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Synthesis and Antibacterial Activity of Urea and Thiourea Derivatives of Anacardic Acid Mixture Isolated from A Natural Product Cashew Nut Shell Liquid (CNSL)

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ABSTRACT

Synthesis and antibacterial activity of some novel urea and thiourea derivatives (8a - 8k, 9a - 9f) of anacardic acid prepared from commercially available anacardic acid which is obtained from natural product Cashew Nut Shell Liquid (CNSL). Compounds (8a - 8k, 9a - 9f) were tested for Gram positive and Gram negative bacterial cultures. Most of the compounds were showed active compared with standard drug ampicilline.

Keywords: Synthesis; Urea and Thiourea Derivatives; Anacardic Acid; Anti-Bacterial Activity

1. Introduction

Anacardic acid mixture (1a-d) isolated from a natural product Cashew Nut Shell Liquid (CNSL) which is a by-product of cashew nut industry and these are salicylic acid derivatives with a nonisoprenoid alk(en)yl side chain [1]. Kubo *et al.* reported the separation of anacardic acid into monoene (15:1), diene (15:2) and triene (15:3) by preparative HPLC and tested against cancer cells, and found to show moderate cytotoxic activity on BT-20 breast and HeLa epithelioid cervix carcinoma cells [2]. The emergence of drug resistant strains in clinical applications [3-5] especially to Gram positive bacteria [6,7] has created a problem of global proportions [8,9]. This phenomenon has led in creating novel antibacterial agents distinct from existing classes of compounds. Anacardic acid (pentadecyl salicylic acid) is a phenolic constituent present in Cashew Nut Shell Liquid (CNSL); (*Anacardium occidentale L.*) and exhibits antimicrobial properties [10,11] which have led to the preparation of various analogues [12,13]. Anacardic acid and its derivatives exhibits biological activities like antimicrobial activity [10,11] and soybean lipoxygenase-1 inhibitory activity[14,15]. G. C. Reddy *et al.* reported the synthesis of benzamide derivatives of anacardic acid [16], sildenafil analogues [17], dihydropyridine analogues [18] as calcium channel blockers, isonicotinoylhydrazones for antimycobacterial activity [19] starting from anacardic acid. Recently, a few anacardic acid derivatives exhibited various activities like affect the structure of the enzyme [20], anacardic acid is a specific activator of kinase activity of Aurora Kinase A [21], suppresses expression of nuclear factor-kB regulated gene products leading to potentiation of apoptosis [22] inhibitor of the HAT activity of recombinant Plasmodium falciparum GCN5 [23] and as modulators of histone acetyltransferases [24].

In the present work we wish to report to synthesize novel cell permeable urea and thiourea compounds from cheaply available anacardic acid which was a major constituent of Cashew Nut Shell Liquid (CNSL) natural source to evaluate their biological activity by various antibacterial strains. This report describes the synthesis, spectroscopic identification and antibacterial activity of some novel urea and thiourea anacardic acid derivatives against *Escherichia coli*, *Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus pyogenes* bacterial strains.

2. Results and Discussion

Here we described the synthesis of various biologically active novel urea and thiourea derivatives using anacardic acid mixture as starting material and various reagents in the given below conditions (**Scheme 1**).

The anacardic acid mixture (1a-d) was isolated from commercially available CNSL by a reported method [25].

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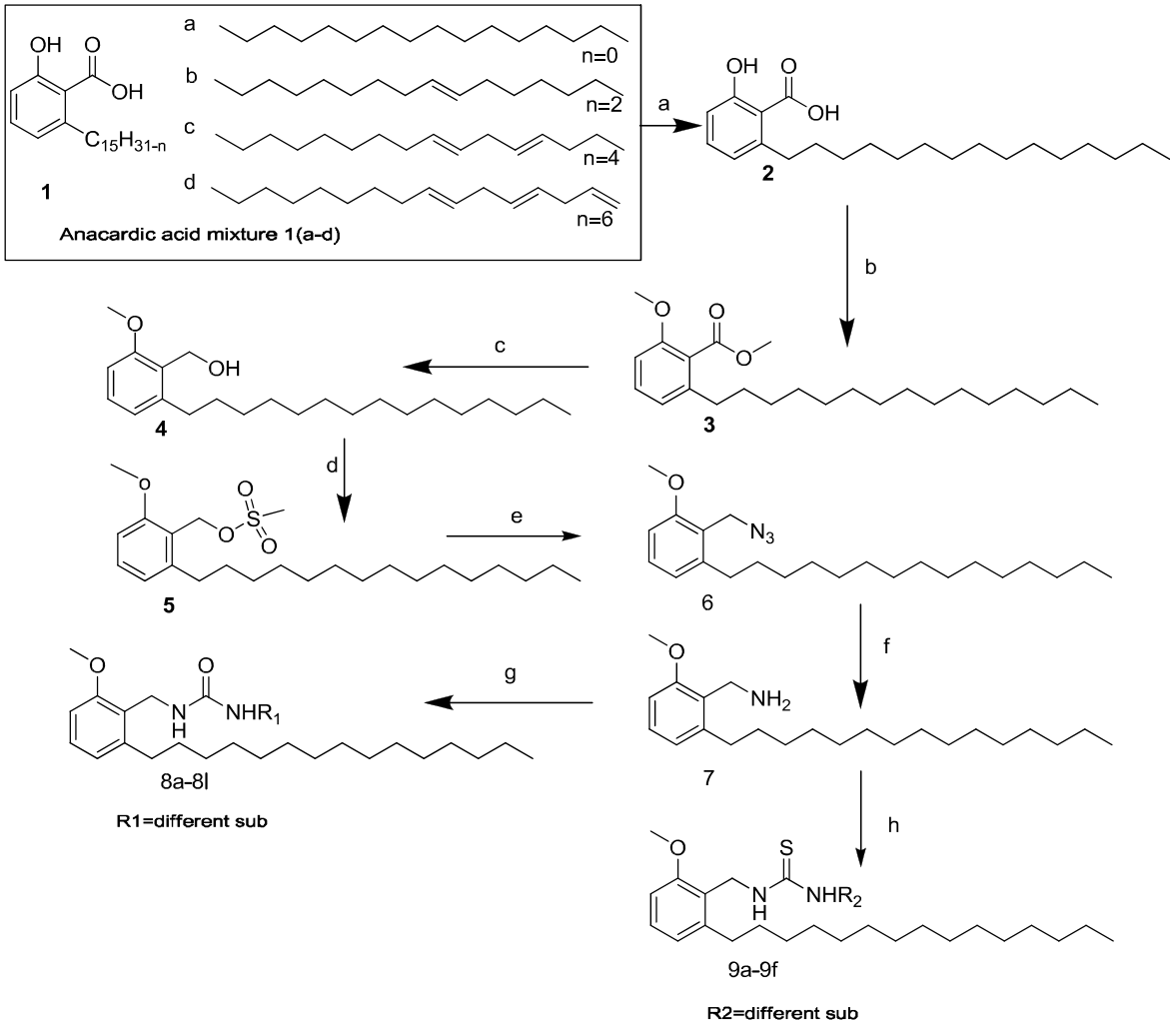


