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Short communication

Synthesis and cardio protective biological applications of glucodendrimers by H9C2 cell studies

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ABSTRACT

Novel glucodendrimers scaffolds containing α-D-glucopyranoside at the surface and triazole as bridging unit have been synthesized. Cardiomyocytes were exposed to normal and high glucose level in the presence and absence of glucodendrimers. Cytotoxicity studies were also carried out and the expression of metalloproteinases mainly MMP-2 and 9 was confirmed with gelatine zymography and RT-PCR gene expression studies. Cardio protective efficiency of the synthesized glucodendrimers against high glucose induced toxicity on mettalloproteinase-2 and 9 and also on H9C2 cell lines revealed that higher generation glucodendrimers **6** and **8** are more effective than the lower generation glucodendrimers.

1. Introduction

The incidence of diabetes mellitus is increasing worldwide and currently over 240 million people suffer from diabetes, which is projected to rise to 380 million by 2025 (Kengne, Amoah, & Mbanya, 2005; Wild, Roglic, Green, Sicree, & King, 2004). Cardiovascular diseases are responsible for 80% of deaths among diabetic patients. Diabetic cardiomyopathy can be characterized functionally by ventricular dilation, myocyte hypertrophy, prominent interstitial fibrosis (Scherpereel & Tavernier, 2001). Several lines of study suggests that hyperglycemia is the main cause for the development of diabetic complications. However the mechanism responsible for the high glucose induced alterations are not well understood. Increased oxidative stress, depletion of antioxidant levels, increased inflammatory cytokines and altered activity of matrix metalloproteinase could be the root cause for cardiac failure in diabetic patients (Hayashi, Mori, Yamashita, & Miyamura, 2008) cadiac muscular function is highly influenced by the activities of metalloproteinase -2 and 9. Furthermore, the altered activity of matrix metalloproteinases (MMP) particularly MMP-2 and MMP-9 could be due to increased accumulation of extra cellular matrix (ECM) protein which are the causal agents for the cardiac fibrosis and organ damage in diabetes (Basta, Schmidt, & De Caterina, 2004; Mohamad et al., 2011) Dys-regulation of metalloproteinases especially MMP-2 and MMP-9 was noticed under diabetic condition. Though the cellular activity of MMPs are regulated by several factors, any compound which have inhibitory activity over the MMPs may have beneficial effects in the treatment of high glucose induced cardiovascular diseases (Sang et al., 2006). Hence there is a need for the development of new drugs which can exhibit hypoglycemic, anti-oxidant efficacy and beneficial effects in the treatment of complications associated with diabetes (Chin, Balunas, Chai, & Kinghorn, 2006).

The glucodendrimer (Camponovo et al., 2009; Kikkeri, Liu, Adibekian, Tsai, & Seeberger, 2010; Moses & Moorhouse, 2007; Rajakumar & Ananthan, 2011; Rajakumar, Anandhan, & Kalpana, 2009; Roy, 2003) has been known to enhance binding to cognate receptors. Since many key biological processes involve binding of sugars to receptors (Andrea et al., 2001), multivalent glucose residues in dendrimeric sugars can be exploited in therapeutic processes. Carbohydrates either bind to proteins or lipids (Imberty, Chabre, & Roy, 2008; Kikkeri, Hossain, & Seeberger, 2008; Roy, Baek, & Olson, 2001; Wu et al., 2008), to play the essential role as communication molecules in many inter and intracellular signaling pathways of many cellular processes. In particular, glucodendrimers are important mediators in cell–cell recognition (Doores, Gamblin, & Davis, 2006; Rabinovich et al., 2006), and cell signaling regulation (Camby, Mercier, Lefranc, & Kiss, 2006; Collins & Paulson, 2004), cellular differentiation (Nishiwaki et al., 2004) and immune response processess (Lowe, 2001). Recent studies show that free radicals produced by high glucose levels in diabetic patients causes organ damage especially heart. The exact mechanism is underlying

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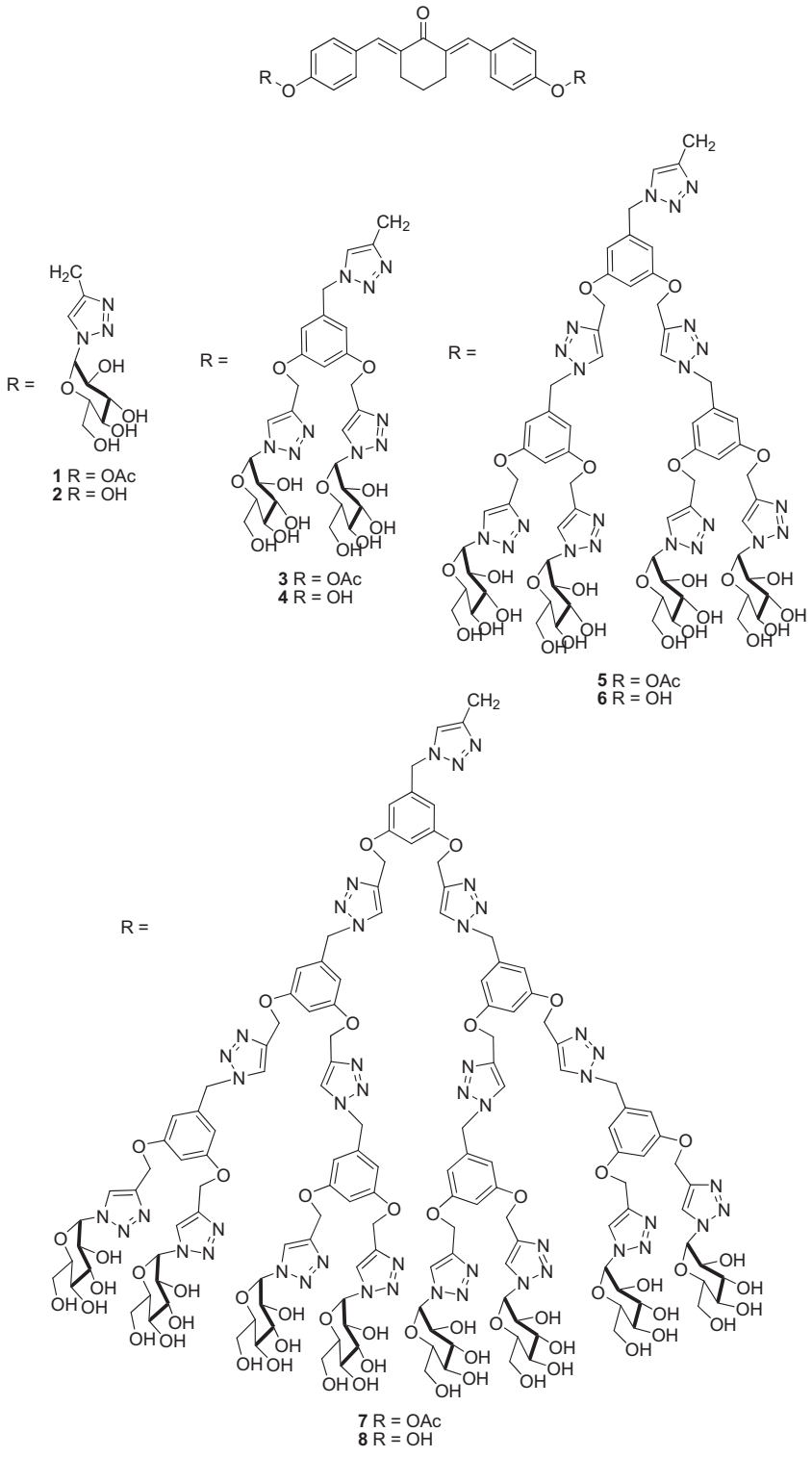


Fig. 1. Structure of glucodendrimers.

the high glucose induced toxicity, and factors playing significant role in inducing pathological alterations in cardiac muscles of diabetic patients needs further more understanding. In order to understand the mechanism of high glucose induced pathological changes, in vitro study has to be carried out using H9C2 cardiac myocytes. The present study designed mainly to investigate the effect of glucodendrimers **2**, **4**, **6** and **8** (Fig. 1) against high glucose induced toxicity on MMP-2 and 9.

2. Experimental

2.1. Materials

Analytical TLC was performed on commercial Merk plates coated with Silica Gel GF254. Analytical samples were obtained from flash silica gel chromatography, using silica gel of 100–200 mesh and elution with solvent system as mentioned order each

experiment. ^{1}H and ^{13}C NMR spectra were recorded on a 300 MHz BRUKER AVANCE (75 MHz for ^{13}C NMR) spectrometer. All chemical shifts values are reported in δ_{ppm} relative to internal standard tetramethylsilane (TMS, δ 0.00). ^{13}C chemical shifts are reported relative to $CDCl_3$ (center of triplet, δ 77.23) or relative to DMSO-d_6 (center of septet, δ 39.51). The spin multiplicities are indicated by the symbols s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br (broad), dd (doublet of doublets). The coupling constants *J*, are reported in Hertz (Hz). Elemental analysis data was recorded on Vario EL III (CDRI, Lucknow) instrument. Mass spectra (M.S) were recorded obtained using Fast Atom Bombardment and MALDI-TOF. Fourier transform infrared (FTIR) were recorded using Perkin–Elmer RX1 Spectrophotometer. Dulbecco modified Eagle medium (DMEM), Trypsin-EDTA, Fetal Bovine Serum, and Antibiotic solution from HIMEDIA (Mumbai, India); TRIsol reagent were obtained from Bangalore GeNei (Bangalore, India). Whole cell lysis buffer was purchased from Thermo Scientific. All other chemicals used were of reagent grade (SRL-Mumbai).

2.2. General procedure for the synthesis of Cu(I) catalyzed click reaction (procedure A)

A mixture of acetylenic derivative (1.0 mmol) and acetylated glycodendritic azide (2.1 mmol, 2.1 equiv.) was dissolved in *t*-BuOH/H_2O (1:1; 8 mL) and added sodium ascorbate (0.4 mmol, 0.4 equiv.) followed by the addition of $CuSO_4$ (0.2 mmol, 0.2 equiv.). The reaction mixture was stirred overnight at room temperature. The solvent was evaporated and the crude product was dissolved with EtOAc (2 × 100 mL), washed with NH_4Cl solution (50 mL) and brine solution (50 mL), dried over Na_2SO_4 and concentrated to give a residue, which was purified by column chromatography (SiO_2), using the eluent as mentioned under each compound.

2.3. General procedure for conversion of dendritic chloride to azide (procedure B)

Dendritic chloride (1.0 mmol, 1.0 equiv.) was dissolved in a mixture of acetone/water (4:1; 8 mL) and added NaN_3 (1.5 mmol, 1.5 equiv.), and the mixture was heated to 60 °C for 3 h. The reaction mixture was cooled to room temperature, acetone evaporated, diluted with water (100 mL), and extracted with EtOAc (2 × 100 mL). The organic layer was washed with saturated NaCl (100 mL), dried over Na_2SO_4, and evaporated to give the dendritic azide.

2.4. General procedure for synthesis of De-O-acetylation (Zemplen reaction) (procedure C)

Acetylated glucodendrimer (1.0 mmol, 1.0 equiv.) was dissolved in a mixture of dry MeOH/dry THF/dry CH_2Cl_2 (6:1:1, 40 mL) and added a solution of sodium methoxide (1 M NaOMe) until pH 9–10 is reached. The reaction mixture was stirred at room temperature for 48 h. At the end, water was added for the entire solubilization of the product and the solution was neutralized to pH 6–7 by the addition of ion-exchange resin (Amberlite IR 120H^+) and filtered, and the solvent was removed in vacuo with rotary evaporator. The residue was then lyophilized to yield the fully deprotected glucodendrimer.

