Supporting information for:

Small-Molecular Adjuvants with Weak Membrane Perturbation Potentiate Antibiotics against Gram-Negative Superbugs

Geetika Dhanda[a], Riya Mukherjee[a], Debajyoti Basak[a] and Jayanta Haldar[a], [b]

[a]Antimicrobial Research Laboratory, New Chemistry Unit, Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR), Jakkur, Bengaluru 560064, Karnataka, India

[b]School of Advanced Materials, Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR), Jakkur, Bengaluru 560064, Karnataka, India

E-mail: jayanta@jncasr.ac.in

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Characterization details

***N*^{1}-Boc-*N*^{3}-(3-(Boc-amino) propyl) propane-1,3-diamine (1a):** Yield-50%, ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 5.178 (s, -*NH*Boc, 2H), 3.205-3.098 (m, NH(-*CH*$_2$-$(CH_2)_2$-NHBoc)$_2$, 4H), 2.658-2.625 (m, NH(-$(CH_2)_2$-*CH*$_2$-NHBoc)$_2$, 4H), 1.672-1.607 (m, NH(-CH_2-*CH*$_2$-CH_2-NHBoc)$_2$, 4H), 1.443 (s, NH(-$(CH_2)_3$-NH-COO-C*(CH*$_3$*)*$_3$)$_2$, 18H). Formula: $C_{16}H_{33}N_3O_4$, HRMS (m/z): 332.2539 [$(M+H)^+$] (Observed), 332.2549 [$(M+H)^+$] (Calculated).

***N*,*N*-bis(3-(Boc-amino)propyl) decanamide (1b):** Yield-78%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 5.388 (s, -*NH*Boc, 1H), 4.707 (s, -*NH*Boc, 1H), 3.386-3.000 (m, R-CO-N(-*CH*$_2$-CH_2-*CH*$_2$-NHBoc)$_2$, 8H), 2.267 (t, *J* = 7.6 Hz, CH_3-$(CH_2)_7$-*CH*$_2$- of R group, 2H), 1.777-1.611 (m, CH_3-$(CH_2)_6$-*CH*$_2$-CH_2- of R group, 6H), 1.407 (s, R-CO-N(-$(CH_2)_3$-NH-COO-C*(CH*$_3$*)*$_3$)$_2$, 18H), 1.241 (bs, CH_3-*(CH*$_2$*)*$_6$-CH_2-CH_2- of R group, 12H), 0.854 (t, *J* = 7.08 Hz, *CH*$_3$-$(CH_2)_7$-CH_2- of R group, 3H). Formula: $C_{26}H_{51}N_3O_5$, HRMS (m/z): 486.3899 [$(M+H)^+$] (Observed), 486.3907 [$(M+H)^+$] (Calculated).

***N*,*N*-bis(3-(Boc-amino)propyl) dodecanamide (1c):** Yield-90%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 5.377 (s, -*NH*Boc, 1H), 4.654 (s, -*NH*Boc, 1H), 3.393-3.006 (m, R-CO-N(-*CH*$_2$-CH_2-*CH*$_2$-NHBoc)$_2$, 8H), 2.271 (t, *J* = 7.44 Hz, CH_3-$(CH_2)_9$-*CH*$_2$- of R group, 2H), 1.783-1.570 (m, CH_3-$(CH_2)_8$-*CH*$_2$-CH_2-of R group, 6H), 1.422 (s, R-CO-N(-$(CH_2)_3$-NH-COO-C*(CH*$_3$*)*$_3$)$_2$, 18H), 1.242 (bs, CH_3-*(CH*$_2$*)*$_8$-CH_2-CH_2- of R group, 16H), 0.863 (t, *J* = 7.2 Hz, *CH*$_3$-$(CH_2)_9$-CH_2- of R group, 3H). Formula: $C_{28}H_{55}N_3O_5$, HRMS (m/z): 514.4208 [$(M+H)^+$] (Observed), 514.4220 [$(M+H)^+$] (Calculated).

***N*,*N*-bis(3-(Boc-amino)propyl) naphthyl ethanamide (1d):** Yield-55%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 7.936-7.771 (m, *H*$_{Ar}$, 3H), 7.553-7.331 (m, *H*$_{Ar}$, 4H), 5.268 (s, -N*H*Boc, 1H), 4.484 (s, -N*H*Boc, 1H), 4.133 (s, Ar*CH*$_2$- of R group, 2H), 3.461-3.037 (m, R-CO-N(-*CH*$_2$-CH_2-*CH*$_2$-NHBoc)$_2$, 8H), 1.709-1.679 (m, R-CO-N((-CH_2-*CH*$_2$-CH_2-NHBoc)$_2$, 4H), 1.4075 (s, R-CO-N(-$(CH_2)_3$-NH-COO-C*(CH*$_3$*)*$_3$)$_2$, 18H). Formula: $C_{28}H_{41}N_3O_5$, HRMS (m/z): 522.2935 [$(M+Na)^+$] (Observed), 522.2944 [$(M+Na)^+$] (Calculated).

***N*,*N*-bis(3-(Boc-amino)propyl) 3,3-diphenyl propanamide (1e):** Yield-74%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 7.288-7.284 (*H*$_{Ar}$, 3H), 7.252-7.154 (m, *H*$_{Ar}$, 7H), 5.227 (s, -N*H*Boc, 1H), 4.714 (t, *J* = 7.6 Hz, Ar*CH*CH_2- of R group , 1H), 4.560 (s, -N*H*Boc, 1H), 3.337-3.055 and 2.783-2.739 (m, R-CO-N(-*CH*$_2$-CH_2-*CH*$_2$-NHBoc)$_2$, 8H), 3.026 (d, *J* = 7.6 Hz, ArCH*CH*$_2$- of R group, 2H), 1.636-1.439 (m, R-CO-N(-CH_2-*CH*$_2$-CH_2-NHBoc)$_2$, 4H), 1.434

(s, R-CO-N(-$(CH_2)_3$-NH-COO-C*(*$\underline{CH_3}$*)*$_3$)$_2$, 18H). Formula: $C_{31}H_{45}N_3O_5$, HRMS (m/z): 540.3400 [$(M+H)^+$] (Observed), 540.3437 [$(M+H)^+$] (Calculated).

***N*-(Adamantan-2-yl)-2-bromoethanamide (1f):** ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 6.914 (s, Ad-N$\underline{H}$-CO-CH_2Br, 1H), 4.046-4.025 (m, $\underline{H_{Ad}}$, 1H), 3.912 (s, Ad-NH-CO-$\underline{CH_2}$Br, 2H), 1.944-1.612 (m, $\underline{H_{Ad}}$, 14H). Formula: $C_{12}H_{18}BrNO$, HRMS (m/z): 272.0575 [$(M+H)^+$] (Observed), 272.0650 [$(M+H)^+$] (Calculated).

***N*-(Adamantan-2-yl)-2-[*N′,N′*-{bis-(3-Boc-amino) propyl} amino] ethanamide (1g):** ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 4.747 (br, -N$\underline{H}$Boc, 2H), 4.126-4.040 (m, $\underline{H_{Ad}}$, 1H), 3.178-3.087 (br, Ad-NH-CO-$\underline{CH_2}$-N(-$(CH_2)_2$-$\underline{CH_2}$-NHBoc)$_2$, 6H), 2.560 (br, Ad-NH-CO-CH_2-N(-$\underline{CH_2}$-$(CH_2)_2$-NHBoc)$_2$, 4H), 1.903-1.664(br, $\underline{H_{Ad}}$ and Ad-NH-CO-CH_2-N(-CH_2-$\underline{CH_2}$-CH_2-NHBoc)$_2$, 17H), 1.432 (s, Ad-NH-CO-CH_2-N(-$(CH_2)_3$-NH-COO-C*(*$\underline{CH_3}$*)*$_3$)$_2$, 18H). Formula: $C_{28}H_{50}N_4O_5$, HRMS (m/z): 523.3971 [$(M+H)^+$] (Observed), 523.3859 [$(M+H)^+$] (Calculated).

***N*,*N*-bis((3-amino)propyl)decanamide bis(trifluoroacetate) (NC10):** Yield-Quantitative. ^{1}H-NMR (400 MHz, DMSO-d_6) δ/ppm: 8.589-7.886 (br, R-CO-N(-$(CH_2)_3$-$\underline{NH_3^+}$)$_2$, 6H), 3.131-2.868 (m, R-CO-N(-$\underline{CH_2}$-CH_2-$\underline{CH_2}$-NH_3^+)$_2$, 8H), 2.061 (t, *J* = 7.6 Hz, CH_3-$(CH_2)_7$-$\underline{CH_2}$- of R group, 2H), 1.910-1.464 (m, CH_3-$(CH_2)_6$-$\underline{CH_2}$-CH_2- of R group, 6H), 1.239 (bs, CH_3-*(*$\underline{CH_2}$*)*$_6$-CH_2-CH_2- of R group, 12H), 0.857 (t, *J* = 7.2 Hz, $\underline{CH_3}$-$(CH_2)_7$-CH_2- of R group, 3H). ^{13}C-NMR (100 MHz, DMSO-d_6): 172.7 (R-$\underline{C}$O-N(-CH_2-CH_2-CH_2-NH_3^+)$_2$, 1C), 44.8, (R-CO-N(-$\underline{C}H_2$-CH_2-CH_2-NH_3^+)$_2$, 2C), 44.0 (R-CO-N(-CH_2-CH_2-$\underline{C}H_2$-NH_3^+)$_2$, 2C), 39.5 (($\underline{C}D_3$)$_2$SO), 36.2, 35.5, 35.3, 31.2, 28.9, 28.8, 28.7, 28.6, 26.1, 25.2, 23.8, 22.1, 13.9 (36.2-13.9 signifies aliphatic region). FT-IR (cm^{-1}): 3254 (N-H str. of 1° amine), 3080 (N-H str. of 1° amine), 2909 (sp^3 C-H str.), 2849 (sp^3 C-H str.), 1674 (C=O str. of 3° amide). Formula: $C_{16}H_{35}N_3O$, HRMS (m/z): 286.2850 [$(M+H)^+$] (Observed), 286.2858 [$(M+H)^+$] (Calculated).

