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Synthesis, Characterization and Antibacterial Evaluation of E-N'-(2-methoxy-6-pentadecyl-substituted-benzylidene)benzohydrazide Derivatives

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ABSTRACT

*Anacardic acid, is a natural product found in cashew nut shells and is associated with antiinflammatory, antitumor, molluscicidal, and anti-microbial activities. The synthesis of E-N'-(2-methoxy-6-pentadecyl-substituted-benzylidene)benzohydrazide **(6a-6k)** was accomplished from the key intermediate 2-methoxy-6-pentadecyl-benzaldehyde **4** which is in turn achieved from 2-hydroxy-6-pentadecylbenzoic acid **1** (anacardic acid). All the synthesized compounds were characterized by spectroscopic techniques like ^{1}H NMR, IR and MS analysis. These compounds were further evaluated for antimicrobial screening against Gram negative strains of (i) Escherichia coli (MTCC 443) and (ii) Pseudomonas aeruginosa (MTCC 424) and Gram positive strains of (iii) Staphylococcus aureus (MTCC 96) and (iv) Streptococcus pyogenes (MTCC 442). Compound **6e**, **6i** and **6k** with substitution R = 4-OMe, 3,4,5-OMe and 2,5-dufluoro exhibited good antibacterial activity.*

Keywords: Antibacterial Activity, Anacardic acid, Benzohydrazides, Synthesis, Gram positive bacteria, Gram negative bacteria.

INTRODUCTION

Hydrazones containing an azomethine –NHN=CH- proton are synthesized by heating the appropriate substituted hydrazines/hydrazides with aldehydes and ketones in solvents like ethanol, methanol, tetrahydrofuran, butanol, glacial acetic acid, ethanol-glacial acetic acid. Hydrazide-hydrazones compounds are not only intermediates but also very effective organic compounds. Literature studies revealed that hydrazones and various substituted hydrazones are associated with a broad spectrum of biological activities such as antioxidant, antibacterial, antiviral, analgesic, antiplatelet, antimicrobial, and anticancer activities etc [1].

Infectious diseases caused by bacteria have increased significantly in recent years. In spite of many considerable advances in antibacterial therapy, the extensive use and misuse of antibiotics have caused the

materialization of bacterial resistance to antibiotics, which is a serious hazard to public health [2]. Therefore, the increase of new compounds to deal with resistant bacteria has become one of the most important areas of antibacterial research today.

6-pentadecyl salicylic acid, commonly known as anacardic acid, is a natural product found in cashew nut shells. This compound is often associated with antiinflammatory, antitumor, molluscicidal, and antimicrobial activities [3-5]. In addition, anacardic acid has a number of roles including the inhibition of lipid synthesis, enzyme activity such as lipoxygenase, prostaglandin endoperoxide synthase and histone acetyltransferase and the expression of nuclear factor-κB (NF-κB) as well as the activation of aurora kinase A [6–10]). Additionally, anacardic acid has an antibacterial and anticancer effect [11, 12]. In view of the immense significance of the pharmacological and biological activities associated with hydrazone and anacardic acid derivatives, we describe here in the synthesis, characterization and antibacterial activity of some novel hydrazone derivatives synthesized from anacardic acid.

MATERIALS AND METHODS

The dry solvents and the chemicals available commercially are employed for the chemical process. Silica gel 60 F24 of Merck pre-coated plates are employed for their thin layer chromatography (TLC) analysis and the spots formed are visualized by UV-light. Merck silica gel 60 (230-400) mesh employed for flash column chromatography and the eluting solvents are mentioned in the procedures. Melting point (mp) determined by Mel-temp apparatus.. ^{1}H NMR spectra recorded in Brucker MR-400 MHz devise. Chemical shifts are reported in δ parts per million (ppm) downfield from tetramethylsilane (TMS) with reference to internal standard and the signals reported as s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), m (multiple) and coupling constants in Hz. The data related are mass spectra recorded on Agilent ion trap MS. Infrared (IR) spectra are recorded on a Perkin Elmer FT-IR spectrometer.