2.5. First generation dendritic chloride **11**

Following the general procedure A, the dendritic chloride **11** was obtained as white solid from 3,5-bis(propargyloxy)benzyl chloride **10** (0.3 g, 1.3 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoazide **9** (1.01 g, 2.6 mmol). R_f = 0.6 (Ethylacetate: Hexane, 1:1); M.p.: 115 °C; ^{1}H NMR: (300 MHz, $CDCl_3$) δ = 1.85, 2.03, 2.07 (3× s, 24 H), 4.00–4.04 (m, 2 H), 4.13–4.17 (m, 2 H), 4.28–4.32 (m, 2H), 4.51 (s, 2 H), 5.20 (s, 4 H), 5.22–5.28 (m, 2 H), 5.39–5.49 (m, 4 H), 5.92 (d, *J* = 8.7 Hz, 2 H), 6.64 (s, 3 H), 7.92 (s, 2 H). ^{13}C NMR: (75 MHz, $CDCl_3$) δ = 20.1, 20.5, 20.5, 20.6, 46.0, 61.5, 61.9, 67.7, 70.3, 72.6, 75.2, 85.8, 101.7, 108.2, 121.4, 139.9, 144.6, 159.4, 168.9, 169.4, 169.9, 170.5. MS (FAB): *m/z* = 980 [M^+]. Elemental Anal. Calcd for $C_{41}H_{49}Cl_1N_6O_{20}$: C, 50.18; H, 5.03; N, 8.56%. Found: C, 50.17; H, 5.03; N, 8.60%.

2.6. First generation dendritic azide **12**

Following the general procedure B, the dendritic azide **12** was obtained as white solid from dendritic chloride **11** (4 g, 4.0 mmol) and NaN_3 (0.53 g, 8.16 mmol). R_f = 0.6 (Ethylacetate: Hexane, 1:1); M.p.: 133 °C; ^{1}H NMR: (300 MHz, $CDCl_3$) δ = 1.78, 1.96, 2.00 (3× s, 24H), 3.92–3.97 (m, 2H), 4.04–4.11 (m, 2H), 4.21–4.26 (m, 4H), 5.13 (s, 4H), 5.18–5.21 (m, 2H), 5.32–5.43 (m, 4H), 5.84 (d, *J* = 2.7 Hz, 2H). 6.50 (d, *J* = 2.1 Hz, 2H), 6.57 (d, *J* = 2.1 Hz, 1H), 7.86 (s, 2H). ^{13}C NMR: (75 MHz, $CDCl_3$) δ = 19.1, 19.5 19.5, 19.6, 53.6, 60.6, 60.9, 66.8, 69.4, 71.7, 74.2, 84.8, 100.6, 106.8, 120.3, 137.0, 143.6, 158.6, 167.9, 168.4, 168.9, 169.5. MS (EI): *m/z* = 987 [MALDI-TOF]. Elemental Anal. Calcd for $C_{41}H_{49}N_9O_{20}$: C, 49.85; H, 5.00; N, 12.76%. Found: C, 49.81; H, 4.96; N, 12.51%.

2.7. Second-generation dendritic chloride **13**

Following the general procedure A, the dendritic chloride **13** was obtained as white solid from 3,5-bis (propargyloxy) benzyl chloride **10** (0.45 g, 1.9 mmol) and dendritic azide **12** (3.6 g, 4.02 mmol). Yield: 96%; R_f = 0.72 (Ethylacetate: Hexane, 7:3); M.p.: 148 °C; ^{1}H NMR: (300 MHz, $CDCl_3$) δ = 1.82, 2.02, 2.04, 2.07 (4× s, 48H), 4.01–4.10 (m, 4H), 4.11–4.16 (m, 4H), 4.28–4.32(m, 4H), 4.48 (s, 2H), 5.14 (s, 8H), 5.17 (s, 4H), 5.27 (t, *J* = 9.6 Hz, 4H), 5.39–5.50 (m, 12H), 5.92 (d, *J* = 8.7 Hz, 4H), 6.51(d, *J* = 2.1 Hz, 4H), 6.59 (d, *J* = 2.1 Hz, 1H), 6.62 (d, *J* = 2.4 Hz, 2H), 6.64 (s, 2H), 7.63 (s, 2H), 7.96 (s, 4H). ^{13}C NMR: (75 MHz, $CDCl_3$) δ = 20.1, 20.5, 20.5, 20.6, 46.1,54.0, 60.4, 61.5, 61.8, 62.1, 67.7, 70.3, 72.6, 75.1, 85.7, 101.9, 107.7, 108.1,121.6, 123.0, 137.0, 139.8, 144.2, 144.3, 159.5, 159.7, 168.9, 169.4, 169.9, 170.5. MS (EI): *m/z* = 2210 [M^+]. Elemental Anal. Calcd for $C_{95}H_{109}Cl_1N_{18}O_{42}$: C, 51.60; H, 4.97; N, 11.41%. Found: C, 51.48; H, 4.90; N, 11.35%.

2.8. Second generation dendritic azide **14**

Following the general procedure B, the dendritic azide **14** was obtained as white solid from dendritic chloride **13** (2 g, 0.9 mmol) and NaN_3 (0.16 g, 2.4 mmol). Yield: 99%; R_f = 0.72 (Ethylacetate: Hexane, 7:3); M.p.: 159 °C; ^{1}H NMR: (300 MHz, $CDCl_3$) δ = 1.82, 2.02, 2.05. 2.07 (4× s, 48H), 4.02–4.05 (m, 4H), 4.11–4.17 (m, 4H), 4.24 (d, *J* = 4.8 Hz, 2H), 4.27–4.32 (m, 4H), 5.14 (s, 8H), 5.17 (s, 4H), 5.27 (t, *J* = 9.6 Hz, 4H), 5.39–5.50 (m, 12H), 5.93 (d, *J* = 8.7 Hz, 4H), 6.51 (s, 4H), 6.59 (s, 2H), 6.60 (s, 1H), 6.64 (s, 2H), 7.63 (s, 2H), 7.96 (s, 4H). ^{13}C NMR: (75 MHz, $CDCl_3$) δ = 20.1, 20.5, 20.5, 20.6, 54.0, 54.6, 61.6, 61.8, 62.1, 67.7, 70.4, 72.6, 75.1, 85.8, 101.7, 107.9, 107.7, 107.8, 121.6, 123.0, 137.0, 144.2, 144.3, 159.4, 159.7, 168.9, 169.4, 169.9, 170.5. MS (EI): *m/z* = 2217 [M^+]. Elemental Anal. Calcd for $C_{95}H_{109}N_{21}O_{42}$: C, 51.47; H, 4.96N, 13.27%. Found: C, 51.41; H, 4.90; N, 13.14%.

2.9. Third generation dendritic chloride **15**

Following the general procedure A, the dendritic chloride **15** was obtained as white solid from 3,5-bis(propargyloxy)benzyl chloride **10** (0.08 g, 0.34 mmol) and dendritic azide **14** (1.45 g, 0.71 mmol). Yield: 91%; R_f = 0.62 (Ethylacetate: Hexane, 4:1); M.p.: 167 °C;

^{1}H NMR: (300 MHz, $CDCl_3$) δ = 1.81, 2.02, 2.03, 2.07 (4× s, 96H), 4.03–4.07 (m, 8H), 4.09–4.16 (m, 8H), 4.27–4.32 (m, 8H), 5.10 (s, 30H), 5.28 (t, J = 9.6 Hz, 8H), 5.41–5.53 (m, 28H), 5.94 (d, J = 8.7 Hz, 8H), 6.47 (s, 14H), 6.54–6.61 (m, 7H), 7.64 (s, 4H), 7.65 (s, 2H), 7.99 (s, 8H). ^{13}C NMR: (75 MHz, $CDCl_3$) δ = 20.1, 20.5, 20.5, 20.6, 46.1, 53.9, 61.6, 61.7, 61.9, 67.8, 70.4, 71.8, 72.7, 75.1, 85.7, 101.8, 102.1, 107.7, 108.1, 121.8, 123.3, 123.4, 128.8, 130.9, 137.0, 137.1, 139.7, 143.8, 144.0, 144.2, 159.4, 159.6, 159.7, 168.9, 169.4, 169.9, 170.5. MS (MALDI): m/z = 4687 [M$^+$+Na]. Elemental Anal. Calcd for $C_{203}H_{229}Cl_1N_{42}O_{86}$: C, 52.22; H, 4.94; N, 12.60%. Found: C, 52.01; H, 4.71; N, 12.44%.

2.10. Third generation dendritic azide **16**

Following the general procedure B, the dendritic azide **16** was obtained as white solid from dendritic chloride **15** (0.47 g, 0.1 mmol) and NaN_3 (0.016 g, 0.25 mmol). Yield: 95%; R_f = 0.62 (Ethylacetate: Hexane, 4:1); M.p.: 174 °C; ^{1}H NMR: (300 MHz, $CDCl_3$) δ = 1.81, 2.02, 2.07 (4× s, 96H), 4.05–4.1 (m, 8H), 4.13–4.21 (m, 8H), 4.23–4.32 (m, 8H), 5.10 (s, 30H), 5.28 (t, J = 9.6 Hz, 8H), 5.41–5.53 (m, 28H), 5.95 (d, J = 8.4 Hz, 8H), 6.47 (s, 14H), 6.55–6.61 (m, 7H), 7.65 (s, 6H), 8.00 (s, 8H). ^{13}C NMR: (75 MHz, $CDCl_3$) δ = 20.1, 20.5, 20.6, 20.6, 53.9, 54.0, 54.5, 61.6, 61.7, 67.7, 70.3, 71.8, 72.7, 75.0, 85.7, 101.8, 102.0, 107.7, 114.1, 121.8, 123.4, 128.8, 130.9, 132.4, 137.0, 137.1, 137.9, 143.8, 144.0, 144.2, 159.6, 159.7, 169.0, 169.5, 170.0, 170.5. MS (MALDI): m/z = 4695 [M$^+$+Na]. Elemental Anal. Calcd for $C_{203}H_{229}N_{45}O_{86}$: C, 52.15; H, 4.94; N, 13.48%. Found: C, 52.02; H, 4.83; N, 13.39%.

2.11. Bis(propargyloxy)enone **18**

A mixture of enone **17** (0.5 g, 1.4 mmol), propargyl bromide (0.3 mL, 3.08 mmol) in the presence of K_2CO_3 in DMF (5 mL) at room temperature for 48 h. The reaction mixture was poured into water and extracted with CH_3Cl (3 × 150 mL). The combined organic extract was washed with brine, dried over Na_2SO_4. Evaporation of organic layer gave a residue, which was chromatographed on silica gel using Hexane/$CHCl_3$ (1:20) as a yellow solid bis(propargyloxy)enone **18**. Yield: 83%; M.p.: 153 °C; ^{1}H NMR: (300 MHz, $CDCl_3$) δ = 1.70 (t, J = 5.4 Hz, 2H), 2.47 (s, 2H), 2.81 (d, J = 4.8 Hz, 4H), 4.64 (d, J = 1.8 Hz, 4H), 6.92 (d, J = 8.7 Hz, 4H), 7.37 (d, J = 8.4 Hz, 4H), 7.67 (s, 2H). ^{13}C NMR: (75 MHz, $CDCl_3$) δ = 23.0, 28.5, 55.8, 75.9, 78.3, 114.8, 129.6, 132.1, 134.7, 136.3, 157.8, 190.1. MS (FAB): m/z = 382 [M+]. Elemental Anal. Calcd for $C_{26}H_{22}O_3$: C, 81.65; H, 5.80%. Found: C, 81.58; H, 5.74%.