***N*,*N*-bis((3-amino)propyl)dodecanamide bis(trifluoroacetate) (NC12):** Yield-Quantitative, HPLC Purity- 97%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ/ppm: 7.894 (bs, R-CO-N(-$(CH_2)_3$-$\underline{NH_3^+}$)$_2$, 6H), 3.300-3.265 (m, R-CO-N(-$(CH_2)_2$-$\underline{CH_2}$-NH_3^+)$_2$, 4H), 2.864-2.696 (m, R-CO-N(-$\underline{CH_2}$-$(CH_2)_2$-NH_3^+)$_2$, 4H), 2.276 (t, *J* = 7.2 Hz CH_3-$(CH_2)_9$-$\underline{CH_2}$- of R group, 2H), 1.850-1.481 (m, CH_3-$(CH_2)_8$-$\underline{CH_2}$-CH_2- of R group, 6H), 1.237 (s, CH_3-*(*$\underline{CH_2}$*)*$_8$-CH_2-CH_2- of R group, 16H), 0.850 (t, *J* = 6.8 Hz, $\underline{CH_3}$-$(CH_2)_8$-CH_2-CH_2- of R group, 3H). ^{13}C-NMR (100 MHz, DMSO-d_6): 172.7 (R-$\underline{C}$O-N(-CH_2-$(CH_2)_2$-NH_3^+)$_2$, 1C), 44.8, 44.3, 44.0, 41.8, 39.5

(($\underline{C}$D$_3$)$_2$SO), 36.6, 36.4, 36.2, 35.5, 35.3, 31.9, 31.3, 29.0, 29.0, 28.8, 28.7, 26.6, 26.1, 25.6, 25.2, 25.0, 23.8, 22.1, 13.9 (44.8-13.9 signifies aliphatic region). FT-IR (cm^{-1}): 3458 (N-H str. of 1° amine), 3084 (N-H str. of 1° amine), 2928 (sp^3 C-H str.), 2852 (sp^3 C-H str.), 1677 (C=O str. of 3° amide). Formula: $C_{18}H_{39}N_3O$, HRMS (m/z): 314.3172 [(M+H)$^+$] (Observed), 314.3171 [(M+H)$^+$] (Calculated).

***N*,*N*-bis((3-amino)propyl)naphthyl ethanamide bis(trifluoroacetate) (NNaph):** Yield- Quantitative, HPLC Purity- 99%. ^{1}H-NMR (400 MHz, DMSO-d$_6$) δ/ppm: 7.913-7.824 (m, R-CO-N(-(CH$_2$)$_3$-$N\underline{H_3}^+$)$_2$ and $\underline{H_{Ar}}$, 8H), 7.542-7.329 (m, $\underline{H_{Ar}}$, 5H), 4.176 (s, ArC$\underline{H_2}$- of R group, 2H), 3.556-3.339 (m, R-CO-N(-(CH$_2$)$_2$-C$\underline{H_2}$-NH$_3^+$)$_2$, 4H), 2.901-2.729 (m, R-CO-N-(C$\underline{H_2}$-(CH$_2$)$_2$-NH$_3^+$)$_2$, 4H), 1.987-1.767 (m, R-CO-N(-CH$_2$-C$\underline{H_2}$-CH$_2$-NH$_3^+$)$_2$, 4H). ^{13}C-NMR (100 MHz, DMSo-d$_6$): 170.9-170.6 (R-$\underline{C}$O-N(-(CH$_2$)$_2$-CH$_2$-NH$_3^+$)$_2$, 1C), 133.3-124.1 (C_{Ar}, 7C) , 44.8, 42.2, 39.5 (($\underline{C}$D$_3$)$_2$SO), 36.8, 36.7, 36.5, 26.7, 25.7, 23.8 (44.8-23.8 signifies aliphatic region). FT-IR (cm^{-1}): 3013 (N-H str. of 1° amine), 1674 (C=O str. of 3° amide). Formula: $C_{18}H_{25}N_3O$, HRMS (m/z): 300.2070 [(M+H)$^+$] (Observed), 300.2076 [(M+H)$^+$] (Calculated).

***N*,*N*-bis((3-amino)propyl)3,3-diphenyl propanamide bis(trifluoroacetate) (NDiphe):** Yield- Quantitative, HPLC Purity- 96%. ^{1}H- NMR (400 MHz, DMSO-d$_6$) δ/ppm: 7.845-7.648 (br, R-CO-N(-(CH$_2$)$_3$-$N\underline{H_3}^+$)$_2$, 6H), 7.326-7.140 (m, $\underline{H_{Ar}}$, 10H), 4.536 (t, J = 7.6 Hz, ArC$\underline{H}$CH$_2$- of R group, 1H), 3.384-3.202 (m, R-CO-N(-(CH$_2$)$_2$-C$\underline{H_2}$-NH$_3^+$)$_2$, 4H), 3.109 (d, J = 7.6 Hz, ArCHC$\underline{H_2}$- of R group, 2H), 2.972-2.816 (m, R-CO-N(-C$\underline{H_2}$-(CH$_2$)$_2$-NH$_3^+$)$_2$, 4H), 1.808-1.578 (m, R-CO-N(-CH$_2$-C$\underline{H_2}$-CH$_2$-NH$_3^+$)$_2$, 4H). ^{13}C-NMR (100 MHz, DMSo-d$_6$): 170.8 (R-$\underline{C}$O-N(-CH$_2$-(CH$_2$)$_2$-NH$_3^+$)$_2$, 1C), 144.5-144.1 (C_{Ar}), 128.3-126.1 (C_{Ar}), 46.8, 44.1, 41.8, 39.5 (($\underline{C}$D$_3$)$_2$SO), 37.4, 36.3, 36.3, 36.1, 26.6, 25.4, 23.8 (46.8-23.8 signifies aliphatic region). FT-IR (cm^{-1}): 3428 (N-H str. of 1° amine), 3035 (N-H str. of 1° amine), 1677 (C=O str. of 3° amide). Formula: $C_{21}H_{29}N_3O$, HRMS (m/z): 340.2379 [(M+H)$^+$] (Observed), 340.2389 [(M+H)$^+$] (Calculated).

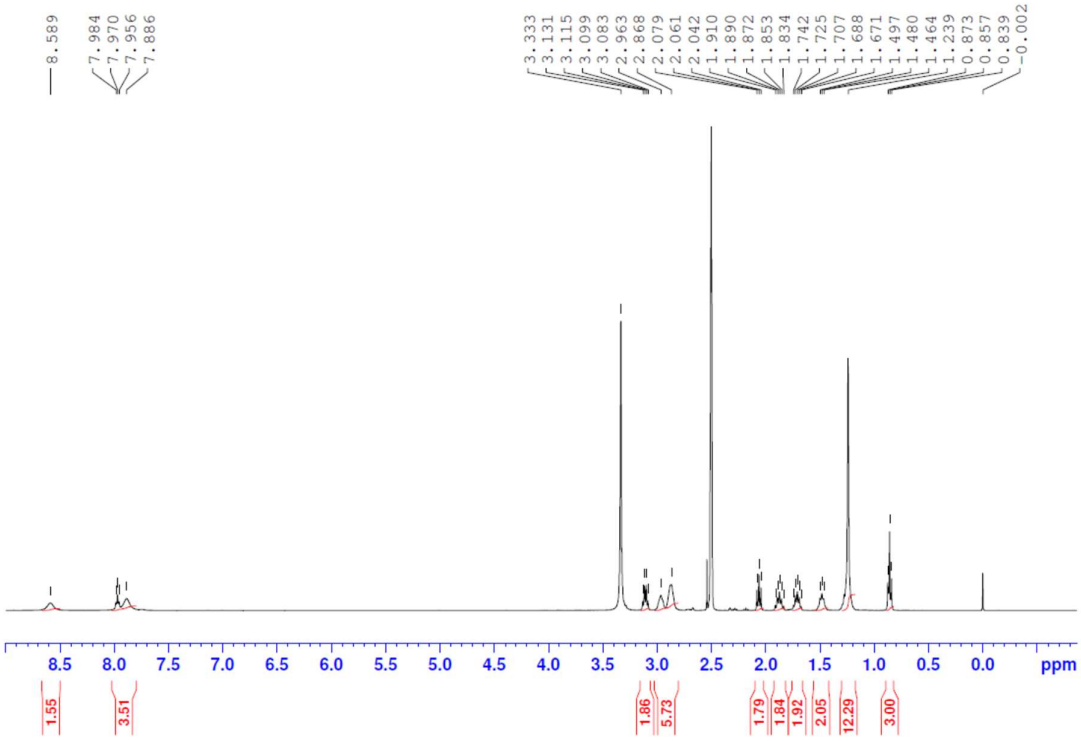
***N*-(Adamantan-2-yl)-2-[*N′*,*N′*-{bis-(3-amino) propyl} amino] ethanamide tris(trifluoroacetate) (NAda):** Yield- Quantitative, HPLC Purity- 96%. ^{1}H-NMR (400 MHz, DMSO-d$_6$) δ/ppm: 8.391 (br, AdN$\underline{H}$COCH$_2$-NH$^+$(-(CH$_2$)$_3$-NH$_3^+$)$_2$, 1H), 7.937 (br, AdNHCOCH$_2$-NH$^+$(-(CH$_2$)$_3$-$N\underline{H_3}^+$)$_2$, 6H), 3.922-3.905 (br, $\underline{H_{Ad}}$, 1H), 2.990-2.860 (br, AdNHCOC$\underline{H_2}$-NH$^+$(-(CH$_2$)$_2$-C$\underline{H_2}$-NH$_3^+$)$_2$, 6H), 1.959-1.704 (m, $\underline{H_{Ad}}$ and AdNHCOCH$_2$-NH$^+$(-C$\underline{H_2}$-(CH$_2$)$_2$-NH$_3^+$)$_2$, 18H), 1.545-1.235 (m, AdNHCOCH$_2$-NH$^+$(-CH$_2$-C$\underline{H_2}$-CH$_2$-