Synthesis of Methyl 2-methoxy-6-pentadecylbenzoate (2): To a solution of 2-hydroxy-6-penta decyl benzoic acid **1** (5 g, 14.34 mmol) in dry CH_3CN (200 mL) was added dimethyl sulphate (5.44 mL, 57.36 mmol) followed by anhydrous K_2CO_3 (10.2 g, 71.70 mmol) and refluxed for 24 h. After the completion of the reaction, the reaction mixture was diluted with EtOAc (150 mL) and water (200 mL). The organic layer was washed with water followed by brine solution (75 mL), dried over Na_2SO_4, filtered and concentrated to obtain methyl 2-methoxy-6-pentadecylbenzoate **3.** Pale yellow solid; Yield: 18.5 g, 85.6 %; M.p: 36-37 °C; FT-IR (KBr pellet): ν_{max} 3004, 2921, 2852, 1732, 1589, 1460, 1266, 1105 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.88 (t, 3H, J = 6.8 Hz), 1.25 (brs, 24H), 1.53-1.60 (m, 2H), 2.53 (t, 2H, J = 8.0 Hz), 3.81 (s, 3H), 3.90 (s, 3H), 6.75 (d, 1H, J = 8.4 Hz), 6.82 (d, 1H, J = 8.0 Hz), 7.25 (t, 1H, J = 8.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.07, 22.66, 29.32, 29.38, 29.48, 29.51, 29.61, 29.65, 31.12, 31.88, 33.45, 52.04, 55.80, 108.30, 121.45, 123.44, 130.17, 141.35, 156.20, 168.88; ESIMS: *m/z* 377 $(M+H)^+$.

Synthesis of (2-Methoxy-6-pentadecylphenyl)methanol (3): To a suspension of $LiAlH_4$ (0.60 g, 15.92 mmol) in dry THF (20 mL) was added methyl 2-methoxy-6-pentadecylbenzoate **2** (4.0 g, 10.62 mmol) in dry THF (25 mL) drop wise over a period of 30 min at 0 °C. The reaction mixture was stirred at room temperature for 18 h. After completion of the reaction, the reaction mixture was quenched with brine solution (10 mL) at 0 °C and diluted with EtOAc (50 mL). The filtrate was washed with brine solution (30 mL), dried over Na_2SO_4 and evaporated under vacuum to obtain (2-methoxy-6-pentadecylphenyl)methanol **3** as an off-white solid (3 g, 81 %). M.P: 60-62 °C; FT-IR (KBr pellet): ν_{max} 3367, 3004, 2924, 2853, 2781, 1689, 1596, 1577, 1472, 1457, 1438, 1409, 1377, 1268, 1180, 1169, 1119, 1080, 823, 788, 650, 474, 466, 418 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.89 (t, 3H, J = 7.2 Hz, D_2O exchangeable OH), 1.27 (brs, 24H), 1.53-1.58 (m, 2H), 2.37 (t, 1H, J = 6.4 Hz), 2.68 (t, 2H, J = 6.4 Hz), 3.87 (s, 3H), 4.75 (d, 2H, J = 6.4 Hz) 6.77 (d, 1H, J = 8.0 Hz), 6.82 (d, 1H, J = 7.6 Hz), 7.20 (t, 1H, J = 8.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.07, 22.66, 29.32, 29.46, 29.57, 29.61, 29.65, 31.88, 32.10, 33.19, 55.37, 57.30, 108.01, 122.28, 126.83, 128.45, 142.62, 158.22; ESIMS: *m/z* 349 $(M+H)^+$.

Synthesis of 2-methoxy-6-pentadecyl-benzaldehyde (4): To a solution of compound **3** (2.5g, 7.18 mmol) in DCM (120 mL) was added pyridinium dichromate (4.05g, 10.77 mmol) at 0°C in ten portions at equal interval of time . The reaction mixture was stirred at room temperature for 5 h. After completion of the reaction, the reaction mixture was diluted with diethyl ether (50mL) and filtered through celite bed and the filtrate was evaporated under reduced pressure. The crude compound was purified by column chromatography using silica gel 60-120 mesh (elluant: 15% ethylacetate: pet ether) to obtain 2-methoxy-6-pentadecyl-benzaldehyde **4.** Yellow solid; Yield: 3.0g (60.3%); m.p: 70-72 ^{o}C; IR (KBr pellet): ν_{max} 3067, 2921, 2848, 1687, 1589, 1466, 1412, 1268,1073cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.25 (brs, 24H), 1.51-1.57 (m, 2H), 2.92 (t, 2H, *J* = 8.0 Hz), 3.88 (s,3H), 6.80 (d, *J* = 8.4 Hz, 2H), 7.39 (t, 1H, *J* = 7.6 Hz), 10.62 (s, 1H); MS (ESI) *m/z* : 347.29 $(M+H)^+$.