2.12. Glucodendrimer **1**

Following the general procedure A, the glucodendrimer **1** was obtained as white solid from bis(propargyloxy)enone **18** (0.25 g, 0.52 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoazide **9** (0.43 g, 1.15 mmol). Yield: 96%; R_f = 0.50 (Chloroform: Hexane, 1:10); M.p.: 96 °C; ^{1}H NMR: (300 MHz, $CDCl_3$) δ = 1.82 (m, 2H), 1.84, 2.03, 2.07, 2.08 (4× s, 24H), 2.92 (t, J = 5.4 Hz, 4H), 4.00–4.05 (m, 2H), 4.13–4.18 (m, 2H), 4.28–4.34 (m, 2H), 5.22–5.28 (s, 6H), 5.39–5.49 (m, 4H), 5.92 (d, J = 5.7 Hz, 2H), 7.02 (d, J = 9 Hz, 4H), 7.46 (d, J = 9 Hz, 4H), 7.75 (s, 2H), 7.89 (s, 2H). ^{13}C NMR: (75 MHz, $CDCl_3$) δ = 20.1, 20.5, 20.7, 23.0, 28.5, 61.5, 61.8, 67.7, 70.3, 72.6, 75.2, 85.8, 114.8, 121.2, 129.4, 132.2, 134.6, 136.3, 144.7, 158.4, 168.9, 169.3, 169.9, 170.5, 190.2. MS (FAB): m/z = 1128 [M$^+$]. Elemental Anal. Calcd for $C_{54}H_{60}N_6O_{21}$: C, 57.44; H, 5.36; N, 7.44%. Found: C, 57.29; H, 5.25; N, 7.40%.

2.13. Glucodendrimer **2**

Following the general procedure C, the glucodendrimer **2** was obtained as pale yellow solid from glucodendrimer **1** using Zemplen reaction. Yield: 92%; ^{1}H NMR: (300 MHz, DMSO-d_6) δ = 1.73 (s, 2H), 2.88 (s, 4H), 3.19–3.27 (m, 2H), 3.66–3.71 (m, 2H), 3.74–3.82 (m, 2H), 4.68 (t, J = 5.4 Hz, 2H), 5.21 (s, 4H), 5.33 (d, J = 4.5 Hz, 2H), 5.44 (d, J = 6.0 Hz, 2H), 5.55 (d, J = 9.0 Hz, 2H), 7.14 (d, J = 8.4 Hz, 4H), 7.53 (d, J = 8.4 Hz, 4H), 7.59 (s, 2H), 8.47 (s, 2H). ^{13}C NMR: (75 MHz, DMSO-d_6) δ = 20.31, 60.7, 61.0, 69.5, 72.0, 76.9, 79.9, 87.5, 115.0, 124.0, 128.2, 134.4, 135.3, 142.3, 158.5, 188.7. MS (FAB): m/z = 792 [M$^+$]. Elemental Anal. Calcd for $C_{38}H_{44}N_6O_{13}$: C, 57.57; H, 5.59; N, 10.60%. Found: C, 57.47; H, 5.43; N, 10.42%.

2.14. Glucodendrimer **3**

Following the general procedure A, the glucodendrimer **3** was obtained as white solid from bis(propargyloxy)enone **18** (0.15 g, 0.31 mmol) and dendritic azide **12** (0.6 g, 0.69 mmol). Yield: 89%; R_f = 0.54 (Chloroform: Hexane, 1:5); M.p.: 236 °C; ^{1}H NMR: (300 MHz, $CDCl_3$) δ = 1.72 (s, 2H), 1.84, 2.03, 2.07, 2.08 (4× s, 48H), 2.90 (t, J = 5.4 Hz, 4H), 4.00–4.04 (m, 4H), 4.13–4.17 (m, 4H), 4.28–4.33 (m, 4H), 5.16 (s, 8H), 5.24–5.30 (m, 8H), 5.39–5.50 (m, 8H), 5.90 (d, J = 8.7 Hz, 4H), 6.52 (d, J = 2.1 Hz, 4H), 6.66 (d, J = 2.1 Hz, 2H), 7.02 (d, J = 8.7 Hz, 4H), 7.45 (d, J = 8.7 Hz, 4H), 7.74 (s, 2H), 7.94 (s, 4H). ^{13}C NMR: (75 MHz, $CDCl_3$) δ = 20.1, 20.5, 20.5, 20.7, 28.5, 29.7, 54.1, 61.8, 62.1, 67.7, 70.4, 72.6, 75.2, 85.8, 101.9, 107.8, 114.1, 114.8, 121.5, 122.9, 129.3, 132.3, 134.6, 136.9, 144.3, 158.6, 159.8, 168.9, 169.4, 169.9, 170.5, 190.2. MS (MALDI-TOF): m/z = 2218 [M$^+$+Na]. Elemental Anal. Calcd for $C_{102}H_{114}N_{12}O_{43}$: C, 55.79; H, 5.23; N, 7.65%. Found: C, 55.70; H, 5.25; N, 7.54%.

2.15. Glucodendrimer **4**

Following the general procedure C, the glucodendrimer **4** was obtained as pale yellow solid from the glucodendrimer **3** using Zemplen reaction. Yield: 81%; ^{1}H NMR: (300 MHz, DMSO-d_6) δ = 1.69 (s, 2H), 2.94 (s, 4H), 3.34 (d, J = 7.8 Hz, 4H), 3.66–3.71 (m, 4H), 3.74–3.82 (m, 4H), 5.20 (s, 12H), 5.27 (s, 4H), 5.63 (s, 12H), 6.70 (s, 4H), 6.85 (s, 2H), 7.21 (d, J = 7.2 Hz, 4H), 7.59–7.65 (m, 4H), 8.43 (s, 2H), 8.53 (s, 4H). ^{13}C NMR: (75 MHz, DMSO-d_6) δ = 30.5, 34.6, 58.0, 65.9, 66.3, 70.3, 74.7, 77.3, 82.1, 85.1, 92.7, 104.9, 106.1, 112.4, 120.1, 129.2, 130.2, 133.4, 137.5, 139.6, 143.4, 145.1, 147.5, 147.9, 163.7, 164.6, 193.8. MS (MALDI-TOF): m/z = 1545 [M$^+$+Na]. Elemental Anal. Calcd for $C_{70}H_{82}N_{12}O_{27}$: C, 54.19; H, 5.43; N, 11.03%. Found: C, 55.01; H, 5.36; N, 10.97%.

2.16. Glucodendrimer **5**

Following the general procedure A, the glucodendrimer **5** was obtained as white solid from bis(propargyloxy)enone **18** (0.13 g, 0.27 mmol) and dendritic azide **14** (1.15 g, 0.57 mmol). Yield: 86%; R_f = 0.65 (Chloroform: Hexane, 1:5); M.p.: 139 °C; ^{1}H NMR: (300 MHz, $CDCl_3$) δ = 1.75 (s, 2H), 1.82, 2.02, 2.04, 2.07 (4× s, 96H), 2.88 (t, J = 5.4 Hz, 4H), 4.04–4.07 (m, 8H), 4.13–4.17 (m, 8H), 4.27–4.32 (m, 8H), 5.12 (s, 26H), 5.28 (t, J = 8.7 Hz, 8H), 5.40–5.52 (m, 30H), 5.93 (d, J = 8.7 Hz, 8H), 6.48 (s, 12H), 6.56–6.63 (m, 8H), 7.00 (d, J = 8.7 Hz, 4H), 7.43 (d, J = 8.7 Hz, 4H), 7.65 (s, 4H), 7.66 (s, 2H), 7.98 (s, 8H). ^{13}C NMR: (75 MHz, $CDCl_3$) δ = 20.1, 20.5, 20.6, 20.7, 28.5, 29.7, 54.0, 61.6, 61.8, 62.0, 67.8, 70.4, 72.7, 75.1, 85.7, 101.8, 102.0, 107.7, 114.8, 121.7, 123.2, 123.3, 129.2, 132.2, 132.3, 134.6, 136.4, 136.9, 137.1, 143.9, 144.2, 144.2, 158.6, 159.5, 159.7, 168.9, 169.4, 169.9, 170.5, 190.1. MS (MALDI-TOF): m/z = 4838 [M$^+$+Na]. Elemental Anal. Calcd for $C_{216}H_{240}N_{42}O_{87}$: C, 53.86; H, 5.02; N, 12.21%. Found: C, 53.82; H, 5.05; N, 12.00%.

2.17. Glucodendrimer **6**

Following the general procedure C, the glucodendrimer **6** was obtained as pale yellow solid from the glucodendrimer **5** using Zemplen reaction. Yield: 84%; ^{1}H NMR: (300 MHz, $CDCl_3$) δ = 1.68 (s, 2H), 2.84 (s, 4H), 3.25 (bs, 8H), 3.43 (bs, 16H), 5.11 (s, 44H), 5.53 (s, 28H), 6.61 (s, 12H), 6.71–6.76 (m, 8H), 7.10 (s, 4H), 7.54 (d, J = 12.6 Hz, 4H), 8.33 (s, 6H), 8.44 (s, 8H). ^{13}C NMR: (75 MHz, $CDCl_3$) δ = 27.9, 31.5, 52.8, 60.5, 60.8, 68.5, 69.2, 72.0, 76.6, 79.4, 87.3, 100.9, 107.1, 115.7, 119.3, 123.8, 124.0, 124.3, 124.9, 126.3, 127.9, 128.8, 129.3, 133.0, 137.6, 138.0, 142.4, 142.7, 153.2, 159.1, 159.2. MS (MALDI-TOF): m/z = 3494 [M$^+$+Na]. Elemental Anal. Calcd for $C_{152}H_{176}N_{42}O_{55}$: C, 52.59; H, 5.11; N, 16.95%. Found: C, 52.50; H, 5.29; N, 17.04%.