$NH_3^+)_2$, 4H). ^{13}C-NMR (100 MHz, DMSO-d_6): 158.6, 53.4 (AdNHCO<u>C</u>H_2-NH^+(-CH_2-$(CH_2)_2$-$NH_3^+)_2$, 1C), , 53.1 (AdNHCOCH_2-NH^+(-<u>C</u>H_2-$(CH_2)_2$-$NH_3^+)_2$, 2C), 51.4, 44.0, 39.5 ((<u>C</u>$D_3)_2$SO), 37.0, 36.7, 36.2, 31.3, 30.9, 26.6, 26.6, 23.9, 23.8 (51.4-23.8 signifies aliphatic region). FT-IR (cm^{-1}): 3410 (N-H str. of primary amine), 3051 (N-H str. of 1° amine), 2916 (sp^3 C-H str.), 2852 (sp^3 C-H str.), 1678 (C=O str. of 2° amide). Formula: $C_{18}H_{34}N_4O$, HRMS (m/z): 323.2791 [$(M+H)^+$] (Observed), 323.2811 [$(M+H)^+$] (Calculated).

Spectra of final compounds

***N*,*N*-bis((3-amino)propyl)decanamide bis(trifluoroacetate) (NC10)**

^{1}H-NMR



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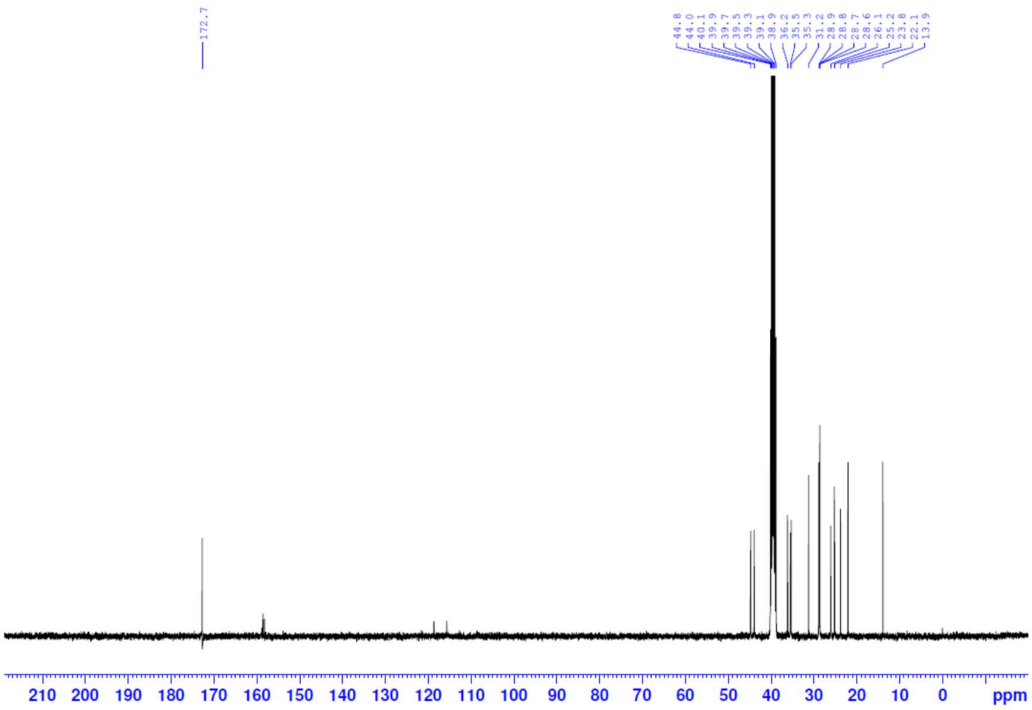
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39.7
39.5
39.3
39.1
38.9
36.2
35.5
35.3
31.2
28.9
28.8
28.7
28.6
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25.2
23.8
22.1
13.9
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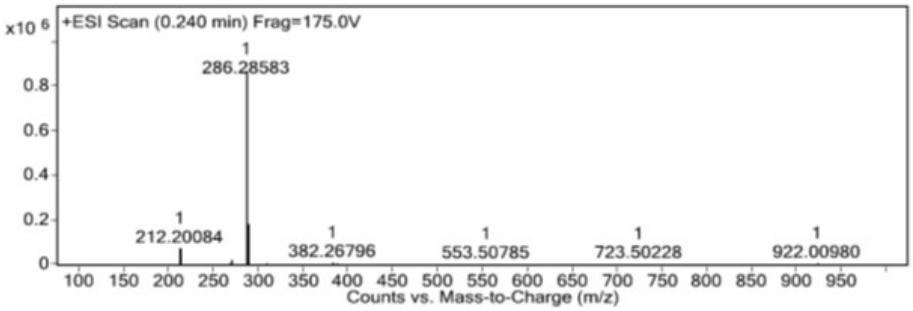
HRMS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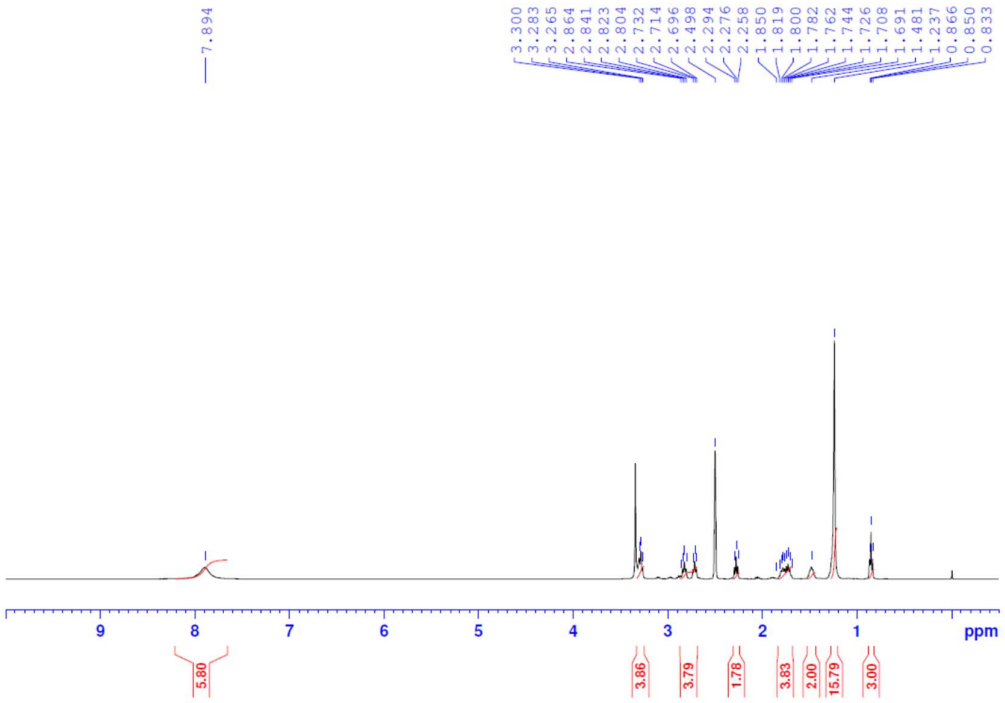
0.4
0.2
0
100 150 200 250 300 350 400 450 500 550 600 650 700 750 800 850 900 950
Counts vs. Mass-to-Charge (m/z)