General Experimental procedure for synthesis of 5a-k [13, 14]: To a stirred solution of benzoic acids (6.42 mmol) in ethanol (3 mL) was added catalytic qty of conc.H_2SO_4 and heated to reflux for 6 – 10 h. The reaction mixture was diluted with ethylacetate followed by water. The organic layer was washed with saturated $NaHCO_3$ followed by water and brine solution. The organic layer was dried over sodium sulphate, filtered and evaporated to obtain respective ethyl benzoates. To a stirred solution of ethyl benzoates (3 mmol) in ethanol was added hydrazine-hydrate (5.44 mmol) and refluxed for 6-12 h. The reaction mixture was diluted with ethylacetate followed by water. The organic layer was dried over sodium sulphate, filtered and evaporated to obtain respective benzohydrazides **5a-k**. The yields of the products varied from 85 – 90%.

General experimental procedure for the preparation of novel hydrazone derivatives of anacardic acid derivatives (6a-6k): To a stirred solution of compound **4** (100 mg, 0.288 mmol) in ethanol was added corresponding benzohydrazides **5a-5k** (0.288 mmol) and refluxed for 1 h. The reaction medium was poured into water and extracted with ethyl acetate. The organic layer was washed with water followed by brine solution, dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure, to obtain corresponding hydrazide-hydrazone derivatives **6a-6k**. Yields of the products varied between 80 to 90%.

(E)-N'-(2-methoxy-6-pentadecylbenzylidene)benzohydrazide (6a): Pale Brown solid; Yield: 80%; M.p.: 92-93 ^{o}C; IR (KBr): υ_{max} 3227, 3064, 2919, 2851, 1653(-C=O strt), 1545, 1466, 1370, 1261, 1145, 1057, 970, 914, 792, 749, 698 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.08 (* 8.60, s, 1H), 8.30 (* 6.80, s, 1H), 7.88 (d, J = 7.2 Hz, 2H), 7.51-7.46 (brm, 3H), 6.74 (d, J = 8.4 Hz, 2H), 7.22 (brs, 1H), 3.84 (s, 3H), 3.05 (* 2.72, brs, 2H), 1.56 (* 1.46, brs, 2H), 1.48-1.20 (m, 24H), 0.86 (t, J = 6.0 Hz, 3H); EI MS: m/z (rel.abund.%) 465.72 (M^+, 100).

(E)-N'-(2-methoxy-6-pentadecylbenzylidene)-4-bromobenzohydrazide (6b): Pale yellow solid; Yield: 84%; M.p.: 88-89 ^{o}C; IR (KBr): υ_{max} 3427, 3276, 3158, 2957, 2919, 2848, 1646 (-CO-NH-), 1590, 1514, 1474, 1406, 1372, 1254, 1179, 1152, 1071, 973, 873, 836, 770, 734 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.81 (s, 1H), 8.77 (s, 1H), 7.88 (d, J = 6.0 Hz, 2H), 7.72 (d, J = 6.4 Hz, 2H), 7.27 (t, J = 6.0 Hz, 1H), 6.90 (d, J = 6.0 Hz, 1H), 6.84 (d, J = 6.4 Hz, 1H), 3.82 (s, 3H), 2.95 (brs, 2H), 1.51 (brs, 4H), 1.32-1.73 (m, 22H), 0.84 (t, J = 5.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.8, 22.0, 28.6, 28.8 (9C), 30.7, 31.2, 33.5, 55.6, 108.7, 120.5, 122.8, 125.2, 129.6, 130.0 (2C), 131.2 (2C), 132.4, 143.4, 145.1, 158.6, 161.5; EI MS: m/z (rel.abund.%) 543.68 (M^+, 100).

