SYNTHESIS AND ANTIMICROBIAL ACTIVITIES OF DENDRIMERS WITH INDAZOLE AS SURFACE UNITS AND MELAMINE AS CORE UNIT

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Abstract

Dendrimers with indazole as surface units and melamine has core unit has been synthesized in good yields. The antimicrobial activities of the newly synthesized dendrimers were evaluated against four human pathogenic bacteria such as, E. coli, S. aureus, P. aeruginosa, B. subtilis and one fungi C. albicans under in vitro conditions by “cup plate method”.

Keywords: Dendrimer, indazole, O-alkylation, LAH, antimicrobial

Introduction

Dendrimers based on melamine can reduce the organ toxicity of solubilized cancer drugs administered by intraperitoneal injection. The solubility of FDA approved\(^1\) hepatotoxins such as methotrexate and 6-mercaptopurine can be increased by mixing them with a dendrimer based on melamine. C3H mice were administered subchronic doses of methotrexate or 6-mercaptopurine with and without a solubilizing dendrimer and the ALT (Alanine Aminotransferase) levels were found to be lower than those of animals treated with the drug alone.\(^2\)-\(^3\)

As Melamine is rich in nitrogen, a property similar to that of protein; combines with cyanuric acid and related compounds to form melamine cyanurate and show related crystal structures, which have been implicated as contaminants or biomarkers in Chinese protein adulterations. Melamine is one of the major components in Pigment Yellow 150,\(^4\) a colorant in inks and plastics. Melamine and its salts are used as fire-retardant additives in paints, plastics, and paper.\(^5\) Melamine is also used as a nitrogen and carbon source for N-doped carbon nanotube. Melamine resin is the main constituent of high-
pressure laminates, such as Formica and Arborite, and of laminate flooring. The adsorption of melamine molecules on Au (III) or Ag (III) surface tend to arrange into honeycomb or closed-packed structures. Such a self-assembly occurs due to the intermolecular hydrogen bond interaction. Being inspired by such results the synthesis of melamine based dendrimers with indazoles as surface units are focused. The antimicrobial activities of compounds were tested using cup plate method. Tested microorganism strains were human pathogenic bacteria such as, *E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis*, and *C. albicans* under *in vitro* condition.

**Results and discussion**

3-(Bromomethyl)-1H-indazole was obtained in good yield from indazole by Vilsmeier Haack formylation followed by reduction with sodium borohydride and then by the reaction of the resulting alcohol with PBr₃ in CHCl₃. 3-(Bromomethyl)-1H-indazole thus obtained was further reacted with 4-hydroxy ethylbenzoate in dry DMF in the presence of K₂CO₃ at RT for 24 h to afford the corresponding ester compound in 85 % yield (Scheme 1). The ¹H NMR spectrum of 4 displayed a triplet at δ 1.36-1.40 for ester methyl protons, a quartet at δ 4.31-4.39 for ester methylene protons and a singlet at δ 5.23 for O-methylene protons respectively in addition to the signals for the aromatic protons. The ¹³C NMR spectrum of 4 displayed the O-methylene carbon at δ 67.2, the ester methyl and methylene carbons at δ 14.4 and 60.7 respectively in addition to the signals for the aromatic carbons.

The ester 4 was then hydrolyzed with 1 N HCl in methanol at reflux for 4 h to afford the corresponding carboxylic acid in 80 % yield (Scheme 1). The ¹H NMR spectrum of the carboxylic acid 5 displayed the O-methylene protons at δ 5.23 in addition to the signals for the aromatic protons. In the ¹³C NMR spectrum the carboxylic acid 5 displayed the O-methylene carbon at δ 71.9 in addition to the signals for the aromatic carbons.
Scheme 1 Reagents and conditions: (i) Ethyl 3,5-dihydroxy benzoate, K$_2$CO$_3$, dry DMF, RT, 24 h, 6 (81 %), 11 (80 %) (ii) LAH, THF, 50 °C, 18 h, 7 (76 %), 12 (77 %) (iii) PBr$_3$, CHCl$_3$, 0 °C to RT, 12 h, 8 (74 %), 13 (71 %) (iv) 4-Hydroxy ethylbenzoate, K$_2$CO$_3$, dry DMF, RT, 24 h, 4 (85 %), 9 (83 %), 14 (83 %) (v) 1N HCl, Methanol, reflux, 4 h, 5 (80 %), 10 (78 %), 15 (75 %)