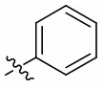
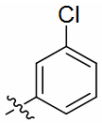
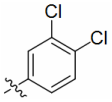
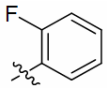
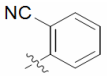
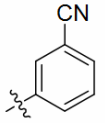
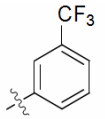
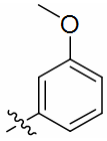
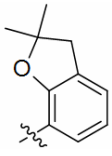
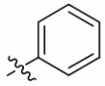
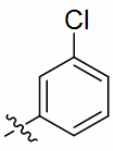
Reagents: a) 10% Pd/C, EtOH, 50psi, RT, 2 hrs; b) Dimethyl sulfate, K_2CO_3, Acetonitrile, 90˚C, 24 hrs; c) LAH, THF, 0˚C-rt, 18 hrs; d) Methane sulphonyl chloride, Et_3N, DCM, 0˚C-rt, 3 hrs; e) NaN_3, DMF; f) 10% Pd/C, 50 psi, 2 hrs; g) different isocynate, $CHCl_3$, h) different isothiocynate, $CHCl_3$.

Scheme 1: Synthesis of various biologically active urea and thiourea derivatives from anacardic acid mixture.

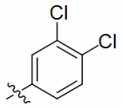
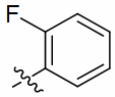
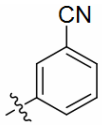
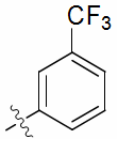
Accordingly CNSL was treated with calcium hydroxide, during which anacardic acid present in CNSL becomes calcium anacardate, which was isolated and hydrolyzed with dil. hydrochloric acid to generate anacardic acid ene mixture, which was a mixture of monoene, diene and triene located at (8'), (8', 11') and (8', 11', 14') of the C15 alkyl chain respectively. Saturated anacardic acid was obtained by hydrogenation of the ene mixture of anacardic acid [17] and was further converted to dialkylated compound by reacting with dimethylsulfate in acetonitrile. Dialkylated anacardic acid was reduced to alcohol by treatment with lithium aluminium hydride in tetrahydrofuran and then protected with methane sulphonyl chloride in dichloromethane. Resultant mesylated compound was reacted with sodium azide followed by reduction with Pd/C under H_2 pressure to obtained amine coupled with various isocynate or iso thiocynate in chloroform to obtain compounds (8a - 8k, 9a - 9f, Scheme 1) of urea and thiourea derivatives were purified by column chromatography to yield title compounds. The structure of urea and thiourea derivatives (8a - 8k, 9a - 9f) was determined by using different spectroscopic techniques ^{1}H NMR, IR, Mass. The resulting compounds are screened for their antibacterial activity.

Biological Activity: The urea and thiourea derivatives (8a - 8k,9a - 9f) were screened for their antibacterial activity[26] against some of the pathogenic bacteria viz. *E.coli* (MTCC443), *P. aeruginosa* (MTCC424), *S. aureus*, (MTCC96) and *S. pyogenes* (MTCC443) using agar well diffusion method according to the literature protocol [26] The anti-bacterial activity of the analogues was compared with standard drug ampicilline and the results of investigation have been presented in **Table 1** and observed that some of the compounds are showed high biological activity. Based on the test results it is evident that several of synthesized anacardic acid analogues

Table 1. Antibacterial activity of Urea and Thiourea derivatives of anacardic acid.

