*)*$_2$)-CH_2-CH_2-N(CO-CH(NHBoc)-CH_2-CH*(C*<u>*H*$_3$</u>*)*$_2$)-CH_2-CH_2-CH_2-N(CO-CH(NHBoc)-CH_2-CH*(C*<u>*H*$_3$</u>*)*$_2$)-CH_2-CH_2-), 18H); HRMS (m/z): 840.61846 [$(M+H)^+$] (Observed), 840.61740 [$(M+H)^+$] (Calculated).

General protocol for synthesizing 1a-8a: About 1.5 equivalents (0.5-1 mmol) of different aliphatic acids (such as hexanoic, Octanoic, decanoic and dodecanoic acids) were first dissolved in dry DCM: anhydrous DMF (4:1) at 0 ºC. Then, 6 equivalents of DIPEA were added to it followed by 1.5 equivalents of HBTU and the reaction mixture was allowed to stir for 10-15 min. After that, 1 equivalent of **A** or **B** was added drop wise to the reaction mixture after dissolving it in dry DCM. The reaction was then allowed to stir for 24 h at RT. At the end, the solvent was removed by using rotary evaporator and crude residue was

diluted in ethyl acetate. The ethyl acetate layer was then washed by using 1N HCl (3 times) and saturated Na_2CO_3 solution (3 times). Finally, ethyl acetate layer was collected through anhydrous Na_2SO_4 and column chromatography was performed on silica gel (60-120 mesh) to obtain the pure compound with 70-80% yield.

1a: Yield-76%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 7.314-7.008 (m, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-CH(NHBoc)-CH_2-*PhH*)-CH_2-CH_2-N(CO-CH(NHBoc)-CH_2-*PhH*)-CH_2-CH_2-CH_2-N(CO-CH(NHBoc)-CH_2-*PhH*)-CH_2-CH_2-), 15H), 5.376-4.609 (m, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-*CH*(*NH*Boc)-CH_2-Ph)-CH_2-CH_2-N(CO-*CH*(*NH*Boc)-CH_2-Ph)-CH_2-CH_2-CH_2-N(CO-*CH*(*NH*Boc)-CH_2-Ph)-CH_2-CH_2-), 6H), 3.707-2.932 (m, cy(-N(CO-R_1)-*CH_2*-CH_2-*CH_2*-N(CO-CH(NHBoc)-*CH_2*-Ph)-*CH_2*-*CH_2*-N(CO-CH(NHBoc)-*CH_2*-Ph)-*CH_2*-CH_2-*CH_2*-N(CO-CH(NHBoc)-*CH_2*-Ph)-*CH_2*-*CH_2*-), 22H), 2.411-2.011 (m, -*CH_2*-CH_2-$(CH_2)_2$-CH_3 of R_1 group, 2H), 1.429-1.362 (m, cy(-N((CO-CH_2-*CH_2*-*$(CH_2)_2$*-CH_3)-CH_2-*CH_2*-CH_2-N(CO-CH(NH-COO-*C(CH_3)$_3$*)-CH_2-Ph)-CH_2-CH_2-N(CO-CH(NH-COO-*C(CH_3)$_3$*)-CH_2-Ph)-CH_2-*CH_2*-CH_2-N(CO-CH(NH-COO-*C(CH_3)$_3$*)-CH_2-Ph)-CH_2-CH_2-), 37H), 0.917-0.893 (t, -CH_2-CH_2-$(CH_2)_2$-*CH_3* of R_1 group, 3H); HRMS (m/z): 1040.64221 [$(M+H)^+$] (Observed), 1040.64362 [$(M+H)^+$] (Calculated).

2a: Yield-78%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 7.312-7.130 (m, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-CH(NHBoc)-CH_2-*PhH*)-CH_2-CH_2-N(CO-CH(NHBoc)-CH_2-*PhH*)-CH_2-CH_2-CH_2-N(CO-CH(NHBoc)-CH_2-*PhH*)-CH_2-CH_2-), 15H), 5.383-4.608 (m, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-*CH*(*NH*Boc)-CH_2-Ph)-CH_2-CH_2-N(CO-*CH*(*NH*Boc)-CH_2-Ph)-CH_2-CH_2-CH_2-N(CO-*CH*(*NH*Boc)-CH_2-Ph)-CH_2-CH_2-), 6H), 3.705-2.905 (m, cy(-N(CO-R_1)-*CH_2*-CH_2-*CH_2*-N(CO-CH(NHBoc)-*CH_2*-Ph)-*CH_2*-*CH_2*-N(CO-CH(NHBoc)-*CH_2*-Ph)-*CH_2*-CH_2-*CH_2*-N(CO-CH(NHBoc)-*CH_2*-Ph)-*CH_2*-*CH_2*-), 22H), 2.411-2.154 (m, -*CH_2*-CH_2-$(CH_2)_4$-CH_3 of R_1 group, 2H), 1.427-1.302 (m, cy(-N((CO-CH_2-*CH_2*-*$(CH_2)_4$*-CH_3)-CH_2-*CH_2*-CH_2-N(CO-CH(NH-COO-*C(CH_3)$_3$*)-CH_2-Ph)-CH_2-CH_2-N(CO-CH(NH-COO-*C(CH_3)$_3$*)-CH_2-Ph)-CH_2-*CH_2*-CH_2-N(CO-CH(NH-COO-*C(CH_3)$_3$*)-CH_2-Ph)-CH_2-CH_2-), 41H), 0.882-0.869 (t, -CH_2-CH_2-$(CH_2)_4$-*CH_3* of R_1 group, 3H); HRMS (m/z): 1068.64697 [$(M+H)^+$] (Observed), 1068.67492 [$(M+H)^+$] (Calculated).

3a: Yield-75%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 7.305-7.108 (m, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-CH(NHBoc)-CH_2-*Ph*<u>*H*</u>)-CH_2-CH_2-N(CO-CH(NHBoc)-CH_2-*Ph*<u>*H*</u>)-CH_2-CH_2-CH_2-N(CO-CH(NHBoc)-CH_2-*Ph*<u>*H*</u>)-CH_2-CH_2-), 15H), 5.380-4.604 (m, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-*C*<u>*H*</u>(*N*<u>*H*</u>Boc)-CH_2-Ph)-CH_2-CH_2-N(CO-*C*<u>*H*</u>(*N*<u>*H*</u>Boc)-CH_2-Ph)-CH_2-CH_2-CH_2-N(CO-*C*<u>*H*</u>(*N*<u>*H*</u>Boc)-CH_2-Ph)-CH_2-CH_2-), 6H), 3.714-3.198 (m, cy(-N(CO-R_1)-*C*<u>*H*₂</u>-CH_2-*C*<u>*H*₂</u>-N(CO-CH(NHBoc)-*C*<u>*H*₂</u>-Ph)-*C*<u>*H*₂</u>-*C*<u>*H*₂</u>-N(CO-CH(NHBoc)-*C*<u>*H*₂</u>-Ph)-*C*<u>*H*₂</u>-CH_2-*C*<u>*H*₂</u>-N(CO-CH(NHBoc)-*C*<u>*H*₂</u>-Ph)-*C*<u>*H*₂</u>-*C*<u>*H*₂</u>-), 22H), 2.407-2.203 (m, -*C*<u>*H*₂</u>-CH_2-$(CH_2)_6$-CH_3 of R_1 group, 2H), 1.418-1.284 (m, cy(-N(CO-CH_2-*C*<u>*H*₂</u>-*(C*<u>*H*₂</u>*)*$_6$-CH_3)-CH_2-*C*<u>*H*₂</u>-CH_2-N(CO-CH(NH-COO-*C(C*<u>*H*₃</u>*)*$_3$)-CH_2-Ph)-CH_2-CH_2-N(CO-CH(NH-COO-*C(C*<u>*H*₃</u>*)*$_3$)-CH_2-Ph)-CH_2-*C*<u>*H*₂</u>-CH_2-N(CO-CH(NH-COO-*C(C*<u>*H*₃</u>*)*$_3$)-CH_2-Ph)-CH_2-CH_2-), 45H), 0.881-0.868 (t, -CH_2-CH_2-$(CH_2)_6$-*C*<u>*H*₃</u> of R_1 group, 3H); HRMS (m/z): 1096.68537 [$(M+H)^+$] (Observed), 1096.70622 [$(M+H)^+$] (Calculated).

4a: Yield-80%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 7.304-7.107 (m, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-CH(NHBoc)-CH_2-*Ph*<u>*H*</u>)-CH_2-CH_2-N(CO-CH(NHBoc)-CH_2-*Ph*<u>*H*</u>)-CH_2-CH_2-CH_2-N(CO-CH(NHBoc)-CH_2-*Ph*<u>*H*</u>)-CH_2-CH_2-), 15H), 5.381-4.617 (m, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-*C*<u>*H*</u>(*N*<u>*H*</u>Boc)-CH_2-Ph)-CH_2-CH_2-N(CO-*C*<u>*H*</u>(*N*<u>*H*</u>Boc)-CH_2-Ph)-CH_2-CH_2-CH_2-N(CO-*C*<u>*H*</u>(*N*<u>*H*</u>Boc)-CH_2-Ph)-CH_2-CH_2-), 6H), 3.712-2.958 (m, cy(-N(CO-R_1)-*C*<u>*H*₂</u>-CH_2-*C*<u>*H*₂</u>-N(CO-CH(NHBoc)-*C*<u>*H*₂</u>-Ph)-*C*<u>*H*₂</u>-*C*<u>*H*₂</u>-N(CO-CH(NHBoc)-*C*<u>*H*₂</u>-Ph)-*C*<u>*H*₂</u>-CH_2-*C*<u>*H*₂</u>-N(CO-CH(NHBoc)-*C*<u>*H*₂</u>-Ph)-*C*<u>*H*₂</u>-*C*<u>*H*₂</u>-), 22H), 2.405-2.168 (m, -*C*<u>*H*₂</u>-CH_2-$(CH_2)_8$-CH_3 of R_1 group, 2H), 1.623-1.268 (m, cy(-N(CO-CH_2-*C*<u>*H*₂</u>-*(C*<u>*H*₂</u>*)*$_8$-CH_3)-CH_2-*C*<u>*H*₂</u>-CH_2-N(CO-CH(NH-COO-*C(C*<u>*H*₃</u>*)*$_3$)-CH_2-Ph)-CH_2-CH_2-N(CO-CH(NH-COO-*C(C*<u>*H*₃</u>*)*$_3$)-CH_2-Ph)-CH_2-*C*<u>*H*₂</u>-CH_2-N(CO-CH(NH-COO-*C(C*<u>*H*₃</u>*)*$_3$)-CH_2-Ph)-CH_2-CH_2-), 49H), 0.892-0.862 (t, -CH_2-CH_2-$(CH_2)_8$-*C*<u>*H*₃</u> of R_1 group, 3H); HRMS (m/z): 1124.74198 [$(M+H)^+$] (Observed), 1124.73752 [$(M+H)^+$] (Calculated).

5a: Yield-76%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 5.232-4.966 (m, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-CH(*N*<u>*H*</u>Boc)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-N(CO-CH(*N*<u>*H*</u>Boc)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-CH_2-N(CO-CH(*N*<u>*H*</u>Boc)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-), 3H), 4.648-4.397 (m, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-*C*<u>*H*</u>(NHBoc)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-N(CO-*C*<u>*H*</u>(NHBoc)-

CH_2-$CH(CH_3)_2$)-CH_2-CH_2-CH_2-N(CO-*CH*(NHBoc)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-), 3H), 3.875-3.126 (m, cy(-N(CO-R_1)-*CH*$_2$-CH_2-*CH*$_2$-N(CO-CH(NHBoc)-CH_2-$CH(CH_3)_2$)-*CH*$_2$-*CH*$_2$-N(CO-CH(NHBoc)-CH_2-$CH(CH_3)_2$)-*CH*$_2$-CH_2-*CH*$_2$-N(CO-CH(NHBoc)-CH_2-$CH(CH_3)_2$)-*CH*$_2$-*CH*$_2$-), 16H), 2.459-1.470 (m, cy(-N(CO-*CH*$_2$-*CH*$_2$-$(CH_2)_2$-CH_3)-CH_2-*CH*$_2$-CH_2-N(CO-CH(NHBoc)-*CH*$_2$-*CH*$(CH_3)_2$)-CH_2-CH_2-N(CO-CH(NHBoc)-*CH*$_2$-*CH*$(CH_3)_2$)-CH_2-*CH*$_2$-CH_2-N(CO-CH(NHBoc)-*CH*$_2$-*CH*$(CH_3)_2$)-CH_2-CH_2-), 17H), 1.408 (bs, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-CH(NH-COO-*C*$(CH_3)_3$)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-N(CO-CH(NH-COO-*C*$(CH_3)_3$)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-CH_2-N(CO-CH(NH-COO-*C*$(CH_3)_3$)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-), 27H), 1.307 (bs, -CH_2-CH_2-*(CH*$_2)_2$-CH_3 of R_1 group, 4H), 0.993-0.872 (m, cy(-N(CO-CH_2-CH_2-$(CH_2)_2$-*CH*$_3$)-CH_2-CH_2-CH_2-N(CO-CH(NHBoc)-CH_2-CH*(CH*$_3)_2$)-CH_2-CH_2-N(CO-CH(NHBoc)-CH_2-CH*(CH*$_3)_2$)-CH_2-CH_2-CH_2-N(CO-CH(NHBoc)-CH_2-CH*(CH*$_3)_2$)-CH_2-CH_2-), 21H); HRMS (m/z): 938.69235 [$(M+H)^+$] (Observed), 938.69357 [$(M+H)^+$] (Calculated).