2.18. Glucodendrimer **7**

Following the general procedure A, the glucodendrimer **7** was obtained as white solid from bis(propargyloxy)enone **18** (0.023 g, 0.048 mmol) and dendritic azide **16** (0.44 g, 1.0 mmol). Yield: 99%; R_f = 0.50 (Chloroform: Hexane, 3:7); M.p.: 140 °C; ^{1}H NMR: (300 MHz, $CDCl_3$) δ = 1.73 (s, 2H), 1.80, 2.02, 2.06 (4× s, 192H), 2.89 (s, 4H), 4.05–4.10 (m, 16H), 4.12–4.16 (m, 16H), 4.26–4.32 (m, 16H), 5.06 (s, 28H), 5.09 (s, 32H), 5.28 (t, J = 9.6 Hz, 16H), 5.41–5.54 (m, 60H), 5.94 (d, J = 8.7 Hz, 16H), 6.44 (s, 12H), 6.46 (s, 18H), 6.56 (s, 6H), 6.60 (s, 8H), 7.00 (dd, J = 7.5 Hz, 4H), 7.43 (d, J = 9.3 Hz, 4H), 7.67 (s, 10H), 7.72 (s, 4H), 8.01 (s, 16H). ^{13}C NMR: (75 MHz, $CDCl_3$) δ = 20.1, 20.5, 20.5, 20.6, 27.7, 29.7, 53.9, 61.6, 61.7, 61.8, 61.8, 67.7, 67.7, 68.6, 70.4, 71.8, 72.7, 75.0, 85.7, 101.8, 102.0, 107.5, 107.7, 114.1, 114.8, 121.9, 123.4, 123.5, 123.5, 128.8, 129.1, 130.9, 132.2, 132.3, 132.4, 136.3, 136.9, 137.0, 137.1, 137.1, 139.3, 143.8, 144.2, 159.5, 159.6, 159.7, 168.9, 169.4, 169.9, 170.5. MS (MALDI-TOF): m/z = 9755 [M$^+$+Na]. Elemental Anal. Calcd for $C_{432}H_{480}N_{90}O_{175}$: C, 53.31; H, 4.97; N, 12.95%. Found: C, 53.25; H, 4.90; N, 13.04%.

2.19. Glucodendrimer **8**

Following the general procedure C, the glucodendrimer **8** was obtained as pale yellow solid from the glucodendrimer **7** using Zemplen reaction. Yield: 63%; ^{1}H NMR: (300 MHz, $CDCl_3$) δ = 1.98 (s, 2H), 3.08–3.12 (m, 4H), 3.45–3.52 (m, 16H), 3.74 (d, J = 10.2 Hz, 16H), 3.85 (t, J = 9 Hz, 16H), 5.18 (s, 76H), 5.61–5.64 (m, 76H), 6.66 (s, 12H), 6.69 (s, 18H), 6.78 (s, 6H), 6.84 (s, 8H), 7.65 (d, J = 5.4 Hz, 4H), 7.97 (d, J = 7.5 Hz, 4H), 8.42 (s, 14H), 8.52 (s, 16H). ^{13}C NMR: (75 MHz, $CDCl_3$) δ = 21.1, 52.7, 52.8, 60.6, 61.1, 61.2, 68.5, 69.4, 72.0, 76.9, 79.0, 79.9, 87.5, 100.8, 100.9, 107.1, 107.2, 124.0, 124.8, 124.9, 125.6, 125.7, 127.0, 128.3, 128.9, 130.6, 130.7, 132.2, 132.8, 133.9, 138.1, 138.2, 142.3, 142.5, 142.6, 159.3, 159.4. MS (MALDI-TOF): m/z = 7063 [M$^+$+Na]. Elemental Anal. Calcd for $C_{304}H_{352}N_{90}O_{111}$: C, 51.85; H, 5.04; N, 17.90%. Found: C, 51.55; H, 4.94; N, 17.62%.

2.20. Cardiac myoblasts (H9C2) cell culture and treatment

Cultured rat cardiac myoblast (H9C2) cells were obtained from National Centre For Cell Science (*NCCS*, *Pune*). The cells were maintained in Dulbecco modified Eagle medium (DMEM) (HIMEDIA, Mumbai) supplemented with 10% Fetal Bovine Serum, 90 U/mL penicillin, 90 μg/mL streptomycin and 5 μg/mL amphotericin B under atmospheric conditions of 95% air–5% CO_2 at 37 °C. Cells were seeded in 6-well plates until semi confluence and then the cells were maintained in 2 mL of DMEM without FBS for 20 h prior stimulation and during the stimulation. The conditioned cell media were collected 24 h after stimulation with glucose concentration ranging from 4.5 mM (normal) and 30 mM (high). One set of cells with the same concentrations were exposed along with glucodendrimer for 48 h time period. At the end of the experiments cells were scrapped and cell lysates were used for further analysis. Effective glucodendrimers used as potent anti diabetic agent at 10 mM concentration. MEFs grown in serum free media for 24 h before treatment planned. MEFs treated with different concentration of glucose ranging from 4.5 mM (normal) and high glucose concentration 30 mM glucose for 48 h time points.

2.21. MTT assay

MTT assay is mainly based on the enzymatic conversion of MTT in the mitochondria. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) is a water soluble tetrazolium salt, which is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by succinate dehydrogenase within the mitochondria. As the formazan product is impermeable to the cell membranes and therefore it accumulates in healthy cells. The MTT assay is based on the protocol described (Mosmann, 1983). Briefly, at the end of the incubation time, cells were incubated for 4 h with 0.8 mg/mL of MTT, dissolved in serum free medium (DMEM for H9C2). Washing with PBS (1 mL) was followed by the addition of DMSO (1 mL), gentle shaking for 10 min so that complete dissolution was achieved. Aliquots (200 μL) of the resulting solutions were transferred in 96-well plates and absorbance was recorded at 560 nm using the micro plate spectrophotometer system. Results were analyzed and are presented as percentage of the control values.

2.22. Determination of extracellular glucose concentration

The concentration of glucose in the culture medium was collected and estimated after incubation for 48 h and glucose level was determined using a colorimetric kit (BioSystems, India) following the manufacturer's instructions. Glucose estimated immediately after collecting the medium from control, high glucose induced, glucodendrimers **2**, **4**, **6** and **8** was treated with cell culture flasks.

2.23. Glucose titration the assay

To prove the effectiveness of the glucodendrimers against glucose toxicity glucose titration using glucodendrimer and glucose was performed. Glucodendrimer **8** was diluted to 2 mL of 0.1 mM and placed into a UV–visible spectrophotometer (TECOMP-6500). UV–vis spectrum was recorded and the λ_{max} was observed at 289 nm. Glucose solution was prepared by dissolving (35 μL, 0.1 M) in 1 mL of DMSO. 5 μL was added to the glucodendrimers test solution and optical density was measured at 289 nm. The measurements were carried out by adding further glucose solution of 5 μL each time and OD was measured. The variation of optical changes on the addition of glucose was monitored.

2.24. Oil Red O staining for cultured cells

Oil Red O staining for cultured cells done by the method (Pittenger et al., 1999) of briefly culture and treated cultured cells in tissue culture plate as needed, once the treatment period over, take the culture plate out of incubator and remove the medium. Add ~2 mL of PBS to wash the cells and remove PBS completely. Add 2 mL of 10% formalin (RT) and incubate for 10 min at RT. Incubate for at least 1 h. Remove formalin with a pipette and wash cells with ddH_2O twice, and with 2 mL of 60% isopropanol for 5 min at RT. Let the cells dry completely at RT. Add 1 mL of Oil Red O working solution and incubate at RT for 10 min. Remove Oil Red O solution and immediately add double distilled water. Washing with ddH_2O

can repeat for 4 times. Acquire images under the light microscope for analysis.

2.25. Zymography for MMP activity

Gelatin zymography was performed to determine gelatinolytic activities of MMP-2 and 9 following by the method (Shimokawa Ki et al., 2002). Protein isolated after H9C2 cells exposed to high glucose for 48 h were loaded at concentration of 40 μg along with sampling buffer (0.5 mol/L Tris–HCl, glycerol, 10% SDS, and 0.1% bromophenol blue) in a final solution of 25 μL. SDS-PAGE was performed using 10% polyacrylamid gel containing 0.1% gelatin at 125 V. SDS was removed with Triton X-100 for 60 min, and the gel was incubated in a developing buffer (Tris-base, Tris–HCl, NaCl, $CaCl_2$, Brij-35, and $ZnCl_2$) for 18 h. Gels were stained for 3 h with 0.5% coomassie G250 and destained for 60 min in 7% acetic acid and 35% methanol. The gelatinolytic activities were detected as a clear band against a blue background (arbitrary unit per centimeter).

2.26. Extraction of RNA and RT-PCR gene expression studies

Total RNA from H9C2 cells was successfully isolated using Trizol (Genie-India) following the manufacturer's protocol (Spier et al., 2001). To remove genomic DNA contamination, RNA samples were treated with RNase-free DNase I (1 unit/μg of RNA) at 37 °C for 30 min. The RNA integrity was confirmed by visualization of distinct 28 S and 18 S bands after electrophoresis on 1.5% agarose gel. RT-PCR was performed with GeNei M-MuLV RT-PCR kit (GeNei, Bangalore, India) with following gene specific oligonucleotide primers: MMP-2 sense primer: 5′-CTATTCTGTCAGCACTTTGG and anti-sense primer 5′-CAGACTTTGGTTCTCCAACTT (product size: 309 bp); MMP-9 sense primer: 5′-AGTTTGGTGTCGCGGAGCAC and anti-sense primer 5′-TACATGAGCGCTTCCGGCAC (product size: 754 bp); GAPDH sense primer: 5′-TCCACCACCCTGTTGCTGTAGC-3′ and anti-sense primer 5′-TGGAAAGCTGTGGCGTGATG-3′ (product size: 401 bp). PCR was performed for 30 cycles (94 °C for 30 s, 57 °C for 30 s, and 72 °C for 30 s) by Eppendorff-programmed thermal cycler and the products were visualized on 2% agarose gels by ethidium bromide staining.

3. Results and discussion

3.1. Synthesis of glucodendrimers

The importance of the current study is to evaluate the antidiabetic and cardio protective nature of glycodendrimers **2**, **4**, **6** and **8** (G0, G1, G2 and G3) against high glucose induced toxicity, up regulation of MMP-2, 9 and to study pathological disorder on H9C2 cardiac cells.

Reaction of 1.0 equiv. of 3,5-bis(propargyloxy)benzyl chloride **10** (Wu et al., 2004) with 2.1 equiv. of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl azide **9** (Singh, 2009) under the Cu(I) catalyzed click reaction conditions gave the dendritic chloride **11** in 96% yield (AB2-type). The use of NaN_3 in a mixture of acetone and water at 60 °C allowed the transformation of the dendritic chloride **11** to the corresponding dendritic azide **12** in 98% yield. In the ^{1}H NMR spectrum of compound **12**, a singlet at δ 5.13 for the —*O*-CH_2 protons and a singlet at δ 7.86 for triazole protons were observed in addition to other protons. The ^{13}C NMR spectrum of compound **12** displayed the —*O*-CH_2 carbon at δ 84.8 and the triazole carbon at δ 158.6. The appearance of molecular ion peak *m/z* 987 also confirmed the structure of the dendritic azide **12**.

A similar synthetic strategy was adopted for the synthesis of second and third generation of dendron using the building block viz, 3,5-bis(propargyloxy)benzyl chloride **10**. Reaction of the dendritic azide G1-N_3 **12** and dendritic azide G2-N_3 **14** with the 3,5-bis(propargyloxy)benzyl chloride **10** in the presence of the Cu(I) catalyzed click reaction conditions gave the dendritic chlorides **13** and **15** (AB_2-type) in 93% and 91% yields respectively. The use of NaN_3 in mixture of acetone and water at 60 °C allowed the desired dendritic chlorides **13** and **15** to the corresponding dendritic azides **14** and **16** in 99% and 95% yields respectively. The ^{1}H NMR spectrum of dendritic azide **16** displayed a singlet at δ 5.10 for the —*O*-CH_2 protons and a singlet at δ 8.00 for triazole protons. The ^{13}C NMR spectrum of compound **16** displayed the —*O*-CH_2 carbon at δ 85.7 and the triazole carbon at δ 159.7. The appearance of molecular ion peak *m/z* 4695 [M^++Na] also confirmed the structure of the dendritic azide **16** (Scheme 1).

