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Synthesis of novel benzylamine analogues of Anacardic acid as potent antibacterial agents

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ABSTRACT

*A series of N-substituted benzyl amine analogues of anacardic acid (**6a-6v**) were synthesized and evaluated for anti-bacterial activities against S. aureus, E. coli, P. aeruginosa and S. Pyogens. Compound **6q** showed excellent activity compared to Ampicillin and Chloramphenicol and comparable activity with Ciprofloxacin (18 mm zone of inhibition). Compounds **6h** and **6n** exhibited comparable to better anti-bacterial activity and Compounds **6k, 6o, 6s, 6u** and **6v** exhibited moderate activity compared to Ampicillin and Chloramphenicol against S. aureus. Similarly molecules **6f**, **6k** and **6r** showed moderate activity and others displayed inferior activity against S.pyogens. Compounds **6a**, **6d**, **6g**, **6i** and **6l** showed comparable activity with Ampicillin and **6b**, **6c**, **6e**, **6j** and **6m** displayed moderate activity against E.Coli. Compounds **6b, 6d, 6g, 6k, 6n, 6o** and **6t** exhibited at par activity with Chloramphenicol against P.aeruginosa. More or less all the compounds displayed inferior activities compared to Ciprofloxacin and Norfloxacin against the test strains.*

Keywords: CSNL, Anacardic acid, benzylamine analogues, Antibacterial activity.

INTRODUCTION

From times immemorial natural products have been used as bioactive molecules for different therapeutic purposes. Weak biological activities often render such molecules useless as potential drug candidates despite their easy availability. Structural modifications carried out on such mild molecules could turn them into potent bioactive compounds. The emergence of drug resistant strains of bacteria has led to an explosion of demand for a new class of anti-bacterial agents. This trend has inspired us to explore the opportunities to develop novel molecules from Anacardic acid (**1a**) to generate the lead candidates as anti-bacterial agents.

Anacardic acid is a natural product that can be isolated from Cashew Nut Shell Liquid (CNSL),

usually available as an ene-mixture (Fig**. 1)**. CNSL contains anacardic acid ene-mixture along with salicylic acid and a non-isoprenoid long alk(en)yl side chain moiety[1-2]. Anacardic acid is thought to possess its anti-bacterial activity due to its long alkyl chain[3]. The anacardic acid ene-mixture is composed of a long C15-alkyl side chain with monoenoic, dienoic, and trienoic at the C-8, C-10, and C-14 positions respectively (Fig. **1**). Anacardic acid and its derivatives have been reported to possess a range of bioactivities such as antibacterial action against Methicillin Resistant *Staphylococcus aureus* (MRSA)[4].

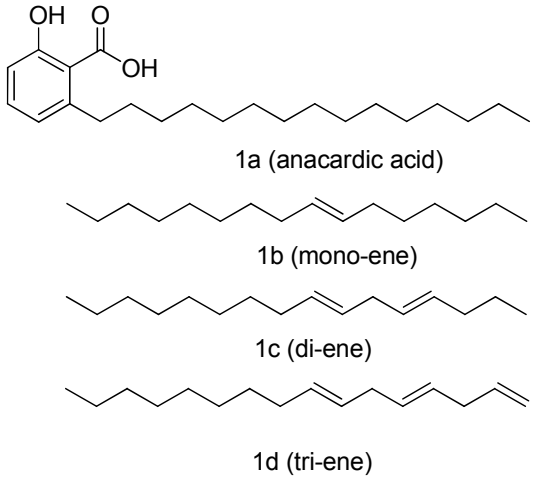


Fig. 1 Anacardic acid and its ene-mixture

The antibacterial activity against Methicillin against MRSA strains was significantly enhanced when combined with C12:0-anacardic acid with a fractional inhibitory concentration index of 0.281. Apart from anti-bacterial action, Anacardic acid was also found to inhibit triglycerides with natural phenolic lipids[5] and have the following activities viz., ureil soybean lipoxygenase-**1** inhibitory activity[6], synthesis of Sildenafil analogues[7], dihydropyridine analogues as Calcium channel blockers[8], Isonicotinoylhydrazones for antimycobacterial activity[9], bioactivity against Colarado Potato beetle[10], modulation of nuclear factor Kappaß signalling pathway by inhibit *acetyl transferase* in suppression of nuclear factorß[11]. The mono-ene, di-ene and tri-ene of anacardic acids were also shown to have moderate cytotoxicity on BT-20 breast and HeLa epithelioid cervix carcinoma cells[12] and Zoosporicidal activity against *Aphanomyces cochlioides*[13]. Further, Anacardic acid is also reported to strongly activate the kinase activity of *Aurora kinase A*, but not *Aurora kinase B*[14]. Recently a few benzamide derivatives from anacardic acid were studied for HAT activation as well[15]. Very recently it was proved that anacardic acid derivative has shown very improved inhibition of the acetyl histone transferase PCAF[16].

Current work aimed to synthesize novel benzyl amine derivatives of Anacardic acid (Fig. 2) as potential anti-bacterial agents. The synthesized targets were screened for their anti-bacterial activity against *E.Coli*, *P.aeruginosa*, *S.aureus* and *S.pyogens*, while using Ampicillin, Chloramphenicol, Ciprofloxacin and Norfloxacin as the standard drugs.