***N*,*N*-bis((3-amino)propyl)dodecanamide bis(trifluoroacetate) (NC12)**

^{1}H-NMR



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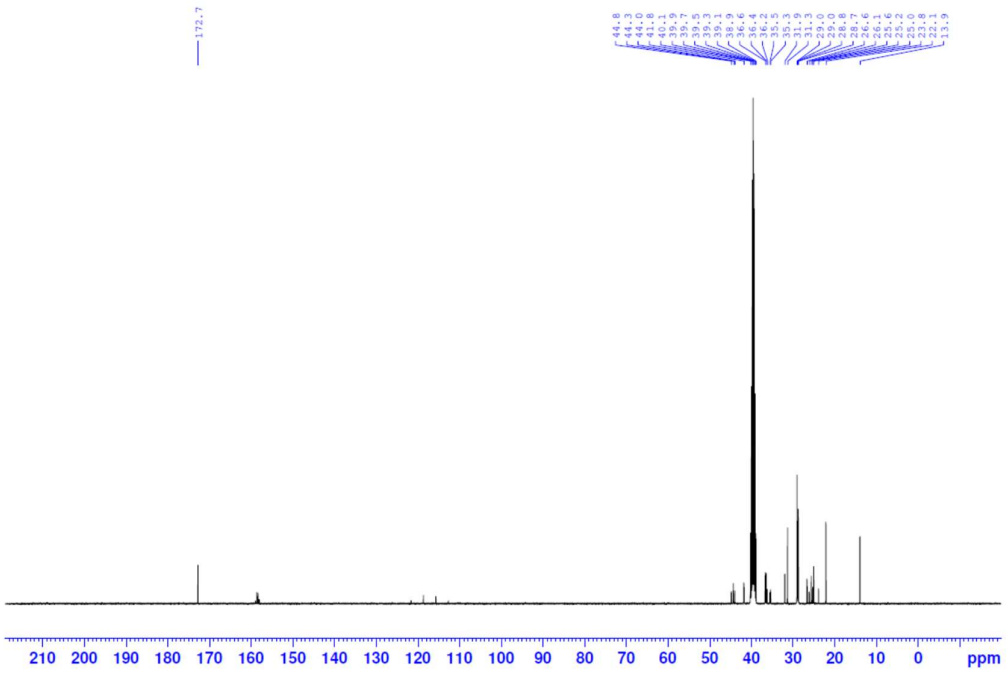
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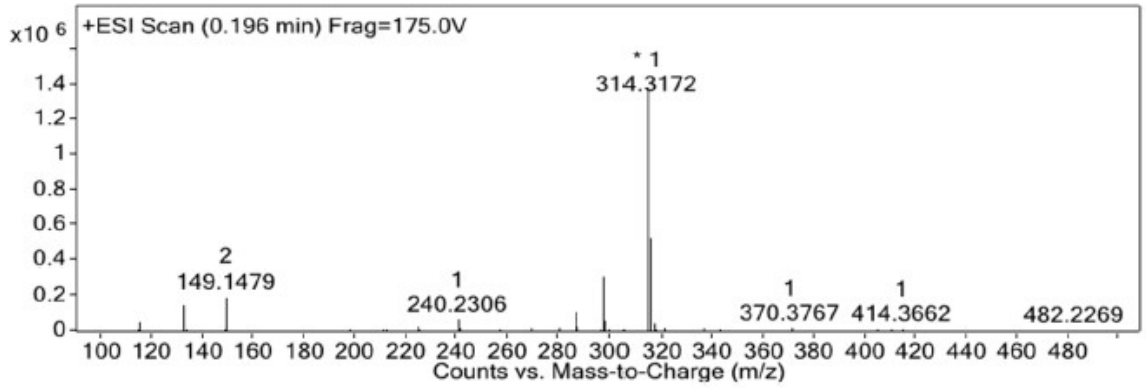
HRMS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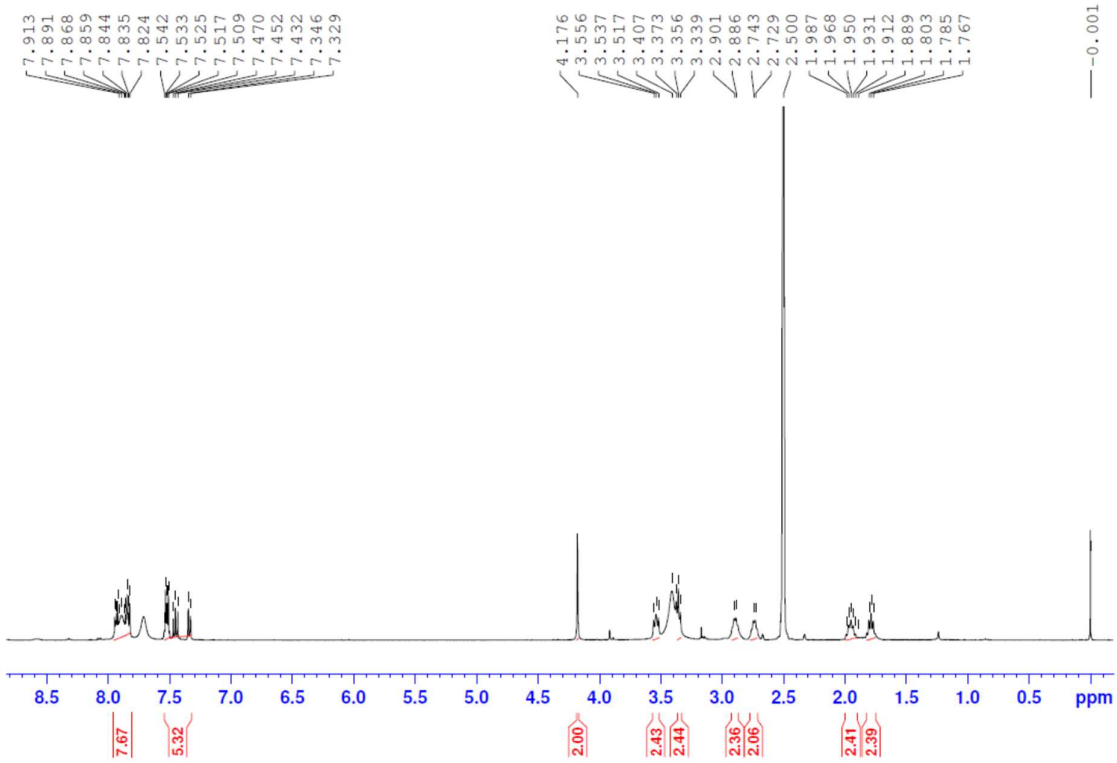
Counts vs. Mass-to-Charge (m/z)

***N*,*N*-bis((3-amino)propyl)naphthyl ethanamide bis(trifluoroacetate) (NNaph)**

^{1}H-NMR



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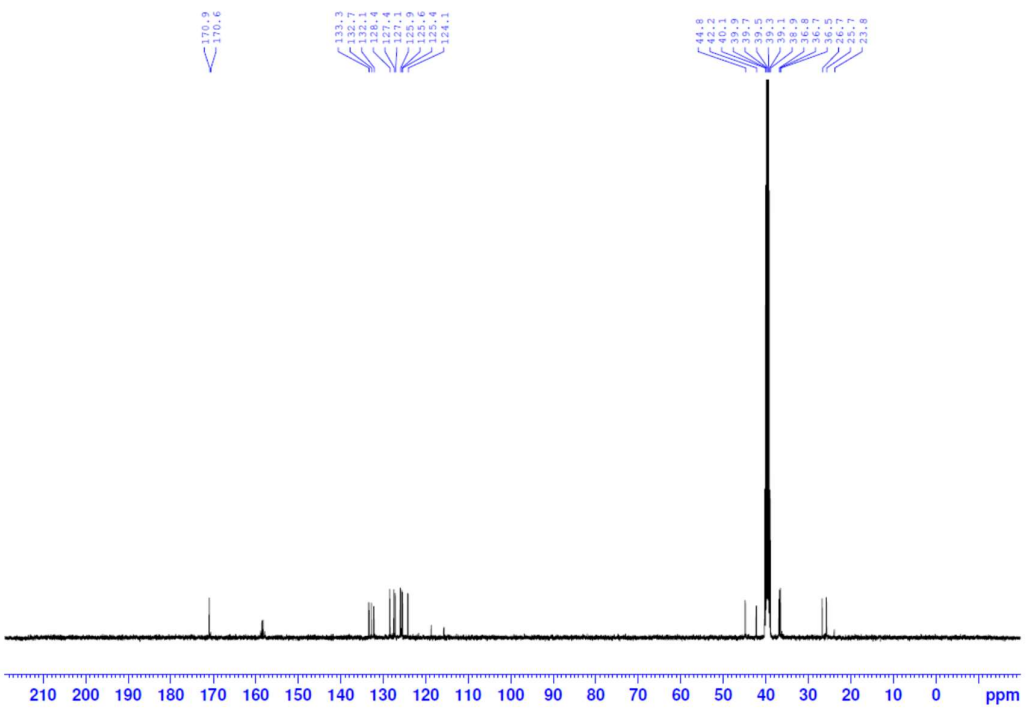
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39.9
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39.1
38.9
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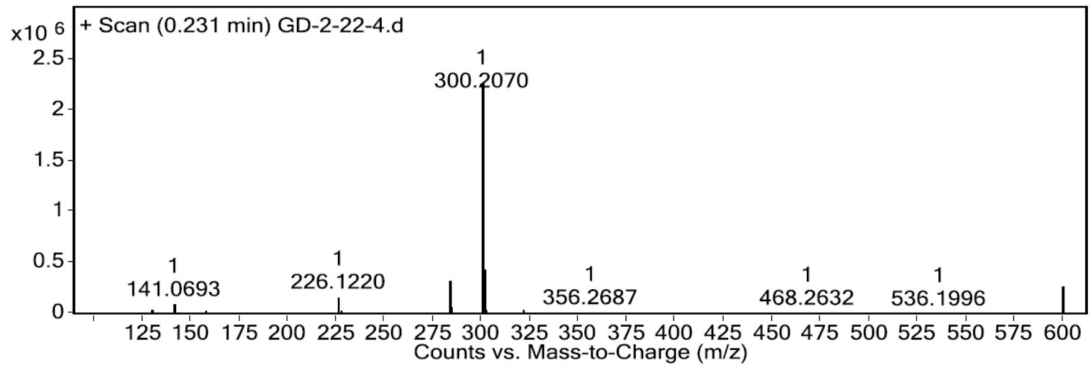
HRMS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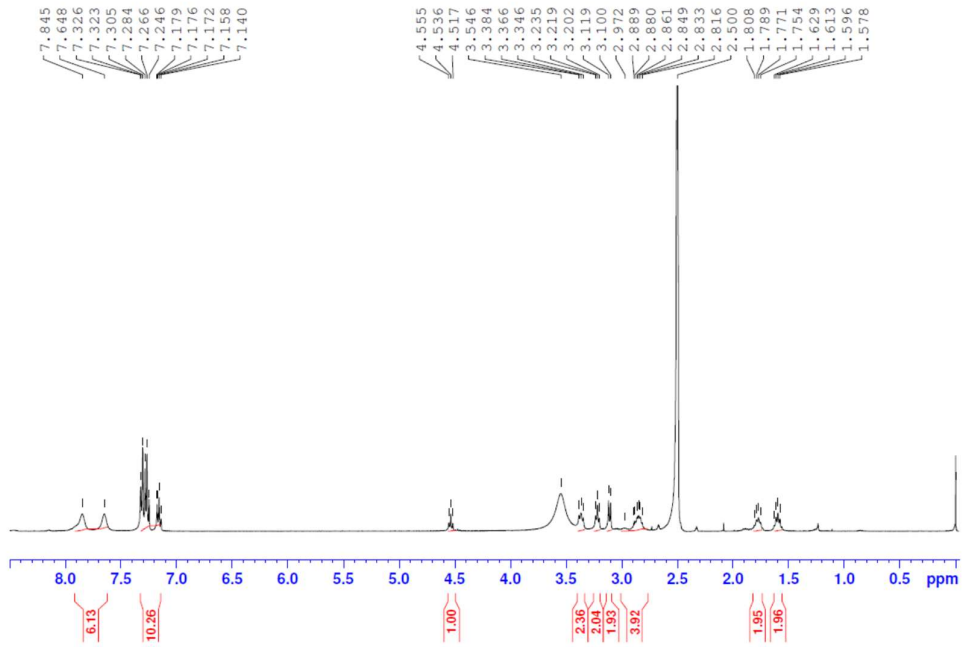
Counts vs. Mass-to-Charge (m/z)