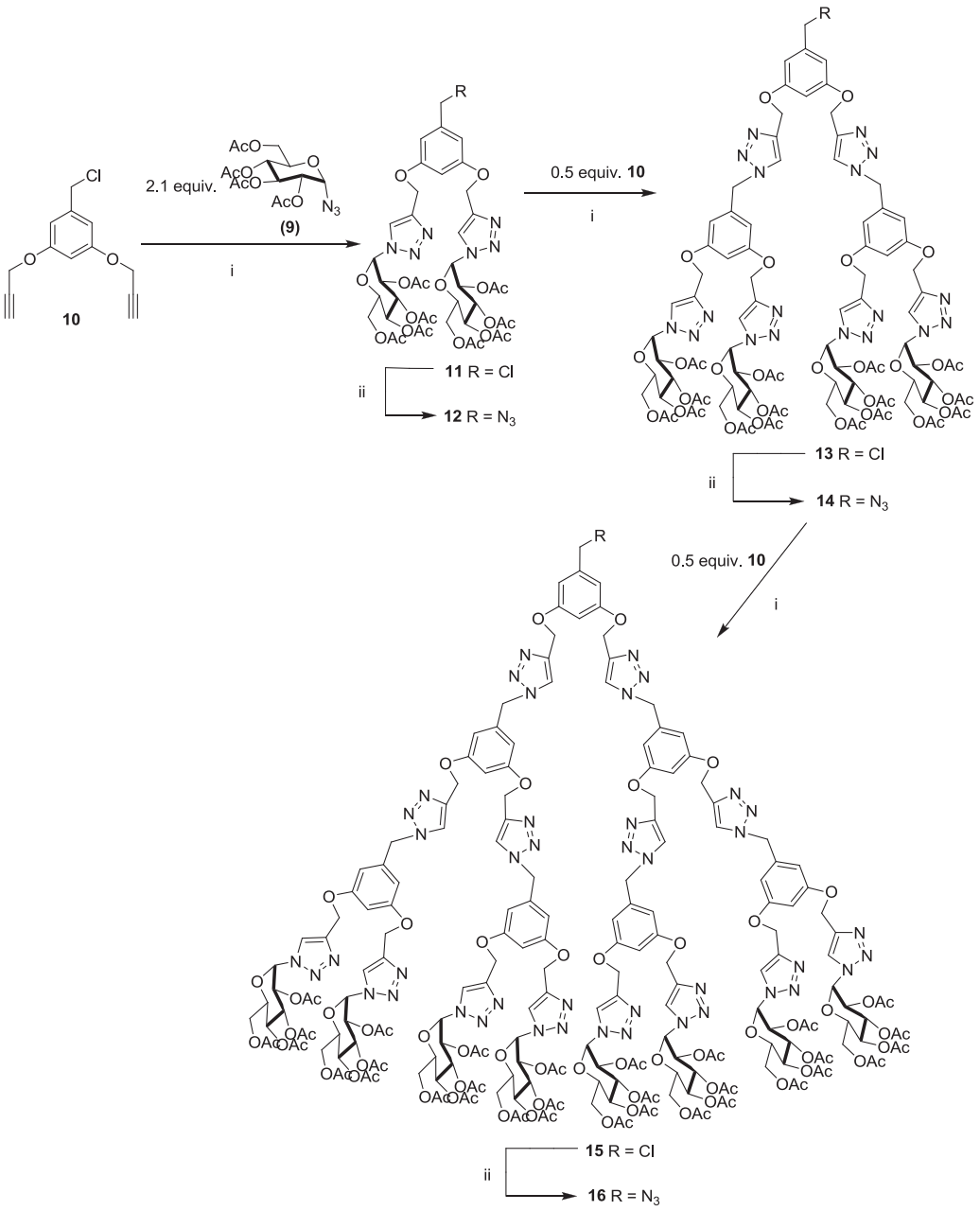
The core unit bis(propargyloxy)enone **18** was obtained in 83% yield by the *O*-alkylation of enone **17** with 2.2 equiv. of propargyl bromide in the presence of K_2CO_3 in DMF The Cu(I) catalyzed click reaction of 1.0 equiv. of bis(propargyloxy)enone **18** with 2.1 equiv. of dendritic azide **9**, **12**, **14** and **16** in a mixture of H_2O and THF afforded the glycodendrimers **1**, **3**, **5** and **7** in 98%, 98%, 95% and 90% yields, respectively. All the glycodendrimers **1**, **3**, **5** and **7** were de-*O*-acetylated in the presence of 0.1 N NaOMe in mixture of MeOH, CH_2Cl_2 and THF to afford the di-, tetra-, octa-valent glycodendrimers **2**, **4**, **6** and **8** in 92, 94, 91 and 86% yields, respectively. The ^{1}H NMR spectrum of glycodendrimer **8** showed sharp singlets at δ 5.18 for —*O*-CH_2 protons and another sharp singlet at δ 8.42 for triazole protons and the disappearance of four different glyco-acetoxy protons. The ^{13}C NMR spectrum of glycodendrimer **8** displayed the —*O*-CH_2 carbons at δ 87.5 and triazole carbons at δ 159.4, with the disappearance of four different glyco-acetoxy carbonyl carbons. The appearance of molecular ion peak at *m/z* 7063 [M^++Na] in mass spectrum also confirmed the structure of the glycodendrimer **8** (Scheme 2).

3.2. Effect of glucodendrimer on the cell survival of H9C2 cardiomyocytes at normal and high glucose levels

High glucose induced cytotoxicity and the effect of added glucodendrimers under high glucose induced toxic condition was performed using MTT assay. MTT assay was carried to find the protective effect of glucodendrimers on the high glucose induced cytotoxicity in H9C2 cells. High glucose level is known to be toxic for cardiac cells and cell death occurred in high glucose medium (Marfella, Esposito, & Giugliano, 2003). High glucose induced cell death could be probably due to the cytotoxicity induced by the free radical damage and oxidative stress (Chan, 2005).

In order to study the effect of concentration of glucodendrimers **2**, **4**, **6** and **8** on glucose induced toxicity various concentrations ranging from 5, 10, 20 and 50 mM of glucodendrimers were used in the current investigation. Fig. 2A–C represents the effect of the glucodendrimers **6** and **8** against normal (5 mM) and high glucose (30 mM) induced cytotoxicity in H9C2 cells. Culture of cardiac cells with high glucose (30 mM) induced toxicity and cell death in cardiac cells (Lablanche et al., 2011). Research studies also supported that incubation of INS-1 cells in the presence of 30 mM glucose or 2.5 mM fructose induced PTP opening and led to cell death (Cai et al., 2002). Our previous studies also demonstrated that high glucose (30 mM) induced collagen accumulation in cardiac cells (Vadla & Vellaichamy, 2012).

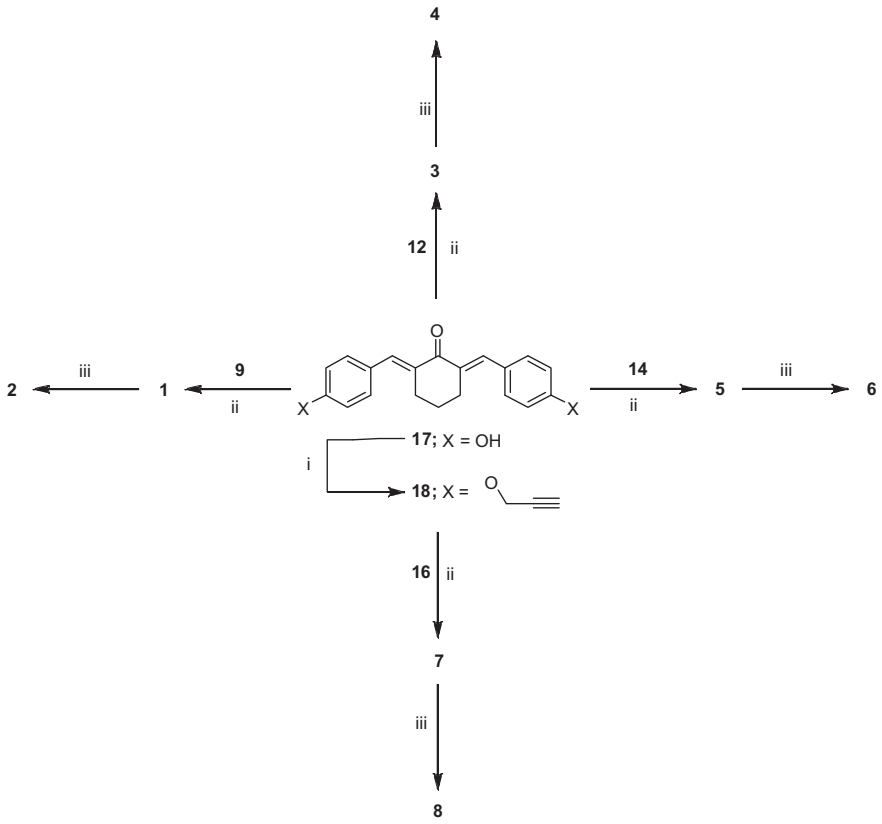
Among all the glucodendrimers investigated for the activity against high glucose induced cytotoxicity, glucodendrimers **6** and **8** showed very prominent effect. From the optical density measurements, cell viability was calculated and normal glucose level of 5 mM concentration was taken as the standard and



Scheme 1. Synthesis of first, second and third generation glucodendrons. *Reagents and conditions*: (i) $CuSO_4$ (5 mol%), sodium ascorbate (10 mol%), H_2O/THF (1:1), rt, 10 h. (ii) 1.5 equiv. NaN_3, CH_3COCH_3/H_2O, 60 °C, 1–3 h.

on increasing the glucose level the percentage of viable cells decreased showing the toxicity at high glucose level (Fig. 2A). To test the effect of glucodendrimer **6** on cell toxicity various concentration of the glucodendrimers viz 5, 10, 20 and 50 mM was used and two different glucose levels normal (5 mM) and high glucose level (30 mM) was chosen and absorption in UV–visible spectrum indicated that at high concentration of the glucodendrimer the toxicity for normal and high glucose level is reduced (Fig. 2B). Similarly glucodendrimer **8** was used at different concentration of 5, 10, 20 and 50 mM under normal (5 mM) and high glucose level (30 mM) condition and the toxicity was reduced for normal and high glucose level by the addition of glucodendrimer **8** (Fig. 2C). In fact the effect of other viz glucodendrimers **2** and **4** on glucose induced toxicity was also tested (supportive information Figure 1S and 2S) and it was observed that glucodendrimers **6** and **8** are effective in controlling the glucose induced toxicity on H9C2 cells. As concentration and generation of glucodendrimer increases the protective nature against high glucose induced toxicity also increases. This can be explained by the binding ability of glucodendrimer with the excess glucose. Glucodendrimer binds with the glucose in the medium at normal and high glucose level. Glucodendrimer **6** and **8** being higher generation dendrimers than **2** and **4**. They are vary effect against high glucose induced cytotoxicity. Based on the MTT assay, 10 mM dose of glucodendrimer **6** and **8** is considered for the further experimental purpose. Glucodendrimers **6** and **8** at a concentration of 10 mM is effective in preventing high glucose induced toxicity.

A slight reduction in the survival rate of the cell was noticed as the glucodendrimer concentration increases to 20 and 30 mM.