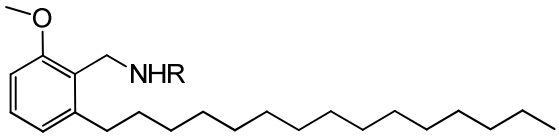


Fig. 2. Compounds 6a-6v

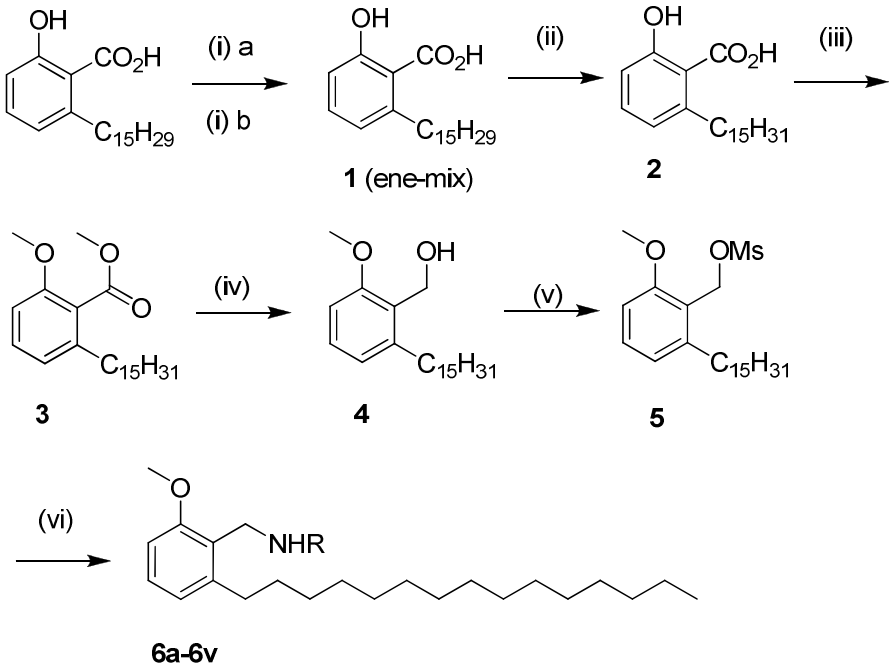
MATERIALS AND METHODS

Chemistry

The reagents and solvents used in this study were of analytical grade and were used without further purification. Melting points were determined in open capillaries and are uncorrected. Thin-layer chromatography (TLC) was performed on silica gel GF254 and spots were detected using iodine chamber or U.V lamp at 254nm. IR were recorded on FTIR Perkin Elmer spectrometer and the 1 H NMR spectra on a Varian EM-360 spectrometer (400 MHz) using TMS as an internal standard. The mass spectra were recorded on Agilent ion trap MS. All the amines (**6a-6v)** were either purchased from commercial sources or prepared in-house.

The synthesis of the benzyl amine analogues of anacardic acid was achieved by (i) Isolation and subsequent saturation of the ene-mixture of anacardic acid from commercially available CSNL; (ii) Alkylation of the phenolic hydroxyl group and esterfication of carboxylic acid group; (iii) Reduction of the ester to alcohol (iv) Transformation of the alcohol to its mesylate (v) Conversion of the mesylate to the benzyl amine derivatives by coupling with a variety of amines followed by screening the benzylamine derivatives of anacardic acid for their anti-bacterial activity.

Isolation of the anacardic acid ene-mixture (Fig. **1**, **1a**–**d**) from commercially available CNSL was carried out by treating with $Ca(OH)_2$ in a MeOH-H_2O mixture at 50 °C for 3 h followed by filtration of calcium anacardate as a brown colored solid. This solid was treated with 6 N HCl to obtain the anacardic acid ene-mixture as a dark brown colored oily liquid. The ene-mixture was saturated by hydrogenolysis using Pd/C-H_2 in EtOH at 60 psi at r.t. and isolated to afford the anacardic acid (**2**) as a pale brown colored solid, which was then alkylated with dimethyl sulphate and K_2CO_3 in acetonitrile at reflux temperature. The 2-methoxy methyl ester of anacardic acid (**3**) was reduced with $LiAlH_4$ in THF at r.t for 16 h to obtain the corresponding benzyl alcohol (**4**), which was then transformed to its methane sulfonate ester (**5**) by treatment with methane sulfonyl chloride and triethylamine in dichloromethane at 0 °C. The mesylate compound was treated with different types of amines in CH_3CN to get various benzyl amines of anacardic acid (**6a-6v)** as depicted in Scheme 1.



Scheme-1

Reagents and conditions: (i) (a) $Ca(OH)_2$, MeOH:H_2O (6:1), 60 °C, 3 h (b) 6 N HCl, rt, 1 h (ii) Pd/C (10%), H_2, EtOH, 60 psi, rt, 2 h (iii) $(CH_3)_2SO_4$, K_2CO_3, CH_3CN, 80 °C, 3 h (iv) $LiAlH_4$, THF, 0 °C-rt, 16 h (v) CH_3SO_2Cl, $(C_2H_5)_3N$, CH_2Cl_2, 0 °C-rt, 3 h (vi) RNH_2, CH_3CN, rt to 80 °C, 1h to 16 h

Preparation of anacardic acid (1a-1d, ene-mix): To a solution of MeOH (6 vol) and H_2O (1 vol) containing commercial grade CSNL (100 g), was added $Ca(OH)_2$ (50 g) and the contents were heated to 60 °C for 3h. The precipitated solid was filtered and the wet cake was washed with MeOH (1 vol) to remove the Cardol and Cardanol. The brown colored precipitate was then suspended in water, pH was adjusted to 2.0 range using 6 N HCl solution under vigorous stirring and extracted with ethyl acetate (3 x 200 mL). The combined organics were washed with H_2O, brine solution, dried over anhydrous Na_2SO_4, concentrated to dryness to afford the anacardic acid ene-mixture as dark-brown colored viscous oil (60 g, crude).