In order to synthesize higher generation carboxylic acid dendritic wedge, two equivalents of 3-(bromomethyl)-1H-indazole was treated with one equivalent of ethyl 3,5-dihydroxy benzoate in dry DMF in the presence of K$_2$CO$_3$ (Scheme 1). In the $^1$H NMR spectrum of G1-ester 6, showed triplet at δ 1.37-1.42 for ester methyl protons, quartet at δ 4.34-4.41 for ester methylene protons and a singlet at δ 5.18 for the O-methylene protons respectively in addition to the signals for the other aromatic protons. The $^{13}$C NMR spectrum of G1-ester 6, showed the ester methyl carbon at δ 14.3, ester methylene carbon at δ 61.3 and O-
methylene carbon peak at $\delta$ 67.5 respectively. The carbonyl carbon appeared at $\delta$ 166.2 in addition to the signals for the aromatic carbons.

The G1-ester 6 was then reduced with LAH in dry THF at 50°C to afford the corresponding hydroxyl compound (G1-CH$_2$OH) 7 in 76% yield followed by bromination with PBr$_3$ to afford the first generation dendritic bromide (G1-CH$_2$Br) 8 in 74% yield (Scheme 1). In the $^1$H NMR spectrum the first generation dendritic hydroxyl compound 7, displayed two distinct O-methylene protons at $\delta$ 4.61 and $\delta$ 5.17 respectively in addition to other the signals for the aromatic protons from $\delta$ 6.45 to $\delta$ 7.48. In the $^{13}$C NMR spectrum the first generation dendritic hydroxyl compound 7, showed two distinct O-methylene carbons at $\delta$ 67.2, 68.0 respectively in addition to the signals for the other aromatic carbons. In the $^1$H NMR spectrum the first generation dendritic bromide (G1-CH$_2$Br) 8, displayed O-methylene protons at $\delta$ 4.64 and bromo methylene proton at $\delta$ 5.14 in addition to the signals for the other aromatic protons from $\delta$ 6.56-7.56. In the $^{13}$C NMR spectrum the first generation dendritic bromide (G1-CH$_2$Br) 8, showed O-methylene carbon at $\delta$ 65.3 and bromo methylene carbon at $\delta$ 67.2 respectively in addition to the signals for the aromatic carbons.

The first generation dendritic carboxylic ester wedge 9 was obtained in 83% yield by reacting equimolar mixture of 4-hydroxy ethylbenzoate and the bromo compound 8 in dry DMF in the presence of K$_2$CO$_3$ (Scheme 1). The first generation dendritic carboxylic acid wedge 10 was obtained in 78% yield by hydrolyzing 9 in 1N HCl at reflux for 4 h. The $^1$H NMR spectrum of the first generation dendritic ester 9 displayed the ester methyl protons as triplet at $\delta$ 1.34 and the ester methylene protons as quartet at $\delta$ 4.29 and two distinct peaks at $\delta$ 4.65, 5.14 for O-methylene protons respectively in addition to the signals for the aromatic protons. In the $^{13}$C NMR spectrum, the dendritic ester 9 displayed the ester methyl carbon at $\delta$ 14.4 and the ester methylene carbon at $\delta$ 60.7 and the two distinct O-methylene carbons at $\delta$ 65.2, 67.2 respectively in addition to the signals for the aromatic carbons. The $^1$H NMR spectrum of the carboxylic acid 10 displayed the two distinct O-methylene protons at $\delta$ 5.09 and 5.16 respectively in addition to the signals for the aromatic protons. In the $^{13}$C NMR spectrum, the carboxylic acid 10 displayed the two distinct O-
methylene carbons at δ 65.3 and 67.2 respectively in addition to the signals for the aromatic carbons.