Compound No.	R	**Name of the Bacteria** (Conc. 250 µg/ml) & Inhibition Zone in mm			
		E. coli **MTCC443**	***P. aeruginosa*** **MTCC424**	***S. aureus*** **MTCC96**	***S. pygenes*** **MTCC442**
S* ampicilline	SD* amplicilline	20	20	18	19
8a		19	18	17	16
8b		22	20	16	17
8c		20	20	16	16
8d		18	16	16	18
8e		24	20	17	17
8f		25	18	17	17
8g		18	17	16	16
8h		23	17	15	19
8i		24	19	17	18
8j		23	17	17	17
8k		15	15	15	16
9a		15	16	17	18
9b		17	19	17	17

Continued

9c		17	18	18	18
9d		16	17	19	17
9e		26	23	18	18
9f		17	18	16	17

possess moderate to good activity against the Gram + ve and Gram – ve bacteria. Of all the compounds prepared entities 8h, 8i, 8j, 9e showed good activity against *E. coli*, 8b, 8c, 8e and 8f of *E. coli* MTCC443, and of *P. aeruginosa* MTCC424, display good to excellent activity while the remaining compounds showed moderate activity. The most active antibacterial agent against *Escherichia coli* found to be compound 8h, 8i, 8j, and 9e having -CN, $-CF_3$ and $-OCH_3$ groups and other compounds in the series exhibited moderate to good activity. The compound 9b, 9c, 9d and 9e showed good activity against almost all the strains and 9f also showed good activity against *S. aureus* MTCC96 and *S. pyogenes* MTCC442. This indicates chloro, dichloro, cyano and methoxy substituted compounds showed better activity when compared to other substituted groups. Here seems to be, thiourea substituted novel compounds are exhibiting better activity than urea substituted compounds. The activity depends to some extent on the R substituent, however all the compounds showed antibacterial activity. So other functionalities in the molecule will contribute to the activity as well. It may be suggested that the anacardic acid derivative with a suitable R may lead to a good antibacterial agent against all the *Escherichia coli*, *Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus pyogenes* bacterial strains.

3. Conclusion

In summary, the present study describes a convenient and efficient protocol for the synthesis of sulfonamide derivatives by using anacardic acid mixture using various reagents and different conditions. We believe that this procedure is convenient, economic and a user- friendly process for the synthesis of these various novel urea and thiourea compounds from anacardic acid mixture. All compounds structures are supported by physico chemical and IR, NMR, Mass spectral data. Urea and thiourea derivatives were screened for their antibacterial activity against few bacterial strains and observed that some of the compounds are showed more biological activity than standards used.

4. Experimental Section

4.1. General Reagents and Equipment

All chemicals and solvents were obtained from Aldrich and Spectrochem., India and used without further purification. Column chromatographic separations were carried out on silica gel 60 - 120 mesh size. Melting points were determined in open glass capillaries on a Mel-temp apparatus and are uncorrected. The IR spectra were recorded on a Thermo Nicolet IR 200 FT-IR spectrometer as KBr pellets and the wave numbers were given in cm^{-1}. The ^1H and ^{13}C NMR spectra of samples were recorded on a Varian EM-360, NMR spectrometer using TMS as an internal standard in $CDCl_3$. The mass spectra were recorded on Jeol JMS-D 300 and Finnigan Mat b at 70 eV with an emission current of 100 μA.

Synthesis of 2-hydroxy-6-pentadecyl-benzoic acid (2): A solution of compound 1 (100 g, 287.35 mmol) in ethanol (500 ml) was taken into a 1L Parr-hydrogenation vessel and added a suspension of 10% Pd/C (10 g, 10%) in 70 ml of ethanol under argon atmosphere and applied H_2-pressure (50 psi) for 2h. Reaction mixture was filtered through celite bed and concentrated the filtrate under reduced pressure to obtained compound recrystallized in pet ether to get 2-hydroxy-6-pentadecyl-benzoic acid (2) as white color solid (70 g); (Yield: 70 g, 68.8%; white solid); M.p: 85˚C - 86˚C; IR (KBr): ν_{max} 3010, 3002,

2918, 2851, 1655, 1450, 1246, 1214 cm^{-1}; H^1-NMR (400 MHz, $CDCl_3$): δ 0.89 (t, 3H, J = 6.8 Hz), 1.27 (brs, 24H), 1.57 - 1.63 (m, 2H), 2.98 (t, 2H, J = 8 Hz), 6.78 (d, 1H, J = 7.6 Hz), 6.88 (d, 1H, J = 8.4 Hz), 7.37 (t, 1H, J = 8 Hz), 11.02 (bs, 1H); EI MS: m/z (rel.abund.%) 349 (M^+, 100).