***N*,*N*-bis((3-amino)propyl)3,3-diphenyl propanamide bis(trifluoroacetate) (NDiphe)**

^{1}H-NMR



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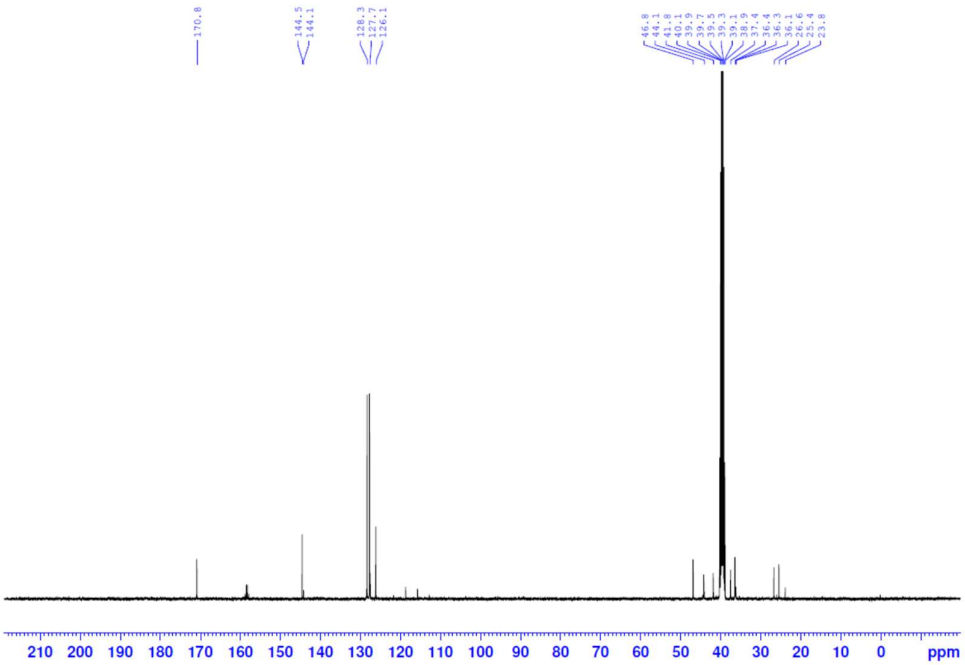
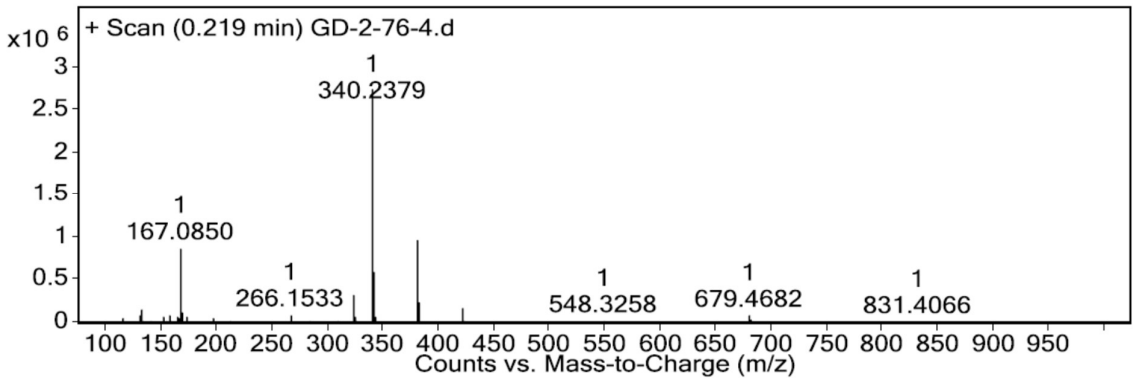
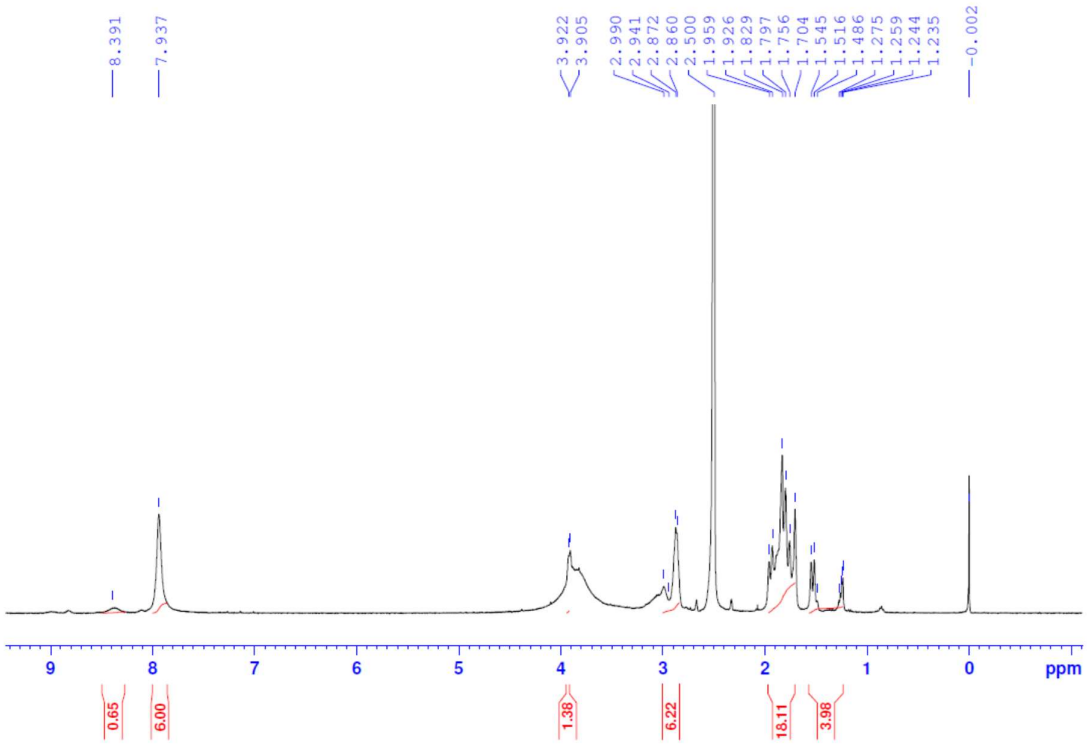