(E)-N'-(2-methoxy-6-pentadecylbenzylidene)-4-chlorobenzohydrazide (6c): White solid; Yield: 84%; M.p.: 112-113 ^{o}C; IR (KBr): υ_{max} 3279, 3160, 2920, 2849, 1647 (-CO-NH- strt), 1592, 1514, 1408, 1374, 1255, 1073, 1014, 974, 920, 874, 840, 779, 740, 723 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.80 (s, 1H), 8.74 (s, 1H), 7.86 (d, J = 6.0 Hz, 2H), 7.70 (d, J = 6.4 Hz, 2H), 7.28 (t, J = 6.0 Hz, 1H), 6.88 (d, J = 6.0 Hz, 1H), 6.84 (d, J = 6.4 Hz, 1H), 3.80 (s, 3H), 2.96 (brs, 2H), 1.51 (brs, 4H), 1.72-1.30 (m, 22H), 0.86

(t, J = 5.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.0, 22.6, 29.3, 29.5, 29.6 (9C), 31.9, 34.0, 55.7, 108.1, 119.8, 123.0, 128.0 (2C), 128.8, 130.4 (3C), 131.3 (2C), 142.7, 144.2, 159.1; EI MS: m/z (rel.abund.%) 499.69 (M^+, 100).

(E)-N'-(2-methoxy-6-pentadecylbenzylidene)-4-fluorobenzohydrazide (6d): White solid; Yield: 82%; M.p.: 106-108 ^oC; IR (KBr): υ_{max} 3208, 2919, 2848, 1651(-CO-NH- strt), 1606, 1548, 1506, 1466, 1435, 1371, 1267, 1229, 1160, 1088, 1055, 975, 914, 847, 789, 747, 721 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.76 (s, 1H), 8.76 (s, 1H), 8.01 (s, 2H), 7.34 (s, 2H), 7.27 (t, J = 6.0 Hz, 1H), 6.90 (d, J = 7.2 Hz, 1H), 6.84 (d, J = 5.2 Hz, 1H), 3.82 (s, 3H), 2.95 (brs, 2H), 1.51 (brs, 5H), 1.32-1.17 (m, 21H), 0.84 (t, J = 5.6 Hz, 3H); EI MS: m/z (rel.abund.%) 483.69 (M^+, 100).

(E)-N'-(2-methoxy-6-pentadecylbenzylidene)-4-methoxybenzohydrazide (6e): Off white solid; Yield: 85%; M.p.: 116-118 ^oC; IR (KBr): υ_{max} 3201, 2920, 2850, 1646(-CO-NH- strt), 1607, 1544, 1510, 1466, 1368, 1292, 1256, 1176, 1116, 1054, 1026, 974, 912, 837, 786, 748, 721 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.62 (s, 1H), 8.75 (s, 1H), 7.92 (s, 2H), 7.26 (t, J = 6.0 Hz, 1H), 7.02 (d, J = 5.2 Hz, 2H), 6.90 (d, J = 6.4 Hz, 1H), 6.84 (d, J = 5.2 Hz, 1H), 3.82 (s, 6H), 2.95 (brs, 2H), 1.50 (brs, 2H), 1.32-1.17 (m, 24H), 0.84 (t, J = 5.6 Hz, 3H); EI MS: m/z (rel.abund.%) 495.67 (M^+, 100).

(E)-N'-(2-methoxy-6-pentadecylbenzylidene)-2-bromobenzohydrazide (6f): White solid; Yield: 85%; M.p.: 94-96 ^oC; IR (KBr): υ_{max} 3178, 3054, 2919, 2849, 1658(-CO-NH strt), 1545, 1467, 1433, 1371, 1301, 1264, 1086, 1057, 916790, 750 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.76 (* 11.64, s, 1H), 8.60 (* 8.44, s, 1H), 7.90 (* 7.82, d, J = 6.4 Hz, 1H), 7.44 (* 7.38, t, J = 6.0 Hz, 1H), 7.28-7.15 (m, 3H), 6.92 (* 6.66, d, J = 6.2 Hz, 1H), 6.88 (d, J = 6.4 Hz, 1H), 3.80 (* 3.78, s, 3H), 2.96 (* 2.44, t, J = 5.6 Hz, 2H), 1.58-0.90 (m, 26H), 0.86 (t, J = 6.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.08, 22.6, 29.3 (2C), 29.7 (9C), 30.1, 34.0, 55.6, 108.0, 119.6, 123.1, 128.6, 130.2, 130.4 (3C), 132.5, 137.5, 142.8, 144.3, 159.2, 169.9; EI MS: m/z (rel.abund.%) 543.75 (M^+, 100).