Scheme 2. Synthesis of glucodendrimers with enone core. *Reagents and conditions*: (i) 2.1 equiv. propargyl bromide, K_2CO_3, DMF, rt, 48 h. (ii) $CuSO_4$ (5 mol%), sodium ascorbate (10 mol%), H_2O/THF (1:1), rt, 10 h. (iii) NaOMe (0.1 N), MeOH/CH_2Cl_2/THF (6:1:1).

This might be due the binding of the available glucose to the glucodendrimer **6** and **8** and thus making the glucose unavailable to the cell. However at the concentration of 5 mM and 10 mM of glucodendrimer no decrease in survival rate was observed. High and excess glucose in the medium which is not utilized by the cells can generate free radicals, which can induce structural, molecular and pathological damages to the cardiac cells (Candido et al., 2003) and such high glucose induced the free radicals and oxidative stress in the diabetes animals (Fowler, 2008; Vincent, Russell, Low, & Feldma, 2004) has been reported earlier.

3.3. Effect of glucodendrimers on the glucose consumption

Fig. 3 shows glucose consumption by H9C2 cardiac myocytes and explaines the percentage of glucose consumed in the presence and absence of glucodendrimers. Glucodendrimers used at the concetration of 10 mM were used against the glucose cultured H9C2 cells. Amount of glucose estimated before and after the incubation time revels exaclty the amount of glucose utilized by the H9C2 cadiac myocytes. A significnat increase in glucose utilization was noticed in the high glucose induced cells (30 mM) when compared to the normal glucose (control). Significnat increase in the glucose consumption was also found in the glucodendrimer treated agaisnt high glucose induced and untreated normal control cells. Amount of glucose consumed by the H9C2 cells was analyzed by the conventional glucose oxidase–peroxidase (GOD–POD) method. Amount of glucose consumed by the cells might be either due to the utilization by the cells or neutralized by the glucodendrimer due to the binding ability of glucodendrimer with the excess unutilized glucose present in the medium. Recent reports suggested that glucodendrimer can bound to the free glucose that is present in the solution (Steiner, Duerkop, & Wolfbeis, 2011). Binding of free glucose in the solution with the glucodendrimer might reduce the free glucose concentration in the solution and thus leads to reduce the high glucose induced osmotic effects, free radical induced toxicity and oxidative stress. Glucose consumption was increased to three fold in high glucose exposed (30 mM) when compare to normal control. Glucose consumption was increased more as the generation of glucodendrimer increases, this might be due to the binding absorption capability with more number of branching triazole unit and α-D-glycopyranosyl units at the surface (multi-valency effect) of glucodendrimer with glucose. Thus added high and excess glucose which is not utilized by cells are effectively removed or neutralized by the added glucodendrimer.

3.4. Glucose titration the assay

Glucose titration studies were carried out in order to find whether the glucodendrimer can bind with added excess glucose through hydrogen bonding. The glucose titration assay was monitored using UV–vis absorption spectrum. In the UV–vis absorption spectrum of the glucodendrimer **8** in DMSO (1×10^{-3} M) λ_{max} was absorbed at 289 nm. However, during titration, absorbance intensity increased linearly on increasing the concentrations of glucose (inset of Fig. 4). Fig. 4 shows that the plot of glucose concentration versus absorption intensity and response of glucodendrimer **8** to the added glucose. The titration curve confirms that the absorbance intensity increases at the λ_{max} 289 nm upon adding 10, 20, 30, 40, 50, 60, 70 nm of glucose to the glucodendrimer **8**. The higher generation glucodendrimer has more number

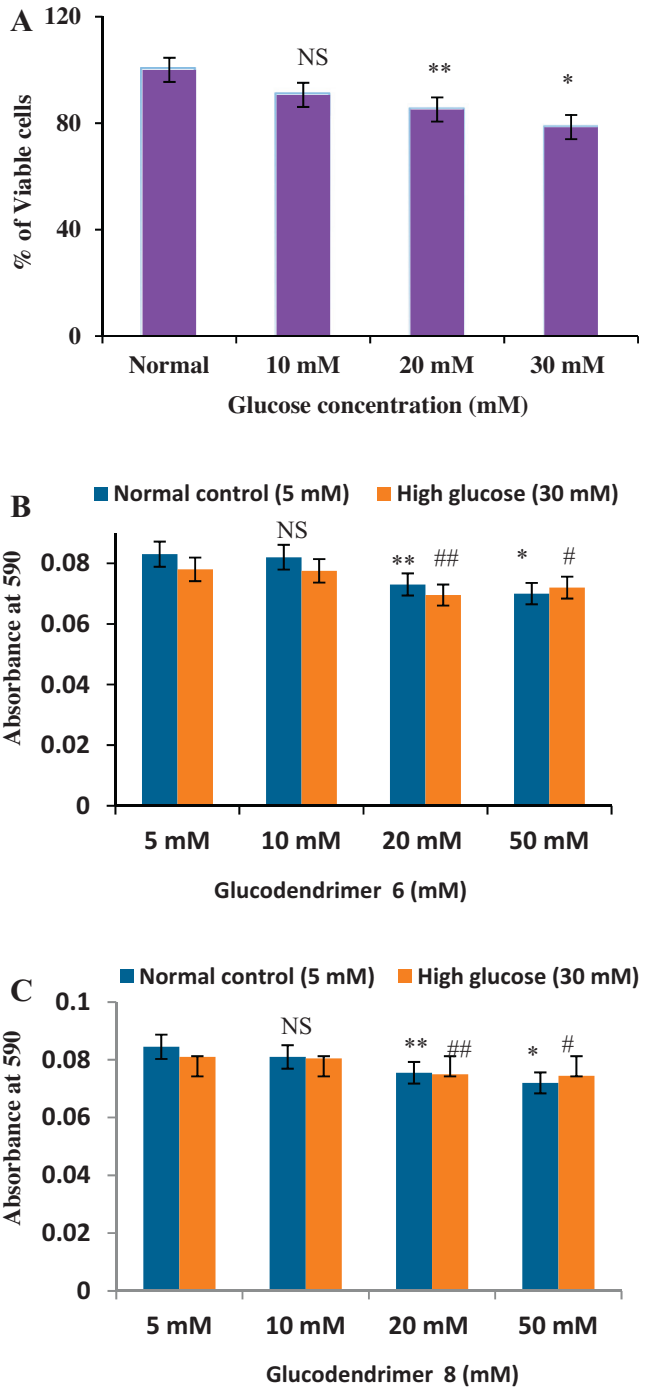


Fig. 2. Cytotoxicity assay of H9C2 cells cultured in normal glucose, high glucose in the presence and absence of glucodendrimers for 48 h. (A) Cytotoxicity assay of H9C2 cells exposed to normal glucose (control) and high glucose cells. (B and C) Effects of glucodendrimer **6** and **8** against the high glucose induced cytotoxicity. Values represented as mean ± S.D; * ($P < 0.05$) represents control vs high glucose (30 mM) and ** ($P < 0.01$) refers to control vs high glucose (20 mM). # ($P < 0.05$) represents cells treated with glucodendrimer compared with untreated cells (30 mM), and ## ($P < 0.01$) compared with cells exposed to glucodendrimer; NS compared with treated cells vs high glucose exposed cells (20 mM).

of triazole branching units and α-D-glycopyranosyl at the surface and hence capable showing strong interaction with the glucose on increasing the concentration from 10, 20, 30, 40, 50, 60 to 70 nm

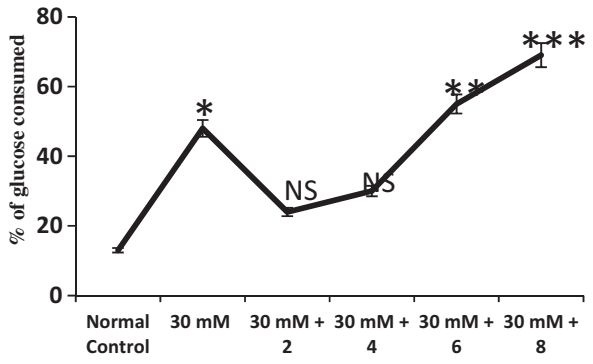


Fig. 3. Glucose consumption by H9C2 cardiac cells in the presence and absence of glucodendrimers for 48 h. All glucodendrimers used at the concetration of 10 mM. Data from triplicate of experiments are expressed as a percentage of glucose consumed. Values represented as mean ± S.D. * Referred as control vs high glucose (30 mM and $P < 0.05$) and ** and *** referred to glucodendrimer-**6** and **8** treated vs high glucose (30 mM, $P < 0.01$).

3.5. *Effects of high glucose and glucodendrimer **6** and **8** on the morphological changes in H9C2 cells*

Morphological changes of the cells are important in order to study remarkable microscopic changes that are associated with the toxicity due to high glucose level and the interaction of the glucodendrimer to reduce the glucose level. Morphological changes were observed in H9C2 cells exposed to medium containing normal glucose (5 mM) (Fig. 5a) and high glucose concentration (30 mM) (Fig. 5b). Fig. 5c and d are the effect of glucodendrimer **6** and **8** at high glucose level. Decrease in total number of cells was observed in high glucose exposed cells when compared to normal control. Decrease in cell number and changes in cellular morphology were noticed in high glucose exposed cells due the high osmotic effects toxicity produced by the high glucose. Upon the treatment with glucodendrimer, loss of cell number and microscopic morphological changes were attenuated in both glucodendrimers **6** and **8** (Fig. 5c and d) when compared to high glucose induced cells. Glucose induced toxicity was neutralized by the interaction of the glucodendrimers **6** and **8** at high glucose level concentration.

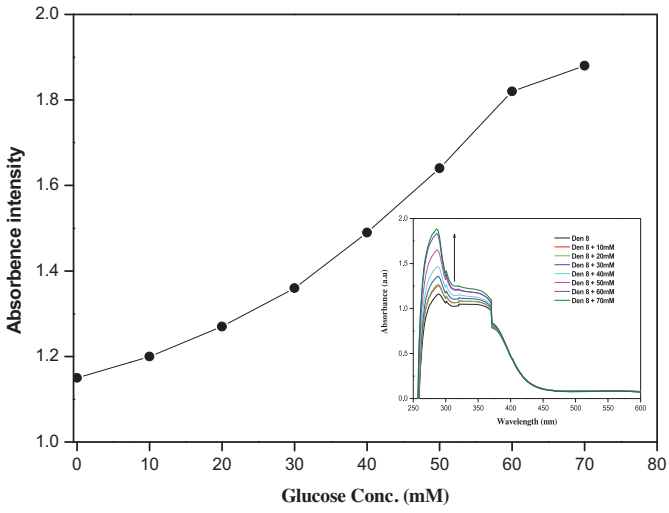


Fig. 4. Variation of absorption peak intensity on increasing the glucose concentration with the glucodendrimer **8**; the spectra is shown as inset within the figure.