6a: Yield-74%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 5.155-4.988 (m, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-CH(*NH*Boc)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-N(CO-CH(*NH*Boc)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-CH_2-N(CO-CH(*NH*Boc)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-), 3H), 4.623-4.395 (m, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-*CH*(NHBoc)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-N(CO-*CH*(NHBoc)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-CH_2-N(CO-*CH*(NHBoc)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-), 3H), 3.875-3.125 (m, cy(-N(CO-R_1)-*CH*$_2$-CH_2-*CH*$_2$-N(CO-CH(NHBoc)-CH_2-$CH(CH_3)_2$)-*CH*$_2$-*CH*$_2$-N(CO-CH(NHBoc)-CH_2-$CH(CH_3)_2$)-*CH*$_2$-CH_2-*CH*$_2$-N(CO-CH(NHBoc)-CH_2-$CH(CH_3)_2$)-*CH*$_2$-*CH*$_2$-), 16H), 2.458-1.455 (m, cy(-N(CO-*CH*$_2$-*CH*$_2$-$(CH_2)_4$-CH_3)-CH_2-*CH*$_2$-CH_2-N(CO-CH(NHBoc)-*CH*$_2$-*CH*$(CH_3)_2$)-CH_2-CH_2-N(CO-CH(NHBoc)-*CH*$_2$-*CH*$(CH_3)_2$)-CH_2-*CH*$_2$-CH_2-N(CO-CH(NHBoc)-*CH*$_2$-*CH*$(CH_3)_2$)-CH_2-CH_2-), 17H), 1.407 (bs, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-CH(NH-COO-*C*$(CH_3)_3$)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-N(CO-CH(NH-COO-*C*$(CH_3)_3$)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-CH_2-N(CO-CH(NH-COO-*C*$(CH_3)_3$)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-), 27H), 1.265 (bs, -CH_2-CH_2-*(CH*$_2)_4$-CH_3 of R_1 group, 8H), 0.991-0.840 (m, cy(-N(CO-CH_2-CH_2-$(CH_2)_4$-*CH*$_3$)-CH_2-CH_2-CH_2-N(CO-CH(NHBoc)-CH_2-CH*(CH*$_3)_2$)-CH_2-CH_2-N(CO-CH(NHBoc)-CH_2-CH*(CH*$_3)_2$)-CH_2-CH_2-CH_2-N(CO-CH(NHBoc)-CH_2-CH*(CH*$_3)_2$)-CH_2-CH_2-), 21H); HRMS (m/z): 966.71739 [$(M+H)^+$] (Observed), 966.72187 [$(M+H)^+$] (Calculated).

7a: Yield-75%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 5.240-5.000 (m, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-CH(*N*<u>*H*</u>Boc)-CH_2-CH(CH_3)$_2$)-CH_2-CH_2-N(CO-CH(*N*<u>*H*</u>Boc)-CH_2-CH(CH_3)$_2$)-CH_2-CH_2-CH_2-N(CO-CH(*N*<u>*H*</u>Boc)-CH_2-CH(CH_3)$_2$)-CH_2-CH_2-), 3H), 4.638-4.413 (m, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-*C*<u>*H*</u>(NHBoc)-CH_2-CH(CH_3)$_2$)-CH_2-CH_2-N(CO-*C*<u>*H*</u>(NHBoc)-CH_2-CH(CH_3)$_2$)-CH_2-CH_2-CH_2-N(CO-*C*<u>*H*</u>(NHBoc)-CH_2-CH(CH_3)$_2$)-CH_2-CH_2-), 3H), 3.878-3.148 (m, cy(-N(CO-R_1)-*C*<u>*H*$_2$</u>-CH_2-*C*<u>*H*$_2$</u>-N(CO-CH(NHBoc)-CH_2-CH(CH_3)$_2$)-*C*<u>*H*$_2$</u>-*C*<u>*H*$_2$</u>-N(CO-CH(NHBoc)-CH_2-CH(CH_3)$_2$)-*C*<u>*H*$_2$</u>-CH_2-*C*<u>*H*$_2$</u>-N(CO-CH(NHBoc)-CH_2-CH(CH_3)$_2$)-*C*<u>*H*$_2$</u>-*C*<u>*H*$_2$</u>-), 16H), 2.469-1.630 (m, cy(-N(CO-*C*<u>*H*$_2$</u>-*C*<u>*H*$_2$</u>-(CH_2)$_6$-CH_3)-CH_2-*C*<u>*H*$_2$</u>-CH_2-N(CO-CH(NHBoc)-*C*<u>*H*$_2$</u>-*C*<u>*H*</u>(CH_3)$_2$)-CH_2-CH_2-N(CO-CH(NHBoc)-*C*<u>*H*$_2$</u>-*C*<u>*H*</u>(CH_3)$_2$)-CH_2-*C*<u>*H*$_2$</u>-CH_2-N(CO-CH(NHBoc)-*C*<u>*H*$_2$</u>-*C*<u>*H*</u>(CH_3)$_2$)-CH_2-CH_2-), 17H), 1.420 (bs, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-CH(NH-COO-*C*(*C*<u>*H*$_3$</u>)<u>$_3$</u>)-CH_2-CH(CH_3)$_2$)-CH_2-CH_2-N(CO-CH(NH-COO-*C*(*C*<u>*H*$_3$</u>)<u>$_3$</u>)-CH_2-CH(CH_3)$_2$)-CH_2-CH_2-CH_2-N(CO-CH(NH-COO-*C*(*C*<u>*H*$_3$</u>)<u>$_3$</u>)-CH_2-CH(CH_3)$_2$)-CH_2-CH_2-), 27H), 1.257 (bs, -CH_2-CH_2-*(C*<u>*H*$_2$</u>)<u>$_6$</u>-CH_3 of R_1 group, 12H), 0.979-0.856 (m, cy(-N(CO-CH_2-CH_2-(CH_2)$_6$-*C*<u>*H*$_3$</u>)-CH_2-CH_2-CH_2-N(CO-CH(NHBoc)-CH_2-CH*(C*<u>*H*$_3$</u>)<u>$_2$</u>)-CH_2-CH_2-N(CO-CH(NHBoc)-CH_2-CH*(C*<u>*H*$_3$</u>)<u>$_2$</u>)-CH_2-CH_2-CH_2-N(CO-CH(NHBoc)-CH_2-CH*(C*<u>*H*$_3$</u>)<u>$_2$</u>)-CH_2-CH_2-), 21H); HRMS (m/z): 994.75708 [$(M+H)^+$] (Observed), 994.75317 [$(M+H)^+$] (Calculated).

8a: Yield-76%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 5.157-4.998 (m, cy(-N(CO- R_1)-CH_2-CH_2-CH_2-N(CO-CH(*N*<u>*H*</u>Boc)-CH_2-CH(CH_3)$_2$)-CH_2-CH_2-N(CO-CH(*N*<u>*H*</u>Boc)-CH_2-CH(CH_3)$_2$)-CH_2-CH_2-CH_2-N(CO-CH(*N*<u>*H*</u>Boc)-CH_2-CH(CH_3)$_2$)-CH_2-CH_2-), 3H), 4.638-4.413 (m, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-*C*<u>*H*</u>(NHBoc)-CH_2-CH(CH_3)$_2$)-CH_2-CH_2-N(CO-*C*<u>*H*</u>(NHBoc)-CH_2-CH(CH_3)$_2$)-CH_2-CH_2-CH_2-N(CO-*C*<u>*H*</u>(NHBoc)-CH_2-CH(CH_3)$_2$)-CH_2-CH_2-), 3H), 3.878-3.144 (m, cy(-N(CO-R_1)-*C*<u>*H*$_2$</u>-CH_2-*C*<u>*H*$_2$</u>-N(CO-CH(NHBoc)-CH_2-CH(CH_3)$_2$)-*C*<u>*H*$_2$</u>-*C*<u>*H*$_2$</u>-N(CO-CH(NHBoc)-CH_2-CH(CH_3)$_2$)-*C*<u>*H*$_2$</u>-CH_2-*C*<u>*H*$_2$</u>-N(CO-CH(NHBoc)-CH_2-CH(CH_3)$_2$)-*C*<u>*H*$_2$</u>-*C*<u>*H*$_2$</u>-), 16H), 2.452-1.631 (m, cy(-N(CO-*C*<u>*H*$_2$</u>-*C*<u>*H*$_2$</u>-(CH_2)$_8$-CH_3)-CH_2-*C*<u>*H*$_2$</u>-CH_2-N(CO-CH(NHBoc)-*C*<u>*H*$_2$</u>-*C*<u>*H*</u>(CH_3)$_2$)-CH_2-CH_2-N(CO-CH(NHBoc)-*C*<u>*H*$_2$</u>-*C*<u>*H*</u>(CH_3)$_2$)-CH_2-*C*<u>*H*$_2$</u>-CH_2-N(CO-CH(NHBoc)-*C*<u>*H*$_2$</u>-*C*<u>*H*</u>(CH_3)$_2$)-CH_2-CH_2-), 17H), 1.420 (bs, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-CH(NH-COO-*C*(*C*<u>*H*$_3$</u>)<u>$_3$</u>)-CH_2-CH(CH_3)$_2$)-CH_2-CH_2-N(CO-CH(NH-COO-*C*(*C*<u>*H*$_3$</u>)<u>$_3$</u>)-CH_2-CH(CH_3)$_2$)-CH_2-CH_2-CH_2-N(CO-CH(NH-COO-*C*(*C*<u>*H*$_3$</u>)<u>$_3$</u>)-CH_2-CH(CH_3)$_2$)-CH_2-CH_2-), 27H), 1.252 (bs, -CH_2-CH_2-*(C*<u>*H*$_2$</u>)<u>$_8$</u>-CH_3 of R_1 group, 16H), 0.978-0.858 (m, cy(-N(CO-CH_2-CH_2-

$(CH_2)_6$-*C<u>H₃</u>*)-CH_2-CH_2-CH_2-N(CO-CH(NHBoc)-CH_2-CH(*C<u>H₃</u>)₂*)-CH_2-CH_2-N(CO-CH(NHBoc)-CH_2-CH(*C<u>H₃</u>)₂*)-CH_2-CH_2-CH_2-N(CO-CH(NHBoc)-CH_2-CH(*C<u>H₃</u>)₂*)-CH_2-CH_2-), 21H); HRMS (m/z): 1022.78362 [$(M+H)^+$] (Observed), 1022.78447 [$(M+H)^+$] (Calculated).

General protocol for synthesizing 1-8: At first, **1a-8a** was dissolved in 2 mL of MeOH followed by 2 mL of 4N HCl was added to it and kept for stirring at RT. At the end of 2h, the reaction solvent was removed and dried to obtain pure **CAM 1-8** with 100% yield.

CAM-1: ^{1}H-NMR (400 MHz, DMSO-d_6) δ/ppm: 8.730-8.421 (m, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-CH(*N<u>H₃</u>*$^+$)-CH_2-PhH)-CH_2-CH_2-N(CO-CH(*N<u>H₃</u>*$^+$)-CH_2-PhH)-CH_2-CH_2-CH_2-N(CO-CH(*N<u>H₃</u>*$^+$)-CH_2-PhH)-CH_2-CH_2-), 9H), 7.391-7.140 (m, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-CH(NH_3^+)-CH_2-*Ph<u>H</u>*)-CH_2-CH_2-N(CO-CH(NH_3^+)-CH_2-*Ph<u>H</u>*)-CH_2-CH_2-CH_2-N(CO-CH(NH_3^+)-CH_2-*Ph<u>H</u>*)-CH_2-CH_2-), 15H), 4.494-4.305 (m, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-*C<u>H</u>*(NH_3^+)-CH_2-Ph)-CH_2-CH_2-N(CO-*C<u>H</u>*(NH_3^+)-CH_2-Ph)-CH_2-CH_2-CH_2-N(CO-*C<u>H</u>*(NH_3^+)-CH_2-Ph)-CH_2-CH_2-), 3H), 3.204-2.669 (m, cy(-N(CO-R_1)-*C<u>H₂</u>*-CH_2-*C<u>H₂</u>*-N(CO-CH(NH_3^+)-*C<u>H₂</u>*-Ph)-*C<u>H₂</u>*-*C<u>H₂</u>*-N(CO-CH(NH_3^+)-*C<u>H₂</u>*-Ph)-*C<u>H₂</u>*-CH_2-*C<u>H₂</u>*-N(CO-CH(NH_3^+)-*C<u>H₂</u>*-Ph)-*C<u>H₂</u>*-*C<u>H₂</u>*-), 22H), 2.331-2.267 (m, -*C<u>H₂</u>*-CH_2-$(CH_2)_2$-CH_3 of R_1 group, 2H), 1.547-1.498 (m, cy(-N(CO-R_1)-CH_2-*C<u>H₂</u>*-CH_2-N(CO-CH(NH_3^+)-CH_2-Ph)-CH_2-CH_2-N(CO-CH(NH_3^+)-CH_2-Ph)-CH_2-*C<u>H₂</u>*-CH_2-N(CO-CH(NH_3^+)-CH_2-Ph)-CH_2-CH_2-), 4H), 1.313 (bs, -CH_2-CH_2-*(C<u>H₂</u>)₂*-CH_3 of R_1 group, 4H), 0.910-0.875 (t, -CH_2-CH_2-$(CH_2)_2$-*C<u>H₃</u>* of R_1 group, 3H).

CAM-2: ^{1}H-NMR (400 MHz, DMSO-d_6) δ/ppm: 8.795-8.460 (m, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-CH(*N<u>H₃</u>*$^+$)-CH_2-PhH)-CH_2-CH_2-N(CO-CH(*N<u>H₃</u>*$^+$)-CH_2-PhH)-CH_2-CH_2-CH_2-N(CO-CH(*N<u>H₃</u>*$^+$)-CH_2-PhH)-CH_2-CH_2-), 9H), 7.376-7.138 (m, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-CH(NH_3^+)-CH_2-*Ph<u>H</u>*)-CH_2-CH_2-N(CO-CH(NH_3^+)-CH_2-*Ph<u>H</u>*)-CH_2-CH_2-CH_2-N(CO-CH(NH_3^+)-CH_2-*Ph<u>H</u>*)-CH_2-CH_2-), 15H), 4.473-4.295 (m, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-*C<u>H</u>*(NH_3^+)-CH_2-Ph)-CH_2-CH_2-N(CO-*C<u>H</u>*(NH_3^+)-CH_2-Ph)-CH_2-CH_2-CH_2-N(CO-*C<u>H</u>*(NH_3^+)-CH_2-Ph)-CH_2-CH_2-), 3H), 3.247-2.733 (m, cy(-N(CO-R_1)-*C<u>H₂</u>*-CH_2-*C<u>H₂</u>*-N(CO-CH(NH_3^+)-*C<u>H₂</u>*-Ph)-*C<u>H₂</u>*-*C<u>H₂</u>*-N(CO-CH(NH_3^+)-*C<u>H₂</u>*-Ph)-*C<u>H₂</u>*-CH_2-*C<u>H₂</u>*-N(CO-CH(NH_3^+)-*C<u>H₂</u>*-Ph)-*C<u>H₂</u>*-*C<u>H₂</u>*-), 22H), 2.324-2.190 (m, -*C<u>H₂</u>*-CH_2-$(CH_2)_4$-CH_3 of R_1 group, 2H), 1.568-

1.408 (m, cy(-N(CO-R_1)-CH_2-*CH_2*-CH_2-N(CO-CH(NH_3^+)-CH_2-Ph)-CH_2-CH_2-N(CO-CH(NH_3^+)-CH_2-Ph)-CH_2-*CH_2*-CH_2-N(CO-CH(NH_3^+)-CH_2-Ph)-CH_2-CH_2-), 4H), 1.238 (bs, -CH_2-CH_2-*$(CH_2)_4$*-CH_3 of R_1 group, 8H), 0.839-0.822 (t, -CH_2-CH_2-$(CH_2)_4$-*CH_3* of R_1 group, 3H).