Preparation of anacardic acid (2): To a solution of ethanol (700 mL) containing the ene-mixture (**1a-1d**) (60 g, crude) 10% Pd/C (6 g) was slowly added under inert atmosphere into a hydrogenation flask. Hydrogenation was carried out under 60 psi of hydrogen pressure for 2 h. Filtered the contents through a pad of celite to remove the catalyst, collected the clear filtrate, concentrated under reduced pressure to afford the crude anacardic acid (**2**), which was recrystallized from petroleum ether (40–60 °C); Yield 36 g ; m.p. 85-86 °C; IR (KBr, cm^{-1}): 3071, 3002, 2917, 1655, 1450, 1246, 1214, 894, 815, 757 ; ^1H NMR (400 MHz, $CDCl_3$): ð 11.02 (br s, 1H), 7.36 (t, *J*= 8.0 Hz, 1H), 6.88 (d, *J* = 8.4 Hz, 1H), 6.78 (d, *J*= 7.6 Hz, 1H), 2.98 (t, *J* = 8.0 Hz, 2H), 1.57-1.63 (m, 3H), 1.27 (br s, 23H), 0.88 (t, *J* = 6.8 Hz, 3H); Mass: m/z: 349 $(M+H)^+$.

Preparationofethyl-2-methoxy-6-pentadecylbenzoate (**3**): To a stirred solution of compound **2** (26 g, 74.71 mmol) in acetonitrile (250 mL) was added powdered anhydrous K_2CO_3 (51 g, 138.2 mmol), followed by dimethyl sulfate (37.6 g, 298.8 mmol) in portions for about 15 min at room temperature. The contents were heated 80 °C for 3 h, cooled to r.t, filtered, collected the filtrate, concentrated under reduced pressure and the residue was diluted with water (150 mL), extracted with EtOAc (2 x 100 mL). The combined organic layer was washed with water, brine solution, dried over anhydrous Na_2SO_4, concentrated to afford the compound **3** as a low-melting solid. m.p. 37-38 °C, Yield: 22 g (80%); IR (KBr, cm^{-1}): 3004, 2921, 2852, 1732, 1589, 1266, 1105; ^1H NMR (400 MHz, $CDCl_3$): ð 7.25 (t, *J*= 8.0 Hz, 1H), 6.82 (d, *J* = 8.0 Hz, 1H), 6.75 (d, *J*= 8.4 Hz, 1H), 3.90 (s, 3H), 3.81 (s, 3H), 2.53 (t, *J* = 8.0 Hz, 2H), 1.53-1.60 (m, 3H), 1.27 (br s, 23H), 0.88 (t, *J* = 6.8 Hz, 3H); Mass: m/z:377 $(M+H)^+$.

Preparation of 2-methoxy-6-pentadecylbenzoic acid (4): To a stirred suspension of $LiAlH_4$ (3.3 g, 87.76 mmol) in dry THF (100 mL) was added the solution of compound **3** (22 g, 58.5 mmol) in dry THF (50 mL) at -20 °C and allowed the contents to r.t and stirred for 16 h. The reaction mixture was quenched with saturated NH_4Cl solution, filtered through a pad of celite, collected the filtrate, concentrated to dryness. The residue was dissolved EtOAc, washed with H_2O, brine solution, dried and concentrated to afford the crude compound **4** as pale brown colored solid (15 g, 73.6%); m.p. 59-60 °C; IR (KBr, cm^{-1}): 3386, 3072, 2916, 2846, 1705, 1461, 1265, 1118 and 1076; ^1H NMR (400 MHz, $CDCl_3$): ð 7.20 (t, *J*= 8.0 Hz,1H), 6.82 (d, *J* = 7.6 Hz, 1H), 6.77 (d, *J*= 8.0 Hz,1H), 4.75 (d, *J*= 6.4 Hz, 2H), 3.87 (s, 3H), 2.67 (t, *J* = 6.4 Hz, 2H), 2.37 (t, *J* = 6.4 Hz, 2H), 1.53-1.58 (m, 3H), 1.27 (br s, 23H), 0.89 (t, *J* = 7.2 Hz, 3H); Mass: m/z: 349 $(M+H)^+$.

Preparation of 2-methoxy-6-pentadecylbenzyl methane sulfonate (5): To a stirred solution of compound **4** (5.0 g, 14.36 mmol) in CH_2Cl_2 (50 mL) was added Et_3N (4.27 g, 43.08 mmol) and cooled to 0 °C. To this mixture was added methanesulfonylchloride (2.42 g, 21.55 mmol) at 0 °C over a period of 15 min. The contents were allowed to warm to r.t and stirred for 4 hrs. The reaction mixture was diluted with water and extracted with CH_2Cl_2 (2 x 50 mL). The organic layer was washed with brine solution, dried over anhydrous Na_2SO_4, concentrated to dryness to obtain crude compound **5** as pale brown coloured solid (5.5 g, 89.8%). The crude product was used as such in the next step without further purification.

General procedure for the preparation of final compounds (6a-6v): To a stirred solution of 2-methoxy-6-pentadecylbenzyl methane sulfonate (**5**) (5 mmol) in acetonitrile (10 vol) and K_2CO_3 was added the solution of amine (5 mmol) in CH_3CN and stirred at r.t to 80 °C for 1 h to 16 h. Upon completion, the reaction mixture was concentrated and the residue was extracted with EtOAc. The combined organic layer was washed with water, brine solution, dried and concentrated to afford the crude amino compounds. The crude products are either recrystalliszed or purified by column chromatography to afford the title compounds (**6a-6v**) in yields ranging from 50 to 85%.

2-(2-Methoxy-6-pentadecyl-benzylamino)benzoic acid (6a); Using **5** and 2-amino benzoic acid as starting materials, the title compound **6a** was obtained as a white solid; m.p. 57-60 °C; IR (KBr, cm^{-1}): 3457, 3342, 3032, 2955, 2920, 2848, 1696, 1614, 1592, 1471, 1288, 1266, 1245, 1107, 1091, 941, 784, and 743; ^1H NMR (400 MHz, $CDCl_3$): ð 7.78 (d, *J* = 8.0 Hz, 1H), 7.27-

7.20 (m, 3H), 6.85 (d, *J* = 7.6 Hz, 1H), 6.78 (d, *J* = 8.4 Hz, 1H), 6.64 (d, *J* = 8.4 Hz, 1H), 6.56 (t, *J* = 7.4 Hz, 1H), 5.71 (br s, 2H), 5.41 (s, 2H), 3.82 (s, 3H), 2.69 (t, *J* = 7.8 Hz, 2H), 1.59-1.55 (m, 3H), 1.31-1.21 (m, 23H), 0.87 (t, *J*=6.8 Hz, 3H); Mass: m/z 468.3 $(M+H)^+$.