Synthesis of 2-methoxy-6-pentadecyl-benzoic acid methyl ester (3): A solution of compound **2** (20 g, 57.471 mmol) in ACN (200 ml) was added K_2CO_3 (40.8 g, 287.35 mmol) and DMS (21.76 ml, 229.88 mmol). The content was heated refluxed for 24 hrs. Reaction mixture was filtered and distilled off filtrate, obtain residue was redissolved in ethyl acetate and washed with water (2 × 200 ml), brine solution (175 ml), dried over anhydrous Na_2SO_4, filtered and evaporated under vacuum to obtain 2-Methoxy-6-pentadecyl-benzoic acid methyl ester (3) as pale yellow solid 18.5 g; Yield: 85.5%; M.p: 36˚C - 37˚C; IR (KBr): ν_{max} 3004, 2921, 2852, 1732, 1589, 1460, 1266, 1105, 1067, 954, 747 cm^{-1}; H^1-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 Hz), 3.81 (s, 3H), 3.90 (s, 3H), 6.75 (d, 1H, J = 8.4 Hz), 6.82 (d, 1H, J = 8 Hz), 7.25 (t, 1H, J = 8 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; EI MS: m/z (rel.abund.%) 377 (M^+, 100).

Synthesis of 2-Methoxy-6-pentadecyl-phenyl)-methanol (4): A suspension of $LiAlH_4$ (3.03 g, 79.787 mmol) in Dry THF (150 ml) was added compound **3** (20 g, 53.19 mmol) in THF (100 ml) drop wise over a period of 40 min at 0˚C. The content was slowly bringing it to room temp., stirred at room temp., for 18h. Reaction mixture was quenched with saturated brine solution (40 ml) at 0˚C diluted with ethyl acetate filtered (200 ml) and washed with ethyl acetate (100 ml), filtrate was washed with brine solution (200 ml) dried over anhydrous Na_2SO_4, filtered and evaporated under vacuum to obtain 2-Methoxy-6-pentadecyl-phenyl)-methanol (4) (15 gm) as off white solid; Yield: 81%; M.p. 60˚C - 62˚C; IR (KBr): ν_{max} 3386, 3073, 2917, 2847, 1589, 1465, 1318, 1263, 1197, 1093, 1001, 739 cm^{-1}; H^1-NMR (400 MHz, $CDCl_3$): δ 0.89 (t, 3H, J = 7.2 Hz), 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 Hz), 6.82 (d, 1H, J = 7.6 Hz), 7.20 (t, 1H, J = 8 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; EI MS: m/z (rel.abund.%) 349 (M^+, 100).

Synthesis of methanesulfonic acid 2-methoxy-6-pentadecyl-benzyl ester (5): To a solution of compound **4** (15 g, 45.181 mmol) in DCM was added TEA (13.92 ml, 99.398 mmol) followed by mesyl chloride (4.2 ml, 54.21 mmol) at 0˚C over a period of 15 mins. The content was stirred at room temp., for 3 hrs. Reaction mixture was washed with water (150 ml), brine solution (200 ml) dried over anhydrous Na_2SO_4, filtered and evaporated under vacuum to obtain methanesulfonic acid 2-methoxy -6-pentadecyl-benzyl ester (**5**) (17 g, 92.59%) as offwhite color solid.

Synthesis of 2-(azidomethyl)-1-methoxy-3-penta-decyl-benzene (6): To a solution of compound **5** (7.5 g, 17.605 mmol) in DMF (30 ml) was added sodium azide (1.49 g, 22.887 mmol), the content was heated at 100˚C for 2 hrs. Reaction mixture was cooled to room temp., and poured into cool water (150 ml) solid compound was precipitated filtered and dried to get Azide (6) (6.2 g, 94.4%) as white color solid; IR (KBr): ν_{max} 3087, 3013, 2924, 2854, 2096, 1589, 1463, 1316, 1262, 1089, 782, 751 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, 3H, J = 6.8 Hz), 1.25 (brs, 24H), 1.52 - 1.56 (m, 2H), 2.63 (t, 1H, J = 7.6 Hz), 3.85 (s, 3H), 4.42 (s, 2H) 6.78 (d, 1H, J = 8 Hz), 6.83 (d, 1H, J = 8 Hz), 7.24 - 7.26 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.09, 22.67, 29.35, 29.48, 29.56, 29.66, 31.56, 31.91, 32.98, 45.14, 55.49, 108.11, 121.55, 121.97, 129.27, 143.70, 158.28; ESIMS(m/z): 331 (M-N3) $(M + H)^+$.

Synthesis of 2-methoxy-6-pentadecyl-benzyl amine (7): A solution of compound 6 (1.5 g, 4.021 mmol) in ethanol (30 ml) was taken into a 500 ml Parr-hydrogenation vessel and added a suspension of 10% Pd/C (250 mg, 10%) in 20 ml of ethanol under argon atmosphere and applied H_2-pressure (60 psi) for 2 hrs. Reaction mixture was filtered through celite bed and concentrated the filtrate under reduced pressure to obtain 2-methoxy-6-pentadecyl-benzyl amine (7) (1.1 g, 78.8%) as a off-white solid. M.p.: 55-56˚C, IR (neat): ν_{max} 3400, 3066, 2924, 2853, 1583, 1464, 1439, 1374, 1259, 1149, 1121, 1079, 879, 787, 742 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.89 (t, 3H, J = 7.2 Hz), 1.26 (brs, 24H), 1.51 - 1.57 (m, 2H), 1.76 (bs, 2H), 2.65(t, 2H, J = 7.6 Hz), 3.84 (s, 5H), 6.74 (d, 1H, J =8 Hz), 6.79 (d, 1H, J = 7.6 Hz), 7.15 (t, 1H, J = 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.06, 22.64, 29.31, 29.49, 29.57, 29.61, 31.88, 32.14, 33.14, 37.54, 55.24, 107.96, 122.02, 127.33, 129.76, 141.62, 157.87; MS (m/z): 348 $(M + H)^+$.

