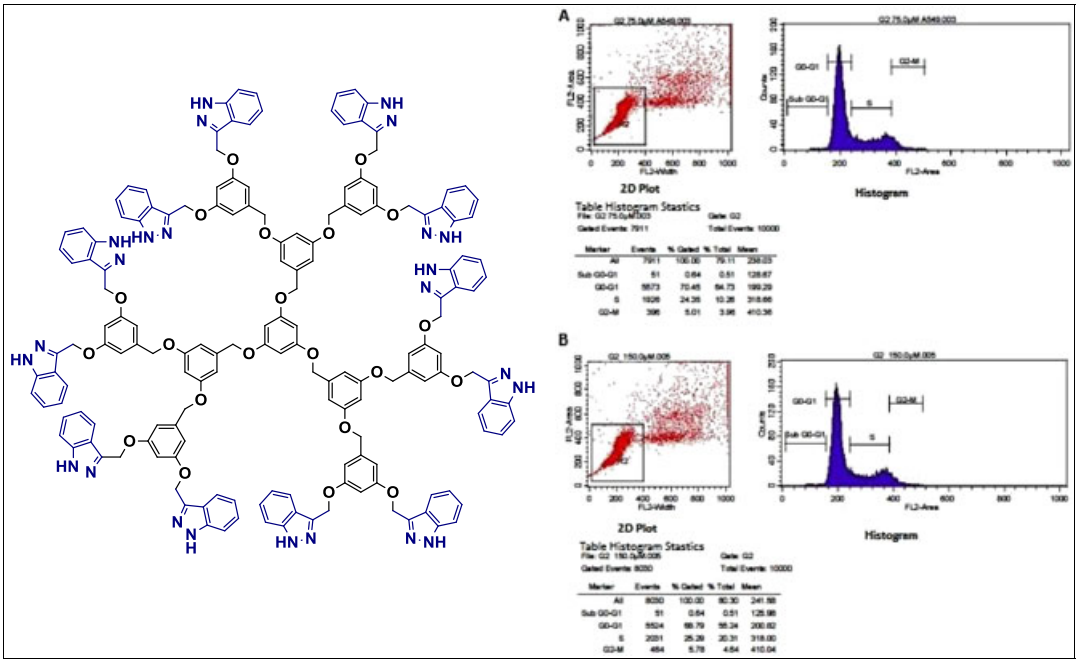
Synthesis, Cell Viability, and Flow Cytometric Fluorescence Pulse Width Analysis of Dendrimers with Indazoles Surface Unit

Mani Jayanthi and Perumal Rajakumar*

Department of Organic Chemistry, University of Madras, Guindy, Chennai 600 025, India
*E-mail: perumalrajakumar@gmail.com

Received June 11, 2016
DOI 10.1002/jhet.2913
Published online 00 Month 2017 in Wiley Online Library (wileyonlinelibrary.com).



Dendrimers with indazole surface units were synthesized up to second generation in good yields, and the MTT cell proliferation assay was carried out with A549 lung adenocarcinoma cell lines. The cell viability and the flow cytometry analysis shows that increased mitochondrial activity was concomitant with increased mitochondrial biomass with no loss of mitochondrial membrane potential or cell death.

INTRODUCTION

Indazole, which is a rare heterocyclic compound in nature [1] possesses broad spectrum of potent pharmacological activities including anti-inflammatory, antiarrhythmic and analgesic [2], anti-tumor [3,4], antifungal [5], antibacterial [6], HIV protease inhibition [7–13], estrogen receptor [14], and antihypertensive properties [15]. So only three natural products based on indazole unit were isolated, and among them, total synthesis of two, namely, nigellicine and nigeglanine, has been reported [16,17]. Because of the much current interest of the indazole ring system, it happens to be the partial structure of vast number of pharmaceutical compounds.

Flow cytometry is the well-known method for assessing the effect of chemotherapeutic agents on cell survival in suspension culture and an ideal means to measure the cell viability by staining with fluorescence diacetate and propidium iodide in isotonic solution. The number of viable cells available per mL of culture is determined by a timed-out count from the flow cytometer sample flow rate. The A549 lung adenocarcinoma cell lines were used as model system.

The MTT assay has demonstrated much potential for the in vitro chemotherapeutic drug screening [18,19], as the best reproducibility is obtained with adherent cell lines in suspension culture. Hence, synthesis of dendrimers with indazole as surface unit would be of great interest. We wish to report herein the synthesis as well as cell viability and flow cytometric fluorescence pulse width analysis of zero, first-generation and second-generation dendrimers **1**, **2,** and **3** with indazole as surface unit.

RESULTS AND DISCUSSION

The indazole was synthesized following the procedure reported by Lukin et al. [20]. The indazole thus obtained

was formylated by Vilsmeier Haack formylation followed by reduction with sodium borohydride, followed by reaction with PBr_3 in $CHCl_3$ to afford 3-(bromomethyl)-1*H*-indazole in good yield.

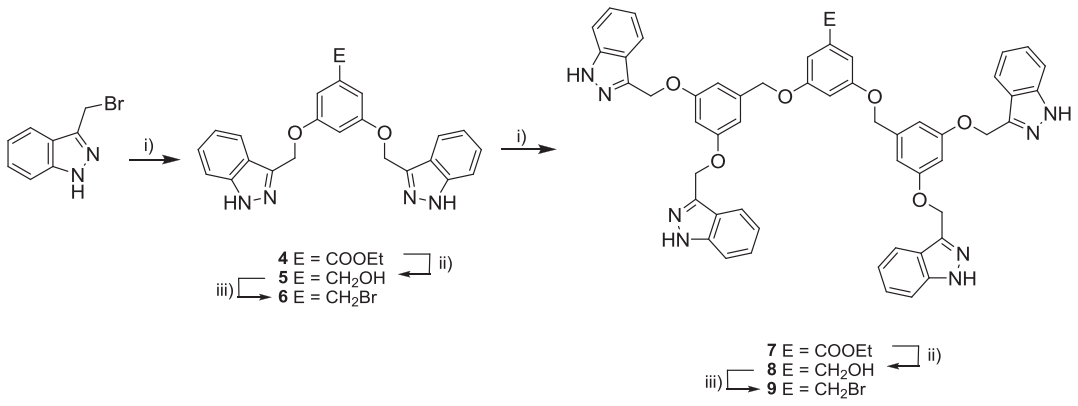
In order to achieve the synthesis of dendritic wedge for the first-generation dendrimer, the dendritic ester G1-COOEt **4** was obtained in 81% yield by reacting two equivalents of 3-(bromomethyl)-1*H*-indazole with one equivalent of ethyl 3,5-dihydroxy benzoate in dry DMF in the presence of K_2CO_3 (Scheme 1). In the ^{1}H NMR spectrum of G1-ester **4** showed triplet at δ 1.37–1.42 for the ester methyl protons and quartet at δ 4.34–4.41 for ester methylene protons and a singlet at δ 5.18 for *O*-methylene protons, respectively, in addition to the signals for the aromatic protons. The ^{13}C NMR spectrum of G1-COOEt **4** showed the ester methyl, methylene, and *O*-methylene carbon peaks at δ 14.3, δ 61.3, and δ 67.5, respectively. The carbonyl carbon appeared at δ 166.2 in addition to the signals for the aromatic carbons.