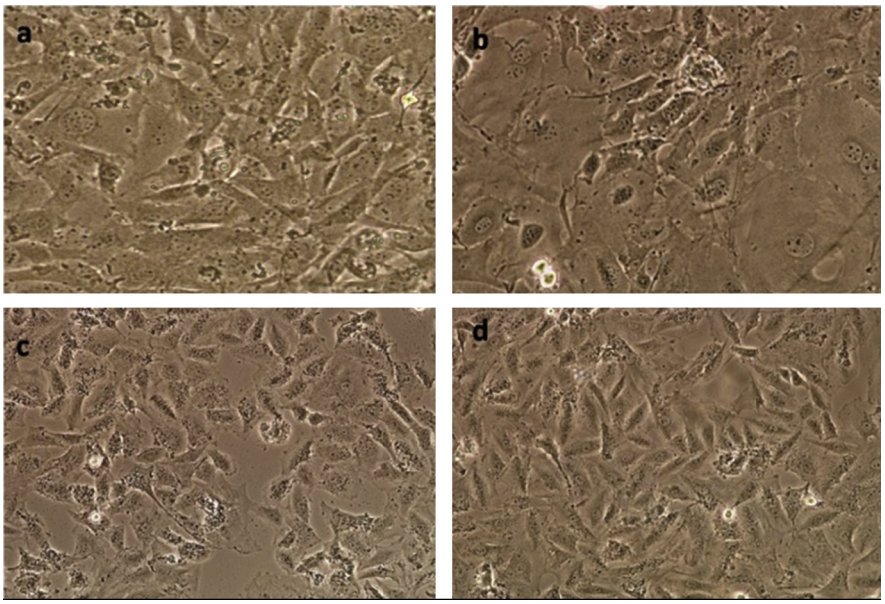


Fig. 5. Morphological changes of H9C2 cells exposed to normal, high glucose and glucodendrimers **6** and **8** for 48 h. (a) Normal control cells exposed to normal glucose, (b) the cells exposed to high glucose (30 mM), (c) the high glucose cultured cells treated with glucodendrimer **6**, (d) and the high glucose cultured cells treated with glucodendrimer **8**. Above shown are microscopic images of H9C2 cells exposed to normal, HG and glucodendrimer treated cells. All images are the same magnification; scale bar = 20 μm.

3.6. Effect of glucodendrimer on lipid accumulation by Oil Red O staining

Intracellular lipid inclusions were assessed with Oil Red O staining. Research reports stated that lipid accumulation was said to be important hall mark of diabetes. High glucose induced lipid accumulation was an index of cellular dys-regulation in the lipid metabolism. Numerous research reports suggested that, high glucose can induce lipid accumulation both in vivo and in vitro models (Anderson & Borlak, 2008; Weijer, Schrauwen-Hinderling, & Schrauwen, 2011). Oil Red O staining was extensively used to study lipid accumulation in various cell lines.

High glucose induced lipid accumulation in H9C2 cells were studied by culturing H9C2 cells for 48 h with 5.0 mM glucose for normal control (Fig. 6a) and high glucose induced toxicity at 30 mM glucose concentration (Fig. 6b). Remarkable changes appeared in

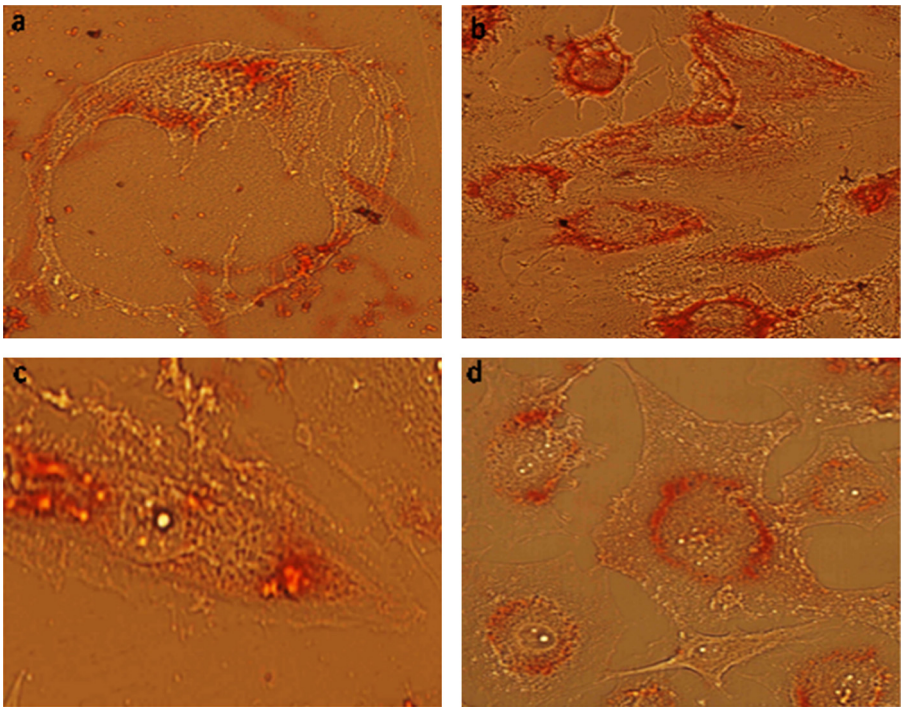


Fig. 6. Effect of glucodendrimers against high glucose induced lipid accumulation by Oil Red O staining. (a) The normal control cells cultures in 5.0 mM glucose, (b) the cells cultured in the high glucose medium (30 mM), (c) the glucodendrimer **6** treated against high glucose induced cells (30 mM), and (d) the glucodendrimer **8** treated against high glucose (30 mM) induced cells.

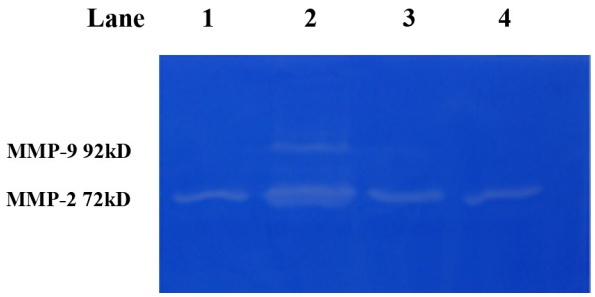


Fig. 7. Gelatin zymographic activity of MMP-2 and 9 in normal control, high glucose induced and glucodendrimers treated cell extracts. Lane-1 represents cellular extracts of normal control. Lane-2 represents extracts of high glucose induced H9C2 cells, Lane-3 and 4 explains the glucodendrimers treated H9C2 extracts. Glucodendrimer **6** and **8** used are at the concentration of 10 mM in the treatment.

the lipid accumulation at high glucose induced H9C2 cell culture (Fig. 6b) when compared to the normal control (Fig. 6a). On treatment of glucodendrimers **6** and **8** with high glucose (Fig. 6c and d) attenuated lipid accumulation in H9C2 cardiac cells, red color droplets in the images appeared indicating the accumulated lipid droplets. Fig. 6c shows the reduction in the lipid accumulation while using the glucodendrimer **6** (10 mM) with cell toxicity induced by 30 mM glucose and Fig. 6d shows the reduction in the lipid accumulation while using the glucodendrimer **8** (10 mM) on the toxicity induced by 30 mM glucose. Hence cell morphological studies also indicated that glucodendrimers **6** and **8** are effective in controlling the high glucose induced toxicity on the cardiac cells.

3.7. Effect of glucodendrimer on the high glucose induced MMP-2 and 9 dys-regulations

Matrix metalloproteinase (MMPs)-2 and 9 was the two important proteins associated with the matrix regulation and has important role in the lipid metabolism. Dys-regulation of MMP-2 and 9 are know to play key role in the abnormal lipid accumulation and also in the extracellular matrix protein accumulation. MMP-2 and 9 also increases STZ induced diabetes in high glucose induced cells.

In the present study increased MMP-2 and MMP 9 activity was observed in the high glucose induced cells (lane 2) when compared to the normal control (lane 1). On treating with glucodendrimer **6** and **8** (lanes 3 and 4) the high glucose induced toxicity on MMP-2 and 9. Cell is effectively controlled. Protective effect of glucodendrimer against high glucose induced MMP-2 and 9 activities were analyzed by the gelatine zymography and RT-PCR gene expression method (Figs. 7 and 8). In general compounds with hypoglycemic nature attenuates the diabetes associated cellular toxicity by osmotic differences, lipid accumulation and attenuated the increased MMPs mainly MMP-2 and 9 which has beneficial role in the treatment of high glucose induced diabetes and its complications (Gruzman, Babai, & Sasson, 2009; Tripathi & Srivastav, 2006). Glucodendrimers **6** and **8** exhibited a pivotal role against high glucose induced toxicity, lipid accumulation and attenuated MMP-2 and 9 activity, which suggested the protective and biologically important role of glucodendrimers against high glucose induced cellular, pathological alterations.

The important finding of this study reveals the anti-diabetic and cardio protective role of glucodendrimers against high glucose induced toxicity, up regulation of MMP-2, 9 and pathological insults to H9C2 cardiac cells. From our studies its is evident that glucodendrimer **6** and **8** provide a better protective effect of the cardiac cells against excessive glucose induced toxicity than the other glucodendrimer **2** and **4**.

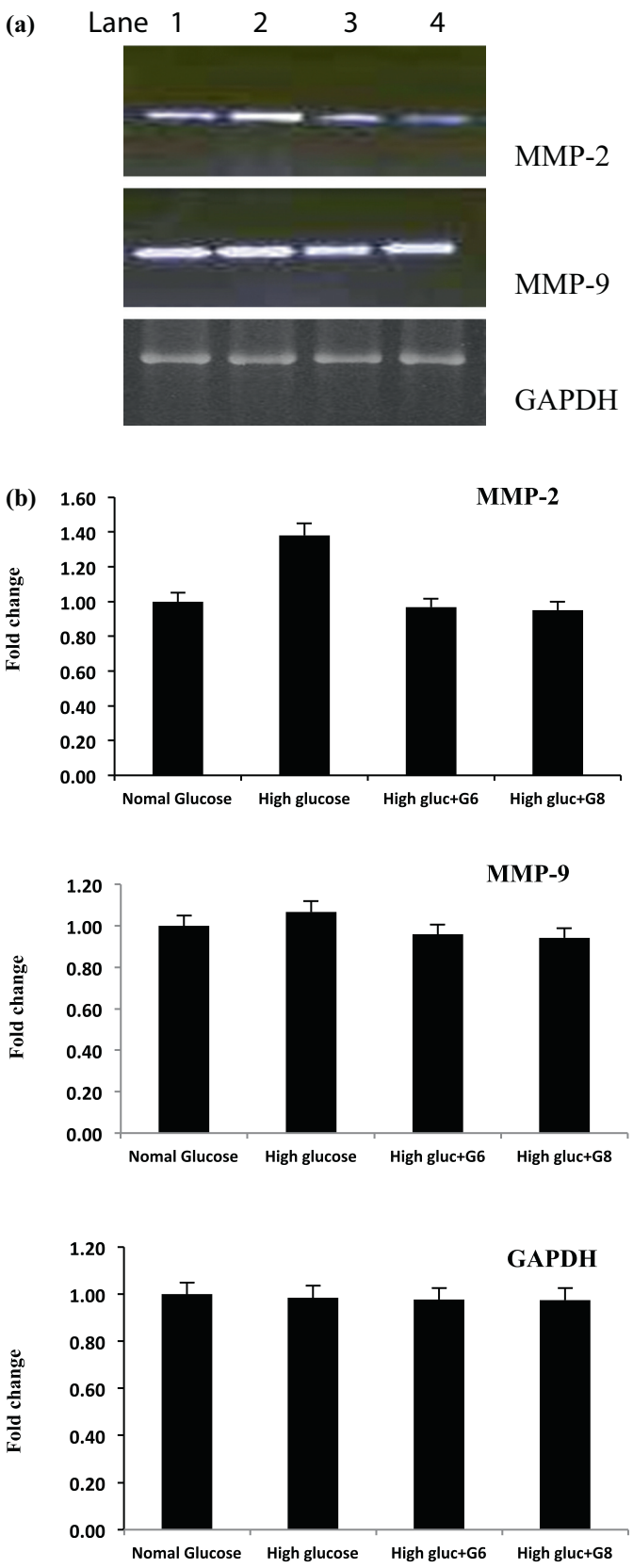


Fig. 8. (a) RT-PCR gene expression analysis of MMP-2 and MMP-9 expression. Lane-1 represents cellular extracts of normal control. Lane-2 represents extracts of high glucose induced H9C2 cells. Lanes-3 and 4 explains the glucodendrimers treated H9C2 extracts. (b) The graphical representation of the quantitated RT-PCR gels for MMP-2, MMP-9 and GAPDH control using Image J software analysis.