Synthesis of urea and thiourea compounds: A solution of amine (1.0 eq) in dry $CHCl_3$ was taken in seal tube was added isocynate or iso thiocynate and stirred at room temperature for 3 hrs to 8 hrs reaction went to completed distilled off solvent crude compound was purified by column chromatography.

Synthesis of 1-ethyl-3-(2-methoxy-6-pentadecylbenzyl) urea (8a): Using 7 and ethyl isocyanate as starting materials, the title compound 8a was obtained as a white solid (Yield = 63.1%); m.p.114˚C - 115˚C; IR (KBr): ν_{max} 3335, 3050, 2967, 2921, 2849, 1622, 1576, 1467, 1260, 1095, 785, 732 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ

0.88 (t, 3H, *J* = 6.8 Hz), 1.12 (t, 3H, *J* = 7.2 Hz), 1.25 (brs, 24H), 1.48 - 1.58 (m, 2H), 2.73 (t, 2H, *J* = 7.8 Hz), 3.17-3.20 (m, 2H), 3.84 (s, 3H), 4.3 (brs, 1H), 4.40 (d, 2H, *J* = 6 Hz), 4.65 (bs, 1H), 6.73 (d, 1H, *J* = 8.4 Hz), 6.82 (d, 1H, *J* = 8 Hz) 7.18 (t, 1H, *J* = 7.6 Hz; ESIMS (m/z): 419 $(M + H)^+$.

Synthesis of 1-cyclopentyl-3-(2-methoxy-6-pentdecyl-benzyl) urea (8b): Using 7 and cyclopentyl isocyanate as starting materials, the title compound 8b was obtained as a white solid (Yield = 37.8%); M.p.: 128˚C - 129˚C; IR (DCM film): ν_{max} 3328, 2921, 2851, 1621, 1573, 1466, 1260, 1092, 782 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.24 - 1.37 (m, 26H), 1.50 - 1.66 (m, 6H), 1.88 - 1.96 (m, 2H), 2.73 (t, 2H, *J* = 7.6 Hz), 3.84 (s, 3H), 3.86 - 3.95 (m, 1H), 4.32 (bs, 1H), 4.40 (d, 2H, *J* = 5.2 Hz), 4.65 (s, 1H), 6.73 (d, 1H, *J* = 8.4 Hz), 6.82 (d, 1H, *J* = 7.6 Hz), 7.18 (t, 1H, *J* = 7.6Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.09, 22.66, 23.56, 29.33, 29.63, 29.66, 31.89, 33.11, 33.58, 35.87, 52.15, 55.43, 107.89, 122.30, 125.19, 128.11, 143.25, 157.71, 157.97; ESIMS (m/z): 459 $(M + H)^+$.

Synthesis of 1-(2-methoxy-6-pentadecylbenzyl)-3-phenylurea (8c): Using 7 and phenyl isocyanate as start- ing materials, the title compound 8c was obtained as a white solid (Yield = 58.3%); M.p.105˚C - 106˚C; IR (KBr): ν_{max} 3324, 3041, 2923, 2851, 1639, 1559, 1465, 1306, 1238, 1088, 912, 782, 729 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.24 (brs, 24H), 1.54 - 1.56 (m, 2H), 2.76 (t, 2H, *J* = 7.6 Hz), 3.82 (s, 3H), 4.49 (d, 2H, J = 5.6 Hz), 5.2 (brs, 1H), 6.25 (bs, 1H), 6.73 (d, 1H, *J* = 8.4 Hz), 6.82 (d, 1H, *J* = 7.6 Hz), 7.05 (m, 1H) 7.18 (t, 1H, *J* = 7.6 Hz), 7.24 - 7.28 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d6): δ 13.90, 22.05, 28.67, 28.87, 29.00, 31.26, 31.56, 32.38, 34.15, 55.54, 108.34, 117.30, 120.80, 121.67, 124.97, 128.11, 128.54, 140.50, 142.29, 154.71, 157.97; ESIMS (m/z): 467 $(M + H)^+$.

Synthesis of 1-(3-chlorophenyl)-3-(2-methoxy-6-pentadecylbenzyl)urea (8d): Using 7 and 3-chlorophenyl isocyanate as starting materials, the title compound 8d was obtained as a off white solid (Yield = 80.8%); M.p.101˚C - 102˚C; IR (DCM film): ν_{max} 3318, 2999, 2922, 2850, 1632, 1582, 1557, 1470, 1260, 1235, 1089, 770 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.87 (t, 3H, *J* = 7.2 Hz), 1.24 - 1.36 (brs, 24H), 1.48 - 1.6 (m, 2H), 2.75 (t, 2H, *J* = 8 Hz), 3.86 (s, 3H), 4.50 (d, 2H, *J* = 6 Hz), 5.16 (brs, 1H), 6.3 (brs, 1H), 6.75 (d, 1H, *J* = 7.6 Hz), 6.83 (d, 1H, *J* = 8 Hz), 6.99 (d, 1H, *J* = 8 Hz), 7.125 - 7.22 (m, 3H), 7.38 (s, 1H); ESIMS (m/z): 501 $(M + H)^+$.