The G1-COOEt **4** was then reduced with LAH in dry THF at 50°C to give the alcohol **5**, G1-CH_2OH in 76% yield which on bromination with PBr_3 to afford the first-generation dendritic bromide G1-CH_2Br **6** in 74% yield (Scheme 1). In the ^{1}H NMR spectrum, the first-generation dendritic alcohol **5** (G1-CH_2OH) displayed the hydroxymethylene and *O*-methylene protons at δ 4.61 and δ 5.17, respectively, in addition to other the signals for the aromatic protons from δ 6.45 to δ 7.47. In the ^{13}C NMR spectrum, the first-generation dendritic alcohol **5** (G1-CH_2OH) showed the hydroxymethylene and *O*-methylene carbons at δ 67.2 and δ 68.0, respectively, in addition to the signals for the other aromatic carbons. In the ^{1}H NMR spectrum, the first-generation dendritic bromide **6** (G1-CH_2Br) displayed the bromo methylene and *O*-methylene protons at δ 4.65 and δ 5.15, respectively, in addition to the signals for the other aromatic protons from δ 6.57–7.55. In the ^{13}C NMR spectrum, the first-generation dendritic bromide **6** (G1-CH_2Br) showed the bromo methylene and *O*-methylene carbons at δ 65.3 and δ 67.2, respectively, in addition to the signals for the other aromatic carbons.

In a similar manner, the second-generation dendritic bromide **9** was synthesized by repeating the reaction sequence [21] (Scheme 1). In the ^{1}H NMR spectrum, the second-generation dendritic ester **7** (G2-COOEt) displayed triplet at δ 1.37–1.41 for ester methyl protons and quartet at δ 4.33–4.40 for ester methylene protons, respectively, and the two *O*-methylene protons appeared at δ 5.15 and δ 5.17, respectively, in addition to the signals for the aromatic protons from δ 6.60 to δ 7.55. In the ^{13}C NMR spectrum, the second-generation dendritic ester **7** (G2-COOEt) showed the ester methyl carbon at δ 14.7 and ester methylene carbon at δ 61.3 and the two *O*-methylene carbons at δ 67.3 and δ 70.1, respectively, in addition to the signals for the aromatic carbons. In the ^{1}H NMR spectrum, the second-generation dendritic alcohol **8** (G2-CH_2OH) displayed three distinct *O*-methylene protons at δ 4.71, δ 4.99, and δ 5.14, respectively, in addition to the signals for the other aromatic protons from δ 6.59 to δ 7.55. In the ^{13}C NMR spectrum, the second-generation dendritic alcohol **8** (G2-CH_2OH) showed three distinct methylene carbons at δ 67.2, δ 67.8, and at δ 69.7, respectively, in addition to the signals for the aromatic carbons. In the ^{1}H NMR spectrum, the second-generation dendritic bromide **9** (G2-CH_2Br) showed the two distinct signals for the *O*-methylene protons at δ 4.40 and δ 5.01 and one bromo methylene protons at δ 5.12 , respectively, in addition to other aromatic protons at δ 6.56 to δ 7.40. In the ^{13}C NMR spectrum, the second-generation dendritic bromide **9** (G2-CH_2Br) showed two *O*-methylene carbons at δ 4.42 and δ 5.03 and one bromo methylene carbon at δ 70.2 , respectively, in addition to the signals for the other aromatic carbons.

The zero-generation G0 dendrimer was obtained in 82% yield by reacting three equivalents of 3-(bromomethyl)-1*H*-indazole with one equivalent of 1,3,5-trihydroxybenzene in

Scheme 1. Reagents and condition: (i) Ethyl 3,5-dihyroxy ethylbenzoate, dry DMF, K_2CO_3, RT, 24 h, **4** (81%), **7** (80%); (ii) LAH, THF, 50°C, 18 h, **5** (76%), **8** (77%); (iii) PBr_3, $CHCl_3$, 0°C to RT, 12 h, **6** (74%), **9** (71%).



dry DMF in the presence of K_2CO_3 (Scheme 2). In ^1H NMR spectrum of dendrimer **1**, the methylene proton appeared at δ 5.13 in addition to the signals for the other aromatic protons from δ 6.31 to δ 7.56.

In ^{13}C NMR spectrum of dendrimer **1**, the methylene carbon appeared at δ 67.3 in addition to signals for the other aromatic carbons.

The first-generation dendrimer **2** was obtained in 72% yield by reacting three equivalents of the first-generation dendritic bromide **6 (**G1-CH_2Br) with one equivalent of the core unit, namely, 1,3,5-trihydroxybenzene in dry DMF in the presence of K_2CO_3 (Scheme 2). In the ^1H NMR spectrum, the first-generation dendrimer **2** showed two distinct *O*-methylene protons at δ 4.63 and δ 5.13 in addition to the signals for the aromatic protons from δ 6.56 to δ 7.55. In the ^{13}C NMR spectrum, the first-generation dendrimer **2** displayed two distinct *O*-methylene carbons at δ 65.2 and δ 67.2, respectively, in addition to the signals for the aromatic carbons.

The second-generation dendrimer **3** was obtained in 70% yield by reacting three equivalents of the second-generation dendritic bromide **9 (**G2-CH_2Br) with one equivalent of the core unit, namely, 1,3,5-trihydroxybenzene in dry DMF in the presence of K_2CO_3 (Scheme 2). In the ^1H NMR spectrum, the second-generation dendrimer **3** displayed three distinct

Scheme 2. Reagents and condition: (i) 3.1 equiv of 3-(bromomethyl)-1*H*-indazole, dry DMF, K2CO3, RT, 24 h, **1** (82%); (ii) 3.1 equiv of **6**, dry DMF, K2CO3, RT, 24 h, **2** (72%); (iii) 3.1 equiv of **9**, dry DMF, K2CO3, RT, 24 h, **3** (70%).