4,5-dimethoxy-2-(2-Methoxy-6-pentadecyl-benzyl amino)-benzoic acid (6b); Using 5 and 2-amino-4,5-dimethoxy benzoic acid as starting materials, the title compound **6b** was obtained as off-white solid; m.p. 100-102 °C; IR (KBr, cm^{-1}): 3477, 3366, 2924, 2853, 1680, 1651, 1585, 1519, 1466, 1247, 1206, 1165, 1005 and 785; ^{1}HNMR (400MHz, $CDCl_3$): ð 7.39-7.21 (m, 2H), 6.84 (t, *J* = 7.4 Hz, 1H), 6.79-6.76 (m, 1H), 6.38 (s, 1H), 5.41(s, 2H), 4.39 (s, 1H), 3.93 (s, 3H), 3.83 (s, 6H), 3.82 (s, 3H), 2.72-2.62 (m, 2H), 1.58-1,54 (m, 3H), 1.41-1.20 (m, 23H), 0.87 (t, *J* =6.4 Hz, 3H); Mass: m/z 528.4 $(M+H)^+$.

4, 5-difluoro-2-(2-Methoxy-6-pentadecyl-benzyl amino)-benzoic acid (6c); Using **5** and 2-amino-4, 5-difluoro benzoic acid as starting materials, the title compound **6c** was obtained as a dark brown solid; m.p. 81-83°C; IR (KBr, cm^{-1}): 3475, 3355, 2918, 2850, 1707, 1645, 1579, 1518, 1469, 1276, 1198, 1153, 1053 and 838; ^{1}H NMR (400 MHz, $CDCl_3$): ð 7.58- 7.57 (m, 1H), 7.30-7,26 (m, 1H), 6.86 (d, *J*= 7.6 Hz, 1H), 6.79 (d, *J*= 8.0 Hz, 1H), 6.43-6.38 (m, 1H), 5.72 (br s, 2H), 5.4 (s, 1H), 3.83 (s, 3H); 2.67 (t, *J*= 7.8 Hz, 2H), 1.56-1.54 (m, 3H), 1.56-1.21 (m, 23H), 0.87 (t, *J* = 6.8 Hz, 3H); Mass: m/z 504.3 $(M+H)^+$.

2-(2-Methoxy-6-pentadecyl-benzylamino)-6-methyl -benzoic acid (6d); Using **5** and 2-amino-6-methyl- benzoic acid as starting materials, the title compound **6d** was obtained as a cream colored solid; m.p. 55-57 °C; IR (KBr, cm^{-1}): 3485, 3375, 2916, 2848, 1701, 1618, 1470, 1266, 1083, 1067, 895, 770; ^{1}H NMR (400 MHz, $CDCl_3$): ð 7.27-7.23 (m, 1H), 7.03 (t, *J*=7.8 Hz, 1H), 6.82 (d, *J*=7.6Hz, 1H), 6.76 (d, *J*= 8.0 Hz, 1H), 6.49-646 (m, 2H), 5.42 (s, 2H), 5.0 (broad exchangeable proton , 1H) 3.83 (s, 3H) 2.69 (t, *J*= 7.8 Hz, 2H), 2.33 (s, , 3H), 1.57-1.51 (m, 3H), 1.41-1.23 (m, 23H), 0.88 (t, *J*= 6.6 Hz, 3H); Mass: m/z 482($(M+H)^+$.

4-methoxy-2-(-(2-Methoxy-6-pentadecyl-benzyl amino)-benzoic acid (6e); Using 5 and 2-amino-4-methoxy benzoic acid as starting materials, the title compound **6e** was obtained as a light brown solid ; m.p. 52-53 °C; IR (KBr, cm^{-1}): 3489, 3361, 2954, 2923, 2848, 1709, 1633, 1559, 1469, 1264, 1154, 1076, 887, 746; ^{1}H NMR (400 MHz, $CDCl_3$): ð 7.71 (d, *J*=9.0 Hz, 1H), 7.28 (m, 1H), 6.85 (d, *J*=7.6Hz, 1H), 6.77 (d, *J*= 8.0Hz, 1H), 6.14 (m, 1H), 6.09 (d, *J*= 2.3 Hz, 1H), 5.38 (s, 2H), 3.82 (s, 3H), 3.76 (s, 3H), 2.68 (t, *J* = 8.0 Hz, 2H), 1.59-1.55 (m, 3H), 1.31-1.21 (m, 23H), 0.88 (t, *J*=6.8 Hz, 3H), Mass: m/z 498 $(M+H)^+$.

2-Fluoro-phenyl)-(2-Methoxy-6-pentadecyl-benzyl)amine (6f). Using 5 and 2-fluoro-phenylamine as starting materials, the title compound **6f** was obtained as a white solid; 84–86 °C, IR (KBr, cm^{-1}): 3429, 2924, 2853, 1735, 1615, 1587, 1258, 1094, 1034, 738; ^{1}H NMR (400MHz, $CDCl_3$): ð 7.23-7.19 (m, 1H), 7.08-7.02 (m, 1H), 6.97-6.90 (m, 2H), 6.78-6.73 (m, 2H), 6.65-6.55 (m, 1H), 4.30 (s, 2H), 3.97 (broad exchangeable proton, 1H), 3.82 (s, 3H), 2.68-2.62 (m, 2H), 1.54- 1.51(m, 3H), 1.29-1.23 (m, 23H), 0.88 (t, *J*=6.8 Hz, 3H); Mass: m/z 442$(M+H)^+$.

