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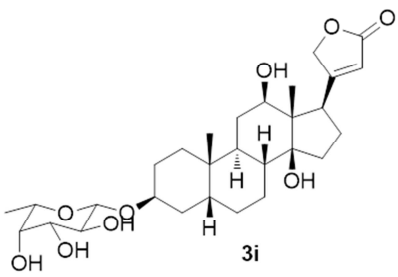

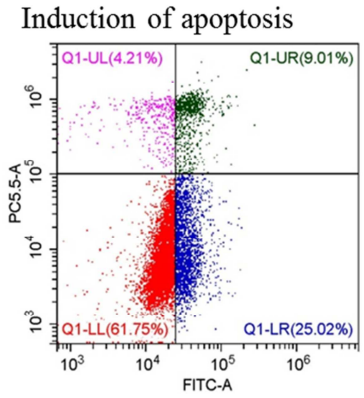

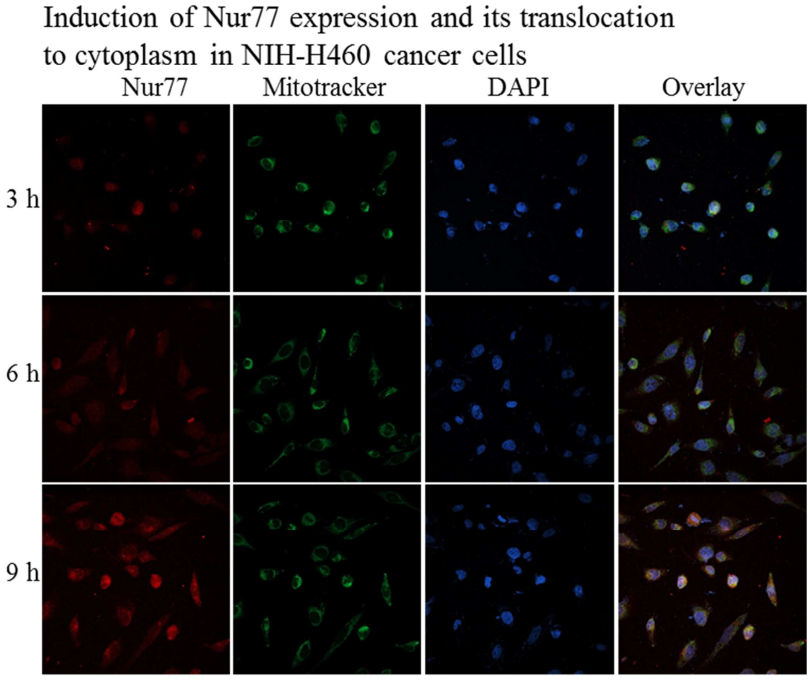

Synthesis of C_3-Neoglycosides of Digoxigenin and Their Anticancer Activities

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ABSTRACT:

Cardiac glycosides exhibit significant anticancer effects and the glycosyl substitution at C_3 position of digoxigenin is pivotal for their biological activity. In order to study the structure-activity relationship (SAR) of cardiac glycosides toward cancers and explore more potent anticancer agents, a series of C_3-*O*-neoglycosides and C_3-MeO*N*-neoglycosides of digoxigenin were synthesized by the Koenigs-Knorr and neoglycosylation method, respectively. In addition, digoxigenin bisdigitoxoside and monodigitoxoside were prepared from digoxin by sodium periodate ($NaIO_4$) oxidation and 6-aminocaproic acid hydrolysis. The SAR analysis revealed that C_3-*O*-neoglycosides of digoxigenin exhibited stronger cytotoxicity and induction of Nur77 expression of tumor cells than C_3-MeO*N*-neoglycosides. Also, 3β-*O*-glycosides exhibited stronger anticancer effects than 3α-*O*-glycosides. Among them, 3β-*O*-(β-L-fucopyranosyl)-digoxigenin (**3i**) showed the highest activity on induction of Nur77 expression and translocation from the nucleus to cytoplasm, leading to cancer cell apoptosis.

Key words: Digoxigenin, *O*-neoglycosylation, MeO*N*-noeglycosylation, cytotoxicity, Nur77 nuclear receptor, anticancer, apoptosis.