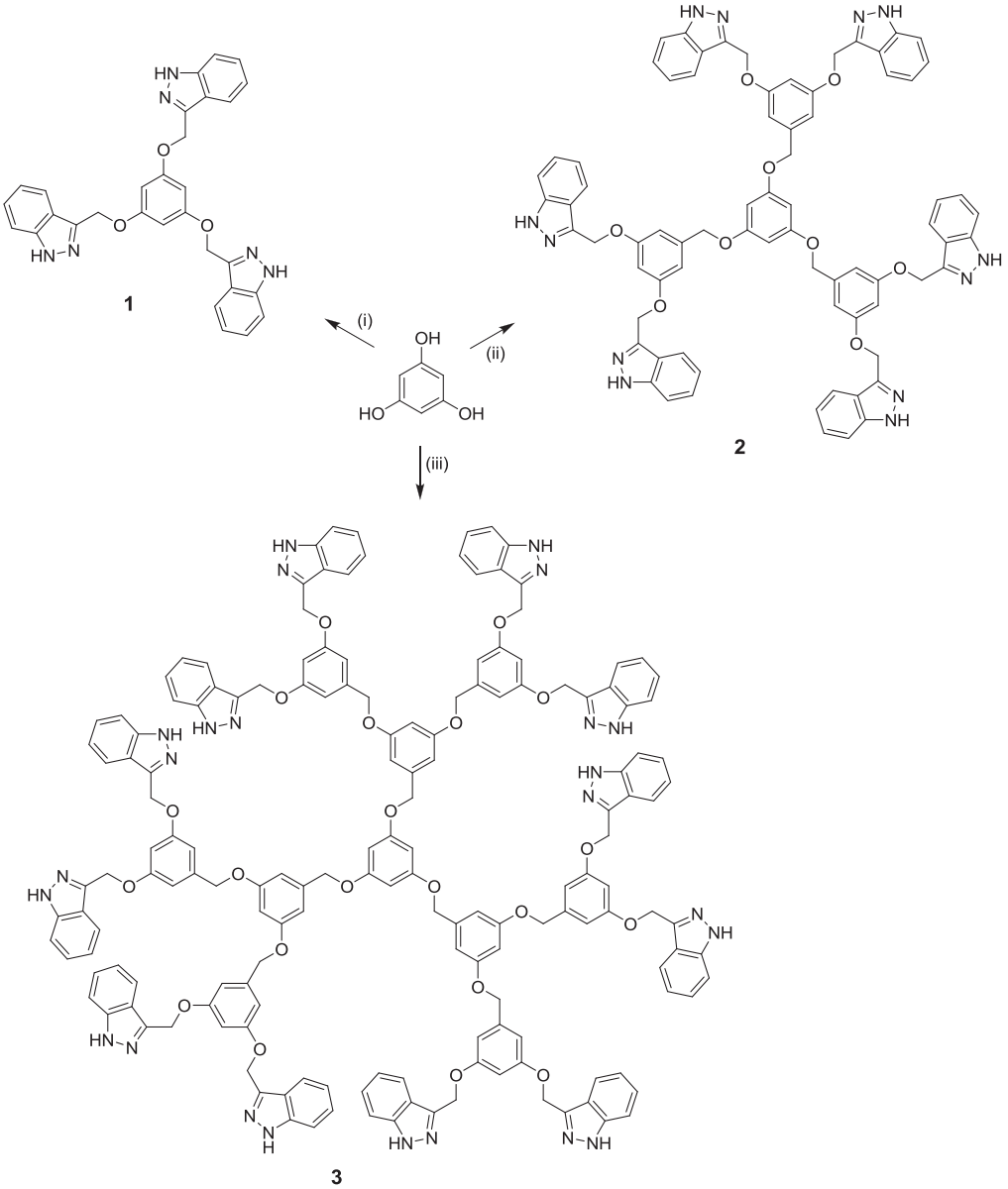


Table 1

Inhibition effect of dendrimers **1** and **3** on A549 lung adenocarcinoma cell lines.

Concentration (μ*M*)	% inhibition of cell growth	
	1	**3**
0.98	18.34 ± 0.61	17.58 ± 0.05
1.95	25.23 ± 0.71	23.00 ± 0.20
3.91	33.18 ± 0.56	24.57 ± 0.35
7.81	39.97 ± 0.56	34.30 ± 0.25
15.63	43.77 ± 3.04	36.78 ± 0.30
31.25	49.59 ± 0.05	38.65 ± 0.15
62.5	68.54 ± 0.15	39.87 ± 0.15
125	87.74 ± 0.71	59.68 ± 0.30
250	91.54 ± 0.35	80.40 ± 0.46
500	92.25 ± 0.15	91.49 ± 1.01

Values were expressed in terms of mean ± SEM (n = 3).

O-methylene protons at δ 4.76, δ 5.07, and δ 5.13, respectively, in addition to the signals for the aromatic protons from δ 6.56 to δ 7.55. In the ^{13}C NMR spectrum, the second-generation dendrimer **3** showed three distinct *O*-methylene carbons at δ 65.2, δ 67.2, and at δ 70.1 in addition to the signals for the other aromatic carbons.

Inhibitory effect of dendrimer 1 and dendrimer 3 against human A549 lung adenocarcinoma cells. The A549 lung adenocarcinoma cells were treated with various concentrations (0.98–500 μ*M*/well) of dendrimer **1** and dendrimer **3** and subjected to MTT assay (Table 1). The results showed that treatment of A549 cells with dendrimer **1** resulted in significant dose-dependent reduction in cell growth ranging from 18.34 ± 0.61 to 92.25 ± 0.15 after 24 h. Similarly, dendrimer **3** showed inhibitory activity against A549 cells ranging from