2-Bromo-5-fluoro-N-(2-Methoxy-6-pentadecylbenzyl)aniline (6g); Using 5 and 2-bromo4-fluoro-phenylamine as starting materials, the title compound **6g** was obtained as a oily mass; IR

(KBr, cm^{-1}): 3409, 2924, 2853, 1588, 1508, 1261, 1086, 857; ^1H NMR (400MHz, $CDCl_3$): ð 7.26 (d, *J*=7.2Hz, 1H), 7.24-7.20 (m, 1H), 7.19-7.16 (m, 2H), 6.99-6.98 (m, 2H), 4.27 (s, 2H), 3.83 (s, 3H), 2.66 (t, *J*=7.8 Hz, 2H), 1.60-1.54 (m, 3H), 1.41-1.25 (m, 23H), 0.88 (t, *J*=6.8 Hz, 3H); Mass: m/z 520$(M+H)^+$.

N-(2-Methoxy-6-pentadecylbenzyl)pyridin-4-amine (6h). Using 5 and pyridine-4-ylamine as starting materials, the title compound **6h** was obtained as a pale brown solid. m.p. 177-178 °C; IR (KBr, cm^{-1}): 3271, 2918, 2849, 1669, 1546, 1254, 1090, 1076, 833; ^1H NMR 400MHz, $CDCl_3$): ð 7.59 (d, *J*=7.6 Hz, 2H), 7.34 (t, *J*=8.0 Hz, 1H), 7.26-7.22 (m, 2H), 6.89 (d, *J*=7.6 Hz, 1H), 6.79 (d, *J*=8.4 Hz, 1H), 5.17 (s, 2H), 3.78 (s, 3H), 2.63 (t, *J*=8.0 Hz, 2H), 1.52-1.47 (m, 3H), 1.33-1.25 (m, 23H),0.88 (t, *J*=6.8 Hz, 3H); Mass: m/z 425$(M+H)^+$.

3-(2-Methoxy-6-pentadecyl-benzylamino)-pyrazine -2-carboxylic acid (6i). Using 5 and 3-amino-pyrazine-2-carboxylic acid as starting materials, the title compound **6i** was obtained as a pale brown solid; m.p. 83-85 °C; IR (KBr, cm^{-1}): 3419, 3278, 3159, 2924, 2849, 1699, 1624, 1527, 1466, 1304, 1192, 1116, 740; ^1H NMR (400MHz, $CDCl_3$): ð 8.14 (d, *J*= 2.0 Hz, 1H), 7.98 (d, *J*= 2.0 Hz, 1H), 7.25-7.23 (m, 1H), 6.83 (d, *J*=7.2 Hz, 1H), 6.75 (d, *J*=8.0 Hz, 1H), 5.53 (s, 2H), 3.82 (s, 3H), 2.69 (t, *J*=8.0 Hz, 1H), 1.61-1.54 (3H), 1.32-1.21(m, 23H), 0.88 (t, *J*=6.6 Hz, 3H); Mass: m/z 470$(M+H)^+$**.**

5-Bromo-N-(2-methoxy-6-pentadecylbenzyl)-6-methylpyridin-2-amine (6j). Using 5 and 5-bromo-6-methyl-pyridin-2-amine as starting materials, the title compound **6j** was as off-white solid; m.p. 88–90 °C; IR (KBr, cm^{-1}): 3429, 2924, 2853, 1615, 1587, 1465, 1258, 1094, 1034, 865, 738; ^1H NMR (400MHz, $CDCl_3$): ð 7.97-7.96 (m. 1H), 7.25-7.23 (m, 1H), 6.90-6.87 (m, 1H), 6.83 (d, *J*=7.6 Hz, 1H), 6.75 (d, *J*=8.4 Hz, 1H), 4.45 (s, 2H), 3.86 (s, 3H), 2.69 (t, *J*=8.0Hz, 1H), 1.63-1.54 (3H), 1.32-1.21(m, 23H), 0.88 (t, *J*=6.8 Hz, 3H); Mass: m/z 517$(M+H)^+$**.**

Furan-2-ylmethyl-(2-Methoxy-6-pentadecyl-benzyl) amine (6k). Using compound **5** and 1-(furan-2-yl)-N-methylmethanmine as starting materials, the title compound **6k** was obtained as a off-white solid; m.p. 85–89 °C; IR (KBr,cm^{-1}): 3054, 2925, 2853, 2305, 1583, 1467, 1422, 1264, 1086, 1012, 896, 734; ^1H NMR (400MHz, $CDCl_3$): ð 7.37 (d, *J*= 1.2 Hz, 1H), 7.14 (t, *J* = 7.8 Hz, 1H), 6.78 (d, *J*= 8.0 Hz, 1H), 6.70 (d, *J*= 8.4 Hz, 1H), 6.33-6.32 (m, 1H), 6.22 (d, *J*= 2.4 Hz, 1H), 3.78 (s, 3H), 3.59 (s, 2H), 3.55 (s, 2H), 2.65-2.61 (m, 2H), 2.18 (s, 3H), 1.61-1.5 (m, 3H), 1.36-1.20 (m, 23H), 0.87 (t, *J*=6.8 Hz, 3H); Mass: m/z 442 $(M+H)^+$.

Benzyl-furan-2-ylmethyl-(2-Methoxy-6-pentadecyl-benzyl amine (6l). Using **5** and benzyl-furan-2-ylmethyl-amine as starting materials, the title compound **6l** as off-white solid; m.p. 60–63 °C; IR (DCM film, cm^{-1}): 3054, 2926, 2854, 2306, 1730, 1583, 1469, , 1264, 1148, 1083, 1012, 895, 741; ^1HNMR (400MHz, $CDCl_3$): ð 7.36 (t, *J* = 0.8 Hz, 1H), 7.28-7.11 (m, 6H), 6.78 (d, *J*= 7.6 Hz, 1H), 6.67 (d, *J*= 8.0 Hz, 1H), 6.32-6.30 (m, 1H), 6.19 (d, *J*= 3.2 Hz, 1H), 3.78 (s, 3H), 3.67 (s, 2H), 3.52 (s, 2H), 3.49 (s, 2H), 2.64-2.60 (m, 2H), 1.55-1.41 (m, 3H), 1.29-1.25 (M, 23H), 0.88 (t, *J*= 6.8 Hz, 3H); Mass: m/z 518 $(M+H)^+$.