1. Introduction

Cardiac glycosides have been used for the treatment of congestive heart failure (CHF) for centuries due to their strong inhibitory activity toward the ubiquitous cell surface Na^+/K^+-ATPase [1-3]. Recent studies demonstrate that cardiac glycosides exhibit potent antiproliferative and apoptotic effects on cancer cells through complex signal transduction mechanisms [4-6]. Our previous studies showed that cardiac glycosides exerted apoptotic effects through the Nur77 dependent apoptotic pathway, a novel signaling pathway for them against cancer cells, which has great potential for novel anticancer agent discovery [7-9]. However, the cardiotoxicity and neurotoxity of cardiac glycosides limit their extensive application in clinical practice [10]. In order to get more potent leading compounds with fewer side effects, it is requisite to study the structural–activity relationship (SAR) of cardiac glycosides against cancers. Preliminary SAR analysis showed that the sugar substitution at the C_3 of digoxigenin (3*β*,12*β*,14-Trihydroxy-5*β*,20(22)-cardenolide) is pivotal for their anticancer effects [11-14]. Recent studies showed that subtle variation of sugars for cardiac glycosides dramatically affected their antiproliferative effects, even the mechanism of action [15-17]. Herein, we report the synthesis of a series of C_3-*O*-neoglycosides and C_3-MeO*N*-neoglycosides of digoxigenin by the Koenigs-Knorr [17] and neoglycosylation methods [15]. Meanwhile, digoxigenin bisdigitoxoside and monodigitoxoside were obtained from digoxin by sodium periodate ($NaIO_4$) oxidation and 6-aminocaproic acid hydrolysis. Also, the anticancer effects of these new cardiac glycosides were evaluated and discussed.

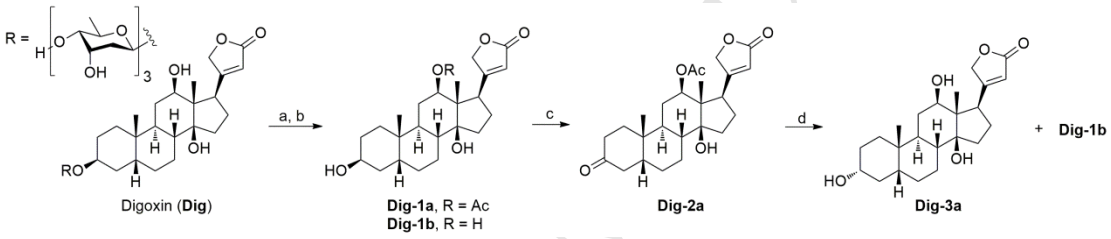
2. Results and discussion

2.1. Chemistry

2.1.1 Synthesis of C_3-O-neoglycosides of digoxigenin by the Koenigs-Knorr method

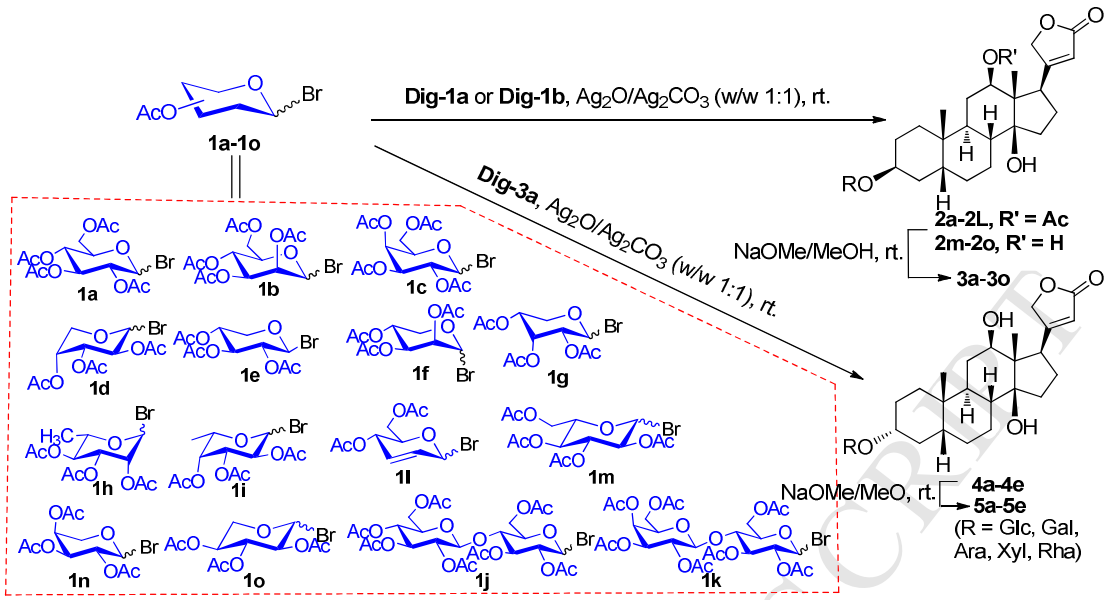
The synthesis of C_3-*O*-neoglycosides of digoxigenin was started from the synthesis of 1-bromo glycosyl donors from commercially available reducing sugars. The sugars were firstly converted to their corresponding peracetylated derivatives under the condition of Ac_2O and anhydrous pyridine at 90°C. The peracetylated derivatives of