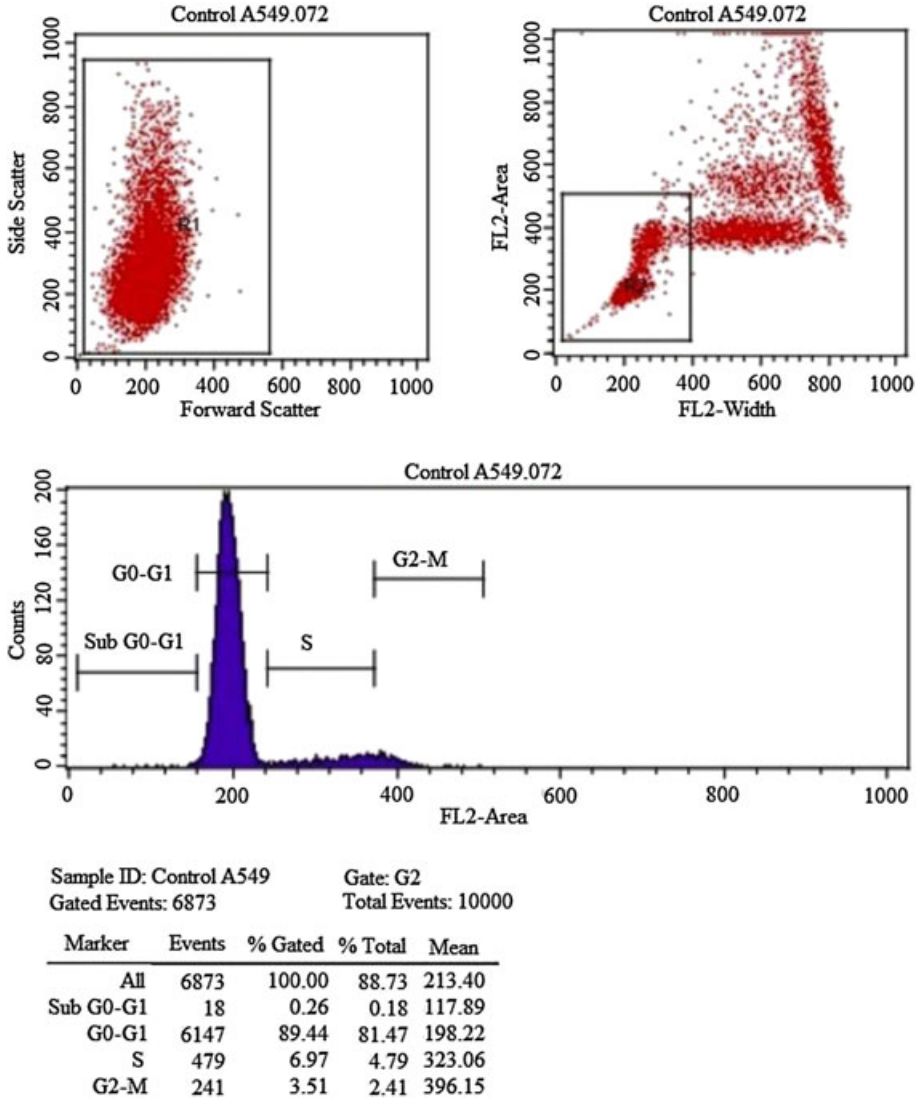


Figure 1. Flow cytometric propidium iodide fluorescence pulse width of control. [Color figure can be viewed at wileyonlinelibrary.com]

17.58 ± 0.05 to 91.49 ± 1.01. The IC_{50}value was found to be 26.11 and 73.39 μ*M* for dendrimer **1** and dendrimer **3**, respectively.

Cell cycle analysis of dendrimers 1 and 3. The effect of the dendrimer **1** and dendrimer **3** was studied on the cell cycle phases of the studied A549 cells. After 24 h of incubation, stability in all the cell cycle population is generally noticed and compared with the control cell line without treatment. Treatment with dendrimer **1** at its two tested-concentration treatments increased the cells in the G2/M phase from 0.18 to 10.54% in 26-μ*M*-treated cells and 23.09% in 52-μ*M*-treated cells, respectively (Fig. 1).

An increased population in the Sub G0-G1 phase with concomitant decrease in the G0/G1 phase suggests that dendrimer **1** inhibited cell cycle progression and subjected the cells to apoptosis, which is evident from cell accumulation in SubG0–G1 phase (Fig. 2).

Similarly, treatment with dendrimer **3** at 75 and 150 μ*M* showed accumulation of cells in the synthesis phase (S phase) with a percentage of 10.26 and 20.31%, respectively. An increased cell population in the S phase with a concomitant decrease in the G0/G1 compared with the untreated cells suggests that dendrimer **3** inhibited the cell cycle progression in S