CAM-3: ^{1}H-NMR (400 MHz, DMSO-d_6) δ/ppm: 8.839-8.462 (m, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-CH(*N*H_3^+)-CH_2-PhH)-CH_2-CH_2-N(CO-CH(*N*H_3^+)-CH_2-PhH)-CH_2-CH_2-CH_2-N(CO-CH(*N*H_3^+)-CH_2-PhH)-CH_2-CH_2-), 9H), 7.378-7.149 (m, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-CH(NH_3^+)-CH_2-*PhH*)-CH_2-CH_2-N(CO-CH(NH_3^+)-CH_2-*PhH*)-CH_2-CH_2-CH_2-N(CO-CH(NH_3^+)-CH_2-*PhH*)-CH_2-CH_2-), 15H), 4.475-4.343 (m, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-*C*H(NH_3^+)-CH_2-Ph)-CH_2-CH_2-N(CO-*C*H(NH_3^+)-CH_2-Ph)-CH_2-CH_2-CH_2-N(CO-*C*H(NH_3^+)-CH_2-Ph)-CH_2-CH_2-), 3H), 3.269-2.720 (m, cy(-N(CO-R_1)-*CH_2*-CH_2-*CH_2*-N(CO-CH(NH_3^+)-*CH_2*-Ph)-*CH_2*-*CH_2*-N(CO-CH(NH_3^+)-*CH_2*-Ph)-*CH_2*-CH_2-*CH_2*-N(CO-CH(NH_3^+)-*CH_2*-Ph)-*CH_2*-*CH_2*-), 22H), 2.326-2.204 (m, -*CH_2*-CH_2-$(CH_2)_6$-CH_3 of R_1 group, 2H), 1.570-1.410 (m, cy(-N(CO-R_1)-CH_2-*CH_2*-CH_2-N(CO-CH(NH_3^+)-CH_2-Ph)-CH_2-CH_2-N(CO-CH(NH_3^+)-CH_2-Ph)-CH_2-*CH_2*-CH_2-N(CO-CH(NH_3^+)-CH_2-Ph)-CH_2-CH_2-), 4H), 1.240 (bs, -CH_2-CH_2-*$(CH_2)_6$*-CH_3 of R_1 group, 12H), 0.841-0.824 (t, -CH_2-CH_2-$(CH_2)_6$-*CH_3* of R_1 group, 3H).

CAM-4: ^{1}H-NMR (400 MHz, DMSO-d_6) δ/ppm: 8.835-8.454 (m, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-CH(*N*H_3^+)-CH_2-PhH)-CH_2-CH_2-N(CO-CH(*N*H_3^+)-CH_2-PhH)-CH_2-CH_2-CH_2-N(CO-CH(*N*H_3^+)-CH_2-PhH)-CH_2-CH_2-), 9H), 7.380-7.173 (m, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-CH(NH_3^+)-CH_2-*PhH*)-CH_2-CH_2-N(CO-CH(NH_3^+)-CH_2-*PhH*)-CH_2-CH_2-CH_2-N(CO-CH(NH_3^+)-CH_2-*PhH*)-CH_2-CH_2-), 15H), 4.485-4.344 (m, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-*C*H(NH_3^+)-CH_2-Ph)-CH_2-CH_2-N(CO-*C*H(NH_3^+)-CH_2-Ph)-CH_2-CH_2-CH_2-N(CO-*C*H(NH_3^+)-CH_2-Ph)-CH_2-CH_2-), 3H), 3.241-2.914 (m, cy(-N(CO-R_1)-*CH_2*-CH_2-*CH_2*-N(CO-CH(NH_3^+)-*CH_2*-Ph)-*CH_2*-*CH_2*-N(CO-CH(NH_3^+)-*CH_2*-Ph)-*CH_2*-CH_2-*CH_2*-N(CO-CH(NH_3^+)-*CH_2*-Ph)-*CH_2*-*CH_2*-), 22H), 2.327-2.268 (m, -*CH_2*-CH_2-$(CH_2)_8$-CH_3 of R_1 group, 2H), 1.573-1.414 (m, cy(-N(CO-R_1)-CH_2-*CH_2*-CH_2-N(CO-CH(NH_3^+)-CH_2-Ph)-CH_2-CH_2-N(CO-CH(NH_3^+)-CH_2-Ph)-CH_2-*CH_2*-CH_2-N(CO-CH(NH_3^+)-CH_2-Ph)-CH_2-CH_2-), 4H), 1.245 (bs, -

CH_2-CH_2-*(C<u>H2</u>)<u>8</u>*-CH_3 of R_1 group, 16H), 0.861-0.828 (t, -CH_2-CH_2-$(CH_2)_8$-*C<u>H3</u>* of R_1 group, 3H).

CAM-5: ^{1}H-NMR (400 MHz, DMSO-d_6) δ/ppm: 8.432 (bs, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-CH(*N<u>H3</u>*$^+$)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-N(CO-CH(*N<u>H3</u>*$^+$)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-CH_2-N(CO-CH(*N<u>H3</u>*$^+$)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-), 9H), 4.251-4.095 (m, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-*C<u>H</u>*(NH_3)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-N(CO-*C<u>H</u>*(NH_3)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-CH_2-N(CO-*C<u>H</u>*(NH_3)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-), 3H), 3.623-3.421 (m, cy(-N(CO-R_1)-*C<u>H2</u>*-CH_2-*C<u>H2</u>*-N(CO-CH(NH_3)-CH_2-$CH(CH_3)_2$)-*C<u>H2</u>*-*C<u>H2</u>*-N(CO-CH(NH_3)-CH_2-$CH(CH_3)_2$)-*C<u>H2</u>*-CH_2-*C<u>H2</u>*-N(CO-CH(NH_3)-CH_2-$CH(CH_3)_2$)-*C<u>H2</u>*-*C<u>H2</u>*-), 16H), 2.328-2.289 (t, -*C<u>H2</u>*-CH_2-$(CH_2)_2$-CH_3 of R_1 group, 2H), 1.795-1.267 (m, cy(-N(CO-CH_2-*C<u>H2</u>*-$(CH_2)_2$-CH_3)-CH_2-*C<u>H2</u>*-CH_2-N(CO-CH(NH_3)-*C<u>H2</u>*-*C<u>H</u>*($CH_3)_2$)-CH_2-CH_2-N(CO-CH(NH_3)-*C<u>H2</u>*-*C<u>H</u>*($CH_3)_2$)-CH_2-*C<u>H2</u>*-CH_2-N(CO-CH(NH_3)-*C<u>H2</u>*-*C<u>H</u>*($CH_3)_2$)-CH_2-CH_2-), 15H), 1.267 (bs, -CH_2-CH_2-*(C<u>H2</u>)<u>2</u>*-CH_3 of R_1 group, 4H), 0.945-0.860 (m, cy(-N(CO-CH_2-CH_2-$(CH_2)_2$-*C<u>H3</u>*)-CH_2-CH_2-CH_2-N(CO-CH(NH_3)-CH_2-CH*(C<u>H3</u>)<u>2</u>*)-CH_2-CH_2-N(CO-CH(NH_3)-CH_2-CH*(C<u>H3</u>)<u>2</u>*)-CH_2-CH_2-CH_2-N(CO-CH(NH_3)-CH_2-CH*(C<u>H3</u>)<u>2</u>*)-CH_2-CH_2-), 21H).

CAM-6: ^{1}H-NMR (400 MHz, DMSO-d_6) δ/ppm: 8.416 (bs, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-CH(*N<u>H3</u>*$^+$)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-N(CO-CH(*N<u>H3</u>*$^+$)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-CH_2-N(CO-CH(*N<u>H3</u>*$^+$)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-), 9H), 4.255-4.101 (m, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-*C<u>H</u>*(NH_3)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-N(CO-*C<u>H</u>*(NH_3)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-CH_2-N(CO-*C<u>H</u>*(NH_3)-CH_2-$CH(CH_3)_2$)-CH_2-CH_2-), 3H), 3.623-3.460 (m, cy(-N(CO-R_1)-*C<u>H2</u>*-CH_2-*C<u>H2</u>*-N(CO-CH(NH_3)-CH_2-$CH(CH_3)_2$)-*C<u>H2</u>*-*C<u>H2</u>*-N(CO-CH(NH_3)-CH_2-$CH(CH_3)_2$)-*C<u>H2</u>*-CH_2-*C<u>H2</u>*-N(CO-CH(NH_3)-CH_2-$CH(CH_3)_2$)-*C<u>H2</u>*-*C<u>H2</u>*-), 16H), 2.358-2.287 (t, -*C<u>H2</u>*-CH_2-$(CH_2)_4$-CH_3 of R_1 group, 2H), 1.793-1.485 (m, cy(-N(CO-CH_2-*C<u>H2</u>*-$(CH_2)_2$-CH_3)-CH_2-*C<u>H2</u>*-CH_2-N(CO-CH(NH_3)-*C<u>H2</u>*-*C<u>H</u>*($CH_3)_2$)-CH_2-CH_2-N(CO-CH(NH_3)-*C<u>H2</u>*-*C<u>H</u>*($CH_3)_2$)-CH_2-*C<u>H2</u>*-CH_2-N(CO-CH(NH_3)-*C<u>H2</u>*-*C<u>H</u>*($CH_3)_2$)-CH_2-CH_2-), 15H), 1.255 (bs, -CH_2-CH_2-*(C<u>H2</u>)<u>4</u>*-CH_3 of R_1 group, 8H), 0.950-0.838 (m, cy(-N(CO-CH_2-CH_2-$(CH_2)_4$-*C<u>H3</u>*)-CH_2-CH_2-CH_2-N(CO-CH(NH_3)-CH_2-CH*(C<u>H3</u>)<u>2</u>*)-CH_2-CH_2-N(CO-CH(NH_3)-CH_2-CH*(C<u>H3</u>)<u>2</u>*)-CH_2-CH_2-CH_2-N(CO-CH(NH_3)-CH_2-CH*(C<u>H3</u>)<u>2</u>*)-CH_2-CH_2-), 21H).

CAM-7: ^{1}H-NMR (400 MHz, DMSO-d_6) δ/ppm: 8.510-8.397 (d, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-CH(*N*H_3^+)-CH_2-CH(CH_3)$_2$)-CH_2-CH_2-N(CO-CH(*N*H_3^+)-CH_2-CH(CH_3)$_2$)-CH_2-CH_2-CH_2-N(CO-CH(*N*H_3^+)-CH_2-CH(CH_3)$_2$)-CH_2-CH_2-), 9H), 4.257-4.106 (m, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-*C*H(NH_3)-CH_2-CH(CH_3)$_2$)-CH_2-CH_2-N(CO-*C*H(NH_3)-CH_2-CH(CH_3)$_2$)-CH_2-CH_2-CH_2-N(CO-*C*H(NH_3)-CH_2-CH(CH_3)$_2$)-CH_2-CH_2-), 3H), 3.624-3.422 (m, cy(-N(CO-R_1)-*C*H_2-CH_2-*C*H_2-N(CO-CH(NH_3)-CH_2-CH(CH_3)$_2$)-*C*H_2-*C*H_2-N(CO-CH(NH_3)-CH_2-CH(CH_3)$_2$)-*C*H_2-CH_2-*C*H_2-N(CO-CH(NH_3)-CH_2-CH(CH_3)$_2$)-*C*H_2-*C*H_2-), 16H), 2.326-2.265 (t, -*C*H_2-CH_2-(CH_2)$_6$-CH_3 of R_1 group, 2H), 1.981-1.484 (m, cy(-N(CO-CH_2-*C*H_2-(CH_2)$_6$-CH_3)-CH_2-*C*H_2-CH_2-N(CO-CH(NH_3)-*C*H_2-*C*H(CH_3)$_2$)-CH_2-CH_2-N(CO-CH(NH_3)-*C*H_2-*C*H(CH_3)$_2$)-CH_2-*C*H_2-CH_2-N(CO-CH(NH_3)-*C*H_2-*C*H(CH_3)$_2$)-CH_2-CH_2-), 15H), 1.243 (bs, -CH_2-CH_2-*(C*H_2*)*$_6$-CH_3 of R_1 group, 12H), 0.953-0.838 (m, cy(-N(CO-CH_2-CH_2-(CH_2)$_6$-*C*H_3)-CH_2-CH_2-CH_2-N(CO-CH(NH_3)-CH_2-CH(*C*H_3*)*$_2$)-CH_2-CH_2-N(CO-CH(NH_3)-CH_2-CH(*C*H_3*)*$_2$)-CH_2-CH_2-CH_2-N(CO-CH(NH_3)-CH_2-CH(*C*H_3*)*$_2$)-CH_2-CH_2-), 21H).