HRMS

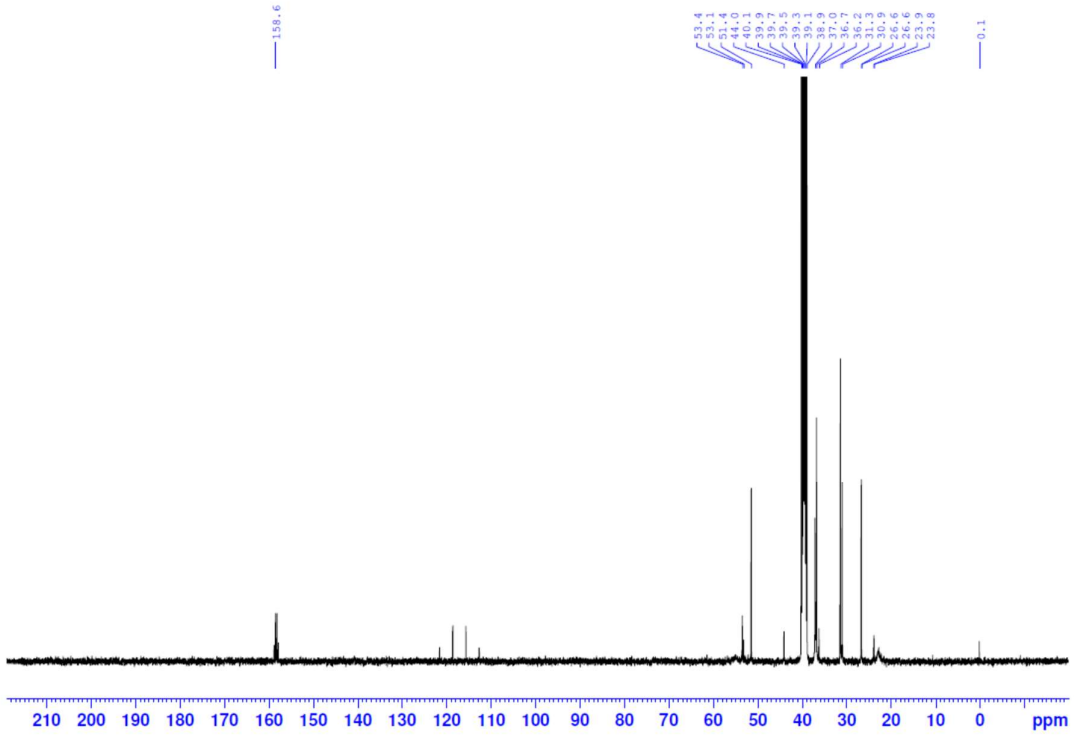


***N*-(Adamantan-2-yl)-2-[*N′*,*N′*-{bis-(3-amino) propyl} amino] ethanamide tris(trifluoroacetate) (NAda)**

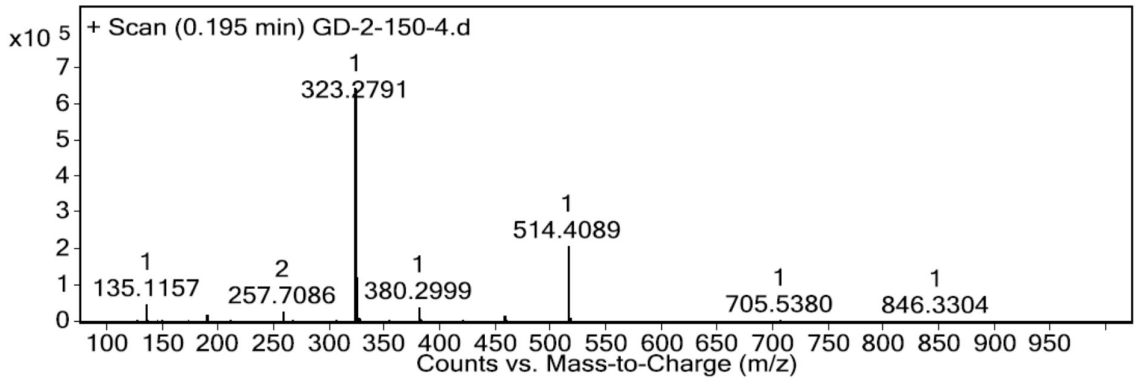
^{1}H-NMR



^{13}C-NMR


158.6
53.4
53.1
51.4
44.0
40.1
39.9
39.7
39.5
39.3
39.1
38.9
37.0
36.7
36.2
31.3
30.9
26.6
26.6
23.9
23.8
0.1
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

HRMS


x10 5
+ Scan (0.195 min) GD-2-150-4.d
1
323.2791
1
514.4089
1
135.1157
2
257.7086
1
380.2999
1
705.5380
1
846.3304
Counts vs. Mass-to-Charge (m/z)

In vitro Antibacterial Assays

a) Antibacterial activity in broth culture media

Water-soluble small molecular adjuvants or antibiotics were assayed in a modified micro-dilution broth format. Stock solutions of all the adjuvants and antibiotics (10 mg/mL) were prepared in autoclaved Millipore water except for rifampicin and erythromycin whose stock solutions were made in dimethyl sulphoxide (DMSO). The dilutions of the adjuvants or antibiotics were done in a 96-well plate using autoclaved water. Bacteria, to be tested, were cultured for 6 h in nutrient broth media. ~10^8 CFU/mL bacteria were diluted to ~10^5 CFU/mL using Mueller Hinton Broth media to perform the experiments. 150 µL of these diluted bacterial solutions were added to 50 µL of serially diluted adjuvants/antibiotics present in a 96 well plate (Polystyrene). 150 µL of media and 50 µL of adjuvant/antibiotic served as the media control with adjuvant/antibiotic, and 150 µL of bacterial solution and 50 µL of water served as the bacterial control. The plate was then incubated at 37 °C in an incubator (without shaking) for a period of 18-24 h and the O.D. value was measured at 600 nm using the microplate Reader. MIC value was determined by observing the minimum concentration at which the O.D. was similar to media control. The MIC values were reported as averages of three independent experiments.

Assay for *in-vitro* mammalian cell toxicity

a) Hemolytic assay

Human red blood cells (hRBCs) were isolated from fresh human blood obtained from a healthy human donor and resuspended in 1X PBS (pH 7.4) to 5% v/v. In a 96-well plate, 150 µL of hRBC suspension was added to 50 µL of serially diluted compound. PBS buffer added to 150 µL of erythrocyte suspension was taken as negative hemolysis control and Triton X-100 (1% v/v), added to the suspension was used as positive hemolysis control. Incubation was done for 1 h at 37 °C. The plate was then centrifuged at 3500 rpm for 5 min. 100 µL of the supernatant from each well was transferred to a new 96-well plate and absorbance was measured at 540 nm using a microplate Reader. Percentage of hemolysis was determined using this formula: $(A - A_0)/ (A_{total} - A_0) \times 100$, where A is the absorbance of the test well, A_0 is the absorbance of the negative controls, and A_{total} the absorbance of wells containing Triton X-100. HC_{50} (concentration which causes 50% hemolysis relative to the positive control) was determined by plotting hemolysis versus compound concentration. The HC_{50} values are reported as averages of three technical triplicates. The HC_{50} values for compounds where no hemolysis

was observed were reported as greater than the maximum concentration used. All the experiments were performed following the guidelines approved by institutional biosafety committee at JNCASR (Project no: JNC/IBSC/2020/JH-12).

b) **Lactic acid Dehydrogenase (LDH) assay**

10^4 cells/well of HEK cells (maintained in complete DMEM media (from Gibco) supplemented with 10% FBS and Penicillin-Streptomycin solution) were seeded in 96 well plates. Cell adherence was allowed to happen overnight. Media and 0.5% Triton-X were used as untreated and positive controls, respectively. The cells were treated with test compound solutions, starting from a concentration of 512 µg/mL. After 24 h of compound treatment, the plates were centrifuged at 1200 rpm for 5 min. 100 µL of the supernatant was then transferred to a fresh 96-well plate and absorbance at 490 nm was measured using a microplate Reader. Percentage of cell death was determined using this formula: $(A - A_0)/ (A_{total} - A_0) \times 100$, where A is the absorbance of the test well, A_0 is the absorbance of the negative controls, A_{total} absorbance of wells treated with triton-X. Percentage of LDH release was plotted versus adjuvant concentration and EC_{50} was determined (concentration which causes 50% LDH release as compared to the positive control).

c) MTT assay

The cells were incubated with MTT for 4 h. After solubilizing the purple crystals formed, the absorbance was measured at 590 nm with a Tecan InfinitePro series M200 Micro plate Reader. Percentage of viable cells was determined as $(A - A_0)/ (A_{total} - A_0) \times 100$, where A is the absorbance of the test well, A_0 is the absorbance of the well containing negative control treated with Triton-X (where no cells are present), A_{total} is the absorbance of the positive control (without any compound treatment). Percentage toxicity was plotted (after subtracting the % cell viability obtained from the assay from 100) as a function of concentration and EC_{50} determined where the cell viability was equal to 50% of the untreated positive control.

Results

Table S1. Concentration of adjuvants required to reduce the MIC of antibiotics as per Figure 1D.

Compound	Concentration required the reduce the MIC of antibiotics as per Figure 1D (μg/mL)		
	Minocycline	Fusidic acid	Rifampicin
NC12	128	64	16
NC10	32	64	64
NAda	128	128	128
NDiphe	16	128	128
NNaph	64	128	128

Table S2. Activity of **NAda** and **NDiphe** against New-Delhi metallo beta-lactamase producing clinical isolates.

Compound	MIC (μg/mL)	
	E. coli R3336	*K. pneumoniae* R3934
NDiphe	>512	>512
NAda	>512	>512

Table S3. Potentiation efficacy of **NAda** with various other antibiotics.

Antibiotic	**Bacteria**	**MIC of antibiotic in absence of adjuvant (µg/mL)**	**MIC of antibiotic in presence of NAda (µg/mL)**		
			+ 32 µg/mL	**+ 64 µg/mL**	**+128 µg/mL**
Tetracycline	*A. baumannii* R674	128	16	16	16
	P. aeruginosa R590	128-256	16	16	8
	K. pneumoniae R3934	>512	4	4	2
	E. coli R3336	16	2	2	2
Doxycycline	*A. baumannii* R674	64	1	0.125	0.125
	P. aeruginosa R590	64	8	8	8
	K. pneumoniae R3934	128	32	16	16
	E. coli R3336	64	1	1	0.5
Erythromycin	*A. baumannii* R674	512	16	16	16
	P. aeruginosa R590	512	32	16	16
	K. pneumoniae R3934	>512	32	16	16
	E. coli R3336	>512	>64	>64	>64
Vancomycin	*A. baumannii* R674	64	32	8	1
	P. aeruginosa R590	64	64	32	16
	K. pneumoniae R3934	>512	>64	>64	>64
	E. coli R3336	512	>64	>64	>64
Chloramphenicol	*A. baumannii* R674	128	64	64	32
	P. aeruginosa R590	256	64	64	32
	K. pneumoniae R3934	256	8	8	4
	E. coli R3336	>512	>64	>64	>64

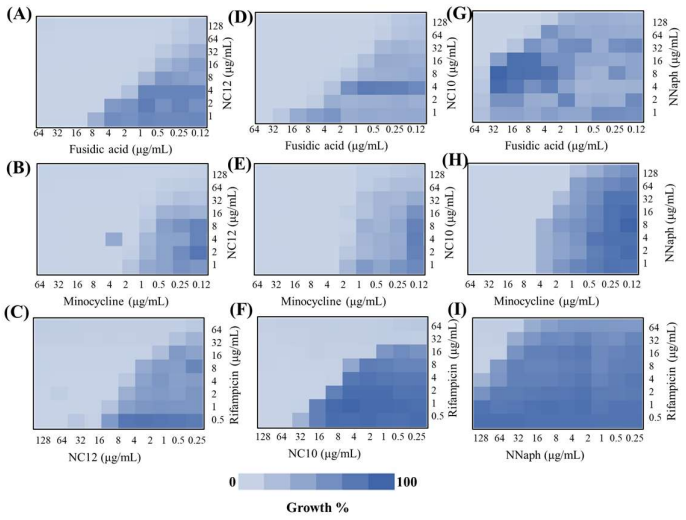


Figure S1. (A)-(C) Chequerboard assays of **NC12** with fusidic acid, minocycline and rifampicin respectively against *A. baumannii* R674. (D)-(F) Chequerboard assays of **NC10** with fusidic acid, minocycline and rifampicin respectively against *A. baumannii* R674. (G)-(I) Chequerboard assays of **NNaph** with fusidic acid, minocycline and rifampicin respectively against *A. baumannii* R674.