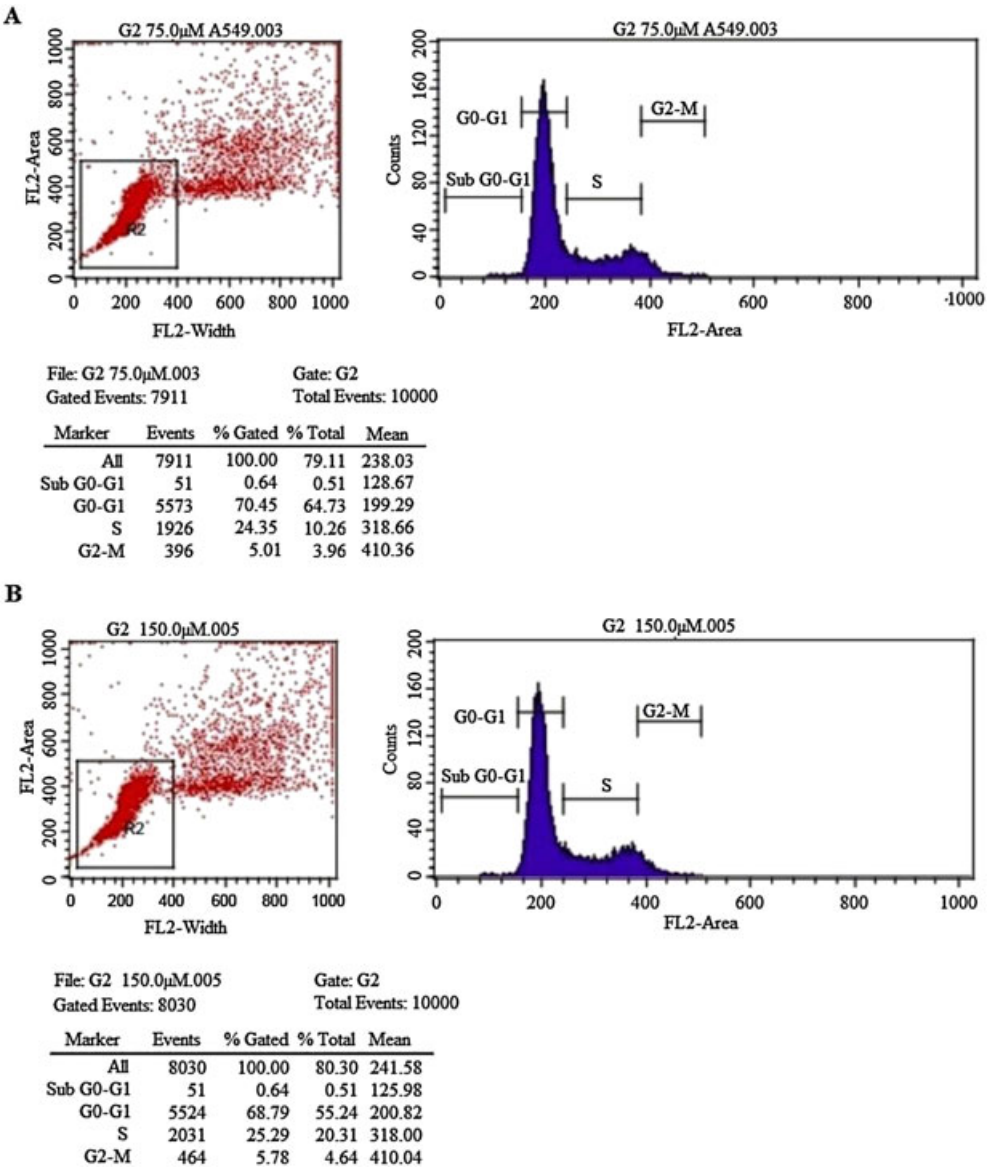


Figure 2. Effect of cell cycle phase on A549 lung adenocarcinoma cell lines. (**A**) FL2-A distribution of A549 lung adenocarcinoma cell lines treated with G0 dendrimer **1** (26 μ*M*) for 24 h, harvested, fixed in 75% ethanol, and stained with propidium iodide (PI); (**B**) FL2-A distribution of A549 lung adenocarcinoma cell lines treated with G0 dendrimer **1** (52 μ*M*) for 24 h, harvested, fixed in 75% ethanol, and stained with PI. [Color figure can be viewed at wileyonlinelibrary.com]

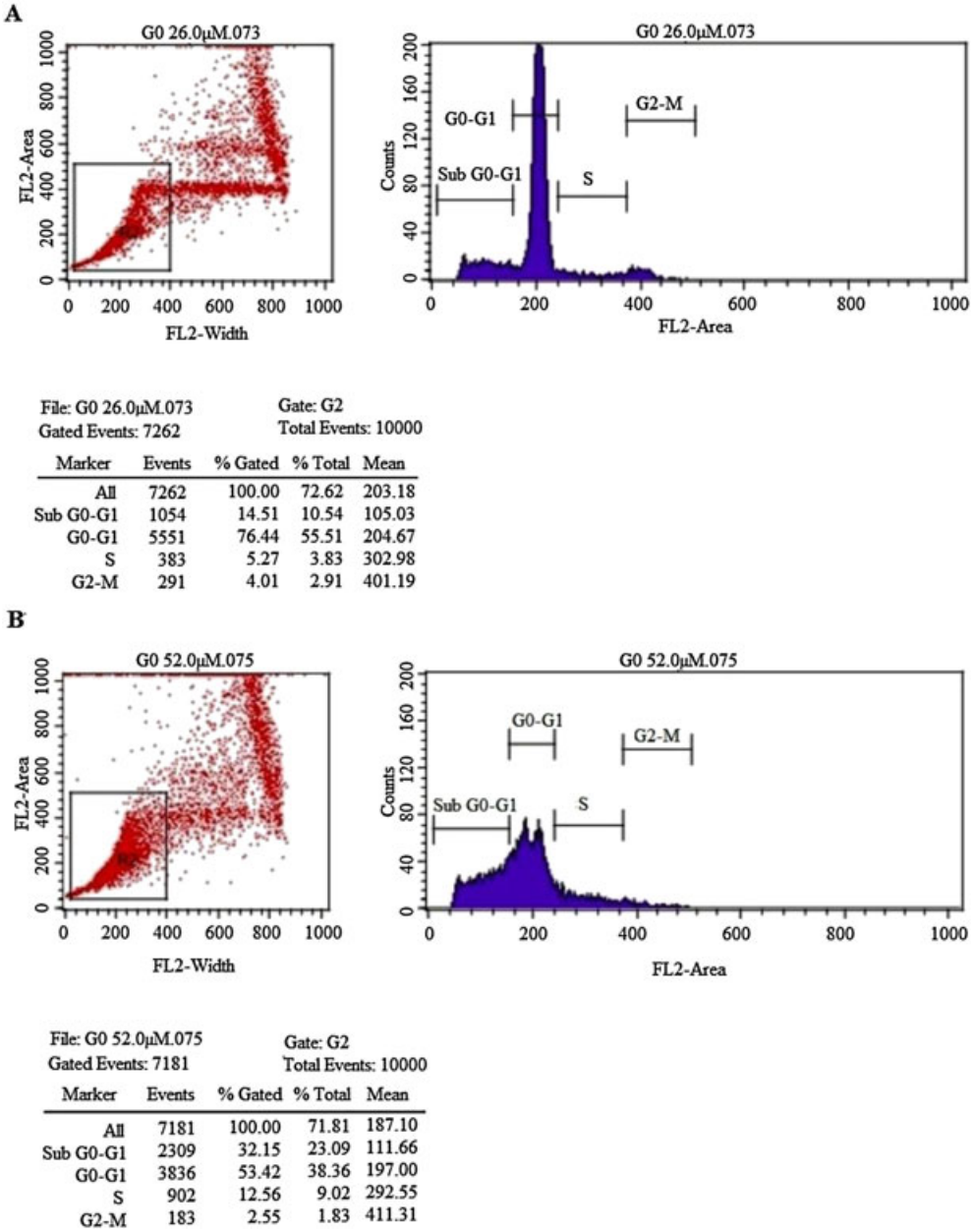


Figure 3. (**A**) FL2-A distribution of A549 lung adenocarcinoma cell lines treated with G2 dendrimer **3** at a concentration of 75 µ*M* for 24 h, harvested, fixed in 75% ethanol, and stained with propidium iodide (PI); (**B**) FL2-A distribution of A549 lung adenocarcinoma cell lines treated with G2 dendrimer **3** at a concentration of 150 µ*M* for 24 h, harvested, fixed in 75% ethanol, and stained with PI. [Color figure can be viewed at wileyonlinelibrary.com]

phase (Fig. 3). These results were summarized in Figure 4.