In a similar manner the second generation dendritic bromide 13 was obtained in 71 % yield by repeating similar reaction sequence (Scheme 1). In the 1H NMR spectrum the second generation dendritic ester (G2-COOEt) 11, displayed triplet at δ 1.35-1.40 for ester methyl protons and a quartet at δ 4.32-4.36 for ester methylene protons respectively. The two O-methylene protons appeared at δ 4.66 and δ 5.06 respectively in addition to the signals for the other aromatic protons from δ 6.57 to 8.00. In the 1H NMR spectrum the second generation dendritic alcohol (G2-CH2OH) 12, displayed three distinct O-methylene protons at δ 4.62, 4.93 and δ 5.07 respectively in addition to the signals for the other aromatic protons from δ 6.45 to δ 7.48. In the 13C NMR spectrum the second generation dendritic alcohol (G2-CH2OH) 12, showed three distinct O-methylene carbons at δ 65.1, 67.4 and at δ 69.2 respectively in addition to the signals for the other aromatic carbons. In the 1H NMR spectrum the second generation dendritic bromide (G2-CH2Br) 13, the three distinct signals for the O-methylene protons appeared at δ 4.42, 5.03 and δ 5.14 respectively in addition to other aromatic protons at δ 6.57 to δ 7.55. In the 13C NMR spectrum the second generation dendritic bromide (G2-CH2Br) 13, showed three O-methylene carbons at δ 65.3, 67.2 and at δ 70.1 respectively in addition to the signals for the other aromatic carbons.

The second generation dendritic carboxylic ester wedge 14 was obtained in 83 % yield by reacting equimolar mixture of 4-hydroxy ethylbenzoate and the bromo compound 13 in dry DMF in the presence of K2CO3 (Scheme 1). The second generation dendritic carboxylic acid wedge 15 was obtained in 75 % yield by hydrolyzing 14 in 1N HCl at reflux for 4 h. The 1H NMR spectrum of the second generation dendritic ester 14 displayed triplet at δ 1.35-1.40 for ester methyl protons and quartet at δ 4.31 4.38 for ester methylene protons and the two distinct O-methylene protons at δ 4.66, 5.15 respectively in addition to the signals for the aromatic protons. The 13C NMR spectrum of the second generation dendritic ester 14 displayed the ester methyl carbon at δ 14.7 and the ester methylene carbon at δ 59.3 and the two distinct O-methylene carbons at δ 65.2, 67.2 respectively in addition to the signals for the aromatic carbons.

The 1H NMR spectrum of the second generation dendritic carboxylic acid 15 displayed two distinct O-methylene protons at δ 4.66 and 5.15 respectively in addition to the signals for
the aromatic protons. The $^{13}$C NMR spectrum of the carboxylic acid 15 displayed two distinct O-methylene carbons at δ 65.2 and 67.2 respectively in addition to the signals for the aromatic carbons.

The zero-generation G0 dendrimer 1 was obtained in 78% yield by reacting three equivalents of the carboxylic acid 5 with one equivalent of melamine in the presence of EDCI.HCl and DMAP in DCM (Scheme 2). In the $^1$H NMR spectrum of the dendrimer 1 the O-methylene protons appeared at δ 5.23 in addition to the signals for the aromatic protons. In the $^{13}$C NMR spectrum of the dendrimer 1 the O-methylene carbon appeared at δ 67.2 in addition to the signals for the aromatic carbons.

The first generation dendrimer 2 was obtained in 75% yield by treating three equivalents of the first generation dendritic carboxylic acid 10 with one equivalent of melamine in the presence of EDCI.HCl and DMAP in DCM (Scheme 2). The $^1$H NMR spectrum of the dendrimer 2 displayed two distinct singlets at δ 5.06 and 5.15 respectively in addition to the signals for the aromatic protons. In the $^{13}$C NMR spectrum of the dendrimer 2 the two O-methylene carbons appeared at δ 67.3, 69.9 respectively in addition to the signals for the aromatic carbons.

The second generation dendrimer 3 was obtained in 73% yield by treating three equivalents of the second generation carboxylic acid 15 with one equivalent of melamine in the presence of EDCI.HCl and DMAP in DCM (Scheme 2). The $^1$H NMR spectrum of the dendrimer 3 displayed two distinct O-methylene protons at δ 5.13, 5.15 respectively in addition to the signals for the aromatic protons. The $^{13}$C NMR spectrum of the dendrimer 3 displayed two distinct O-methylene carbons at δ 65.3, 67.2 respectively in addition to the signals for the aromatic carbons.