CAM-8: ^{1}H-NMR (400 MHz, DMSO-d_6) δ/ppm: 8.566-8.453 (d, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-CH(*N*H_3^+)-CH_2-CH(CH_3)$_2$)-CH_2-CH_2-N(CO-CH(*N*H_3^+)-CH_2-CH(CH_3)$_2$)-CH_2-CH_2-CH_2-N(CO-CH(*N*H_3^+)-CH_2-CH(CH_3)$_2$)-CH_2-CH_2-), 9H), 4.236-4.080 (m, cy(-N(CO-R_1)-CH_2-CH_2-CH_2-N(CO-*C*H(NH_3)-CH_2-CH(CH_3)$_2$)-CH_2-CH_2-N(CO-*C*H(NH_3)-CH_2-CH(CH_3)$_2$)-CH_2-CH_2-CH_2-N(CO-*C*H(NH_3)-CH_2-CH(CH_3)$_2$)-CH_2-CH_2-), 3H), 3.691-3.358 (m, cy(-N(CO-R_1)-*C*H_2-CH_2-*C*H_2-N(CO-CH(NH_3)-CH_2-CH(CH_3)$_2$)-*C*H_2-*C*H_2-N(CO-CH(NH_3)-CH_2-CH(CH_3)$_2$)-*C*H_2-CH_2-*C*H_2-N(CO-CH(NH_3)-CH_2-CH(CH_3)$_2$)-*C*H_2-*C*H_2-), 16H), 2.325-2.269 (t, -*C*H_2-CH_2-(CH_2)$_8$-CH_3 of R_1 group, 2H), 1.991-1.485 (m, cy(-N(CO-CH_2-*C*H_2-(CH_2)$_8$-CH_3)-CH_2-*C*H_2-CH_2-N(CO-CH(NH_3)-*C*H_2-*C*H(CH_3)$_2$)-CH_2-CH_2-N(CO-CH(NH_3)-*C*H_2-*C*H(CH_3)$_2$)-CH_2-*C*H_2-CH_2-N(CO-CH(NH_3)-*C*H_2-*C*H(CH_3)$_2$)-CH_2-CH_2-), 15H), 1.232 (bs, -CH_2-CH_2-*(C*H_2*)*$_8$-CH_3 of R_1 group, 16H), 0.946-0.828 (m, cy(-N(CO-CH_2-CH_2-(CH_2)$_8$-*C*H_3)-CH_2-CH_2-CH_2-N(CO-CH(NH_3)-CH_2-CH(*C*H_3*)*$_2$)-CH_2-CH_2-N(CO-CH(NH_3)-CH_2-CH(*C*H_3*)*$_2$)-CH_2-CH_2-CH_2-N(CO-CH(NH_3)-CH_2-CH(*C*H_3*)*$_2$)-CH_2-CH_2-), 21H).

Protocols for Biological Experiments

Bacterial strains and growth condition: The Gram-negative bacteria, *A. baumannii*-MTCC1425, *P. aeruginosa*-MTCC424, *E.coli*-MTCC443 and Gram-positive bacteria *S. aureus*-MTCC737 were purchased from MTCC (Chandigarh, India). *K. pneumoniae*-ATCC700603 and MRSA-ATCC33591 were obtained from ATCC (Rockville, MD, USA). Clinically isolated bacterial strains; *A. baumannii*-R676*, A. baumannii*-R676, *P. aeruginosa*-R590, *P. aeruginosa*-R596, *P. aeruginosa*-R2477, *E. coli*-R250*, E. coli*-R597*, K. pneumoniae*-R3421, *K. pneumoniae*-R3934, MRSA-R3545, MRSA-R3889 and MRSA-R3890 were obtained from Dr. R. Ravikumar, the Department of Neuromicrobiology, National Institute of Mental Health and Neuro Sciences, Hosur Road, Bangalore 560029, India. All bacterial strains were preserved in nutrient broth (NB) at -80 ºC supplemented with 15% (v/v) glycerol. Before using the bacteria from frozen stock, they were grown overnight on MacConky or Nutrient agar plate and incubated at 37 ºC. Single bacterial colony was then grown for 6h (midlog phase) in 3 mL of nutrient broth and used for antibacterial assay. In order to achieve the stationary phase culture 3 µL of these midlog phase bacteria were added to 3 mL of fresh nutrient broth and allowed to grown for 16 h at 37 ºC. For determining the MIC values, 6h grown midlog phase bacteria were suspended to ~$10^{5\text{-}6}$ CFU/mL and used for the assay. Similarly, stationary phase cultures were diluted to ~$10^{5\text{-}6}$ and assessed for activity test. For ex-vivo corneal infections the *P. aeruginosa*-ATCC27853 and *P. aeruginosa*-L-2026/17 were obtained from the Jhaveri Microbiology laboratory of the L V Prasad Eye Institute.**Antibacterial assay:** Reported protocol was followed for the experiment.[1-5] Briefly, the antibacterial agents (compounds and control antibiotics) were serially diluted by 2-fold in the 96-well plate in sterile Millipore water. Then mid-log phase bacteria (~10^8 CFU/mL) were diluted to ~10^5 CFU/mL in mueller hinton broth and 150 µL of these bacterial suspensions were added to the 96 well plates containing 50 µL of compounds solution. The plates were then incubated under shaking condition for 24 h at 37 ºC. At the end, the OD values of the plates were measured at 600 nm using TECAN (Infinite series, M200 pro) Plate Reader. The experiment was performed

in triplicate and the MIC values were determined by visual observation. MIC value was considered as the lowest concentration of the agent where the OD was less than 0.1.

Hemolytic assay: The experiment was performed by following reported protocol.[1-4] In brief, the test compounds were serially diluted by 2-fold in the 96-well plates in Millipore water. As the negative control same volume of water was placed instead of compound and as a positive control same volume of Triton X-100 (1 vol% solution in 1X PBS) was placed. Freshly collected heparinized human blood was then centrifuged down and plasma was discarded to collect the RBCs. It was then suspended to 5 vol% in 1X PBS (pH = 7.4) and 150 µL of the suspension was added to 96-well plates containing 50 µL compound. After that, the plates were incubated at 37 ºC for 1 h. At the end, the plates were centrifuged at 3500 rpm for 5 min and 100 µL of the supernatant was transferred to the new 96-well plates. The absorbance (OD) at 540 nm was then measured to determine the percentage of hemolysis. The following formula was used: $(A_t - A_{nt})/(A_{TX} - A_{nt})\times100$ where, At is the OD of the compound treated wells, Ant the OD of the negative control, and A_{TX} the OD of the Triton X-100 treated sample. Each concentration had triplicate values and the average of percentage of hemolysis was plotted with standard deviation for each concentration.

Fluorescence microscopy of HEK and HeLa cells: The experiment was performed by following our reported protocol.[4] Briefly, human embryo kidney (HEK 293) and HeLa cells were grown to 70−80% confluency in a 96-well plate (in DMEM media supplemented with 10% fetal bovine serum and 5% penicillin−streptomycin) at 37 ºC under 5% CO_2−95% atmosphere. The cells were then treated with **CAM-8** at 8 µg/mL and incubated for 24 h. Two control experiments were also performed; one containing no compound (untreated cells) and the other one treated with 0.1 vol % Triton-X solution. At the end of 24 h, the cells were then washed once with 1X PBS and stained with calcein AM (2 µM, Fluka) and propidium iodide (PI, 4.5 µM) (Sigma-Aldrich) for 15 min (50 µL of 1:1 calcein AM/PI). Finally, the cells were washed with 1X PBS to remove the excess dyes, and images were captured with a 40X objective in a Leica DM2500 fluorescence microscope. A band-pass

filter for calcein-AM at 500−550 nm and a long-pass filter for PI at 590−800 nm were used while imaging.

Antibacterial assay in presence of human plasma:[1,3] To examine the susceptibility of the new class of compounds towards plasma proteases, the antibacterial activity of the optimized compound, **CAM-8** was tested in presence of 50% of human plasma. Briefly, 250 µL of compound (in 1XPBS) was mixed with 250 µL of fresh human plasma and incubated at 37 ºC. After 3h of incubation, the aliquot was 2-fold diluted in 0.9% saline. Then antibacterial activities were determined against clinical isolates of all four Gram-negative bacteria by following the experimental protocol described above for antibacterial assay.

Time-kill kinetics:[1-3] To investigate the bactericidal efficacy of this new class of compounds, the time-kill kinetic was performed against clinical isolates of all four Gram-negative bacteria. Briefly, the freshly prepared mid-log phase (~10^5 CFU/mL) of bacterial culture in MHB were treated with 16 µg/mL of **CAM-8**. In the negative control experiment, the same volume of Millipore water was added instead of compound. The treated samples were then incubated at 37 °C with shaking and bacterial cell viability was assayed at different time point such as 0, 60, 120, and 240 min. The aliquots were 10-fold serially diluted in saline and 20 µL of these dilutions were spot plated on MacConkey agar plate. The spotted plates were then incubated at 37 ºC for 24 h. At the end of incubation, bacterial colonies were counted and average results are presented in logarithmic scale with standard deviation for each data point. The detection limit for this experiment is 50 CFU/mL.

Efficacy against stationary phase bacteria:[2,3,5] To investigate the antibacterial efficacy further, the activity was assayed against the stationary phase cells of Gram-negative clinical isolates. To obtain the stationary phase culture, 3 µL of midlog phase bacteria were added to 3 mL of fresh nutrient broth and allowed to grown for 16 h at 37 ºC. The resulting

stationary phase culture (~10^9 CFU/mL) was then centrifuged at 9000 rpm for 2 min and re-suspended in 1XPBS and 10-fold serially diluted by to ~10^5 CFU/mL in 1XPBS. The bacterial suspensions were then treated with 16 µg/mL of **CAM-8**. In case of negative control experiment, the same volume of 1XPBS was added instead of test compound. The treated samples were then incubated at 37 °C with shaking and bacterial cell viability was assayed at different time point such as 0, 60, 120 and 240 min. 20 µL of the aliquots were 10-fold serially diluted in saline and 20 µL of these dilutions were spot plated on MacConkey agar plate. The spotted plates were then incubated at 37 ºC. At the end of 24 h incubation, bacterial colonies were counted and average results are presented in logarithmic scale with standard deviation for each data point. The detection limit for this experiment is 50 CFU/mL.

Anti-biofilm assay:[2,3,5] The antibiofilm efficacy of **CAM-8** was evaluated against *A. baumannii*-R674 and *P. aeruginosa*-R596. Briefly, the glass coverslips (18 mm of diameter) were sterilized by soaking them into ethanol followed by drying them in the flame. These sterilized coverslips were then placed into the wells of 6-well plate and allowed to cool to room temperature. After that, 2 mL of mid-log phase bacterial suspension (~10^5 CFU/mL suspended in suitable biofilm forming media) was added to the wells containing coverslips. While *A. baumannii*-R674 suspension was prepared in BM2 media supplemented with 0.5% glucose, 0.5% casamino acids and 200 µM $FeCl_3$, *P. aeruginosa*-R596 was suspended in nutrient broth supplemented with 0.1% glucose and 0.1% NaCl. The plate was incubated at 30-33 ºC under stationary condition to allow the biofilm formation form on the coverslips. At the end of 48 h incubation, the biofilms containing coverslips were washed carefully with 1×PBS and placed into the wells of 6-well plate containing 2 mL of various concentrations of **CAM-8**. In case of negative control experiment, 2 mL of complete medium was added without any antibacterial agent. Post-incubation of 24 h, the treated coverslips were carefully washed with 1×PBS and dried. It was then stained with 0.1% (2 mL of 0.1% crystal-violet solution (prepared in sterile milipore water) and placed into the wells of fresh 6-well plate after washing with 1×PBS.

The CV-stained disrupted biofilm was then dissolved in 2 mL of 95% ethanol in water and the OD of the solution was recorded at 520 nm. The experiment was performed in duplicate and the average percentage of biofilm biomass was calculated by considering 100% for the negative control.

Resistance study:[1-3] For resistance study the last-resort Gram-negative antibiotic colistin was chosen as the control. Briefly, the MIC values of **CAM-8** against four different clinical isolates (*A. baumannii*-R674, *P. aeruginosa*-R596, *E. coli*-R597 and *K. pneumoniae*-R3934) were determined as described in the antibacterial assay. For the next day MIC experiment, the bacterial dilution was made by using the bacteria from sub-MIC (MIC/2) concentration of the **CAM-8** and control antibiotic, colistin. After 24 h incubation period, again bacterial dilution was prepared like the previous day by using the bacterial suspension from sub-MIC concentration and assayed for the next MIC experiment. The process was repeated for 21 (for *A. baumannii*-R674, *P. aeruginosa*-R596, *E. coli*-R597) and 14 passages (*K. pneumoniae*-R3934), respectively. The fold of MIC increase for the test compound, **CAM-8** and the control antibiotic, colistin were the plotted against the number of passages.

Membrane targeting mechanism of action:

Outer membrane permeabilization assay.[6] Reported experimental protocol was followed with little modification. Briefly, ~10^8 CFU/mL bacteria (both actively growing and stationary phase) were centrifuged at 3500 rpm for 5 min and bacterial pellet was suspended into 5 mM glucose and 5 mM HEPES buffer (pH = 7.4) in 1:1 ratio. 10 μM of (NPN) dye was added to the suspension. After that, 190 μL of dye containing bacterial suspension were placed into the wells of black and clear bottom 96-well plates. The fluorescence was monitored for 4 min at emission wavelength of 420 nm by exciting bacterial suspension at 350 nm. 10 μL of test compound, **CAM-8** (16 μg/mL) and colistin (16 μg/mL) were then added to the plate containing bacterial suspension and dye. As a measure of the extent of

membrane outer permeabilization, increase in fluorescence was monitored for another 20 min and demonstrated in the figure. The fluorescence intensity is normalized with respect to control experiment. The intensity of the treated samples was first divided with that of the control sample, followed by multiplication with 100.

Inner membrane permeabilization assay.[1,3] The experiment was performed by following reported experimental protocol. Briefly, ~10^8 CFU/mL bacteria (both actively growing and stationary phase) were centrifuged at 3500 rpm for 5 min and bacterial pellet was suspended into 5 mM glucose and 5 mM HEPES buffer (pH = 7.4) in 1:1 ratio. After that, 10 µM of Propidium iodide (PI) dye was added to the suspension. 190 µL of dye containing bacterial suspension were then placed into the wells of black and clear bottom 96-well plates. The fluorescence was monitored for 4 min at emission wavelength of 617 nm by exciting bacterial suspension at 535 nm. Then, 10 µL of test compound, **CAM-8** (16 µg/mL) colistin (16 µg/mL) were added to the plate containing bacterial suspension and dye. As a measure of the extent of membrane permeabilization, increase in fluorescence was monitored for another 20 min and demonstrated in the figure. The fluorescence intensity is normalized with respect to control experiment. The intensity of the treated samples was first divided with that of the control sample, followed by multiplication with 100.

***Ex-vivo* efficacy in human corneal infection:**[7] Human corneas were incubated overnight in antibiotic free DMEM media at 37 °C. Next day, corneas were washed thrice with sterile 1XPBS and 200 µL of bacterial suspension in 1XPBS from bacterial cultures having concentration of ~10^8 CFU/mL in PBS was injected intra-stromally (by using a 26-gauge needle) and were incubated in 3 mL of antibiotic free DMEM media for 24 h at 37 °C for the development of infection. The infected corneas were washed again with 1XPBS and used for further experiments. The metal ring was placed on the corneosclera button, creating a water tight seal. Into the centre of the ring 200 µL of the various antibacterial agents were added and the set up were incubated in 3 mL of antibiotic free DMEM media

for 24h at 37 °C. In case of control experiment 200 µL of 1XPBS was dispensed into the metal ring placed over the infected cornea. At the end of experiment, the corneas were then be homogenized and the resulting suspension was 10-fold serially diluted and 10 µL of these diluted solution was spotted on agar plates. The colonies were counted visually and the result was expressed in terms of log (CFU/mL). .