CONCLUSIONS

Dendrimers with indazole surface units were synthesized up to second generation in good yields by *O*-alkylation method. The cell proliferation A549 cells were inhibitory activity against human A549 lung adenocarcinoma cells in suspension culture. The cell cycle distribution assessed by flow cytometry suggests that cell cycle progression is inhibited in the G0/G1.

EXPERIMENTAL

Chemistry. ***General.*** All the chemicals and solvents were purchased commercially and used as such without further purification. All melting points of those synthesized

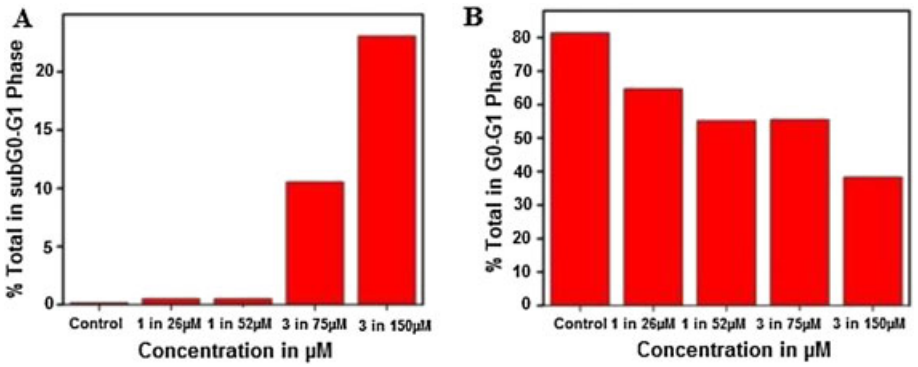


Figure 4. (A) Cell population in SubG0–G1 phase; (B) cell populationin G0–G1. [Color figure can be viewed at wileyonlinelibrary.com]

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 reference. The column chromatography was performed on silica gel (ACME, 100–200 mesh). Routine monitoring of the reaction was made by using thin-layer chromatography (TLC) developed on 0.25-mm glass plates coated with silica gel-G (ACME) 0.25-mm thick and visualized with iodine.

General procedure for O-alkylation reaction (procedure A). A solution containing the hydroxyl compound (1.0 equiv) and the tribromide (3.1 equiv) was stirred with K_2CO_3 (5.1 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 × 150 mL), dried over Na_2SO_4, and 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.

Dendritic ester 4 (G1-COOEt). White solid; mp: 84–87°C; yield: 81%; ^{1}H NMR: (300 MHz, $CDCl_3$) δ_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), 6.82–6.83 (m, 1H), 7.26–7.29 (m, 5H), 7.30–7.34 (m, 3H), 7.36–7.42 (m, 2H), 7.54–7.57 (m, 2H); ^{13}C NMR: (75 MHz, $CDCl_3$) δ_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 $C_{25}H_{22}N_4O_4$: C, 67.86; H, 5.01; found: C, 67.52; H, 4.79; *m*/*z*: 443 [M + 1].

Dendritic ester 7 (G1-COOEt). White solid; mp: 92–95°C; yield: 80%; ^{1}H NMR: (300 MHz, $CDCl_3$) δ_H 1.37–1.41 (t, J = 7.2 Hz, 3H), 4.33–4.40 (q, J_1 = 7.2 Hz, J_2 = 14.4 Hz, 2H), 5.15 (s, 4H), 5.17 (s, 8H), 6.60 (s, 2H), 6.71–6.80 (m, 4H), 7.26–7.29 (m, 13H), 7.37–7.39 (m, 6H), 7.53–7.55 (m, 4H); ^{13}C NMR: (75 MHz, $CDCl_3$) δ_C 14.7, 61.3, 67.3, 70.1, 101.7, 106.7, 114.1, 127.0, 128.9, 129.1, 129.4, 132.7, 134.5, 159.6, 159.9; *Anal*. Calcd for $C_{55}H_{46}N_8O_8$: C, 69.76; H, 4.90; found: C, 69.53; H, 4.62; *m*/*z*: 947 [M + 1].

General procedure for LAH reduction (procedure B). Ester (1.0 equiv) in THF (30 mL) was added to a stirred suspension of LAH (1.2 equiv) in dry THF (100 mL) at 0°C under inert atmosphere [19]. 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 (3 × 50 mL), and the combined THF layers were evaporated. The residue was dissolved in $CHCl_3$ and extracted with $CHCl_3$ (3 × 100 mL), washed with brine (100 mL), dried over Na_2SO_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.

Dendritic hydroxyl compound 5 (G1-CH$_2$OH). Off-white solid; mp: 91–94°C; yield: 76%; ^{1}H NMR: (300 MHz, $CDCl_3$) δ_H 4.61 (s, 2H), 5.17 (s, 4H), 6.45 (s, 1H), 6.59 (s, 2H), 7.08–7.12 (m, 4H), 7.13–7.16 (m, 4H), 7.45–7.47 (m, 2H); ^{13}C NMR: (75 MHz, $CDCl_3$) δ_C 67.2, 68.0, 101.1, 105.9, 127.0, 128.0, 128.8, 129.2, 129.4, 132.7, 134.6, 144.0, 159.9; *Anal*. Calcd for $C_{25}H_{20}N_4O_3$: C, 68.99; H, 5.03; found: C, 68.56; H, 4.83; *m*/*z*: 401 [M + 1].

Dendritic hydroxyl compound 8 (G2-CH$_2$OH). Off-white solid; mp: 101–103°C; yield: 77%; ^{1}H NMR: (300 MHz, $CDCl_3$) δ_H 4.71 (s, 2H), 4.99 (s, 4H), 5.14 (s, 8H), 6.59–6.51 (m, 2H), 6.58–6.64 (m, 2H), 6.68–6.70 (m, 6H), 7.23–7.31 (m, 9H), 7.37–7.40 (m, 6H), 7.53–7.55 (m, 4H); ^{13}C NMR: (75 MHz, $CDCl_3$) δ_C 67.2, 67.8, 69.7, 100.8, 101.4, 105.6, 106.5, 114.0, 126.9, 128.5, 128.9, 129.1, 129.3, 132.6, 134.3, 139.6, 144.5, 159.8; *Anal*. Calcd for $C_{53}H_{44}N_8O_7$: C, 70.34; H, 4.90; found: C, 70.17; H, 4.59; *m*/*z*: 905 [M + 1].

General procedure for bromination (procedure C). 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 $CHCl_3$ (3 × 100 mL), washed with aq., $NaHCO_3$ (100 mL), and brine (100 mL), dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography using $CHCl_3$/hexane (2:3) as eluent.