sugars were then reacted with HBr (33%, w/w) in AcOH at room temperature to yield 1-bromo glycosyl donors (**1a**-**1o**). On the other hand, the synthesis of digoxigenin aglycones was initiated from commercially available digoxin. Peracetylation of digoxin (**Dig**) with Ac_2O in pyridine and subsequent regioselective acid-catalyzed hydrolysis of the sugar chain at C_3 led to digoxigenin aglycones **Dig-1a** and **Dig-1b** [18]. In order to study the effects of C_3 configuration on their anticancer bioactivity, we prepared *3α-OH-digoxigenin* (**Dig-3a**) as well from intermediate **Dig-1a**. Briefly, intermediate **Dig-1a** was oxidized to yield ketone **Dig-2a** with Dess-Martain periodinane in high yield, which was subsequently reduced to yield *3α*-OH-digoxigenin (**Dig-3a**) and *3β*-OH-digoxigenin (**Dig-1b**) with $NaBH_4$ (Scheme 1).



Scheme 1. Synthesis of 3*α*- and 3*β*-OH-digoxigenin. Reagents and conditions: a. Ac_2O, DMAP, CH_2Cl_2/pyr, r.t., 5 h; b. 1*M* H_2SO_4, MeOH, 90°C, 3 h; c. Dess-Martin Periodinane, CH_2Cl_2, r.t., 13 h, 84%; d. [i] $NaBH_4$, MeOH, 0°C, 2h , [ii] H_2O, r.t.