Supplementary Figures

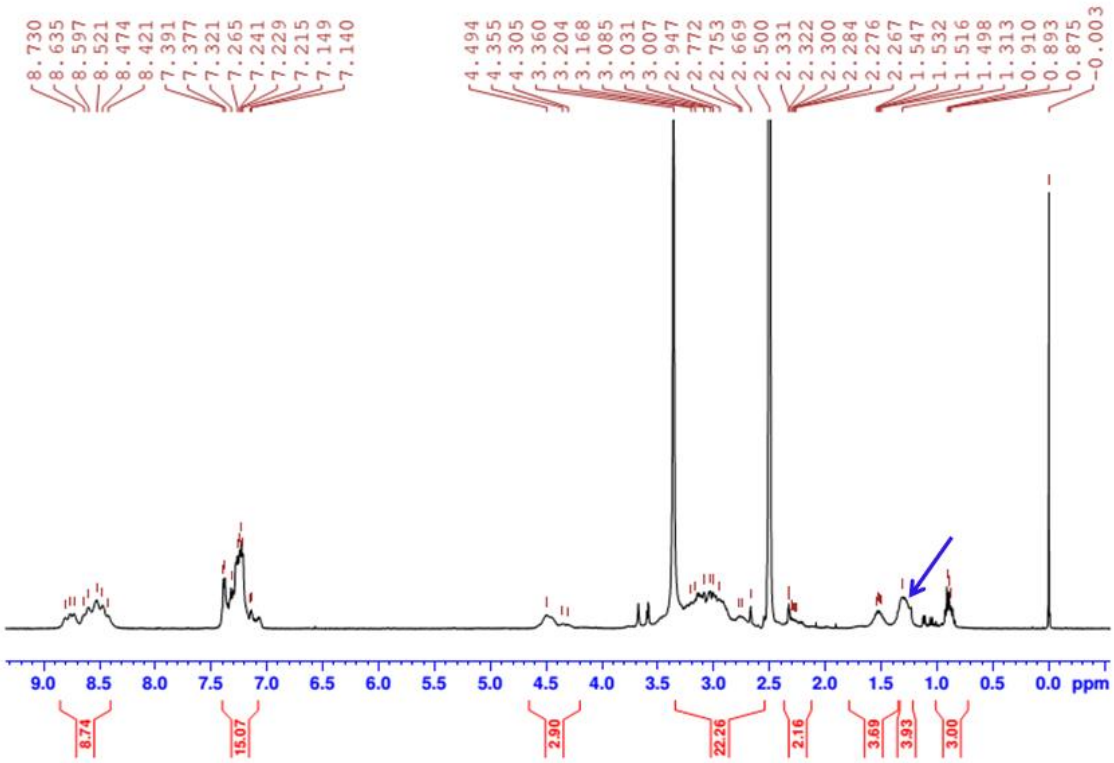


Fig. S1: ^{1}H-NMR spectrum of **CAM-1**. The NMR was taken in DMSO-d6 and the solvent peak was calibrated at δ value of 2.5000 ppm. The arrow corresponds to CH_3-$(CH_2)_2$-CH_2-CH_2-, which confirming the presence of long chain.

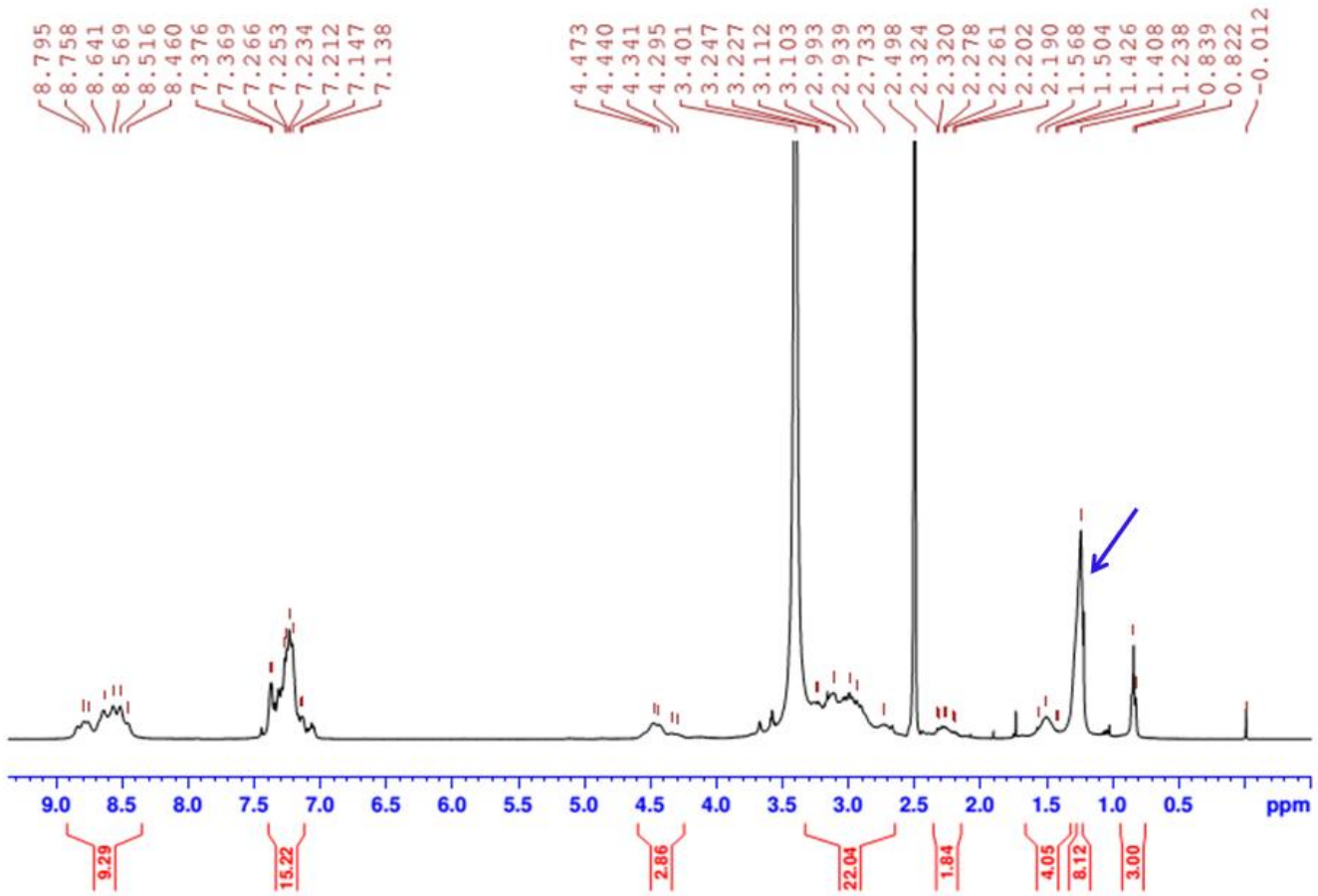


Fig. S2: ^{1}H-NMR spectrum of **CAM-2**. The NMR was taken in DMSO-d6 and the solvent peak was calibrated at δ value of 2.5000 ppm. The arrow corresponds to CH_3-$(CH_2)_4$-CH_2-CH_2-, which confirming the presence of long chain.

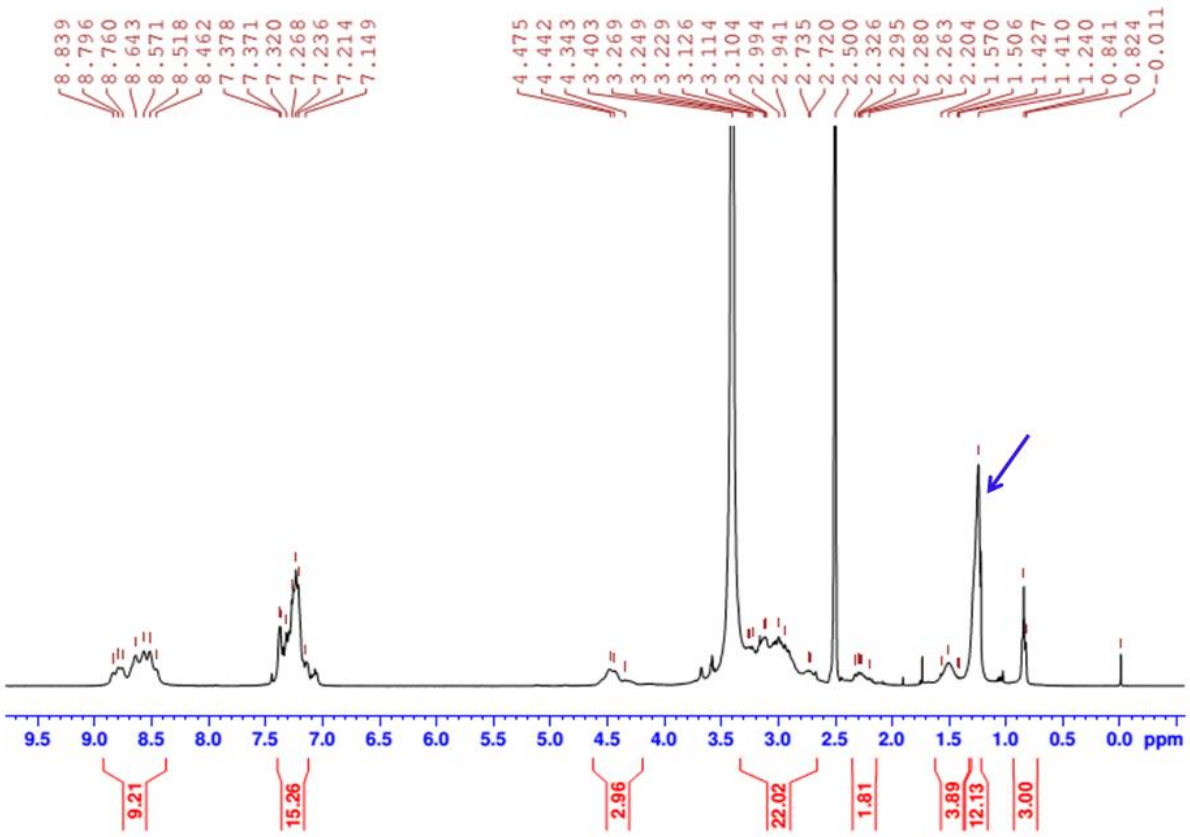


Fig. S3: ^{1}H-NMR spectrum of **CAM-3**. The NMR was taken in DMSO-d6 and the solvent peak was calibrated at δ value of 2.5000 ppm. The arrow corresponds to CH_3-$(CH_2)_6$-CH_2-CH_2-, which confirming the presence of long chain.

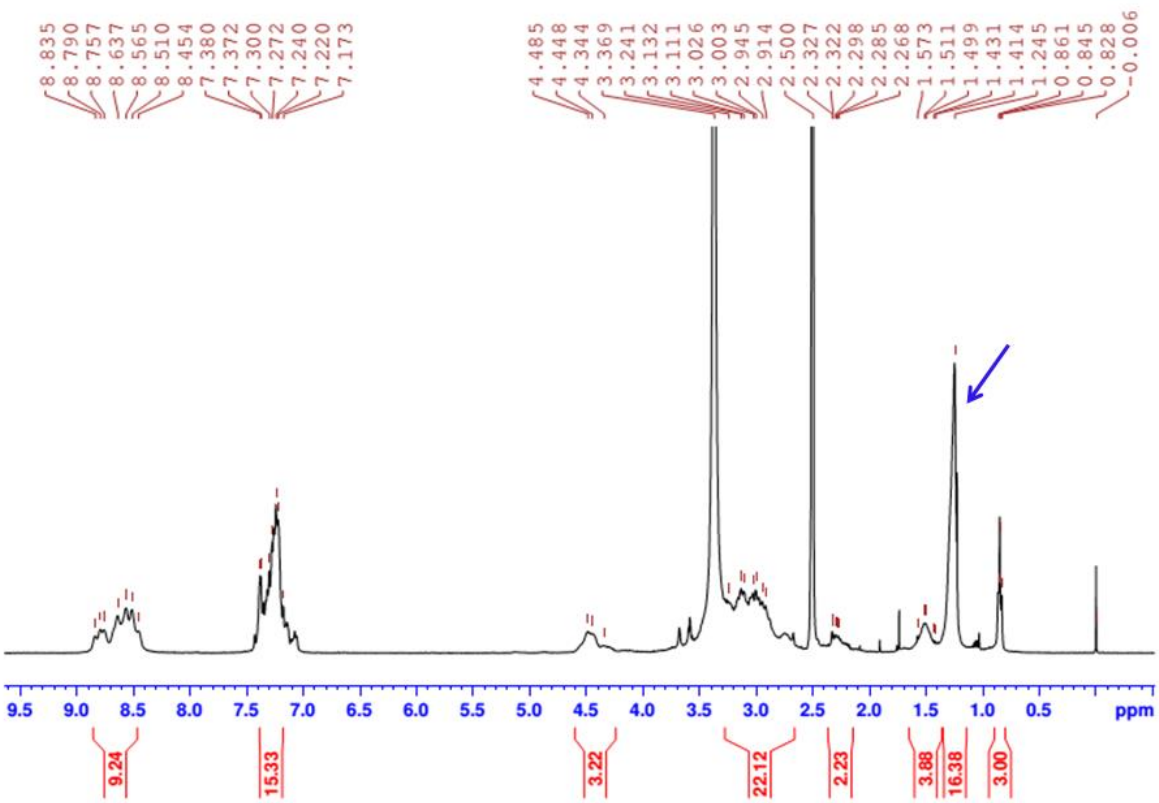


Fig. S4: ^{1}H-NMR spectrum of **CAM-4**. The NMR was taken in DMSO-d6 and the solvent peak was calibrated at δ value of 2.5000 ppm. The arrow corresponds to CH_3-$(CH_2)_8$-CH_2-CH_2-, which confirming the presence of long chain.