(E)-N'-(2-methoxy-6-pentadecylbenzylidene)-2-iodobenzohydrazide (6g): Pale yellow solid; Yield: 90%; M.p.: 69-70 ^oC; IR (KBr): υ_{max} 3177, 3052, 2917, 2850, 1654 (-C=O-NH strt), 1605, 1545, 1466, 1431, 1370, 1300, 1263, 1065, 1017, 915, 790, 749, 722 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.77 (* 11.67, s, 1H), 8.59 (* 8.46, s, 1H), 7.92 (* 7.80, d, J = 6.4 Hz, 1H), 7.47 (* 7.40, t, J = 6.0 Hz, 1H), 7.29-7.12 (m, 3H), 6.90 (* 6.69, d, J = 6.2 Hz, 1H), 6.86 (d, J = 6.4 Hz, 1H), 3.79 (* 3.78, s, 3H), 2.95 (* 2.40, t, J = 5.6 Hz, 2H), 1.58-0.90 (m, 26H), 0.86 (t, J = 6.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.09, 22.6 , 29.3, 29.6 (9C), 29.7, 30.2, 34.1, 55.7, 92.6, 108.09, 119.6, 123.1, 127.6, 128.2 (2C), 130.3 (2C), 139.03, 142.8, 144.3, 159.3, 171.1; EI MS: m/z (rel.abund.%) 591.68 (M^+, 100).

(E)-N'-(2-methoxy-6-pentadecylbenzylidene)-3-nitrobenzohydrazide (6h): Yellow solid; Yield: 95%; M.p.: 119-120 ^oC; IR (KBr): υ_{max} 3196, 3064, 2921, 2849, 1651(-C=O-NH strt), 1532, 1468, 1434, 1346, 1262, 1154, 1117, 1054, 905, 867, 818, 791, 751, 700 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.08 (s, 1H), 8.80 (d, J = 9.6 Hz, 2H), 8.50-8.35 (m, 2H), 7.83 (d, J = 6.4 Hz, 1H), 7.31 (d, J = 6.8 Hz, 1H), 6.92 (d, J = 6.0 Hz, 1H), 6.87 (d, J = 6.0 Hz, 1H), 3.83 (s, 3H), 2.97 (brs, 2H), 1.51 (brs, 4H), 1.73-1.32 (m, 22H), 0.86 (t, J = 6.0 Hz, 3H); EI MS: m/z (rel.abund.%) 510.70 (M^+, 100).

(E)-N'-(2-methoxy-6-pentadecylbenzylidene)-3,4,5-trimethoxybenzohydrazide (6i): Off white solid; Yield: 88%; M.p.: 97-98 ^oC; IR (KBr): υ_{max} 3188, 2919, 2849, 1646(CO-NH strt),, 1582, 1542, 1503, 1466, 1436, 1436, 1371, 1336, 1265, 1235, 1183, 1133, 1056, 845, 719 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.63 (s, 1H), 8.77 (s, 1H), 7.27 (s, 3H), 6.92 (d, J = 7.2 Hz, 1H), 6.85 (d, J = 5.6 Hz, 1H), 3.86 (s, 9H), 3.73 (s, 3H), 2.97 (brs, 2H), 1.52 (brs, 2H), 1.34 (brs, 2H), 1.18-1.14 (m, 22H), 0.85 (s, 3H); EI MS: m/z (rel.abund.%) 501.74 (M^+, 100).