3-(Bromomethyl)-1H-indazole. Colorless liquid; lachrymatory. Yield: 72%; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 4.79 (s, 2H), 7.22–7.31 (m, 2H), 7.35–7.38 (m, 1H), 7.47–7.49 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 62.9, 127.0, 128.8, 128.9, 129.4, 132.8, 138.2; *m*/*z*: 212 [M + 1].

Dendritic bromo compound 6 (G1-CH$_2$Br). White solid; mp: 76–79°C; yield: 74%; ^{1}H NMR: (300 MHz, $CDCl_3$) δ_H 4.65 (s, 2H), 5.15 (s, 4H), 6.57 (s, 1H), 6.66 (s, 2H), 7.26–7.31 (m, 5H), 7.38–7.41 (m, 3H), 7.54–7.55 (m, 2H); ^{13}C NMR: (75 MHz, $CDCl_3$) 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 $C_{23}H_{19}BrN_4O_2$: C, 69.62; H, 4.13; found: C, 59.51; H, 3.94; *m*/*z*: 464 [M + 1].

Dendritic bromo compound 9 (G2-CH$_2$Br). White solid; mp: 94–98°C; yield: 71%; ^{1}H NMR: (300 MHz, $CDCl_3$) δ_H 4.40 (s, 4H), 5.01 (s, 2H), 5.12 (s, 8H), 6.56–6.57 (m, 2H), 6.65–6.66 (m, 5H), 7.25–7.26 (m, 8H), 7.27–7.28 (m, 9H), 7.37–7.40 (m, 5H); ^{13}C NMR: (75 MHz, $CDCl_3$) δ_C 65.5, 67.3, 70.2, 102.2, 108.4, 127.1, 127.6, 128.2, 128.7, 128.9, 129.2, 129.5, 132.7, 134.4, 140.0, 159.9; *Anal*. Calcd for $C_{53}H_{43}BrN_8O_6$: C, 65.77; H, 4.48; found: C, 65.52; H, 4.22; *m*/*z*: 968 [M + 1].

Following the general procedure A, the dendrimers **1**–**3** were synthesized in good yield by reacting three equivalents the corresponding dendritic bromide, namely, 3-(bromomethyl)-1*H*-indazole, **6** and **9** with one equivalent of 1,3,5-trihydroxybenzene in DMF at RT for 24 h.

G0 dendrimer 1. Off-white solid; mp: 88–90°C; yield: 82%; ^{1}H NMR: (300 MHz, $CDCl_3$) δ_H 5.13 (s, 6H), 6.31 (s, 3H), 7.25–7.31 (m, 6H), 7.38–7.41 (m, 3H), 7.53–7.56 (m, 3H); ^{13}C NMR: (75 MHz, $CDCl_3$) δ_C 67.3, 95.1, 126.9, 128.9, 129.0, 129.4, 132.7, 134.5, 160.4, 162.6; *Anal*. Calcd for $C_{30}H_{24}N_6O_3$: C, 69.76; H, 4.68; found: C, 69.52; H, 4.34; *m*/*z*: 517 [M + 1].

G1 dendrimer 2. Off-white solid; mp: 96–98°C; yield: 72%; ^{1}H NMR: (300 MHz, $CDCl_3$) δ_H 4.63 (s, 6H), 5.13 (s, 12H), 6.56 (s, 3H), 6.65 (s, 6H), 7.22–7.27 (m, 13H), 7.37–7.40 (m, 7H), 7.52–7.55 (m, 7H); ^{13}C NMR: (75 MHz, $CDCl_3$) δ_C 65.2, 67.2, 101.3, 106.0, 127.0, 128.9, 129.1, 129.4, 132.7, 134.6, 143.6, 160.0; *Anal*. Calcd for $C_{75}H_{60}N_{12}O_9$: C, 70.74; H, 4.75; found: C, 70.49; H, 4.48; *m*/*z*: 1273 [M + 1].

G2 dendrimer 3. Off-white solid; mp: 88–91°C; yield: 70%; ^{1}H NMR: (300 MHz, $CDCl_3$) δ_H 4.76 (s, 12H), 5.07 (s, 6H), 5.13 (s, 24H), 6.56 (s, 6H), 6.65 (s, 14H), 7.22–7.29 (m, 27H), 7.33–7.40 (m, 27H), 7.52–7.55 (m, 16H); ^{13}C NMR: (75 MHz, $CDCl_3$) δ_C 65.2, 67.2, 70.1, 101.3, 106.0, 127.0, 127.6, 128.0, 128.6, 128.9, 129.1, 129.4, 132.7, 134.6, 143.7, 159.9; *Anal*. Calcd for $C_{165}H_{132}N_{24}O_{21}$: C, 71.11; H, 4.77; found: C, 70.81; H, 4.52.

BIOLOGY

Materials and methods. ***Cell preparation and culturing.*** The A549 lung adenocarcinoma cell line was procured from the National Centre for Cell Science (NCCS), Pune, India, with the passage number of 11. Cells were maintained in Dulbecco's Minimum Essential Media (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), with 100 units/mL penicillin and 100 μg/mL streptomycin. Cells were cultured in a humidified atmosphere with 5% CO_2 at 37°C. Cells were grown in 75 cm^2 culture flask, and after a few passages, cells were seeded for experiments. The experiments were carried out at 70 to 80% confluence. Upon reaching confluence, cells were detached using 0.25% Trypsin-EDTA solution.

Cell proliferation assay or MTT assay. Proliferation of A549 cells was assessed by MTT assay [22]. The proliferation test is based on the color reaction of mitochondrial dehydrogenase in living cells by MTT. Cells were plated in 96-well plate at a concentration of 5×10^4 cells/well 24 h after plating. After 24 h of cells incubation, the medium was replaced with 100-μL medium containing MJ–MU–G0 and MJ–MU–G2 at different concentrations (0.98–500 μ*M*/well) and incubated for 24 h. Untreated cells served as control and received only 0.1% DMSO in which the drug was prepared. At the end of treatment period, media from control and drug-treated cells was discarded and 20 μL of MTT (5 mg/mL PBS) was added to each well. Cells were then incubated for 4 h at 37°C in CO_2 incubator. MTT was then discarded and the coloured crystals of produced formazan were dissolved in 200 μL of DMSO and mixed effectively by pipetting up and down. Spectrophotometrical absorbance of the purple blue formazan dye was measured using an ELISA reader (BIORAD) at 570 nm. Optical density of each sample was compared with control optical density and graphs were plotted.