N^1-Furan-2-ylmethyl-N^1-(2-Methoxy-6-pentadecyl-benzyl)-N^2,$N^{2'}$-dimethyl-ethane-1,2-diamine (6m): Using 5 and N^1-furan-2ylmethyl)-N^2,N^2-dimethylethane-1,2-diamine as starting materials, the title compound **6m** was obtained as a dark brown solid. m.p. 90–92 °C; IR (DCM

film, cm^{-1}): 2925, 2853, 1598, 1582, 1467, 1264, 1084, 1012, 736; ^1H NMR (400 MHz, $CDCl_3$): ð 7.69-7.26 (m, 2H), 6.96-6.93 (m, 3H), 6.83-6.80 (m, 1H), 4.6 (s, 2H), 3.88 (s, 3H), 3.86 (s, 2H), 3.66-3.60 (m, 4H), 2.38 (s, 6H), 1.59-1.54 (m, 3H), 1.21-1.37 (m, 23H), 0.88 (t, *J*=6.8 Hz, 3H); Mass: m/z 499 $(M+H)^+$.

1-(2-Methoxy-6-pentadecyl-benzyl)-2,3-dihydro-1H-tetrazole (6n); Using **5** and 1H-tetrazole as starting materials, the title compound **6n** was obtained as a off-white solid; m.p. 57-60°C; IR (KBr, cm^{-1}): 3010, 2928, 2848, 1598, 1467, 1248, 1100, 787; ^1H NMR (400 MHz, $CDCl_3$): ð 8.2 (s, 1H), 7.26 (t, *J* = 7.6 Hz, 1H), 6.88 (d, *J* = 7.4 Hz, 1H), 6.78 (d, *J* = 7.4 Hz, 1H), 5.4 (s, 2H), 3.81 (s, 3H), 2.75-2.62 (m, 2H), 1.60-1.54 (m, 3H), 1.38-1.2 (m, 23H), 0.87 (t, *J*=6.8 Hz, 3H); Mass: m/z 401 $(M+H)^+$.

Furan-2ylmethyl-bis-(2-Methoxy-6-pentadecyl-benzyl)amine (6o); Using 5 and Furan-2-ylmethanamine as starting materials, the title compound **6o** was obtained as a white solid; m.p. 100-102°C; IR (KBr, cm^{-1}): 3060, 2928, 2848, 1725, 1582, 1462, 1256, 1164, 1080, 890, 742; ^1H NMR (400MHz, $CDCl_3$): ð 7.30 (s, 1H), 7.12 (t, *J*= 8.0 Hz, 2H), 6.76 (d, *J* = 7.2 Hz, 2H), 6.67 (d, *J* = 8.0 Hz, 2H), 6.27 (s, 1H), 6.17 (s, 1H), 3.78 (s, 6H), 3.56 (s, 4H), 3.46 (s, 2H), 2.41 (t, *J*=8.0 Hz, 4H), 1.54-1.02 (m, 52H), 0.88 (t, *J*=6.8 Hz, 6H); Mass spectrum: m/z 758.6 $(M+H)^+$.

5-(2-Methoxy-6-pentadecyl-benzyl amino)-1H-pyrazole-4-carboxylic acid ethyl ester (6p); Using **5** and ethyl 5-amino-1H-pyrazole-4-carboxylate as starting materials, the title compound **6p** was obtained as a dark brown solid. m.p. 81-83°C; IR (KBr, cm^{-1}): 3354, 3028, 2924, 2852, 1708, 1517, 1274, 1152, 836; ^1H NMR (400 MHz, $CDCl_3$): ð 8.26 (s, 1H), 7.15 (t, *J*= 8.0 Hz, 1H), 6.76 (d, *J* = 8.0 Hz, 1H), 6.71 (d, *J* = 7.6 Hz, 1H), 4.29 (q, *J* = 7.6 Hz, 2H), 3.79 (s, 3H), 3.67 (s, 2H), 2.70-2.67 (m, 2H), 1.59-1.54 (m, 3H), 1.37-1.21 (m, 26H), 0.88 (t, *J*=6.8 Hz, 3H); Mass: m/z 486.3 $(M+H)^+$.

(2-Methoxy-6-pentadecyl-benzyl) pyrimidin-2-ylamine (6q); Using **5** and pyrimidine-2-amine as starting materials, the title compound **6q** was obtained as a cream colored solid; m.p. 55-57°C; IR (KBr, cm^{-1}): 3484, 3374, 2918, 2848, 1700, 1614, 1466, 1267, 1078, 773, 645; ^1H NMR (400 MHz, $CDCl_3$): ð 8.32 (s, 2H), 7.24 (t, *J* = 7.6 Hz, 1H), 6.92-6.88 (m, 1H), 6.86 (d, *J* = 7.4 Hz, 1H), 6.76 (d, *J* = 7.4 Hz, 1H), 4.41 (s, 2H), 3.78 (s, 3H), 2.75-2.62 (m, 2H), 1.60-1.54 (m, 3H), 1.38-1.2 (m, 23H), 0.88 (t, *J*=6.8 Hz, 3H) Mass: m/z 426.3 $(M+H)^+$

1-(2-Methoxy-6-pentadecyl-benzyl)-1H-Indole (6r); Using 5 and 1H-Indole as starting materials, the title compound **6r** was obtained as a light brown solid; m.p. 52-53°C; IR (KBr, cm^{-1}): 3349, 3011, 2920, 2850, 1733, 1671, 1579, 1513, 1463, 1411, 1321, 1235, 1100, 800; ^1H NMR (400 MHz, $CDCl_3$): ð 7.94 (s, 1H), 7.67 (d, *J*= 8.0 Hz, 1H), 7.49 (d, *J*=8.4 Hz, 1H), 7.32-7.28 (m, 2H), 7.24-7.20 (m, 1H), 7.08 (t, *J*= 7.6 Hz, 1H), 6.83 (d, *J*=8.0 Hz, 1H), 6.76 (d, *J*= 8.4 Hz, 1H), 5.63 (s, 2H), 3.78 (s, 3H), 2.77-2.64 (m, 2H), 1.60-1.52 (m, 3H), 1.30-1.20 (m, 23H), 0.88 (t, *J*=6.8 Hz, 3H), Mass: m/z 449.3 $(M+2)^+$.