(E)-N'-(2-methoxy-6-pentadecylbenzylidene)-2,4-dichlorobenzohydrazide (6j): Off white solid; Yield: 90%; M.p.: 132-134 ^oC; IR (KBr): υ_{max} 3197, 2919, 2850, 1653(-CO-NH- Strt), 1465, 1435, 1367, 1294,

1264, 1056, 968, 914, 847, 787, 750 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 9.08 (s, 1H), 8.33 (s, 1H), 7.42 (s, 1H), 7.35 (d, J = 8.0 Hz, 1H), 7.30 (d, J = 7.8 Hz, 1H), 7.20 (t, J = 8.0 Hz, 1H), 6.76-6.71 (m, 2H), 3.86 (s, 3H), 2.50 (t, J = 7.2 Hz, 2H), 1.52 (* 1.46, brs, 2H), 1.48-1.20 (brs, 24H), 0.86 (t, J = 6.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.08, 22.6, 29.6 (9C), 29.7, 30.2, 31.9, 34.0, 55.69, 108.0, 119.5, 123.0, 126.8, 129.4, 129.6, 130.4, 132.1, 133.8, 135.8, 143.4, 144.27, 159.3, 168.4; EI MS: m/z (rel.abund.%) 555.74 (M^+, 100).

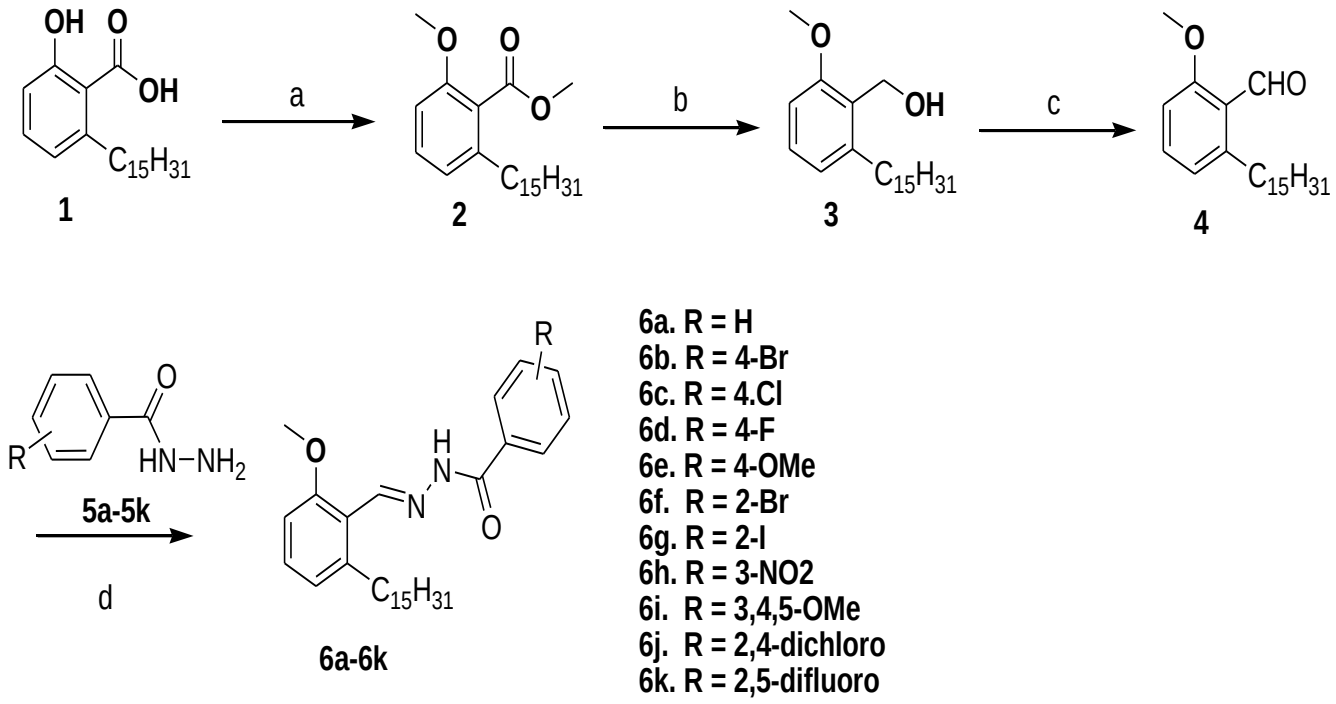
(E)-N'-(2-methoxy-6-pentadecylbenzylidene)-2,5-difluorobenzohydrazide (6k): Off white solid; Yield: 90%; M.p.: 125-126 ^oC; IR (KBr): υ_{max} 3199, 2917, 2849, 1657(-C=O strt), 1493, 1468, 1423, 1370, 1303, 1268, 1190, 1125, 1059, 972, 886, 821, 788, 747 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 9.60 (s, 1H), 8.60 (s, 1H), 7.26-7.14 (m, 4H), 6.86 (d, J = 8.0 Hz, 1H), 6.74 (d, J = 8.0 Hz, 1H), 3.86 (s, 3H), 2.50 (t, J = 7.2 Hz, 2H), 1.52 (* 1.46, brs, 2H), 1.48-1.20 (brs, 24H), 0.86 (t, J = 6.0 Hz, 3H); EI MS: m/z (rel.abund.%) 501.74 (M^+, 100).
Note: * Indicates the presence of rotamers in ^1H NMR.