**Effect of indazole dendrimers 1-3 on the growth of human pathogens**

The anti-bacterial studies were carried out aseptically under *in vitro* conditions by “cup plate method”. All the three synthesized dendrimers exhibited different levels of antibacterial activity against the four tested human pathogenic bacteria and fungi compared to DMSO as control. The antimicrobial activities of three different newly synthesized dendrimers 1-3 were evaluated against four human pathogenic bacteria such as, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and
Candida. albicans. The biological screening results of dendrimers 1-3 with 10% DMSO as control and with commercial
Scheme 2: Reagents and conditions: (i) 5 (3.0 equiv), EDCI.HCl/DMAP, DCM, RT, 18 h, 1 (78 %) (ii) 10 (3.0 equiv), EDCI.HCl/DMAP, DCM, RT, 18 h, 2 (75 %) (iii) 15 (3.0 equiv), EDCI.HCl/DMAP, DCM, RT, 18 h, 3 (73 %)

antibiotics viz., Tetracycline for E.coli, S. aureus, P. aeruginosa and B.subtilis are tabulated (Table 1) in the form of percentage of zone of inhibition. The antibacterial activity of the test compound was dose dependent and it was remarkable at higher concentration. The percentage of the zone of inhibition of dendrimers 1-3 against bacterial human pathogens are determined by the cup plate method between 75 to100 µg/mL. All the three dendrimers 1-3 shows better activity against all the four human pathogenic bacteria such as, E. coli, S. aureus, P. aeruginosa, B. subtilis and one fungi C. albicans (Figure 1).

Organisms used

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A- Standard (tetracycline)

Experimental

General: All chemicals and solvents were purchased commercially and used as such without further purification. All melting points of those synthesized compounds are uncorrected and the $^1$H NMR and $^{13}$C NMR spectra were recorded on Bruker 300-MHz instrument in CDCl$_3$ and DMSO-d$_6$ solvent with tetramethylsilane (TMS) as an internal
Column chromatography was performed on silica gel (ACME, 100–200 mesh). Routine monitoring of the reaction was made using thin-layer chromatography (TLC) developed on 0.25 mm glass plates coated with silica gel-G (ACME) 0.25mm thick and visualized with iodine.

*Figure 1* Zone of inhibition of dendrimers 1-3 by cup plate method

**General Procedure for O-alkylation reaction**
A solution containing the hydroxyl compound (1.0 equiv) and the bromocompound (1.1/2.1/3.1 equiv) was stirred with $\text{K}_2\text{CO}_3$ (5.1/10.1/15.1 equiv) in dry DMF at 50 °C for 24 h after which the reaction mixture was stirred with water and then extracted with CHCl$_3$ (3 x 150 mL), dried over Na$_2$SO$_4$, evaporated to dryness to give the O-alkylated compounds. The crude thus obtained was then purified by column chromatography using hexane: CHCl$_3$ (3:2) as eluent.

**Ester Compound 4**
White solid; m.p: 110-113 °C; Yield: 85 %; $^1$H NMR: (300 MHz, CDCl$_3$) $\delta_H$ 1.36-1.40 (t, $J$ = 7.2 Hz, 3H), 4.31-4.39 (q, $J_1$ = 7.2 Hz, $J_2$ = 14.4 Hz, 2H), 5.23 (s, 2H); 7.00 (d, $J$ = 8.7 Hz, 2H); 7.26-7.30 (m, 3H); 7.40-7.43 (m, 1H); 7.51-7.55 (m, 1H); 8.00 (d, $J$ = 8.7 Hz, 2H); $^{13}$C NMR: (75 MHz, CDCl$_3$) $\delta_C$ 14.4, 60.7, 67.2, 114.4, 123.5, 127.0, 128.8, 129.2, 129.5, 131.6, 132.7, 134.1, 162.1, 166.3; anal. calcd for C$_{17}$H$_{16}$N$_2$O$_3$: C, 68.91; H, 5.44; found: C, 68.67; H, 5.17; m/z: 297 [M+1]