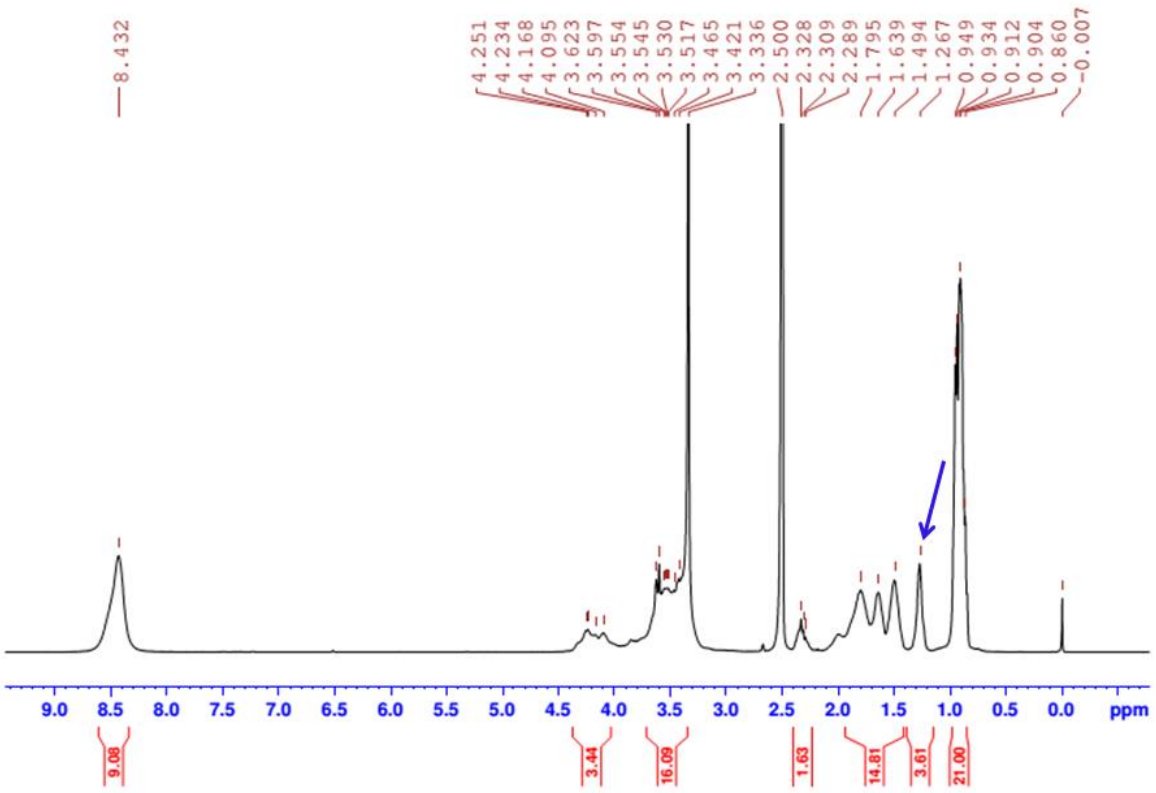


Fig. S5: ^{1}H-NMR spectrum of **CAM-5**. The NMR was taken in DMSO-d6 and the solvent peak was calibrated at δ value of 2.5000 ppm. The arrow corresponds to CH_3-$(CH_2)_2$-CH_2-CH_2-, which confirming the presence of long chain.

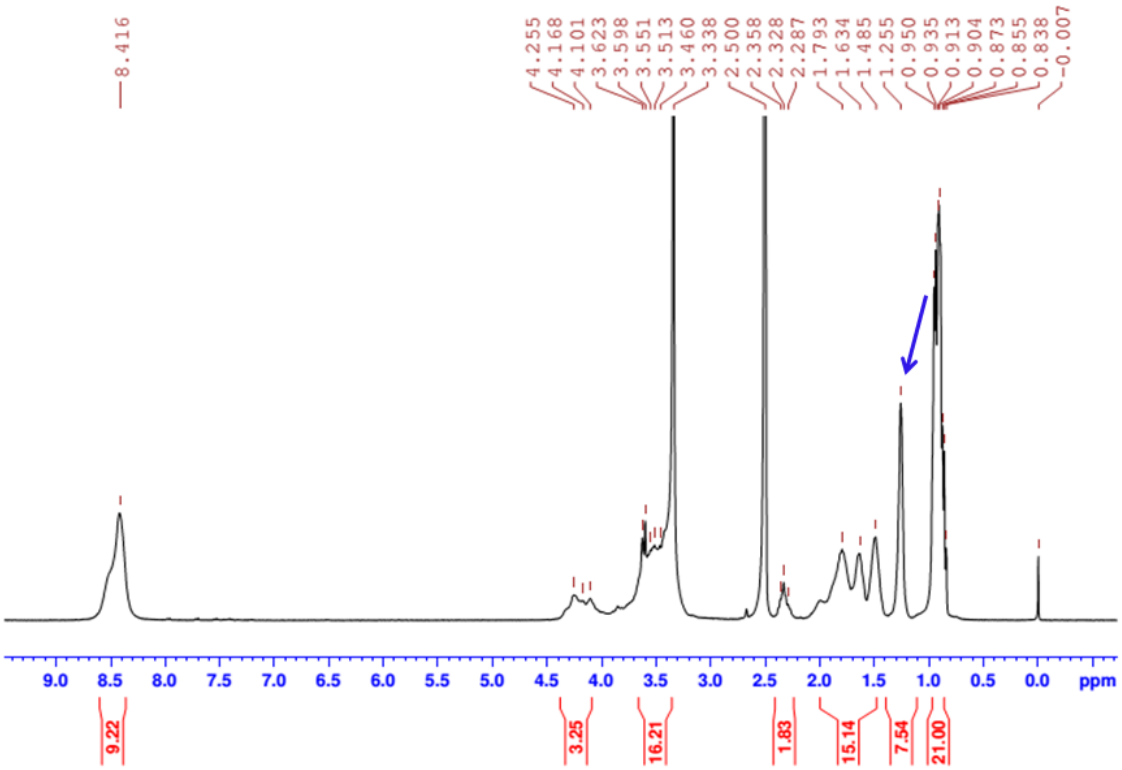


Fig. S6: ^{1}H-NMR spectrum of **CAM-6**. The NMR was taken in DMSO-d6 and the solvent peak was calibrated at δ value of 2.5000 ppm. The arrow corresponds to CH_3-*(*CH_2*)*$_4$-CH_2-CH_2-, which confirming the presence of long chain.

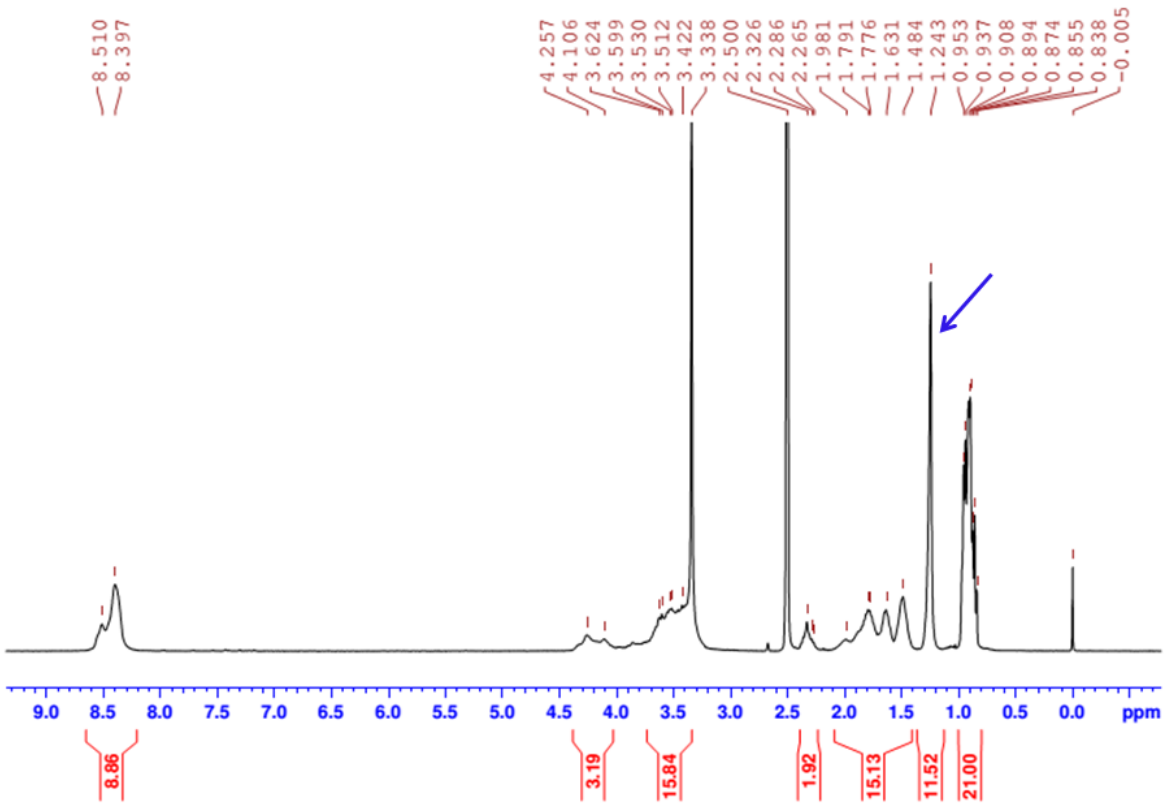


Fig. S7: ^{1}H-NMR spectrum of **CAM-7**. The NMR was taken in DMSO-d6 and the solvent peak was calibrated at δ value of 2.5000 ppm. The arrow corresponds to CH_3-$(CH_2)_6$-CH_2-CH_2-, which confirming the presence of long chain.

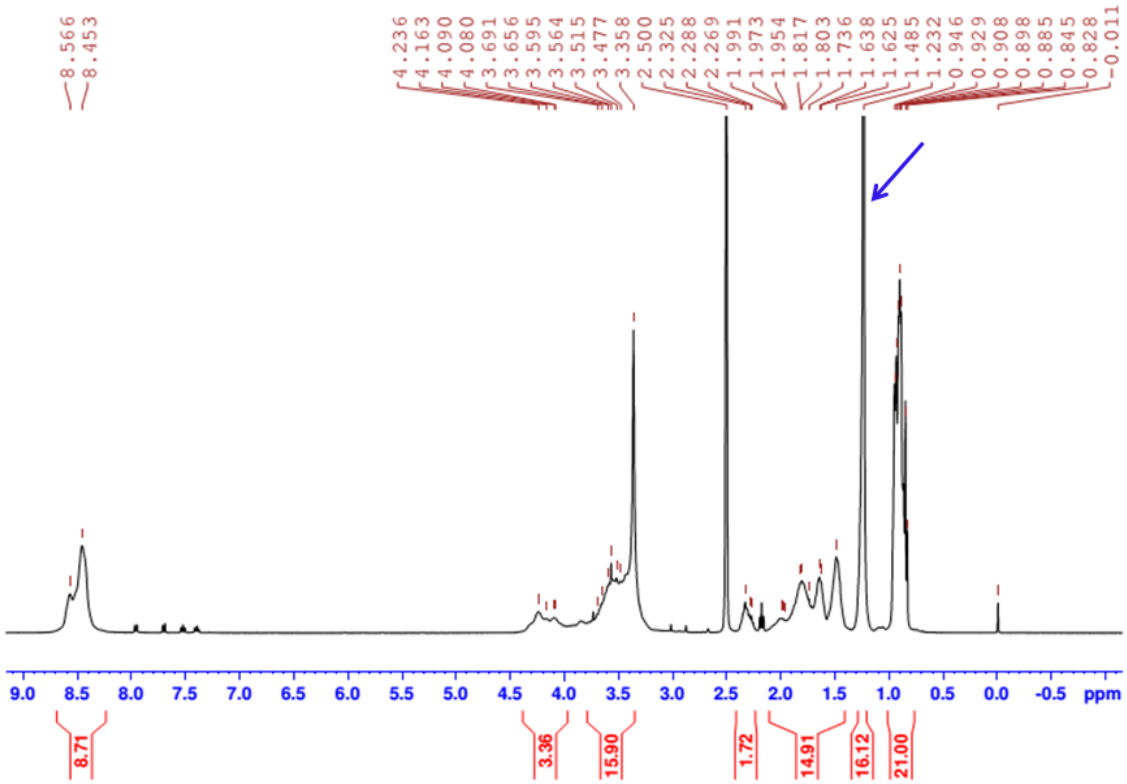


Fig. S8: ^{1}H-NMR spectrum of **CAM-8**. The NMR was taken in DMSO-d6 and the solvent peak was calibrated at δ value of 2.5000 ppm. The arrow corresponds to CH_3-$(CH_2)_8$-CH_2-CH_2-, which confirming the presence of long chain.

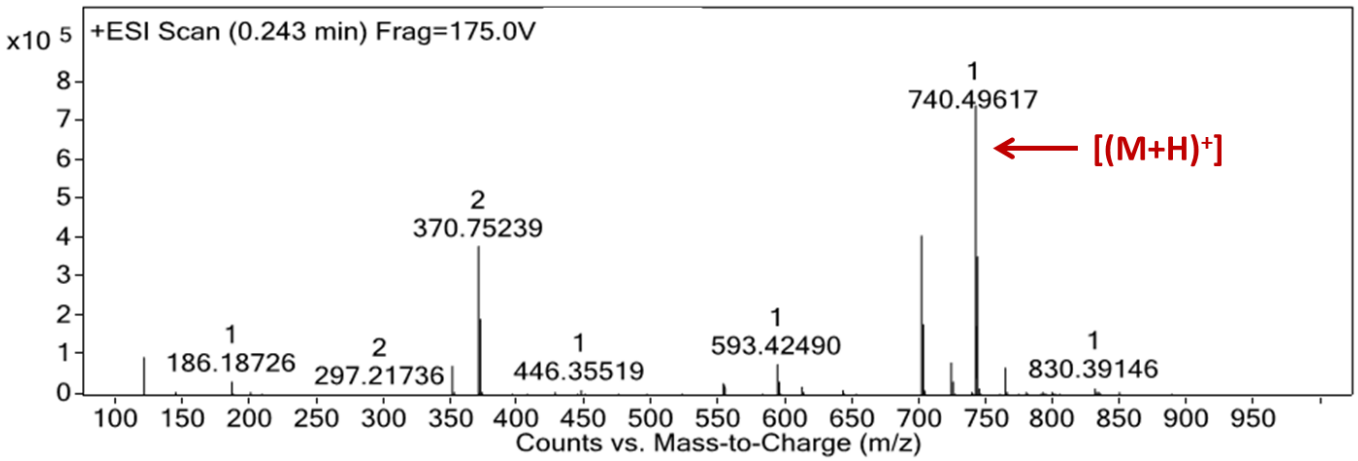


Fig. S9: HR-MS spectrum of **CAM-1**.