Antimicrobial Bioassay: Using the agar well diffusion method [15, 16], the synthesized (E)-N'-(2-methoxy-6-pentadecyl-substituted-benzylidene)benzohydrazide **(6a-6k)** were tested against Gram negative strains of (*i) Escherichia coli* (MTCC 443) and *(ii) Pseudomonas aeruginosa* (MTCC 424) and Gram positive strains of (iii) *Staphylococcus aureus* (MTCC 96) and (*iv) Streptococcus pyogenes* (MTCC 442). The compounds were dissolved in dimethylsulphoxide at 250 μg mL^{-1} concentration and Chloramphenicol was used as the standard antibacterial drug. Growth inhibition was calculated with reference to positive control. Antibacterial activity of the compounds was determined by zones showing complete inhibition (mm). All the samples were taken in triplicate.

RESULTS AND DISCUSSION

The starting material *viz.*, 2-hydroxy-6-pentadecylbenzoic acid **1** (anacardic acid) was isolated as per the reported literature procedure [17]. The reaction scheme for the preparation of hydrazone derivatives **6a-6k**, from anacardic acid is depicted in **scheme 1**. Methylation of 2-hydroxy-6-pentadecylbenzoic acid **1** in presence of dimethyl sulpate and potassium carbonate in anhydrous acetonitrile gave methyl 2-methoxy-6-pentadecylbenzoate **2**. $LiAlH_4$ reduction of methyl ester **2**, followed by oxidation of (2-methoxy-6-penta decylphenyl) methanol **3** in presence of pyridinium dichromate in DCM resulted in the formation of a key intermediate 2-methoxy-6-pentadecyl-benzaldehyde **4.** Condensation of aldehydes **4** with various benzohydrazides **5a-5k** [13, 14] in ethanol at reflux for 1h, resulted in the formation of E-N'-(2-methoxy-6-pentadecyl-substituted-benzylidene)benzohydrazide **(6a-6k)**.

Experimental conditions: *Reagents and Conditions:* a) $(CH_3)_2SO_4$, K_2CO_3, CH_3CN, reflux, 24 h; b) $LiAlH_4$,THF, 0 °C-RT, 18 h; c) Pyridium dichromate, DCM, 0 °C - RT, 5 h; d) Benzohydrazides **5a-5k**, EtOH, reflux, 1h



Scheme-1: Synthesis of E)-N'-(2-methoxy-6-pentadecyl-substituted-benzylidene)benzohydrazide **(6a-6k)**