Synthesis of 1-(3,4-dichlorophenyl)-3-(2-methoxy-6-pentadecylbenzyl)urea (8e): Using 7 and 3,4-dichlorophenyl isocyanate as starting materials, the title compound 8e was obtained as a white solid (Yield = 68.1%); M.p.118˚C - 119˚C; IR (DCM film): ν_{max} 3355, 3288, 2955, 2920, 2848, 1634, 1582, 1549, 1466, 1381, 1264, 1120, 1088, 776 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.02 (t, 3H, *J* = 7.2 Hz), 1.38-1.5 (m, 24H), 1.62-1.67 (m, 2H), 2.88 (t, 2H, *J* = 7.6 Hz), 4.01 (s, 3H), 4.63 (d, 2H, *J* = 5.6 Hz), 5.25 (s, 1H), 6.5 (s, 1H), 6.91 (d, 1H, *J* = 8 Hz), 6.98 (d, 1H, *J* = 8 Hz), 7.29 - 7.45 (m, 3H), 7.67 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 13.88, 22.07, 28.69, 28.92, 29.03, 31.26, 31.62, 32.41, 34.16, 55.54, 108.32, 117.30, 118.32, 121.69, 122.02, 124.62, 128.20, 130.30, 130.93, 140.68, 142.32, 154.28, 157.96; ESIMS (m/z): 535 $(M + H)^+$.537 (chloro).

Synthesis of 1-(2-fluorophenyl)-3-(2-methoxy-6- pentadecylbenzyl)urea (8f): Using 7 and 2-fluorohenyl isocyanate as starting materials, the title compound 8f was obtained as a white solid (Yield = 40.5%); M.p.: 110˚C - 111˚C; IR (DCM film): ν_{max} 3321, 3066, 3002, 2923, 2851, 1639, 1601, 1560, 1462, 1310, 1258, 1092, 750 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.87 (t, 3H, *J* = 6.8 Hz), 1.24 - 1.36 (m, 24H), 1.51-1.56 (m, 2H), 2.76 (t, 2H, *J* = 8 Hz), 3.86 (s, 3H), 4.52 (d, 2H, *J* = 5.6 Hz), 5.16 (s, 1H), 6.4 (s, 1H), 6.75 (d, 1H, *J* = 8.4 Hz), 6.83 (d, 1H, *J* = 7.2 Hz), 6.85 - 7.08 (m, 3H), 7.20 (t, 1H, *J* = 8 Hz), 8.01-8.10 (m, 1H); ESIMS (m/z): 485 $(M+H)^+$.

Synthesis of 1-(2-cyanophenyl)-3-(2-methoxy-6-pentadecylbenzyl)urea (8g): Using 7 and 2-cyanophenyl isocyanate as starting materials, the title compound 8g was obtained as a white solid (Yield = 45.8%); M.p.: 140˚C - 141˚C; IR (DCM film): ν_{max} 3325, 3037, 2918, 2847, 2225, 1702, 1641, 1550, 1468, 1298, 1240, 1087, 756 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.87 (t, 3H, *J* = 6.8 Hz), 1.24 - 1.4 (m, 24H), 1.49-1.57 (m, 2H), 2.78 (t, 2H, *J* = 8 Hz), 3.89 (s, 3H), 4.53 (d, 2H, *J* = 5.2 Hz), 5.39 (s, 1H), 6.76-6.83 (m, 3H), 7.02 (t, 1H), 7.19 - 7.21 (m, 1H), 7.47 - 7.51 (m, 2H), 8.31 (d, 1H, *J* = 8.4 Hz); ESIMS (m/z): 492 $(M + H)^+$.

Synthesis of 1-(3-cyanophenyl)-3-(2-methoxy-6-pentadecylbenzyl)urea (8h): Using 7 and 3-cyanophenyl isocyanate as starting materials, the title compound 8 hrs was obtained as a cream color solid (Yield = 70.6%); M.p.: 100˚C - 101˚C; IR (DCM film): ν_{max} 3324, 3196, 3078, 2921, 2850, 2230, 1636, 1559, 1467, 1239, 1091, 882, 786, 731 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.24 (brs, 24H), 1.45 - 1.56 (m, 2H), 2.75 (t, 2H, *J* = 8 Hz), 3.89 (s, 3H), 4.50 (d, 2H, *J* = 5.2 Hz), 5.14 (brs, 1H), 6.5 (bs, 1H), 6.77 (d, 1H, *J* = 8 Hz), 6.84 (d, 1H, *J* = 8 Hz), 7.19 - 7.28 (m,2H), 7.35 (t, 1H, *J* = 7.6 Hz), 7.55 (d, 1H, *J* = 7.2 Hz), 7.69 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.08, 22.65, 29.32, 29.54, 29.59, 29.66, 31.88, 33.10, 35.69, 55.50, 108.03, 112.49, 118.80, 121.89, 122.39, 123.30, 124.34, 125.81, 128.43, 129.72, 140.26, 143.02, 154.86, 157.93; ESIMS (m/z): 492 $(M + H)^+$.