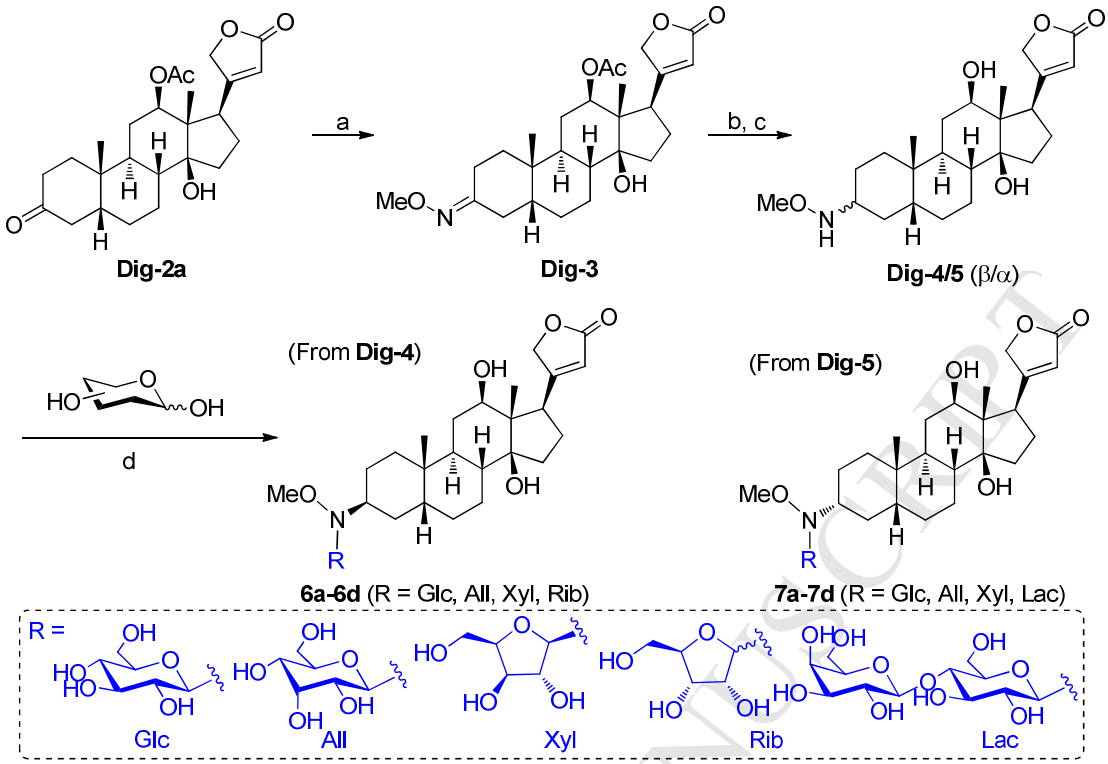
With the glycosyl donors (**1a**-**1o**) and acceptors (**Dig-1a**, **Dig-1b**, and **Dig-3a**) in hands, the C_3-*O*-neoglycosides of digoxigenin (**3a**-**3o** and **5a**-**5e**) were synthesized by the Koenigs-Knorr reaction method and subsequent deacetylation of the acetyl groups of C_{12} and sugars under the condition of CH_3ONa/CH_3OH (Scheme 2). We found that when the aglycone **Dig-1b** was used as glycosyl acceptor, only C_3-*O*-neoglycosides (**3m**-**3o**) were obtained, indicating the regioselective glycosylation of C_3-OH. Therefore, the aglycone **Dig-3a** was directly subjected to glycosylation with 1-bromo glycosyl donors to get C_3-*O*-neoglycosides (**5a**-**5e**). Overall, a series of C_3-*O*-neoglycosides of pyranosides including ribose, xylose, lyxose, arabose were obtained in high yield [19-21]. The configuration of glycosidic bond was identified by the *J* value of $J_{H1'\text{-}H2'}$ and ROESY or NOESY spectrum (Supporting Information, Table S1).



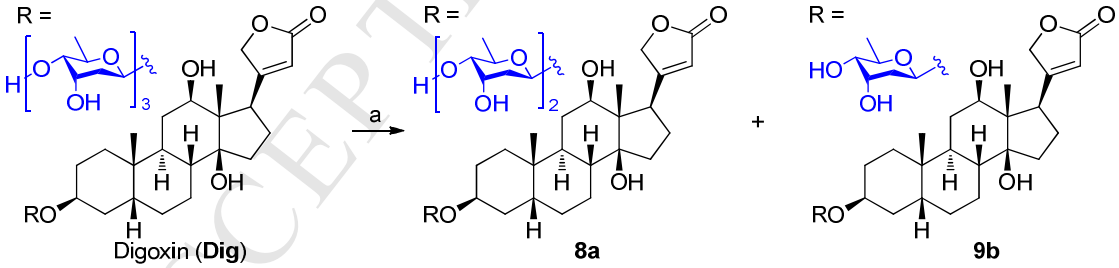
Scheme 2. Synthesis of C_3-*O*-neoglycosides of digoxigenin by the Koenigs-Knorr method.