4. Conclusion

In conclusion, we have designed and synthesized novel glucodendrimers with enone core and triazole bridging unit using click chemistry approach. The cardio protective and anti diabetic effect of glucodendrimers against high glucose induced toxicity and molecular changes in H9C2 cardiac cell lines reveal that the higher generation glucodendrimer is more effective than the lower generation glucodendrimer in protecting the cardiac cells against high glucose level toxicity. High glucose induced changes of MMP-2 and MMP-9 levels attain to normality after treatment with glucodendrimers **6** and **8**, which indicates the cardio protective role of glucodendrimers against high glucose induced dysregulation of metalloproteinase activity.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2013.08.040.

References

Anderson, N., & Borlak, J. (2008). Molecular mechanisms and therapeutic targets in steatosis and steatohepatitis. *Pharmacological Reviews*, *60*, 311.
Andrea, S., Pieters, R. J., Vrasidas, I., Kaltner, H., Kuwabara, I., Liu, F. T., et al. (2001). Wedgelike glycodendrimers as inhibitors of binding of mammalian galectins to glycoproteins, lactose maxiclusters, and cell surface glycoconjugates. *ChemBioChem*, *2*, 822.
Basta, G., Schmidt, A. M., & De Caterina, R. (2004). Advanced glycation end products and vascular inflammation: Implications for accelerated atherosclerosis in diabetes. *Cardiovascular Research*, *63*, 582.
Cai, L., Li, W., Wang, G., Guo, L., Jiang, Y., & Kang, Y. J. (2002). Hyperglycemia-induced apoptosis in mouse myocardium: Mitochondrial cytochrome C-mediated caspase-3 activation pathway. *Diabetes*, *6*, 1938.
Camby, I., Mercier, M. L., Lefranc, F., & Kiss, R. (2006). Galectin-1: A small protein with major functions. *Glycobiology*, *16*, 137R.
Camponovo, J., Hadad, C., Ruiz, J., Cloutet, E., Gatard, S., Muzart, J., et al. (2009). "Click" glycodendrimers containing 27, 81, and 243 modified xylopyranoside termini. *Journal of Organic Chemistry*, *74*, 5071.
Candido, R., Forbes, J. M., Thomas, M. C., Thallas, V., Dean, R. G., Burns, W. C., et al. (2003). A breaker of advanced glycation end products attenuates diabetes-induced myocardial structural changes. *Circulation Research*, *92*, 785.
Chan, W. H. J. (2005). Effect of resveratrol on high glucose-induced stress in human leukemia K562 cells. *Journal of Cellular Biochemistry*, *94*, 1267.
Chin, Y. W., Balunas, M. J., Chai, H. B., & Kinghorn, A. D. (2006). Drug discovery from natural sources. *AAPS Journal*, *8*, E239.
Collins, B. E., & Paulson, J. C. (2004). Cell surface biology mediated by low affinity multivalent protein–glycan interactions. *Current Opinion in Chemical Biology*, *8*, 617.
Doores, K. J., Gamblin, D. P., & Davis, B. G. (2006). Exploring and exploiting the therapeutic potential of glycoconjugates. *Chemistry – A European Journal*, *12*, 656.
Fowler, M. J. (2008). Microvascular and macrovascular complications of diabetes. *Clinical Diabetes*, *26*, 77.
Gruzman, A., Babai, G., & Sasson, S. (2009). Erratum to: Adenosine monophosphate-activated protein kinase (AMPK) as a new target for antidiabetic drugs: A review on metabolic, pharmacological and chemical considerations. *Review of Diabetic Studies*, *6*, 13.
Hayashi, T., Mori, T., Yamashita, C., & Miyamura, M. (2008). Regulation of oxidative stress and cardioprotection in diabetes mellitus. *Current Cardiology Reviews*, *4*, 251.
Imberty, A., Chabre, Y. M., & Roy, R. (2008). Glycomimetics and glycodendrimers as high affinity microbial anti-adhesins. *Chemistry – A European Journal*, *14*, 7490.
Kengne, A. P., Amoah, A. G. B., & Mbanya, J. C. (2005). Cardiovascular complications of diabetes mellitus in sub-Saharan Africa. *Circulation*, *112*, 3592.
Kikkeri, R., Hossain, L. H., & Seeberger, P. H. (2008). Supramolecular one-pot approach to fluorescent glycodendrimers. *Chemical Communications*, *18*, 2127.
Kikkeri, R., Liu, X., Adibekian, A., Tsai, Y.-H., & Seeberger, P. H. (2010). Facile synthesis of size dependent Ru(II) – carbohydrate dendrimers *via* click chemistry. *Chemical Communications*, *46*, 2197.
Lablanche, S., Cottet-Rousselle, C., Lamarche, F., Benhamou, P. Y., Halimi, S., Leverve, X., et al. (2011). Protection of pancreatic INS-1 β-cells from glucose- and fructose-induced cell death by inhibiting mitochondrial permeability transition with cyclosporin A or metformin. *Cell Death & Disease*, *2*, e134.
Lowe, J. B. (2001). Glycosylation, immunity, and autoimmunity. *Cell*, *104*, 804.
Marfella, R., Esposito, K., & Giugliano, D. (2003). Effect of high glucose on vasculature. *Circulation*, *108*, e74 (author reply)
Mohamad, H. E., Askar, M. E., & Hafez, M. M. (2011). Management of cardiac fibrosis in diabetic rats; the role of peroxisome proliferator activated receptor gamma (PPAR-gamma) and calcium channel blockers (CCBs). *Diabetology & Metabolic Syndrome*, *3*, 4.
Moses, J. E., & Moorhouse, A. D. (2007). The growing applications of click chemistry. *Chemical Society Reviews*, *36*, 1249.
Mosmann, T. J. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Immunological Methods*, *65*, 55.
Nishiwaki, K., Kubota, Y., Chigira, Y., Roy, S. K., Suzuki, M., Schvarzstein, M., et al. (2004). An NDPase links ADAM protease glycosylation with organ morphogenesis in *C. elegans*. *Nature Cell Biology*, *6*, 31.
Pittenger, M. F., Mackay, A. M., Beck, S. C., Jaiswal, R. K., Douglas, R., Mosca, J. D., et al. (1999). Multilineage potential of adult human mesenchymal stem cells. *Science*, *284*, 143.
Rabinovich, G. A., Cumashi, A., Bianco, G. A., Ciavardelli, D., Iurisci, I., Degidio, M., et al. (2006). Synthetic lactulose amines: Novel class of anticancer agents that induce tumor-cell apoptosis and inhibit galectin-mediated homotypic cell aggregation and endothelial cell morphogenesis. *Glycobiology*, *16*, 210.
Rajakumar, P., Anandhan, R., & Kalpana, V. (2009). Click chemistry approach for the synthesis of water-soluble glycodendrimer with triazole as building unit. *Synlett*, *9*, 1417.
Rajakumar, P., & Ananthan, R. (2011). Synthesis and in-vitro anti-inflammatory activity of novel glycodendrimers with benzene 1, 3, 5 carboxamide core and triazole as branching unit. *European Journal of Medicinal Chemistry*, *46*, 4687.
Roy, R. (2003). Click chemistry: Diverse chemical function from a few good reactions. *Angewandte Chemie International Edition*, *85*, 291.
Roy, R., Baek, M. G., & Olson, K. R. (2001). Synthesis of N,N′-bis(acrylamido)acetic acid-based T-antigen glycodendrimers and their mouse monoclonal IgG antibody binding properties. *Journal of the American Chemical Society*, *123*, 1809.
Sang, Q. X., Jin, Y., Newcomer, R. G., Monroe, S. C., Fang, X., Hurst, D. R., et al. (2006). Matrix metalloproteinase inhibitors as prospective agents for the prevention and treatment of cardiovascular and neoplastic diseases. *Current Topics in Medicinal Chemistry*, *6*, 289.
Scherpereel, P. A., & Tavernier, B. (2001). Perioperative care of diabetic patients. *European Journal of Anaesthesiology*, *18*, 277.
Shimokawa Ki, K., Katayama, M., Matsuda, Y., Takahashi, H., Hara, I., Sato, H., et al. (2002). Matrix metalloproteinase MMP-2 and MMP-9 activities in human seminal plasma. *Molecular Human Reproduction*, *8*, 32.
Singh, R. J. (2009). 1, 2, 3-triazole derivatives as possible anti-inflammatory agents *Rasayan*. *Journal of Chemistry*, *2*, 706.
Spier, A. W., Meurs, K. M., Coovert, D. D., Lehmkuhl, L. B., O'Grady, M. R., Freeman, L. M., et al. (2001). Use of western immunoblot for evaluation of myocardial dystrophin, alpha-sarcoglycan, and beta-dystroglycan in dogs with idiopathic dilated cardiomyopathy. *American Journal of Veterinary Research*, *62*, 67.
Steiner, M. S., Duerkop, A., & Wolfbeis, O. S. (2011). Optical methods for sensing glucose. *Chemical Society Reviews*, *40*, 4805.
Tripathi, B., & Srivastav, A. (2006). Diabetes mellitus: Complications and therapeutics. *Medical Science Monitor*, *12*, RA130.
Vadla, G. P., & Vellaichamy, E. (2012). Anti-fibrotic cardio protective efficacy of aminoguanidine against streptozotocin induced cardiac fibrosis and high glucose induced collagen up regulation in cardiac fibroblasts. *Chemico-Biological Interactions*, *197*, 119.
Vincent, M., Russell, J. W., Low, P., & Feldma, E. L. (2004). Oxidative stress in the pathogenesis of diabetic neuropathy. *Endocrine Reviews*, *25*, 612.
Weijer, T., Schrauwen-Hinderling, V. B., & Schrauwen, P. (2011). Lipotoxicity in type 2 diabetic cardiomyopathy. *Cardiovascular Research*, *92*, 10.
Wild, S., Roglic, G., Green, A., Sicree, R., & King, H. (2004). Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. *Diabetes Care*, *27*, 1047.
Wu, P., Chen, X., Hu, N., Tam, U. C., Blixt, O., Zettl, A., et al. (2008). Biocompatible carbon nanotubes generated by functionalization with glycodendrimers. *Angewandte Chemie International Edition*, *47*, 5022.
Wu, P., Feldman, A. K., Nugent, A. K., Hawker, C. J., Scheel, A., Voit, B., et al. (2004). Efficiency and fidelity in a click-chemistry route to triazole dendrimers by the copper(I)-catalyzed ligation of azides and alkynes. *Angewandte Chemie International Edition*, *43*, 3928.