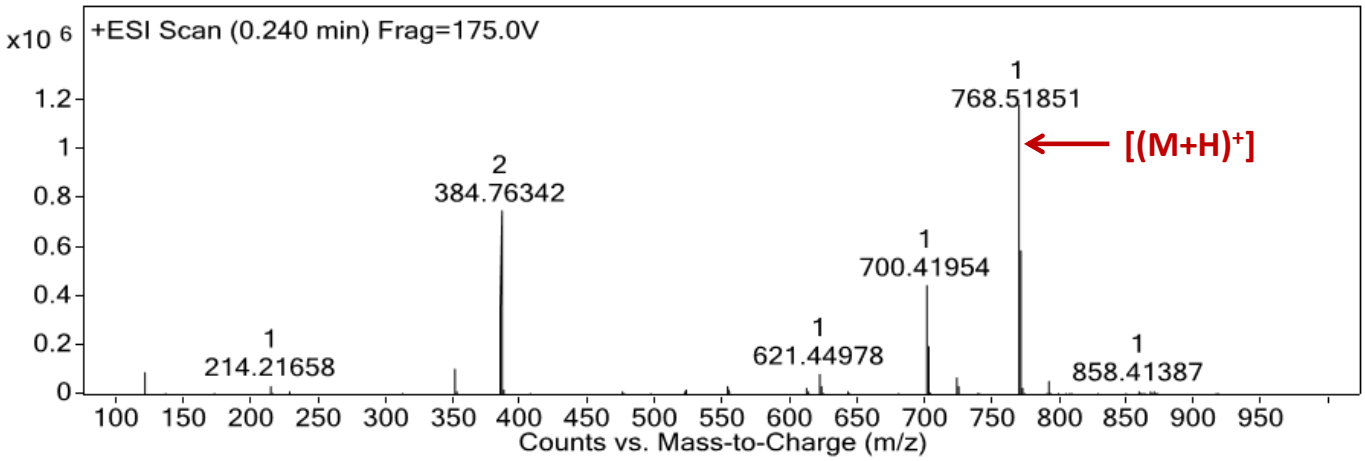


Fig. S10: HR-MS spectrum of **CAM-2**.

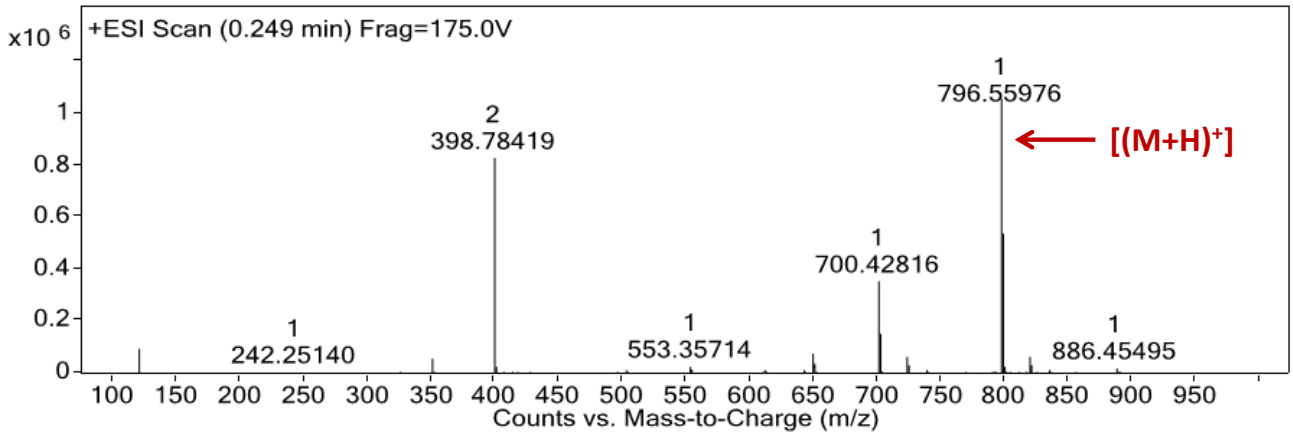


Fig. S11: HR-MS spectrum of **CAM-3**.

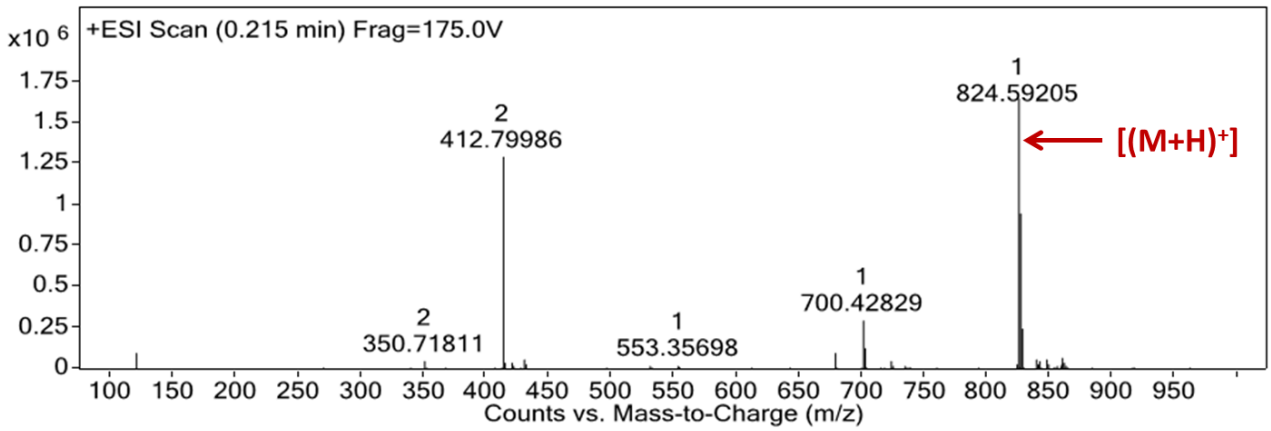


Fig. S12: HR-MS spectrum of **CAM-4**.

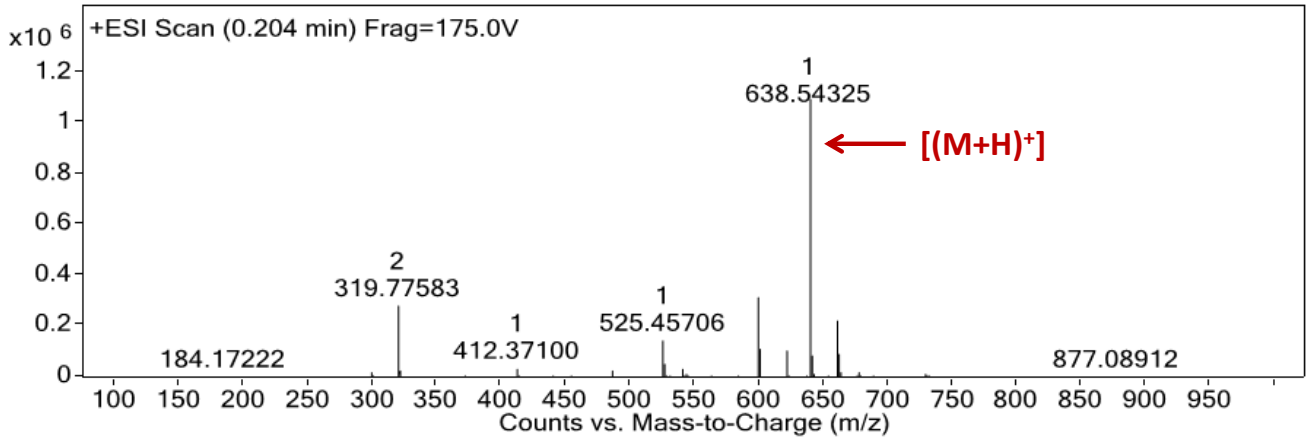


Fig. S13: HR-MS spectrum of **CAM-5**.

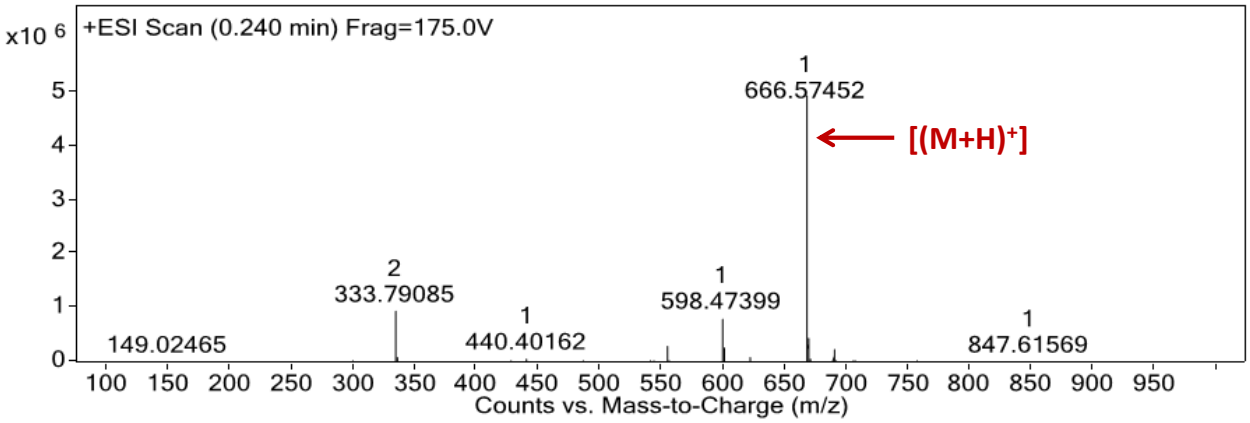


Fig. S14: HR-MS spectrum of **CAM-6**.

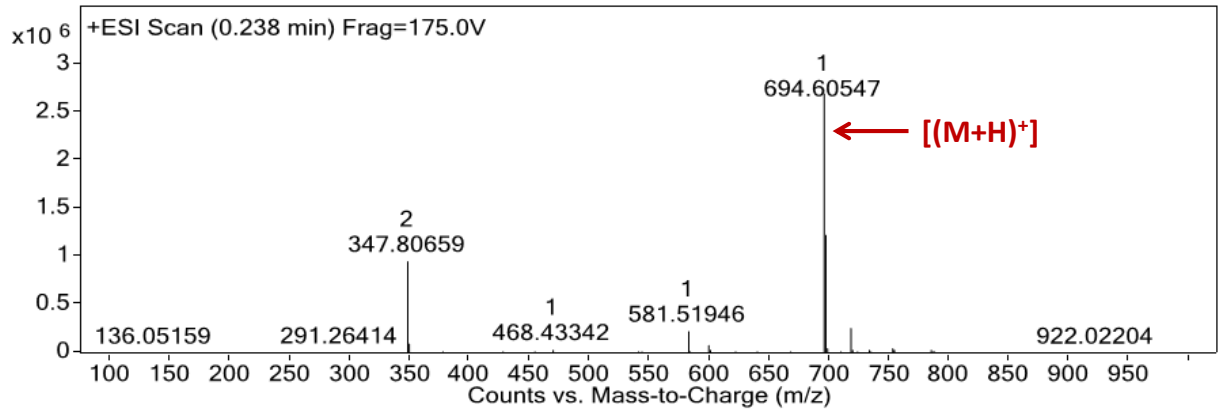


Fig. S15: HR-MS spectrum of **CAM-7**.

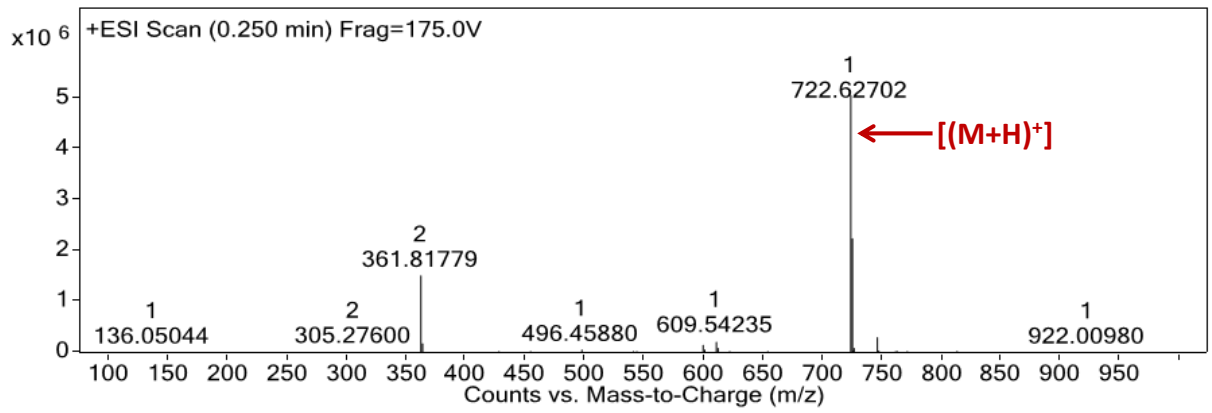


Fig. S16: HR-MS spectrum of **CAM-8**.