2.1.2 Synthesis of C_3-MeON-neoglycosides of digoxigenin by neoglycosylation

The synthesis of C_3-MeO*N*-neoglycosides was initiated by preparation of MeO*N*-digoxigenin as glycosyl acceptor. Briefly, the ketone **Dig-2a** was reacted with methoxyamine hydrochloride salt to produce corresponding methoxyimines at C-3 (**Dig-3**, *Z*/*E*), which was subsequently reduced by $NaCNBH_3$ and then deacetylation of the C_{12}-OAc with K_2CO_3 to give a separable 1:1 mixture of MeO*N*-digoxigenin **Dig-4/5** (β/α, 1/1). Then, glycosyl acceptors **Dig-4**/**5** were reacted with reducing sugar in DMF/AcOH (3:1) at 50 °C for 48 h to obtain C_3-MeO*N*-neoglycosides **6a**-**6d** and **7a**-**7d** (Scheme 3) [15]. It is interesting that furanosides were obtained for ribose and xylose [19-21]. The glycosidic bond configuration of all the C_3-MeO*N*-neoglycosides was characterized according to the ^{1}H NMR spectral analysis (see Support Information Table 1). In addition, the digoxigenin bisdigitoxoside (**8a**) and monodigitoxoside (**9b**) were obtained *via* sodium periodate ($NaIO_4$) oxidation and 6-aminocaproic acid hydrolysis from digoxin (Scheme 4) [22].



Scheme 3. Synthesis of C_3-MeO*N*-neoglycosides of digoxigenin by the neoglycosylation method. Reagents and conditions: a. $MeONH_2$·HCl, MeOH/pyr, r.t., 13 h; b. $NaCNBH_3$, MeOH, r.t.; c. K_2CO_3, MeOH, r.t., 4h; d. DMF/AcOH (v/v 3:1), 50°C, 48 h.



Scheme 4. Synthesis of digoxigenin bisdigitoxoside (**8a**) and monodigitoxoside (**9b**) from Digoxin (**Dig**). Reagents and conditions: a.[i] $NaIO_4$, MeOH, r.t., 3h; [ii] 6-Aminocaproic acid, MeOH, r.t., 4h.

2.2. Biology

Our previous study suggested that some cardiac glycosides showed strong inhibitory effects on the proliferation of cancer cells [8,9]. Further study demonstrated that induction of Nur77 expression and its subsequent translocation from the nucleus

to the cytoplasm are critical events in apoptosis induction by cardiac glycosides in cancer cells, which underlay the new mechanism of action for cardiac glycosides toward cancer cell [8,9,12]. In this study, the cytotoxicity of all new glycosides toward NIH-H460 cancer cells was assessed by MTT assay. Digoxin was used as positive control. As a result, glycosides **3a**, **3g**, **3h** and **3i** showed significant inhibitory activities on the proliferation of NIH-H460 cancer cells at a concentration of 50 nM (Fig. 1). Also, SAR analysis revealed that neoglycosides with *O*-linkage exhibited stronger cytotoxic activities than Me*O*/*N*-neoglycosides, especially for the 3β-*O*-neoglycosides.