The structural determination of all the compounds was done by ^{1}H NMR, ^{13} C NMR, IR and MS spectroscopic techniques. As an example the spectral assignment for the following benzohydrazide derivative of anacardic acid is discussed, *interpretation of ^{1}H NMR spectra of (E)-N'-(2-methoxy-6-pentadecylbenzylidene)-4-fluorobenzohydrazide 6d*: The protons resonating at 11.76 ppm and 8.76 ppm as a singlet with one proton integration corresponds to Ph-CO-**NH**- and –N=**CH**-Ph groups respectively. The singlet signals resonating at 8.01 ppm and 7.34 ppm as singlets with two proton integration corresponds to the para-fluoro phenyl ring while the protons resonating at 7.27 ppm (triplet, 1H), 6.90 (doublet, 1H) and 6.84 ppm (doublet, 1H) represents to the phenyl ring of the ancardic acid moiety. The singlet resonating at 3.82 ppm is due to the methoxy group and the remaining aliphatic protons of the anacardic appeared in the expected region. *IR interpretation (E)-N'-(2-methoxy-6-pentadecylbenzylidene)-4-fluorobenzohydrazide 6d*: A strong characteristic band in the region 1651cm^{-1} is due to the C=O stretching vibrations of amide functional group and the peaks in the region 1371-1607 cm^{-1} are due to C=N and aromatic ring stretching vibrations. The peaks in the regions 1267-1305 cm^{-1} are due to C-N stretching vibrations while the N-H stretching vibrations of the compounds appeared in the region 3447 cm^{-1}. The remaining aliphatic and aromatic stretching bands appeared in the expected region. The mass spectral assignments of all the synthesized compounds are in agreement with their molecular formulae showing (M+1) peaks.

Antibacterial activity: The antibacterial evaluation data of hydrazone derivatives **6a-6k** is tabulated is table -1. Compound **6e**, **6i** and **6k** with substitution R = 4-OMe, 3,4,5-OMe and 2,5-dufluoro exhibited good antibacterial activity with zone of inhibition 18-22 mm and the compounds **6a**, **6d** and **6h** with R = H, 4-F and 3-NO_2 showed moderate antibacterial activity with zone of inhibition 14-17 mm. The remaining compounds **6b**, **6c**, **6f**, **6g** and **6j** with R = 4-Br, 4-Cl, 2-Br, 2-I and 2,5-dichloro showed nil antibacterial activity. In general, hydrazone derivatives with R = bromo and chloro substitution showed no bacterial activity against the tested organism.

Table-1 Results of Antibacterial Bioassay of Compounds **6a-6k**
(Concentration Used 250 μg mL^{-1} of DMSO).

Compound No.	*Gram negative*		*Gram positive*	
	E.coli ***MTCC 443***	*P.aeruginosa* ***MTCC 424***	*S.pyogenes* ***MTCC 442***	*S.aureus* ***MTCC 96***
	Zones of Inhibition of compounds 6a – 6k in mm			
6a (R = H)	15	16	16	17
6b (R = 4-Br)	-	-	-	-
6c (R = 4-Cl)	-	-	-	-
6d (R = 4-F)	17	15	17	16
6e (R = 4-OMe)	19	18	18	18
6f (R = 2-Br)	-	-	-	-
6g (R = 2-I)	-	-	-	-
6h (R = 3-NO2)	14	16	14	15
6i (R = 3,4,5-OMe)	22	20	19	20
6j (R = 2,4-dichloro)	-	-	-	-
6k (R = 2,5-difluoro)	18	19	19	18
* Standard Drug	23	21	20	21

* Chloramphenicol (at concentration 250 μg mL^{-1})

APPLICATIONS

Some of the newly synthesized novel E-N'-(2-methoxy-6-pentadecyl-substituted-benzylidene) benzohydrazide derivatives was found to possess antibacterial activity and may lead to a promising active pharmacophore. Further exploration of various biological activity is the future scope of the work.

CONCLUSIONS

In summary we have synthesized new E-N'-(2-methoxy-6-pentadecyl-substituted-benzylidene) benzohydrazide **(6a-6k)** from the key intermediate 2-methoxy-6-pentadecyl-benzaldehyde **4.** Condensation of aldehydes **4** with various benzohydrazides **5a-5k** in ethanol at reflux for 1h, resulted in the formation of E-N'-(2-methoxy-6-pentadecyl-substituted-benzylidene)benzohydrazide **(6a-6k).** The antibacterial screening results against the standard drug chloramphenicol (at concentration, 250 μg mL^{-1}), revealed that compound **6e**, **6i** and **6k** with substitution R = 4-OMe, 3,4,5-OMe and 2,5-dufluoro exhibited good antibacterial activity with zone of inhibition 18-22m and the compounds **6a**, **6d** and **6h** with R = H, 4-F and 3-NO_2 showed moderate antibacterial activity with zone of inhibition 14-17 mm.

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