2-Bromo-5-Fluoro-phenyl)-(2-Methoxy-6-pentadecyl-benzyl)amine(6s). Using 5 and 2-bromo-5-fluoroaniline as starting materials, the title compound **6s** was obtained as a white solid; m.p. 83–85 °C, IR (KBr, cm^{-1}): 3429, 2924, 2853, 1735, 1615, 1587, 1258, 1094, 1034, 738; ^1H

NMR (400MHz, $CDCl_3$): ð 7.32 (t, *J*= 4.2 Hz, 1H), 7.29-7.21 (m, 1H), 6.85 (d, *J*= 7.6 Hz, 1H), 6.78 (d, *J*= 8.4 Hz, 1H), 6.56-6.52 (m, 1H), 6.29 (d, *J*= 2.4 Hz, 1H), 4.28 (s, 2H), 3.84 (s, 3H), 2.65 (t, *J*= 8.0 Hz, 2H), 1.51-1.41 (m, 3H), 1.29-1.23 (m, 23H), 0.94-0.86 (m, 3H); Mass Spectrum: m/z 520 $(M+H)^+$.

(2-Methoxy-6-pentadecyl-benzyl)-(4-methyl-5-nitro-pyridin-2-yl)amine (6t); Using 5 and 4-methyl-5-nitropyridin-2-amine as starting materials, the title compound **6t** was obtained as a yellow solid, m.p. 197-200 °C; IR (KBr, cm^{-1}): 2924, 2853, 1598, 1547, 1507, 1463, 1327, 1288, 1264, 1087, 831; ^1H NMR (400MHz, $CDCl_3$): ð 9.0 (s, 1H), 7.16 (t, *J*=8.0 Hz, 1H), 6.76 (d, *J*= 7.6 Hz, 1H), 6.69 (d, *J*= 8.0 Hz, 1H) 6.33 (s, 1H), 4.80 (s, 2H), 3.68 (s, 3H), 2.48 (s, 3H), 2.48-2.41 (m, 2H), 1.53-1.21 (m, 26H), 0.88 (t, *J*=6.8 Hz, 3H); Mass Spectrum: m/z 484 $(M+H)^+$.

4-Bromo-6-methyl-pyridin-2-yl)-(2-Methoxy-6-pentadecyl-benzyl)-amine (6u). Using 5 and 4-bromo-6-methylpyridine-2-amine as starting materials, the title compound **6u** was obtained as a grey solid. m.p. 177-178 °C; IR (DCM film, cm^{-1}): 3456, 2923, 2853, 1586, 1495, 1466, 1259, 1089, 891, 779; ^1H NMR 400MHz, $CDCl_3$): ð 8.05 (s, 1H), 7.27-7.20(m, 2H), 6.83 (d, *J*= 7.6 Hz, 1H), 6.77 (d, *J*= 8.4Hz, 1H), 4.65 (d, *J*= 4.8 Hz, 2H), 3.84 (s, 3H), 2.73 (t, *J*= 8.0 Hz, 2H), 1.98 (s, 3H), 1.54-1.51 (m, 3H), 1.31-1.22 (m, 23H), 0.88 (t, *J*=6.8 Hz, 3H); Mass Spectrum: m/z 517.2 $(M+H)^+$.

1-(2-Methoxy-6-pentadecyl-benzyl)-1H-Indazole (6v). Using 5 and 1H-Indazole as starting materials, the title compound **6v** was obtained as a light brown solid; m.p. 83-85 °C; IR (KBr, cm^{-1}): 2923, 2852, 1586, 1464, 1364, 1314, 1259, 1089, 900, 744; ^1H NMR (400MHz, $CDCl_3$): ð 7.93 (s, 1H), 7.66 (d, *J* = 8.0 Hz, 1H), 7.48 (d, *J*= 8.4 Hz, 1H), 7.29-7.08 (m, 2H), 6.83 (d, *J*= 7.6 Hz, 1H), 6.75 (d, *J*= 8.4 Hz, 1H), 5.63 (s, 2H), 3.78 (s, 3H), 2.79 (t, *J*= 7.8 Hz, 2H), 1.36-1.20 (m, 26H), 0.88 (t, *J*=6.6Hz, 3H); Mass: m/z 449$(M+H)^+$**.**

RESULTS AND DISCUSSION

To generate the lead molecules chosen different amines such as substituted anthranilic acids, substituted pyridines, substituted pyrazines, furanyl amines, tetrazole, substituted pyrazole, substituted pyrimidine, indole and indazoles. The generated benzylamines were screened for their anti-bacterial activity. All compounds were purified by either column chromatography or recrystallization techniques and were characterized by IR, NMR and Mass spectroscopy.

In order to evaluate the anti-bacterial activity, compounds **6a-6v** were screened for in vitro activity against *S. aureus* and *S. Pyogens* (Gram positive (G +ve)) and *E. Coli* and *P. Aeruginosa* (Gram negative (G –ve)) groups of bacteria.