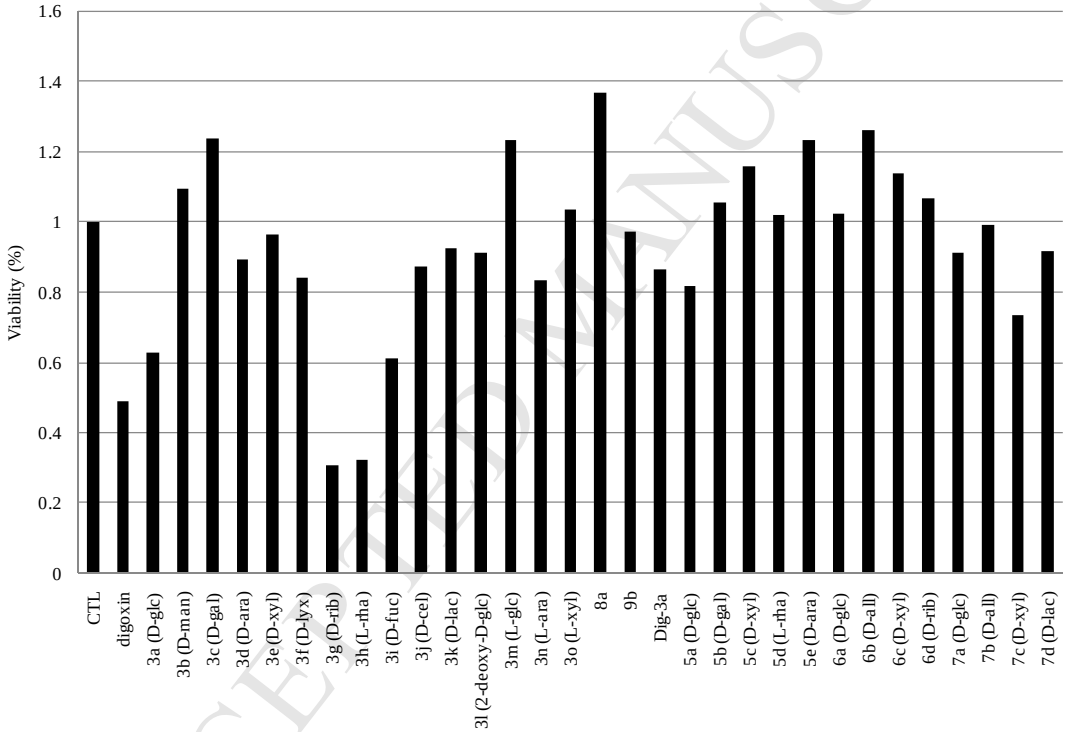


Fig. 1. Antiproliferative effects of neoglycosides on NIH-H460 cancer cells. NIH-H460 cells were incubated with neoglycosides (50 nM) for 48h and cell viability was assayed by the MTT method.

Next, the induction of Nur77 expression for the newly synthesized neoglycosides was evaluated by western blot. As a result, most of C_3-*O*-neoglycosides, especially for **3c**, **3g**-**3k**, **5b** and **8a**, **Dig-3a** exhibited significant induction of Nur77 expression at a concentration of 20 nM compared to digoxin (Fig. 2).

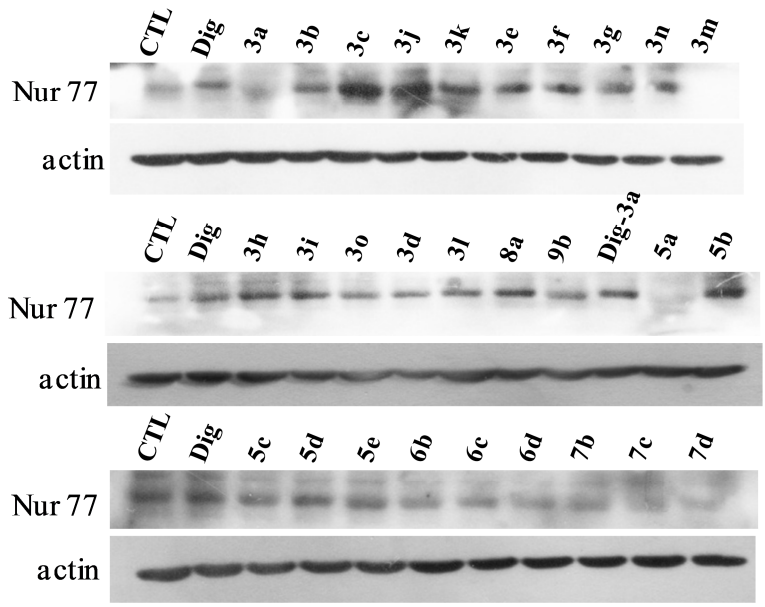


Fig. 2. Induction of Nur77 expression by neoglycosides. NIH-H460 cancer cells were incubated with neoglycosides (20 nM) for 3 h, and Nur77 expression was determined by Western blotting using anti-Nur77 antibody.

Previous study suggested that subcellular localization of induced Nur77 determines its biological function. We chose 3*β*-*O*-neoglycosides **3g**, **3h**, and **3i** that showed significant inhibitory effects on the proliferation of NIH-H460 cancer cells to evaluate their subcellular localization of the induced Nur77. We found that these compounds could significantly induce the translocation of the induced Nur77 from the nucleus to cytoplasm after 9 h treatment. Meanwhile, they could induce the Nur77 expression of cancer cells in a time dependent manner (Supporting Information Fig. S3). Among them, 3*β*-*O*-neoglycoside **3h** could significantly induce the translocation of the induced Nur77 from the nucleus to cytoplasm after 6 h (Fig. 3). We then tested the apoptotic level of NIH-H460 cells after treating with **3g**, **3h** and **3i** by Flow Cytometry after staining with PI and Annixin V. Results showed apoptotic ratio of **3g** was about 14.79%, **3h** was about 14.85% and **3i** was 34.03% (Fig. 4). These results demonstrate **3g**, **3h** and **3i** was good cancer cell apoptosis inducer.