**Ester Compound 6**
White Solid; mp: 84-87 °C; Yield: 81 %; $^1$H NMR : (300 MHz, CDCl$_3$) $\delta_H$ 1.37-1.42 (t, $J$ = 7.2 Hz, 3H); 4.34-4.41 (q, $J_1$ = 7.2 Hz, $J_2$ = 14.4 Hz, 2H); 5.18 (s, 4H); 6.81-6.83 (m, 1H); 7.26-7.30 (m, 5H); 7.32-7.36 (m, 3H); 7.39-7.42 (m, 2H); 7.54-7.57 (m, 2H); $^{13}$C NMR: (75 MHz, CDCl$_3$) $\delta_C$ 14.3, 61.3, 67.5, 106.9, 108.7, 127.0, 129.0, 129.2, 129.5, 132.6, 132.9, 134.2, 159.6, 166.2; anal. calcd for C$_{25}$H$_{22}$N$_4$O$_4$: C, 67.86; H, 5.01; found: C, 67.52; H, 4.79; m/z: 443 [M+1]

**Ester Compound 9**
White solid; m.p: 101-104 °C; Yield: 83 %; $^1$H NMR: (300 MHz, CDCl$_3$) $\delta_H$ 1.34-1.39 (t, $J$ = 7.2 Hz, 3H); 4.29-4.37 (q, $J_1$ = 7.2 Hz, $J_2$ = 14.4 Hz, 2H); 4.65 (s, 2H); 5.14 (s, 4H); 6.57-6.58 (d, $J$ = 2.1 Hz, 1H); 6.65-6.66 (d, $J$ = 2.1 Hz, 2H); 6.85-6.87 (d, $J$ = 8.7 Hz, 2H); 7.26-7.29 (dd, $J_1$ = 3.6 Hz, $J_2$ = 7.2 Hz, 4H); 7.38- 7.41 (m, 2H); 7.53-7.56 (m, 2H); 7.92-7.95 (d, $J$ = 8.7 Hz, 2H); $^{13}$C NMR: (75 MHz, CDCl$_3$) $\delta_C$ 14.4, 60.7, 65.2, 67.2, 101.3, 106.0, 115.2, 127.0, 128.9, 129.5, 131.8, 132.7, 134.5, 143.6, 159.9; anal. calcd for C$_{32}$H$_{28}$N$_4$O$_5$: C, 70.06; H, 5.14; found: C, 69.83; H, 4.87; m/z: 549 [M+1]

**Ester Compound 11**
White Solid; mp: 92-95 °C; Yield: 80 %; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$H 1.35-1.40 (t, $J$= 7.2 Hz, 3H); 4.32-4.36 (q, $J$= 3.3 Hz, $J_2$= 7.2 Hz, 2H); 4.66 (s, 4H); 5.06 (s, 8H); 6.57 (d, $J$= 1.8 Hz, 2H); 6.66 (d, $J$= 1.8 Hz, 4H); 6.84 (d, $J$= 8.4 Hz, 1H); 7.26-7.29 (m, 11H); 7.38-7.41 (m, 5H); 7.53-7.56 (m, 5H); 7.93-8.00 (m, 1H); $^{13}$C NMR: (75 MHz, CDCl$_3$) $\delta$C 14.4, 60.7, 65.3, 67.2, 101.3, 106.0, 115.1, 127.0, 128.9, 129.1, 129.4, 131.8, 132.7, 134.5, 143.6, 159.9; anal. calcd for C$_{55}$H$_{46}$N$_8$O$_9$: C, 69.76; H, 4.90; found: C, 69.53; H, 4.62; m/z: 947 [M+1]

Ester Compound 14

White Solid; m.p: 98-101 °C; Yield: 83 %; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$H 1.35-1.40 (t, $J$= 7.2 Hz, 3H); 4.31-4.38 (q, $J$= 7.2 Hz, $J_2$= 14.4 Hz, 2H); 4.66 (s, 4H); 5.15 (s, 10H); 6.58 (d, $J$= 2.1 Hz, 2H); 6.66 (d, $J$= 2.1 Hz, 4H); 6.84 (d, $J$= 8.7 Hz, 2H); 7.26-7.30 (m, 10H); 7.38-7.41 (m, 5H); 7.53-7.56 (m, 4H); 7.95 (d, $J$= 8.7 Hz, 2H); $^{13}$C NMR: (75 MHz, CDCl$_3$) $\delta$C 14.7, 59.3, 65.2, 67.2, 106.0, 115.0, 118.1, 121.0, 127.0, 128.9, 129.1, 129.4, 134.5, 154.9; anal. calcd for C$_{65}$H$_{52}$N$_8$O$_9$: C, 70.71; H, 4.98; found: C, 70.57; H, 4.62; m/z: 1053 [M+1]