Synthesis of 1-(2-methoxy-6-pentadecylbenzyl)-3-(3-(tri-fluoromethyl) phenyl) urea (8i): Using 7 and 3-(tri-fluoromethyl)phenyl isocyanate as starting materials, the

title compound 8i was obtained as a off white solid (Yield = 90.9%); M.p.: 88˚C - 89˚C; IR (DCM film): ν_{max} 3323, 3088, 2922, 2851, 1638, 1560, 1454, 1332, 1253, 1169, 1122, 1076, 888, 755 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.87 (t, 3H, J = 6.8 Hz), 1.23 (brs, 24H), 1.48 - 1.57 (m, 2H), 2.74 (t, 2H, J = 7.2 Hz), 3.85 (s, 3H), 4.50 (d, 2H, J = 5.6 Hz), 5.19 (brs, 1H), 6.6 (bs, 1H), 6.75 (d, 1H, J = 8.4 Hz), 6.83 (d, 1H, J = 7.2 Hz), 7.19 (t, 1H, J =8 Hz), 7.24 - 7.26 (m, 1H), 7.36 (t, 1H, J = 8 Hz), 7.52 - 7.56 (m, 2H); ESIMS(m/z): 535 $(M + H)^+$.

Synthesis of 1-(2-methoxy-6-pentadecylbenzyl)-3-(3-methoxyphenyl) urea (8j): Using 7 and 3-methoxyphenyl isocyanate as starting materials, the title compound 8j was obtained as a off white solid (Yield = 69.9%); M.p.: 77˚C - 78˚C; IR (DCM film): ν_{max} 3318, 2997, 2923, 2851, 1629, 1604, 1562, 1467, 1264, 1212, 1165, 1091, 844,779 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, 3H, J = 6.8 Hz), 1.24 (brs, 24H), 1.51 - 1.57 (m, 2H), 2.75 (t, 2H, J = 8 Hz), 3.76 (s, 3H), 3.82 (s, 3H), 4.49 (d, 2H, J = 5.2 Hz), 5.27 (t, 1H, J = 5.6 Hz), 6.31 (s, 1H), 6.59 - 6.62 (m, 1H), 6.72 - 6.82 (m, 3H), 6.94 (s, 1H), 7.14 - 7.20 (m, 2H); ESIMS(m/z): 497 $(M+H)^+$.

Synthesis of 1-(2,2-dimethyl-2,3-dihydrobenzofuran-7yl)-3-(2-methoxy-6- pentadecylbenzyl) urea (8k): Using 7 and 2,2-dimethyl-2,3-dihydrobenzofuran-7yl isocyanate as starting materials, the title compound 8k was obtained as a light yellow solid (Yield = 66.8%); M.p.: 112˚C - 113˚C; IR (DCM film): ν_{max} 3356, 3299, 3045, 2923, 2854, 1692, 1635, 1556, 1448, 1323, 1304, 1263, 1228, 1132, 1099, 1056, 879,759 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.87 (t, 3H, J = 7.2 Hz), 1.23 - 1.36 (m, 24H), 1.42 (s, 6H), 1.49 - 1.55 (m, 2H), 2.75 (t, 2H, J = 8 Hz), 3.00 (s, 2H), 3.83 (s, 3H), 4.50 (d, 2H, J = 5.2 Hz), 5.16 (bs, 1H), 6.13 (brs, 1H), 6.72 - 6.82 (m, 4H), 7.17 (t, 1H, J = 8.4 Hz), 7.60 (d, 1H, J =7.6 Hz); ESIMS (m/z): 537 $(M + H)^+$.

Synthesis of 1-(2-methoxy-6-pentadecylbenzyl)-3-phenylthiourea (9a): Using 7 and phenyl thioisocyanate as starting materials, the title compound **9a** was obtained as a off white solid (Yield = 86.3%); M.p.: 71˚C - 72˚C; IR (DCM film): ν_{max} 3387, 3192, 3005, 2924, 2853, 1590, 1529, 1461, 1309, 1253, 1171, 1081, 750, 695 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, 3H, J = 6.8 Hz), 1.25 - 1.38 (m, 24H), 1.53 - 1.57 (m, 2H), 2.83 (t, 2H, J = 8 Hz), 3.65 (s, 3H), 4.92 (d, 2H, J = 3.6 Hz), 6.67 (d, 1H, J = 8 Hz), 6.82 (d,1H, J = 7.6 Hz), 6.9 (bs, 1H), 7.13 - 7.19 (m, 3H), 7.23 - 7.29 (m, 1H), 7.36 - 7.40 (m, 2H), 7.52 (s, 1H); ESIMS (m/z): 483 $(M + H)^+$.

Synthesis of 1-(3-chlorophenyl)-3-(2-methoxy-6- pentadecylbenzyl)thiourea (9b): Using 7 and 3-chloro phenyl thioisocyanate as starting materials, the title compound 9b was obtained as a off white solid (Yield = 76.6%); M.p.: 81˚C - 82˚C; IR (DCM film): ν_{max} 3332, 3089, 3029, 2921, 2849, 1634, 1583, 1558, 1469, 1259, 1088, 894, 860, 779 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, 3H, J = 6.8 Hz), 1.24 - 1.40 (m, 24H), 1.50 - 1.56 (m, 2H), 2.75 (t, 2H, J = 8 Hz), 3.85 (s, 3H), 4.49 (d, 2H, J = 6 Hz), 5.2 (s, 1H), 6.40 (s, 1H), 6.75 (d, 1H, J = 8 Hz), 6.82 (d, 1H, J = 7.2 Hz), 6.98 - 7.02 (m, 1H), 7.12 - 7.22 (m, 3H), 7.36-7.39 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.11, 22.68, 29.35, 29.62, 29.69, 31.91, 33.15, 35.81, 55.50, 108.00, 117.91, 119.89, 122.41, 122.99, 124.52, 128.37, 129.95, 134.59, 140.28, 143.15, 154.98, 157.96; ESIMS (m/z): 517 $(M + H)^+$.