Flow cytometry. To investigate the effect of dendrimer **1** and dendrimer **3** on the cell cycle distribution, A549 cells (1×10^5 cells/mL) were treated with 26 and 52 μ*M* of dendrimer **1** and 75 and 150 μ*M* of dendrimer **3** cultured for 24 h. The treated cells were harvested, washed with phosphate-buffer saline (PBS), and fixed in 75% ethanol at 4°C overnight. After washing twice with cold PBS, cells were suspended in PBS containing 40 μg/mL propidium iodide (PI) and 0.1 mg/mL RNase A followed by shaking at37°C for 30 min. The stained cells

were analyzed with flow cytometer (Becton-Dickinson San Jose, CA, USA), and the data were consequently calculated using WINMDI 2.9 software (TSRI, La Jolla, CA, USA) [23].

Acknowledgments. The authors thank the DAT-FIST, New Delhi, for providing NMR spectral analysis to the department, and Herbal and Indian Medicine Research Laboratory, Sri Ramachandra University, Chennai-600 116 for in vitro anti-cancer studies.

REFERENCES AND NOTES

[1] Ali, Z.; Ferreira, D.; Carvalho, P.; Avery, M. A.; Khan, I. A. J. Nat Prod 2008, 71, 1111.

[2] Mosti, L.; Menozzi, G.; Fossa, P.; Filippelli, W.; Gessi, S.; Rinaldi, B.; Falcone, G. Arzneimittelforschung 2000, 50, 963.

[3] Showalter, H. D. H.; Angelo, M. M.; Leopold, L. S. J Med Chem 1988, 31, 1527.

[4] Jakupec, M. A.; Reisner, E.; Eichinger, A.; Pongratz, M.; Arion, V. B.; Galanski, M.; Hartinger, C. G.; Keppler, B. K. J. Med Chem 2005, 48, 2831.

[5] Raffa, D.; Daidone, G.; Maggio, B.; Schillaci, D.; Plescia, F. Arch Pharm Pharm Med Chem 1999, 332, 317.

[6] Vishnu, K. T.; Dharmendra, B. Y.; Praveen, K. S. Bioorg Med Chem Lett 2005, 15, 3288.

[7] Stefan, B.; Carmen, G.; Kerstin, K. Bioorg Med Chem 2002, 10, 2415.

[8] Rodgers, J. D.; Chang, C. H. Bioorg Med Chem Lett 1996, 6, 2919.

[9] Vicente, J. A.; Eduardo, E. C. Bioorg Med Chem 2005, 13, 3197.

[10] Michel, B.; Elodie, L.; Sylvain, R. Bioorg Med Chem Lett 2007, 17, 3177.

[11] Han, W.; Pelletier, J. C.; Hodge, C. N. Tricyclic ureas: a new class of HIV-1 protease inhibitors Bioorg Med Chem Lett 1998, 8, 3615.

[12] Patel, M.; Rodgers, J. D.; McHugh, R. J. Jr.; Johnson, B. L.; Cordova, B. C.; Klaba, R. M.; Bacheler, L. T.; Erickson-Viitanen, S.; Ko, S. S. Unsymmetrical cyclic ureas as HIV-1 protease inhibitors: novel biaryl indazoles as P 2/P2′ substituents Bioorg Med Chem Lett 1999, 9, 3217.

[13] Sun, J.-H.; Teleha, C. A.; Yan, J.-S.; Rodgers, J. D.; Nugiel, D. A. Efficient synthesis of 5-(bromomethyl) and 5-(aminomethyl)-1-THP-indazole J Org Chem 1997, 62, 5627.

[14] Stefan, R. J.; Edward, M.; Douglas, C. H. Synthesis and activity of substituted 4-(indazol-3-yl) phenols as pathway-selective estrogen receptor ligands useful in the treatment of rheumatoid arthritis J Med Chem 2004, 47, 6435.

[15] Goodman, K. B.; Cui, H.; Dowdell, S. E.; Gaitanopoulos, D. E.; Ivy, R. L.; Sehon, C. A.; Stavenger, R. A.; Wang, G. Z.; Viet, A. Q.; Xu, W. Development of dihydropyridone indazole amides as selective rho-kinase inhibitors J Med Chem 2007, 6 *Molbank* 2012 M770 (Page 4).

[16] Elliott, E. L.; Bushell, S. M.; Cavero, M.; Tolan, B.; Kelly, T. R. Total synthesis of nigellicine and nigeglanine hydrobromide Org Lett 2005, 7, 2449.

[17] Inamoto, K.; Katsuno, M.; Yoshino, T.; Arai, Y.; Hiroya, K.; Sakamoto, T. Synthesis of 3-substituted indazoles and benzoisooxazoles via Pd-catalyzed cyclization reactions: application to the synthesis of nigellicine Tetrahedron 2007, 63, 2695.

[18] Marie, J.-P.; Ziltoin, R.; Delmer, A.; Thevenin, D.; Suberville, A.-M. Prognostic value of clonogenic assay for induction and duration of complete remission in acute myelogenous leukemia Leukemia 1987 121.

[19] Griffen, J. D.; Lowenberg, B. Clonogenic cells in acute myeloblastic leukemia Blood 1986, 68, 1185.

[20] Lukin, K.; Hsu, M. C.; Fernando, D.; Leanna, M. R. New practical synthesis of indazoles via condensation of *O*-fluorobenzaldehydea and their *O*-methyloximes with hydrazine J Org Chem 2006, 71, 8166.

[21] Rajakumar, P.; Jayanthi, M. Synthesis and analysis of in vitro anti-arthritic activity of dendrimers with methyl, ethyl and isopropyl salicylates as surface groups RSC Adv 2014, 4, 63208.

[22] Safadi, F. F.; Xu, J.; Smock, S. L.; Kanaan, R. A.; Selim, A. H.; Odgren, P. R.; Marks, S. C.Jr.; Owen, T. A.; Popoff, S. N. Expression of connective tissue growth factor in bone: its role in osteoblast proliferation and differentiation in vitro and bone formation in vivo J Cell Physiol 2003, 196, 51.

[23] Tu, L. C.; Chou, C. K.; Chen, C. Y.; Chang, Y. T.; Shen, Y. C.; Yeh, S. F. Characterization of the cytotoxic mechanism of Mana-Hox, an analog of manzamine alkaloids Biochim Biophys Acta 2004, 1672, 148.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.