**General Procedure for LiAlH$_4$ reduction**

Ester (1.0 equiv) in THF was added to a stirred suspension of LiAlH$_4$ (1.2 equiv/ ester group) in dry THF (100 mL) at 0 °C under inert atmosphere$^{11}$. The reaction mass then allowed to reach RT and then heated to 50 °C for 8 h. It was then cautiously quenched with 10 % NaOH solution at 0 °C. The reaction mixture was then filtered, and the residue obtained was agitated with THF (3x50mL) and the combined THF layers were evaporated. The residue was dissolved in CHCl$_3$ and extracted with CHCl$_3$ (3x100mL), washed with brine (100 mL), dried over Na$_2$SO$_4$ and evaporated to give the diol as crude product, which was purified using silica gel 100-200 using CHCl$_3$:hexane (3:5) as eluent.

Hydroxyl Compound 7

Off-White Solid; mp: 91-94 °C; Yield: 76 %; $^1$H NMR: (300 MHz, CDCl$_3$) $\delta$H 4.61 (s, 2H), 5.17 (s, 4H), 6.45 (s, 1H), 6.59 (s, 2H), 7.08-7.11 (m, 4H), 7.13-7.16 (m, 4H), 7.45-7.47 (m, 2H); $^{13}$C NMR: (75 MHz, CDCl$_3$) $\delta$C 67.2, 68.0, 101.1, 105.9, 127.0, 128.0, 129.1, 128.8, 129.2, 129.4, 132.7, 134.6, 144.0, 159.9; anal. calcd for C$_{25}$H$_{20}$N$_4$O$_3$: C, 68.99; H, 5.03; found: C, 68.56; H, 4.83; m/z: 401 [M+1]
Hydroxyl Compound 12
Off-White Solid; mp: 101-103 °C; Yield: 77 %; \(^1\)H NMR: (300 MHz, CDCl\(_3\)) \(\delta_H\) 4.62 (s, 8H), 4.93 (s, 2H), 5.07 (s, 4H), 6.45 (s, 5H), 6.61-6.62 (m, 4H), 7.23-7.29 (m, 11H), 7.31-7.38 (m, 9H); \(^1^\)C NMR: (75 MHz, CDCl\(_3\)) \(\delta_C\) 65.1, 67.4, 69.2, 101.3, 105.9, 127.2, 128.9, 129.1, 129.4, 132.3, 134.5, 143.6, 160.0; \(m/z:\) 905 [M+1]

General Procedure for bromination
To a solution of hydroxy compound (1.0 equiv) in CHCl\(_3\) (50 mL) was added an excess of PBr\(_3\) (5.0 equiv) at 0 °C and stirred for 1 h. The reaction mixture was allowed to warm up to RT and stirred for further 4-5 h. After the completion of the reaction, it was extracted with CHCl\(_3\) (3x100 mL), washed with aq., NaHCO\(_3\) (100 ml), brine (100 ml), dried over Na\(_2\)SO\(_4\) and evaporated. The residue was purified by column chromatography using CHCl\(_3\)/hexane (2:3) as eluent.

Bromo Compound 8
White Solid; mp: 76-79 °C; Yield: 74 %; \(^1\)H NMR: (300 MHz, CDCl\(_3\)) \(\delta_H\) 4.65 (s, 2H), 5.15 (s, 4H), 6.57 (s, 1H), 6.66 (s, 2H), 7.26-7.31 (m, 5H), 7.39-7.41 (m, 3H), 7.54-7.55 (m, 2H); \(^1^\)C NMR: (75 MHz, CDCl\(_3\)) \(\delta_C\) 65.3, 67.2, 70.1, 101.3, 105.9, 126.7, 128.7, 128.9, 129.1, 129.4, 132.7, 134.6, 143.6, 159.9; \(m/z:\) 464 [M+1]

Bromo Compound 13
White Solid; mp: 94-98 °C; Yield: 71 %; \(^1\)H NMR: (300 MHz, CDCl\(_3\)) \(\delta_H\) 4.42 (s, 4H), 5.03 (s, 2H), 5.14 (s, 8H), 6.57-6.58 (m, 2H), 6.67 (s, 5H), 7.25-7.31 (m, 9H), 7.37-7.38 (m, 10H), 7.53-7.55 (m, 4H); \(^1^\)C NMR: (75 MHz, CDCl\(_3\)) \(\delta_C\) 65.3, 67.2, 70.1, 101.3, 105.9, 126.7, 128.7, 128.9, 129.2, 132.7, 134.6, 143.6, 160.1; \(m/z:\) 968 [M+1]