Synthesis of 1-(3,4-dichlorophenyl)-3-(2-methoxy-6-pentadecylbenzyl) thiourea (9c): Using 7 and 3,4-dichloro phenyl thioisocyanate as starting materials, the title compound 9c was obtained as a off white solid (Yield = 73.5%); M.p.:92˚C - 93˚C; IR (DCM film): ν_{max} 3262, 3052, 3003, 2924, 2853, 1587, 1529, 1469, 1313, 1255, 1128, 1084, 1031, 775 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, 3H, J = 6.8 Hz), 1.25 - 1.40 (m, 24H), 1.52 - 1.60 (m, 2H), 2.8 (brs, 2H), 3.77 (s, 3H), 4.90 (s, 2H), 6.72 - 7.01 (m, 4H), 7.18 - 7.3 (m, 2H), 7.41 - 7.51 (m, 2H); ESIMS (m/z): 551 $(M + H)^+$. 553 (chloro).

Synthesis of 1-(2-fluorophenyl)-3-(2-methoxy-6-pentadecylbenzyl)thiourea (9d): Using 7 and 2-fluoro-phenyl thioisocyanate as starting materials, the title compound **9d** was obtained as a off white solid (Yield = 87.9%); M.p.: 80˚C - 81˚C; IR (DCM film): ν_{max} 3276, 3224, 3000, 2923, 2852, 1585, 1531, 1462, 1318, 1257, 1085, 860, 752 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, 3H, J = 6.8 Hz), 1.25 - 1.40 (m, 24H), 1.51 - 1.60 (m, 2H), 2.8 (bs, 2H), 3.69 (s, 3H), 4.91 (s, 2H), 6.63 - 6.66 (m, 1H), 6.82 - 6.90(m, 2H), 7.14 - 7.3 (m, 5H); ESIMS (m/z): 501 $(M + H)^+$.

Synthesis of 1-(2-methoxy-6-pentadecylbenzyl)-3- cyano phenylthiourea (9e): Using 7 and 3-cyano phenyl thioisocyanate as starting materials, the title compound 9e was obtained as a yellow solid (Yield = 82.1%); M.p.: 81˚C - 82˚C; IR (DCM film): ν_{max} 3349, 2929, 2853, 2228, 1587, 1529, 1463, 1258, 1085, 757 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, 3H, J = 6.8 Hz), 1.25 - 1.38 (m, 24H), 2.50 - 2.60 (m, 2H), 2.79 (bs, 2H), 3.78 (s, 3H), 4.92 (s, 2H), 6.74 (d, 1H, J =7.6 Hz), 6.85 (d, 1H, J = 7.2 Hz), 7.20 - 7.29 (m, 3H), 7.55 - 7.66 (m, 3H); ESIMS (m/z): 508 $(M + H)^+$.

Synthesis of 1-(2-methoxy-6-pentadecylbenzyl)-3-(3-(trifluoromethyl) phenyl) thiourea (9f): Using 7 and 3-(trifluoromethyl)phenyl thioisocyanate as starting materials, the title compound 9f was obtained as a off white solid (Yield = 84.2%); IR (DCM film): ν_{max} 3374, 3204, 2922, 2852, 1586, 1530, 1459, 1328, 1256, 1126, 1075, 894, 780 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, 3H, J = 6.8 Hz), 1.25 - 1.38 (m, 24H), 1.54 - 1.56 (m, 2H), 2.82 (brs, 2H), 3.69 (s, 3H), 4.92 (s, 2H), 6.65-6.84 (m, 3H), 7.18 - 7.21 (m, 1H), 7.28 - 7.6 (m, 4H); ESIMS (m/z): 551 $(M+H)^+$.

Antibacterial Bioassay [26]: Urea and thiourea derivatives of Anacardic acid (8a - 8k, 9a - 9f) were dissolved in dimethyl sulphoxide at 250 µg/ml concentration. The composition of nutrient agar medium was Bactotryptone (10 g), yeast extract (5 g), NaCl (10 g), final pH 7.4. After 18 h the exponentially growing cultures of the six bacteria in nutrient broth at 37˚C were diluted in sterile broth. From each of these diluted cultures, 1mL was added to 100 mL sterilized and cooled nutrient agar media to give a final bacterial count of 1×10^6 cell/ml. The plates were set at room temperature and later dried at 37˚C for 20 hrs. Paper discs (6 mm, punched from Whatmann no. 41 paper) were ultraviolet sterilized and used for the assays. Discs were soaked in different concentration 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 samples were taken in triplicates. The plates were incubated at 37˚C in an inverted fashion. Activity was determined by zones showing complete inhibition (mm). Growth inhibition was calculated with reference to positive control.

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