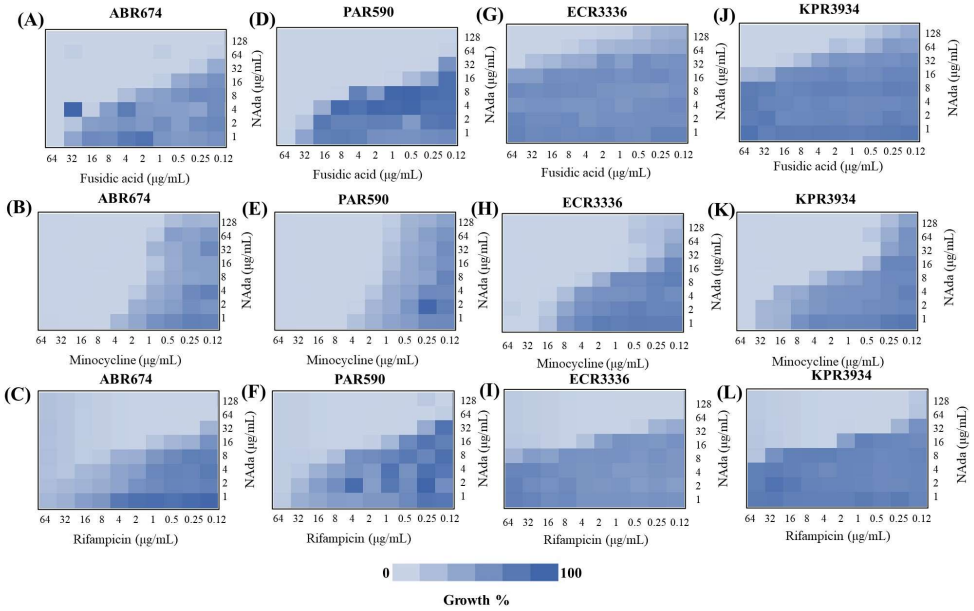


Figure S2. Chequerboard assays of **NAda** with fusidic acid, minocycline and rifampicin respectively against (A)-(C) *A. baumannii* R674, (D)-(F) *P. aeruginosa* R590, (G)-(I) *E. coli* R3336, (J)-(L) *K. pneumoniae* R3934.

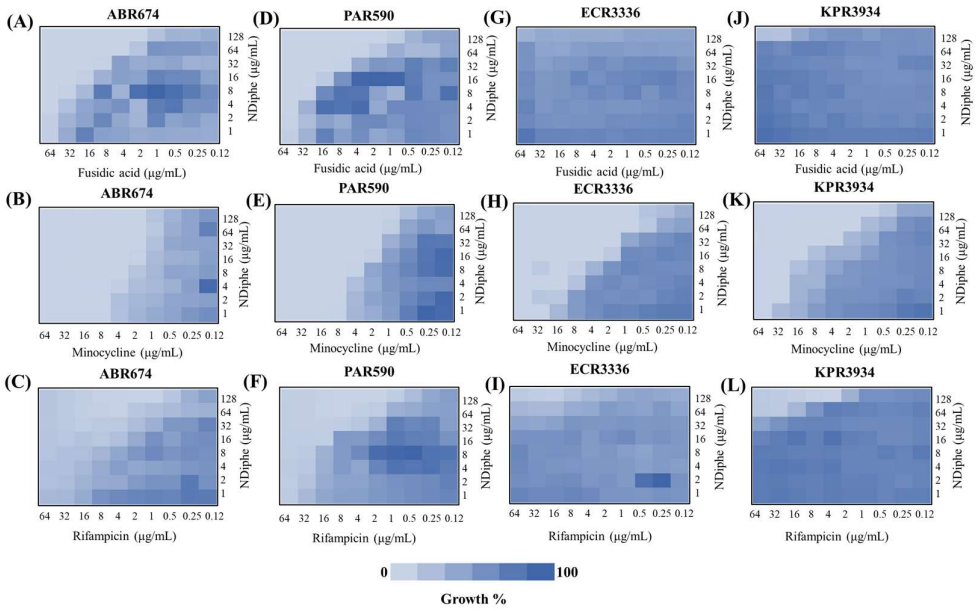


Figure S3. Chequerboard assays of **NDiphe** with fusidic acid, minocycline and rifampicin respectively against (A)-(C) *A. baumannii* R674, (D)-(F) *P. aeruginosa* R590, (G)-(I) *E. coli* R3336, (J)-(L) *K. pneumoniae* R3934.

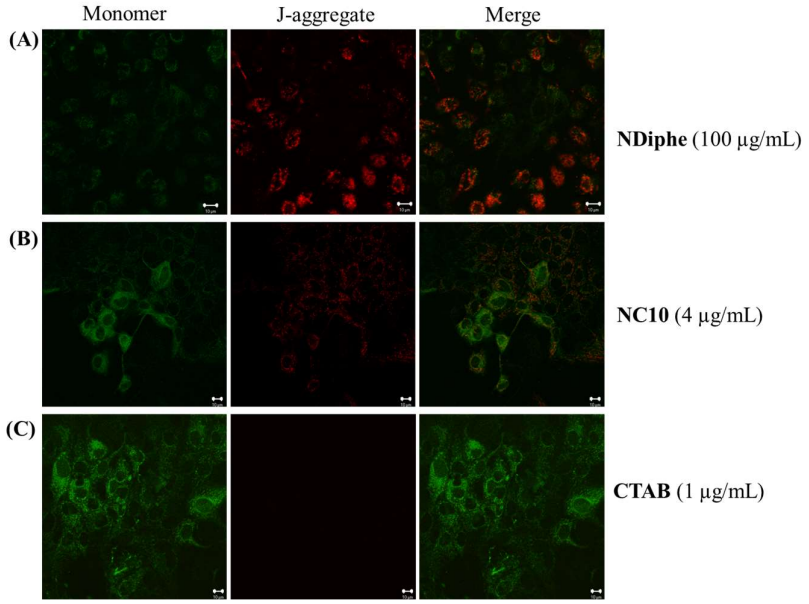


Figure S4. Analysis of mitochondrial membrane potential of HEK 293 cells by studying the formation of J-aggregates through confocal microscopy when treated with (A) **NDiphe** (100 µg/mL). (B) **NC10** (4 µg/mL). (C) CTAB (1 µg/mL). Note: Scale bar is 10 µm.

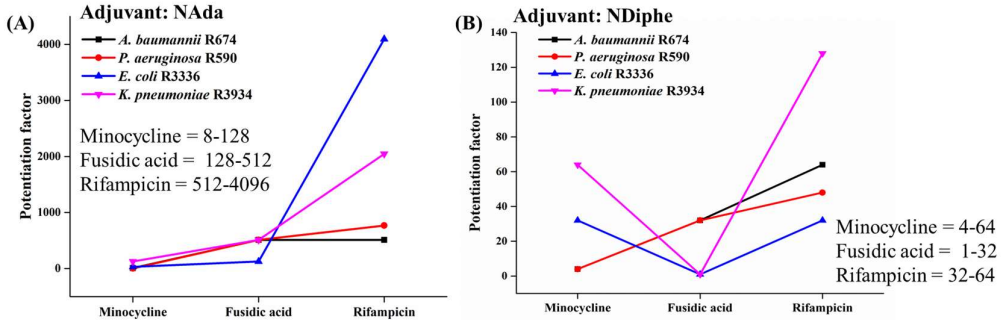


Figure S5. (A) Potentiation factors of different antibiotics in presence of 128 μg/mL of **NAda**. (B) Potentiation factors of different antibiotics in presence of **NDiphe**. Potentiation factor = $MIC_{antibiotic\ alone}/MIC_{antibiotic\ in\ presence\ of\ adjuvant}$.

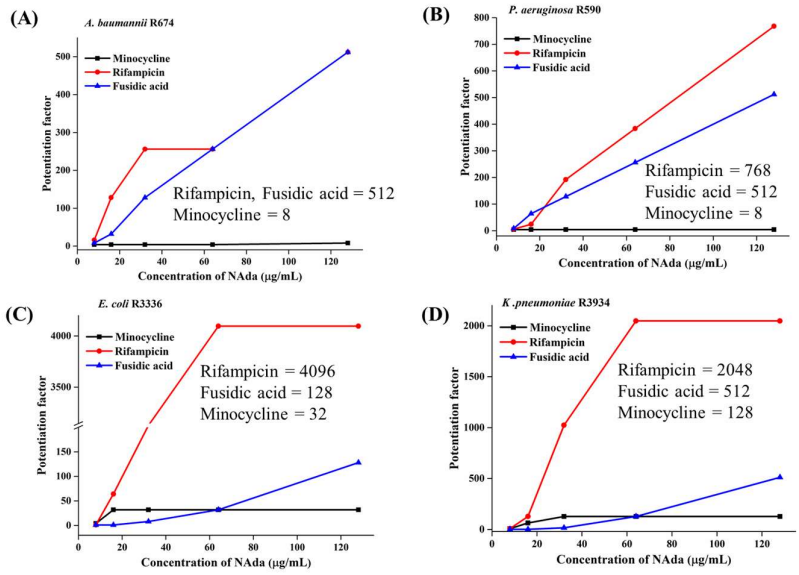


Figure S6. Trend of potentiation of antibiotics with concentration of **NAda** against (A) *A. baumannii* R674. (B) *P. aeruginosa* R590. (C) *E. coli* R3336. (D) *K. pneumoniae* R3934.