The anacardic acid analogues (**6a-6v**) were dissolved in dimethyl sulphoxide at 250µg/mL concentration. The composition of nutrient agar medium was Bactotryptone (10 g), yeast extract (5 g), NaCl (10 g), final pH was 7.4. After 18 h the exponentially growing cultures of the bacteria in nutrient broth at 37 °C were diluted in sterile broth. From each of these diluted cultures, 1 mL was added to 100 mL of sterilized and cooled nutrient agar media to give the final bacterial count of 1 $x10^6$ cell/ml. Paper discs (6 mm, punched from Whatmann no.41 paper) were ultraviolet and used for assays. Discs were soaked in different concentrations of the test

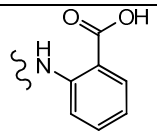
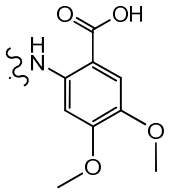
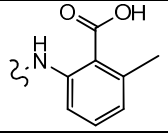
solution and placed on the inoculated agar media at regular intervals of 6-7 cm. Care was taken to ensure that excess solution was not on the discs. All the pure fractions were collected, concentrated under reduced pressure to afford samples were taken in triplicates. The plates were incubated at 37 °C in an inverted fashion. Activity was determined by measuring the zones showing the inhibition (in mm). Growth inhibition was calculated with reference to positive control.

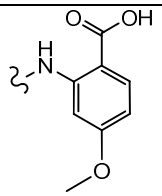
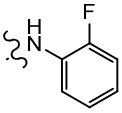
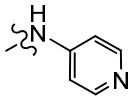
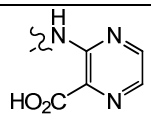
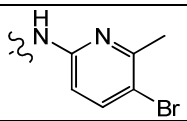
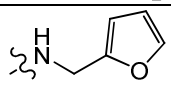
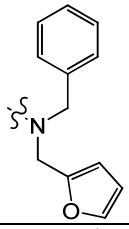
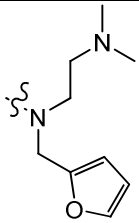
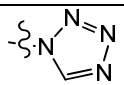
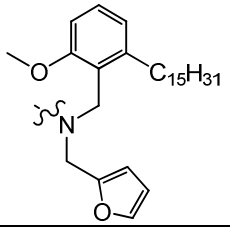
When the compounds were screened against *E.Coli* molecules **6a, 6d**, **6g**, **6i** and **6l** displayed very good activities (15 mm zone of inhibition) which was found to be at par with ampicillin while molecules **6b**, **6c**, **6e**, **6j** and **6m** exhibited moderate activity (14 mm zone of inhibition). In general substituted anthranilic acids and pyridines displayed better activity compared to other amines against *E.Coli*. Similarly, when the compounds were screened against *P. aeruginosa*, molecules **6b**, **6d**, **6g**, **6k**, **6n**, **6o**, **6t** and **6v** exhibited moderate activity compared to ampicillin while the remaining molecules exhibited inferior activity compared to the standard drugs.

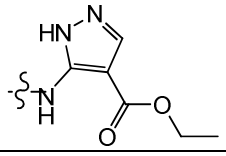
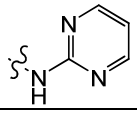
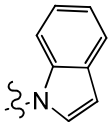
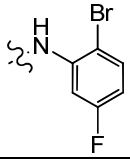
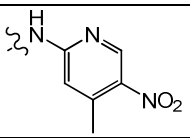
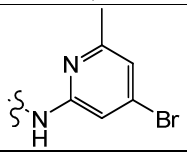
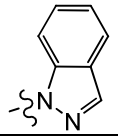
On screening against *S.aureus*, molecule **6q** showed excellent activity compared to Ampicillin and Chloramphenicol and comparable activity with Ciprofloxacin (18 mm zone of inhibition). Likewise, molecule **6t** displayed more activity compared Ampicillin and Chloramphenicol and molecules **6k**, **6o**, **6s**, **6u** and **6v** exhibited activity which was at par with Chloramphenicol. Compounds **6h** and **6n** had activity at par with amplicillin against *S.aereus.*

When the samples were screened against *S.pyogens*, compounds **6f**, **6k** and **6r** showed activities that were at par activity with ampicillin and slightly inferior to Chloramphenicol, while rest of the samples exhibited moderate activity against *S.pyogens.* The in-vitro results are summarized in table **1**.

Table-I-Antimicrobial activity of compounds 6a-6v
Antimicrobial activity (Zone of inhibition in mm)

S. No	*Structure*	*S.A*	*S.P*	*E. Coli*	*P.A*
6a		11	12	15	11
6b		12	11	14	13
6c		12	12	14	11
6d		11	10	15	13

6e		10	12	14	12
6f		10	13	13	11
6g		12	10	15	13
6h		13	10	13	10
6i		12	12	15	12
6j		11	12	14	10
6k		14	14	12	13
6l		10	12	15	12
6m		11	10	14	11
6n		13	12	13	14
6o		14	10	12	13

6p		12	12	11	10
6q		18	12	12	10
6r		12	13	13	11
6s		14	12	11	10
6t		15	12	12	13
6u		14	10	12	11
6v		14	10	13	13
Ampicillin	Zone of Inhibition	13	14	15	15
Chloramphenicol	Zone of Inhibition	14	13	17	17
Ciprofloxacin	Zone of Inhibition	19	19	23	23
Norfloxacin	Zone of Inhibition	22	19	25	19

Zone of Inhibition (DMSO as solvent);

Standard drugs: Ampicillin, Chloramphenicol, Ciprofloxacin, Norfloxacin
S.A**: Staphycoccous aureus-**MTCC-96**; **S.P**: Streptococcus Pyogens-**MTCC-443**; **E.Coli**: Eischria Coil-**MTCC-442**; **P.A**: Pseudomonas Aeruginosa- **MTCC-441

CONCLUSION

Several benzylamine analogues of anacardic acid were synthesized and screened for anti-bacterial activity. Few analogues displayed activities at par with Ampicillin and Chloramphenicol. Compound **6q** exhibited activity at par with ciprofloxacin.

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