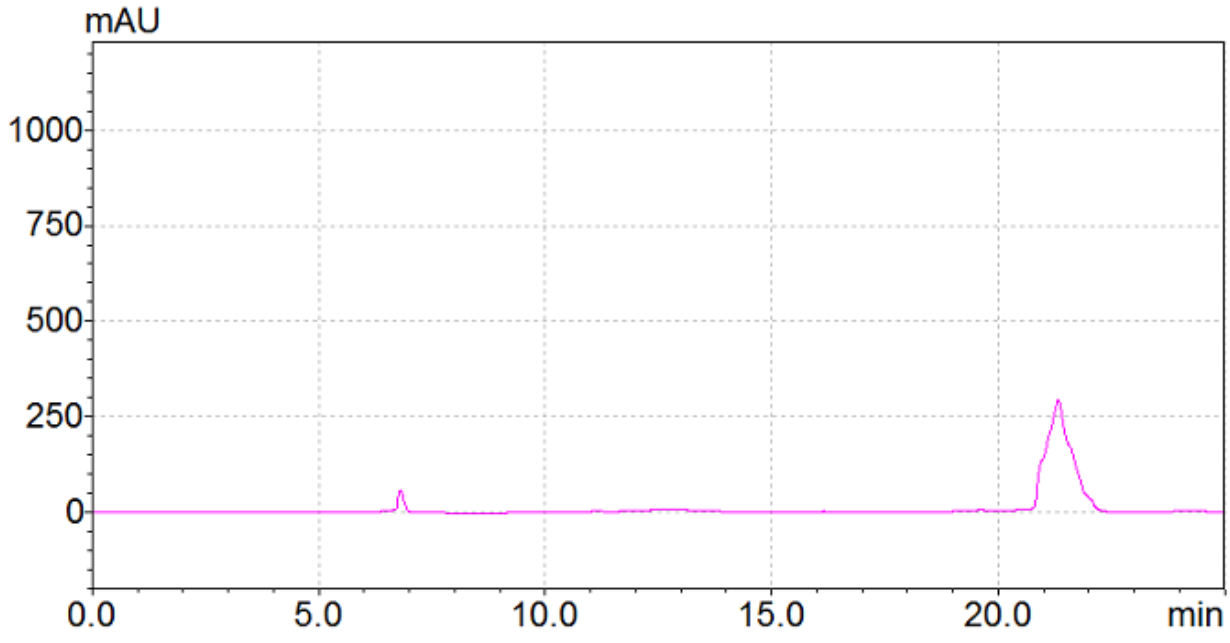


Fig. S17: HPLC trace of **CAM-8**. (The peak at 6.8 min corresponds to acetonitrile, resulting from the binary mobile phase)

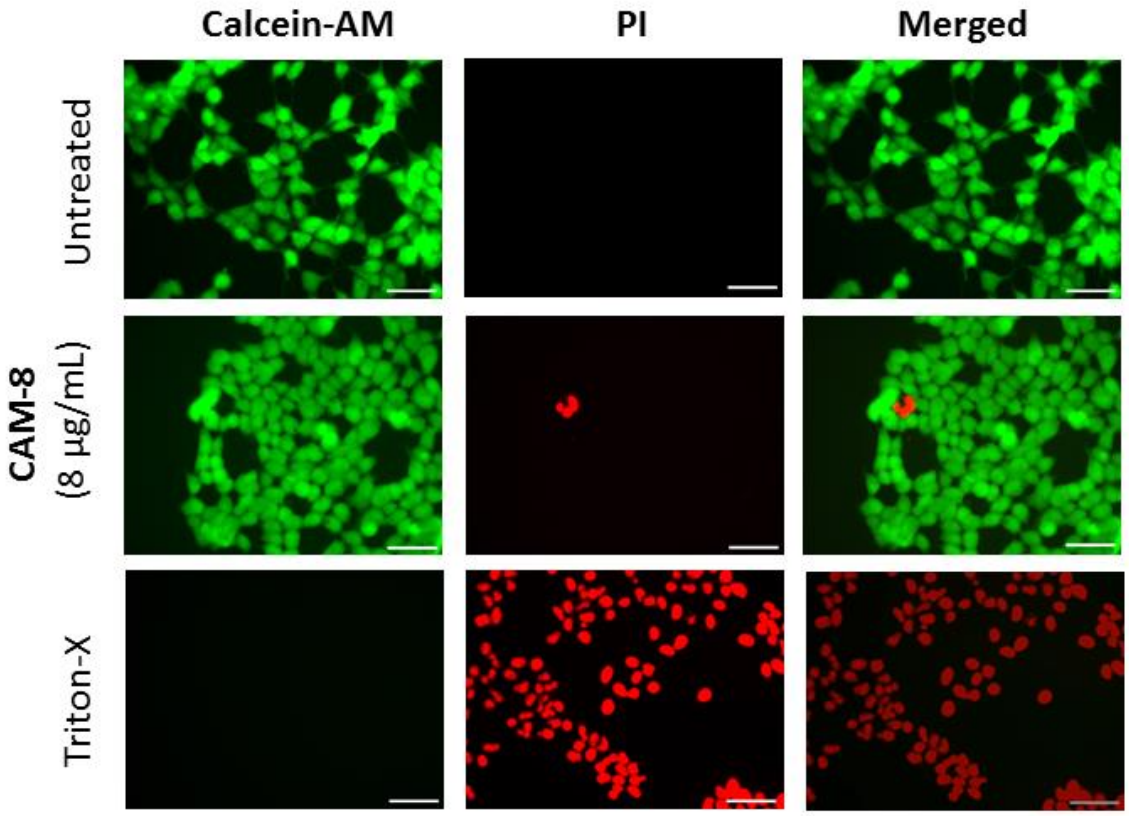


Fig. S18: Fluorescence microscopy images of HEK 293 cells treated with **CAM-8** (8 µg/mL). Scale bar: 50 µm.

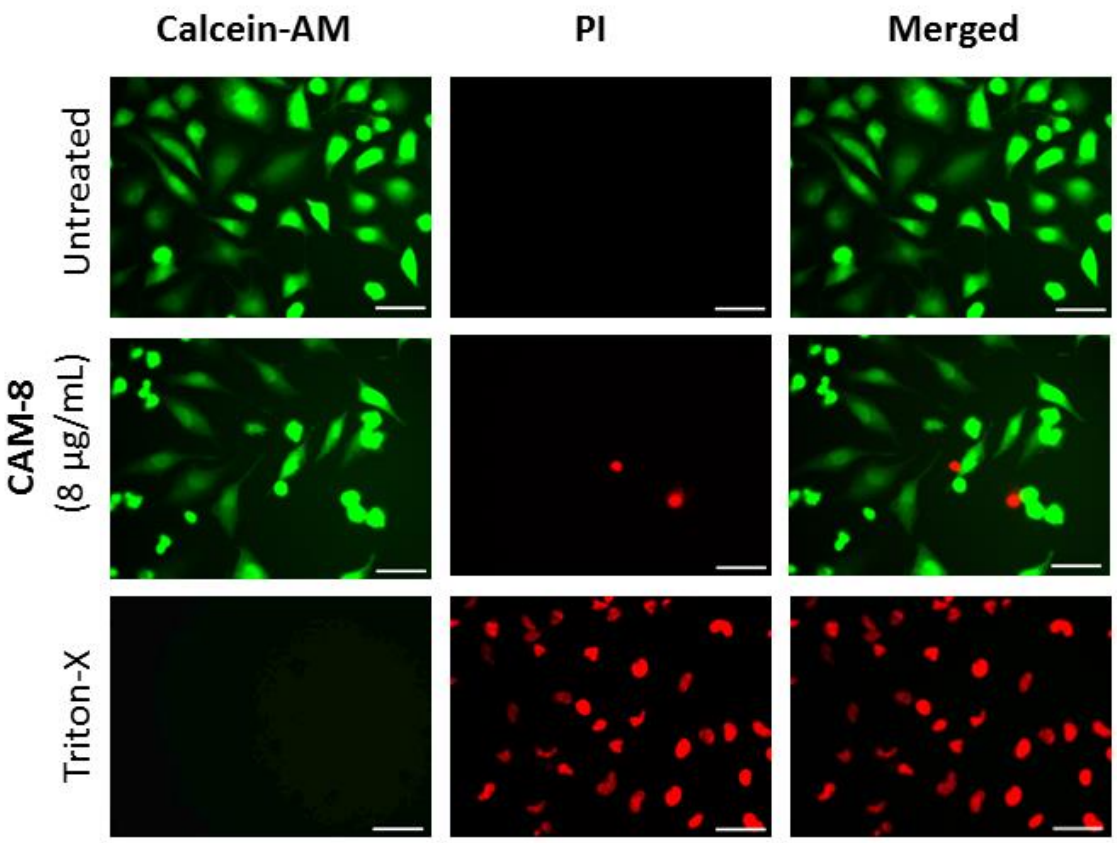


Fig. S19: Fluorescence microscopy images of HeLa cells treated with **CAM-8** (8 µg/mL). Scale bar: 50 µm.

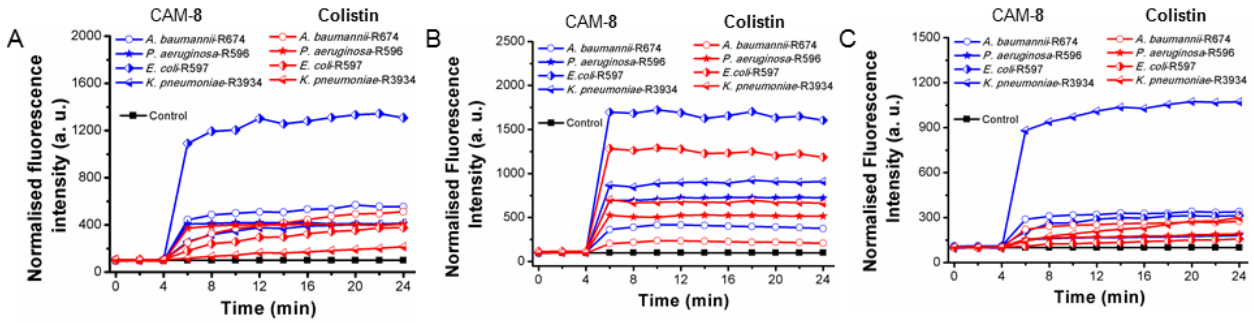


Fig. S20: Comparison of membrane activity of **CAM-8** and colistin, (A) inner membrane permeabilization of growing planktonic cells, (B) outer membrane permeabilization of stationary phase cells, (C) inner membrane permeabilization of stationary phase cells.

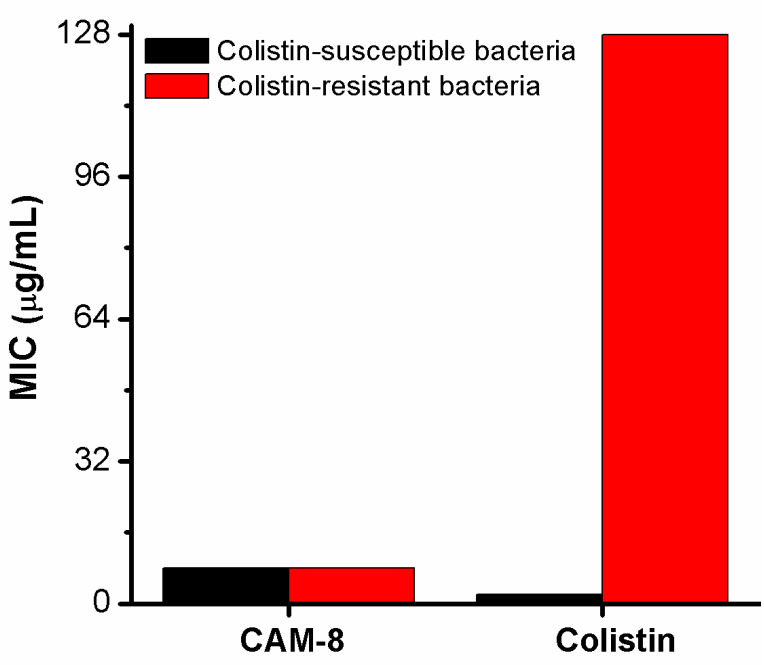


Fig. S21: Comparison of antibacterial activity of **CAM-8** against colistin-susceptible and colistin-resistant *K. pneumoniae*-R3934. Colistin-resistant strain was obtained from the sub-culture of the resistant bacteria generated resistance studies.

Table S1. HR-MS data of **CAM 1-8**.

Compounds	m/z of ($M+H^+$) [Calculated]	m/z of ($M+H^+$) [Observed]
CAM-**1**	740.48633	740.49617
CAM-**2**	768.51763	768.51851
CAM-**3**	796.54893	796.55976
CAM-**4**	824.58023	824.59205
CAM-**5**	638.53328	638.54325
CAM-**6**	666.56458	666.57452
CAM-**7**	694.59588	694.60547
CAM-**8**	722.62718	722.62702

Table S2: Antibacterial activity against drug-sensitive strains of Gram-negative bacteria.

Compound	Minimum Inhibitory Concentration (µg/mL)			
	A. baumannii-MTCC1425	*P. aeruginosa*-MTCC424	*E. coli*-MTCC443	*K. pneumoniae*-ATCC700603
CAM-**1**	>64	>64	>64	>64
CAM-**2**	64	64	32	>64
CAM-**3**	8	16	8	16
CAM-**4**	2	32	4	16
CAM-**5**	>64	>64	>64	>64
CAM-**6**	>64	>64	>64	>64
CAM-**7**	32	64	12	64
CAM-**8**	4	8	2	4
Meropenem	1	0.1	<0.1	0.1
Colistin	1	0.5	0.5	1

Table S3: Antibacterial activity against drug-resistant clinical isolates of Gram-negative bacteria.

Bacterial strains	Minimum Inhibitory Concentration (μg/mL)			
	Tetracycline	Meropenem	Colistin	CAM-**8**
A. baumannii-R674	128	128	1	4
A. baumannii-R676	128	128	1	4
P. aeruginosa-R590	>128	>128	1	8
P. aeruginosa-R596	128	128	1	8
P. aeruginosa-R2477	128	>128	1	8
E. coli-R250	8	32	2	4
E. coli-R597	>128	128	0.5	2
K. pneumoniae-R3421	32	>128	1	8
K. pneumoniae-R3934	>128	>128	2	8

Table S4: Antibacterial activity against Gram-positive bacteria, MRSA.

Bacterial strains	Minimum Inhibitory Concentration (μg/mL)		
	Methicillin	Colistin	CAM-**8**
MRSA-ATCC33591	>16	64	2
MRSA-R3545	>16	32	2
MRSA-R3889	>16	32	2
MRSA-R3890	>16	64	2

Reference

1. Konai, M. M.; Ghosh, C.; Yarlagadda, V.; Samaddar, S.; Haldar, J. *J. Med. Chem.* **2014**, *57*, 9409.

2. Konai, M. M.; Haldar, J. *ACS Infect. Dis.* **2015**, 1, 469.

3. Konai, M. M.; Adhikary, U.; Samaddar, S.; Ghosh, C.; Haldar, J. *Bioconjug. Chem.* **2015**, *26*, 2442.

4. Konai, M. M.; Adhikary, U.; Haldar, J. *Chem. Eur. J.* **2017**, *23*, 12853.

5. Konai, M. M.; Haldar, J. *Bioconjug. Chem.* **2017**, *28*, 1194.

6. Yarlagadda, V.; Sarkar, P.; Samaddar, S.; Manjunath, G. B.; Mitra, S. D.; Paramanandham, K.; Shome, B. R.; Haldar, J. *ACS Infect. Dis.* **2018**, *4*, 1093.

7. Pinnock, A.; Shivshetty, N.; Roy, S.; Rimmer, S.; Douglas, I.; MacNeil, S.; Garg, P. *Graefes. Arch. Clin. Exp. Ophthalmol.* **2017**, *255*, 333.