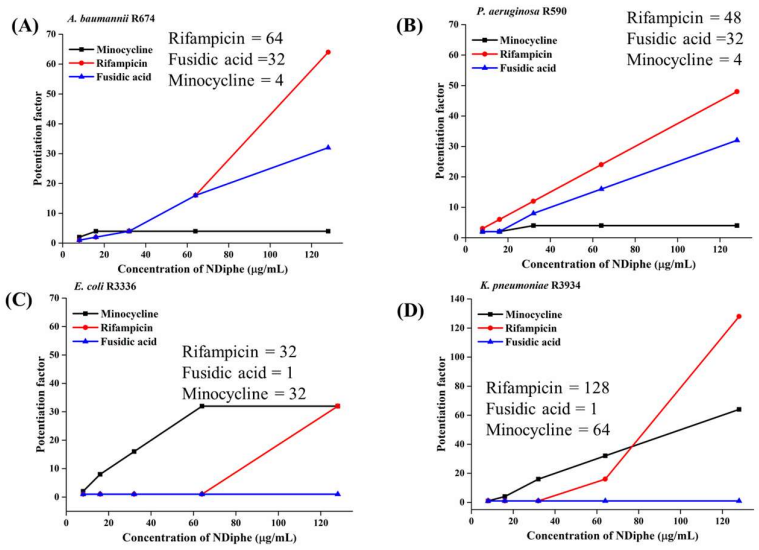


Figure S7. Trend of potentiation of antibiotics with concentration of **NDiphe** against (A) *A. baumannii* R674. (B) *P. aeruginosa* R590. (C) *E. coli* R3336. (D) *K. pneumoniae* R3934. For MIC > 64 μg/mL; potentiation factor is taken to be 1.

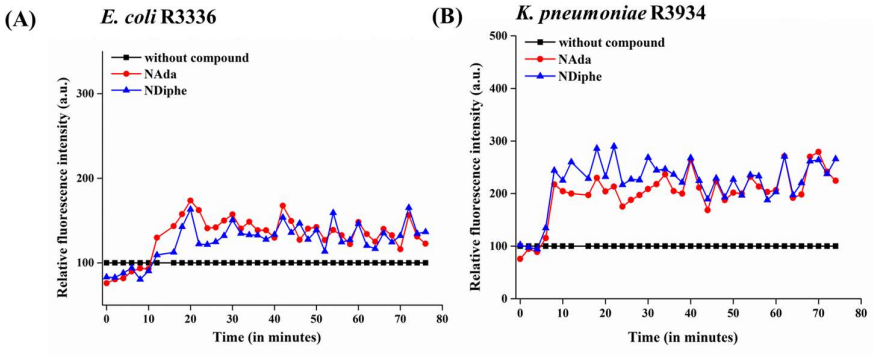


Figure S8. Temporal accumulation of minocycline in presence of **NAda** (100 μg/mL) and **NDiphe** (100 μg/mL) in (A) *E. coli* R3336 and (B) *K. pneumoniae* R3934.

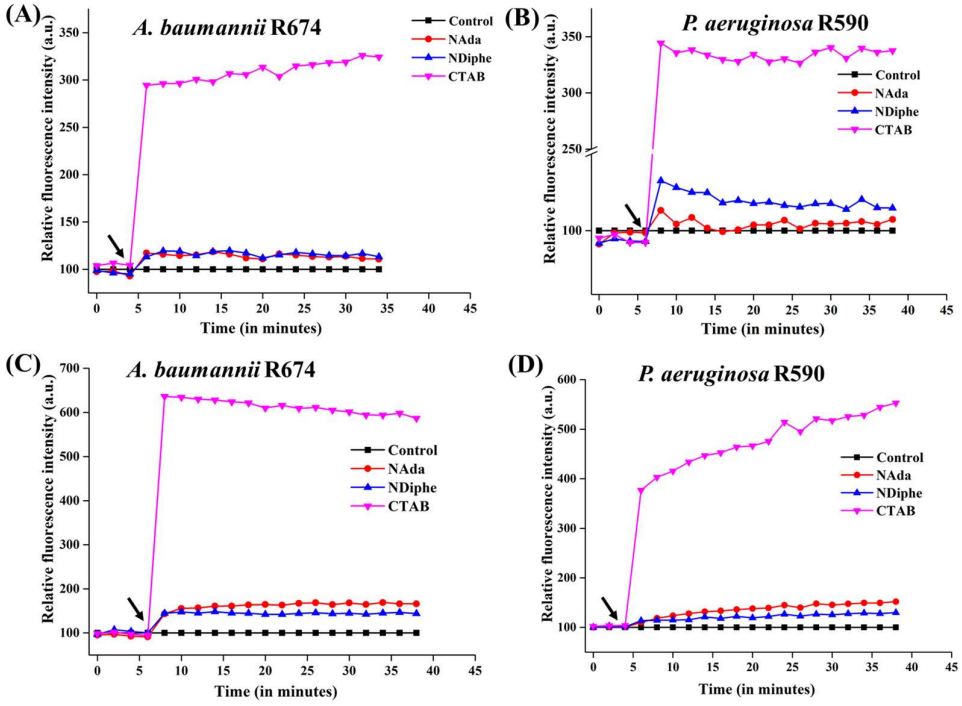


Figure S9. (A) Outer membrane permeabilization in *A. baumannii* R674; and (B) Outer membrane permeabilization in *P. aeruginosa* R590; in presence of **NAda** (100 µg/mL), **NDiphe** (100 µg/mL) (C) Membrane depolarization in *A. baumannii* R674; (D) Membrane depolarization in *P. aeruginosa* R590; in presence of **NAda** (100 µg/mL) and **NDiphe** (100 µg/mL). CTAB was used as control. Arrow indicates the time of compound addition.

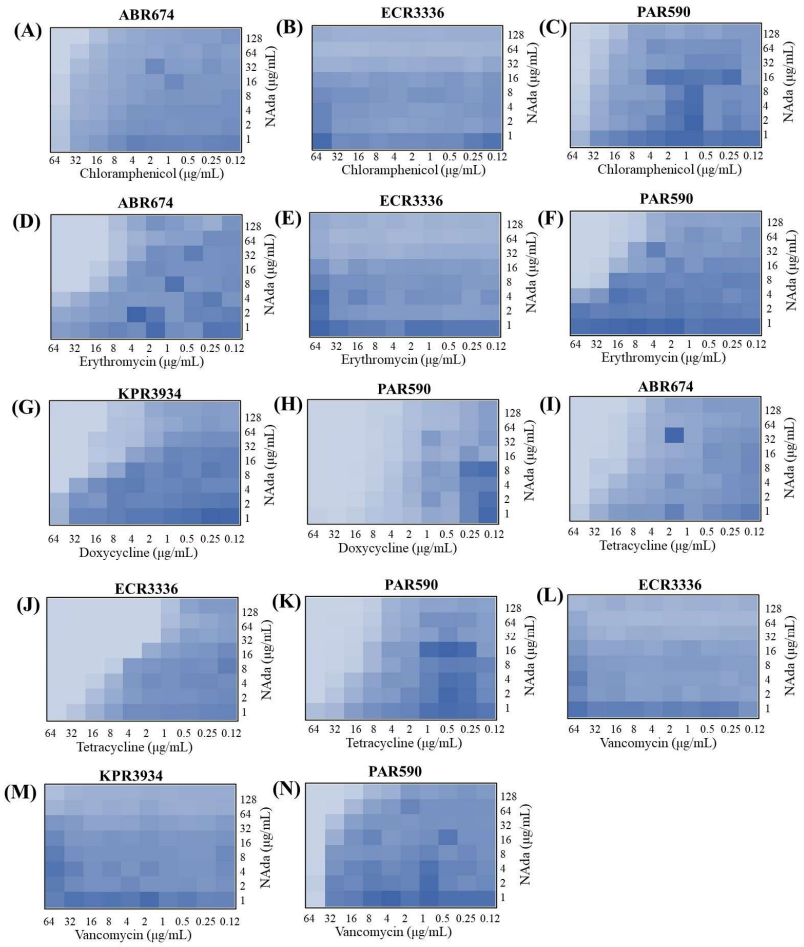


Figure S10. Chequerboard assays of **NAda** with chloramphenicol against (A) *A. baumannii* R674; (B) *E. coli* R3336; (C) *P. aeruginosa* R590; Chequerboard assays of **NAda** with erythromycin against (D) *A. baumannii* R674; (E) *E. coli* R3336; (F) *P. aeruginosa* R590; Chequerboard assays of **NAda** with doxycycline against (G) *K. pneumoniae* R3934; (H) *P. aeruginosa* R590; Chequerboard assays of **NAda** with tetracycline against (I) *A. baumannii* R674; (J) *E. coli* R3336; (K) *P. aeruginosa* R590; Chequerboard assays of **NAda** with vancomycin against (L) *E. coli* R3336; (M) *K. pneumoniae* R3934; (N) *P. aeruginosa* R590.