General Procedure for ester hydrolysis
To the ester compound in methanol was added 1N HCl in drops at 0-5° C and then refluxed at 60 °C for 4 h. The reaction mixture was evaporated to dryness and the residue
obtained was purified by column chromatography on silica gel (100-200 mesh) by using MeOH/CHCl₃ (1:19) as eluent.

**Carboxylic acid 5**

White Solid; m.p: 181-184 °C; Yield: 80 %; (300 MHz, DMSO-D₆): δ_H 5.23 (s, 2H); 7.12 (d, J= 8.7 Hz, 2H); 7.39-7.44 (m, 2H); 7.52-7.55 (m, 1H); 7.60-7.63 (m, 1H); 7.92 (d, J= 8.7 Hz, 2H); ^13^C NMR: (75 MHz, DMSO-D₆): δ_C 71.9, 119.0, 128.6, 131.8, 133.6, 134.0, 134.2, 136.5, 137.4, 138.7, 166.7, 172.9; anal. calcd for C₁₅H₁₂N₂O₃: C, 67.16; H, 4.51; found: C, 66.82; H, 4.23; m/z: 269 [M+1]

**Carboxylic acid 10**

White solid; m.p: 163-165 °C; Yield: 78 %; (300 MHz, CDCl₃): δ_H 5.09 (s, 2H); 5.16 (s, 4H); 6.61 (s, 1H); 6.69 (s, 2H); 7.01 (d, J = 8.7 Hz, 2H); 7.26-7.30 (m, 5H); 7.38-7.41 (m, 3H); 7.52-7.55 (m, 2H); 8.05 (d, J = 8.7 Hz, 2H); ^13^C NMR: (75 MHz, CDCl₃): δ_C 65.3, 67.2, 101.3, 106.0, 127.0, 128.9, 129.1, 129.4, 132.7, 134.6, 143.6, 159.9; anal. calcd for C₃₀H₃₄N₄O₅: C, 69.22; H, 4.65; found: C, 68.89; H, 4.23; m/z: 521 [M+1]

**Carboxylic acid 15**

Off-white solid; m.p: 154-157 °C; Yield: 75 %; (300 MHz, CDCl₃): δ_H 3.87 (s, 2H); 4.65 (s, 4H); 5.15 (s, 8H); 6.57(s, 2H); 6.57-6.65 (m, 6H); 6.66-6.70 (m, 2H); 7.26-7.31 (m, 8H); 7.38-7.41 (m, 5H); 7.53-7.56 (m, 6H); ^13^C NMR: (75 MHz, CDCl₃): δ_C 60.8, 67.1, 71.0, 101.4, 105.5, 115.8, 120.7, 123.9, 125.5, 126.5, 126.9, 127.8, 128.9, 129.1, 129.2, 129.3, 129.4, 132.9, 134.2, 134.6, 140.1, 154.0, 159.6; anal. calcd for C₆₀H₄₈N₈O₉: C, 70.30; H, 4.72; found: C, 70.07; H, 4.32; m/z: 1025 [M+1]

**General Procedure for Amide formation**

To a solution of carboxylic acid (3.0 equiv) in DCM (100 mL) was added EDCI.HCl/DMAP (4.5 equiv) followed by the addition of amine (1.0 equiv) and stirred at RT for 24 h. The reaction mixture was extracted with CHCl₃ (3 x 150 mL), washed with brine solution (100 mL), dried over anhydrous Na₂SO₄. The organic layer was evaporated to dryness and the residue obtained was purified by column chromatography on silica gel (100-200 mesh) by using EtOAc/hexane (1:19) as eluent.

**Dendrimer 1**

Off-white solid; m.p: 108-110 °C; Yield : 78 %; ^1^H NMR (300 MHz, CDCl₃): δ_H 5.23 (s, 6H); 7.01 (d, J = 7.8 Hz, 6H); 7.26-7.32 (m, 8H), 7.39-7.43 (m, 4H); 7.52-7.55 (m, 3H);
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8.03 (dd, J\textsubscript{1} = 1.8 Hz, J\textsubscript{2} = 7.2 Hz, 6H); \textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}): δ\textsubscript{C} 67.2, 114.4, 114.5, 123.5, 127.1, 128.8, 129.2, 129.5, 131.6, 131.7, 132.7, 134.1, 162.1, 166.3; anal. calcd for C\textsubscript{48}H\textsubscript{36}N\textsubscript{12}O\textsubscript{6}: C, 65.75; H, 4.14; found: C, 65.42; H, 3.83; m/z: 877 [M+1]

Dendrimer 2
Off-white solid; m.p: 106-109 °C; Yield : 75 %; \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}): δ\textsubscript{H} 5.06 (s, 6H); 5.15 (s, 12H); 6.60-6.61 (d, J = 2.1 Hz, 3H); 6.68-6.69 (d, J = 2.1 Hz, 6H), 6.95-6.98 (d, J = 8.7 Hz, 6H); 7.26-7.28 (q, J\textsubscript{1} = 3.6 Hz, J\textsubscript{2} = 7.2 Hz, 11H); 7.37-7.40 (m, 7H); 7.51-7.55 (m, 6H); 7.96-7.99 (d, J = 8.7 Hz, 6H); \textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}): δ\textsubscript{C} 67.3, 69.9, 101.7, 106.5, 114.5, 122.9, 127.0, 128.9, 129.1, 129.4, 131.6, 132.7, 134.4, 138.9, 160.0, 162.3, 166.8; anal. calcd for C\textsubscript{93}H\textsubscript{72}N\textsubscript{18}O\textsubscript{12}: C, 68.37; H, 4.44; found: C, 68.04; H, 4.13; m/z: 1633 [M+1]

Dendrimer 3
Off-white solid; m.p: 115-118 °C; Yield : 73 %; \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}): δ\textsubscript{H} 5.13 (s, 18H); 5.15 (s, 24H); 6.55-6.57 (m, 7H); 6.64-6.66 (m, 13H), 7.24-7.29 (m, 33H); 7.38 (s, 18H); 7.53 (m, 16H); \textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}): δ\textsubscript{C} 65.3, 67.2, 70.6, 105.9, 108.6, 115.7, 127.0, 128.9, 129.1, 129.4, 134.8, 137.1, 140.1, 141.3, 143.1, 145.1, 153.9, 159.8; anal. calcd for C\textsubscript{183}H\textsubscript{144}N\textsubscript{30}O\textsubscript{24}: C, 69.84; H, 4.61; found: C, 69.61; H, 4.13

Antimicrobial Susceptibility Testing

Well diffusion method
The well diffusion test\textsuperscript{13} was performed using MHA and PDA medium. The medium was prepared and autoclaved at 15lbs pressure (121°C) for 15minutes immediately cooled in a 50-55°C water bath after removal from the autoclave. The cooled medium was poured into sterile petri plates to a uniform depth of 4mm; this is equivalent to approximately 25mL in a 90mm plate. Once the medium had solidified then the culture was inoculated on the medium. Within 15minutes of adjusting the density of the inoculums, a sterile cotton swab was dipped into the standardized bacterial and yeast suspension or inoculated with 1mL of the organism suspension. The sterile swab was used to streak on the surface of the MHA and PDA medium to ensure an even distribution of the inoculums. The plates were allowed undisturbed for 3 to 5 minutes to absorption of excess moisture. Sterilized 9mm cork borer
was used to make agar wells, 100 μL of the diluted test compound stock solutions were placed into each wells and 100% DMSO as a control. The plates were incubated at 35-37 °C for 24 hours. However NCCLS disc diffusion and MIC standard breakpoints were used for the interpretative results.

The percentage of inhibition was calculated by the formula,

\[
\text{% of inhibition} = \frac{I \times (\text{Diameter of the inhibition zone} \times 100)}{90 \times (\text{Diameter of the Petri-plate in mm})}
\]

**Conclusion**

Melamine based dendrimers with indazole as surface units were synthesized up to second generation and the zero, first and second generation carboxylic acid dendrons were obtained in good yields. The antimicrobial studies of these synthesized dendrimers shows better antibacterial activities compared to the standard.

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**References**