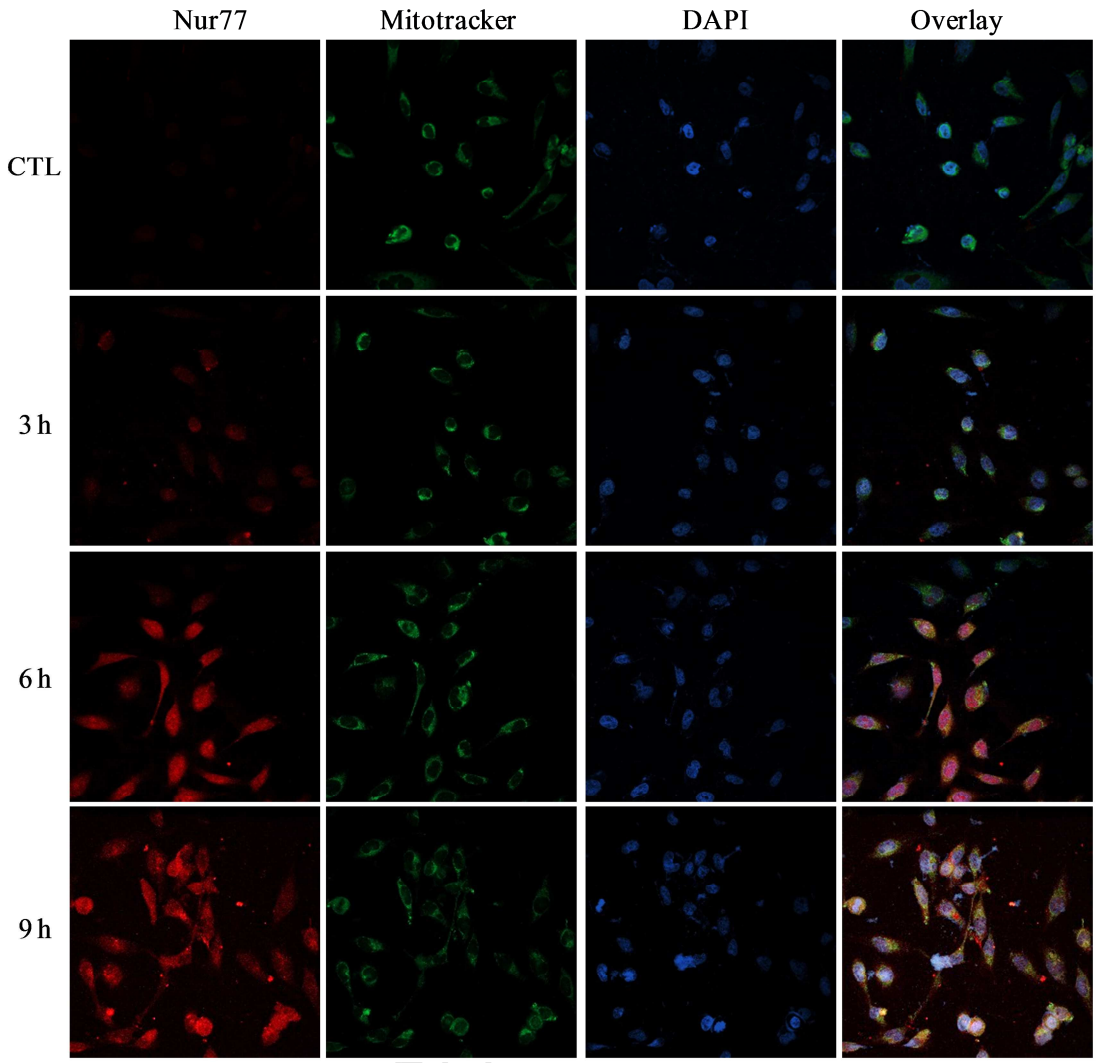


Fig. 3. Effects of 3*β*-*O*-neoglycosides **3h** on translocation of the induced Nur77 protein from the nucleus to the cytoplasm. NIH-H460 cancer cells were treated with 3*β*-*O*-neoglycosides **3h** (20 nM) for 3, 6, and 9 h. Endogenous Nur77 expression was immunostained with anti-Nur77 antibody. Cells were costained with DAPI to visualize the nuclei.

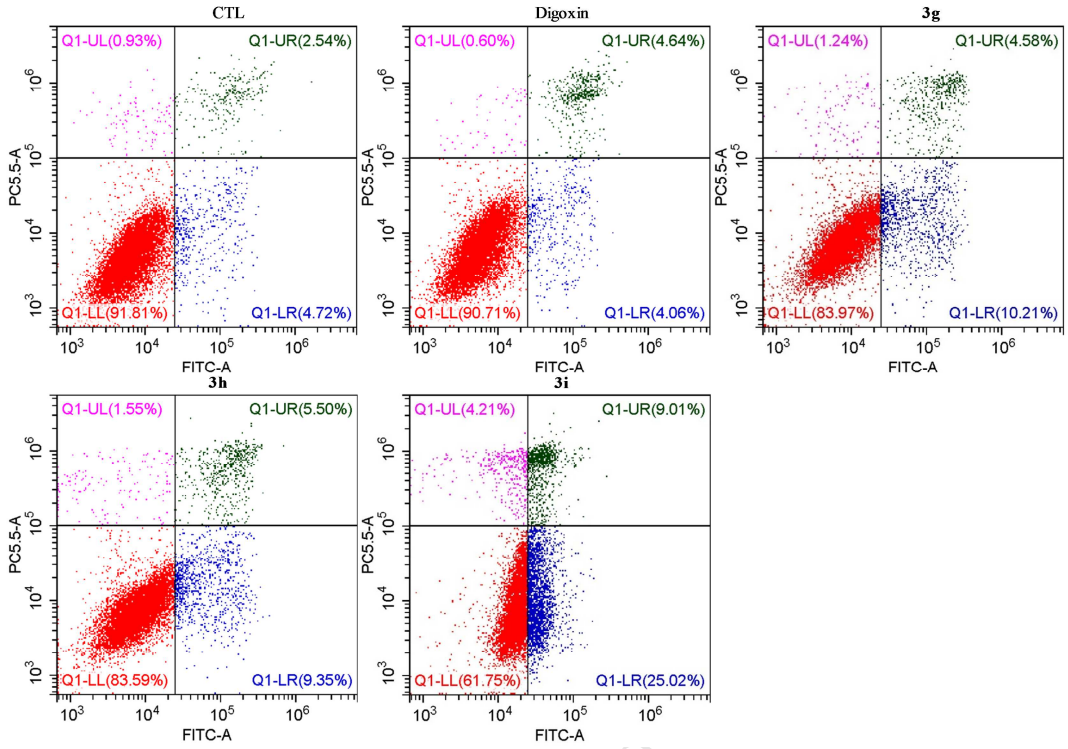


Fig. 4. Pro-apoptotic effects of 3*β*-*O*-neoglycosides **3g**, **3h**, and **3i** toward NIH-H460 cancer cells. NIH-H460 cancer cells were treated with 3*β*-*O*-neoglycosides **3g**, **3h**, and **3i** (20 nM) for 12 h.

3. Conclusions

In conclusion, we have successfully synthesized a series of C_3-*O*-glycosides of digoxigenin by the Koenigs-Knorr reaction and a series of C_3-MeO*N*-neoglycosides of digoxigenin by neoglycosylation. Their anticancer activities were investigated by examine the induction of Nur77 expression and its translocation from the nucleus to cytoplasm together with cytotoxicity toward NIH-H460 cancer cells. Overall, we found that 3*β*-*O*-neoglycosides showed the strongest cytotoxicity. Preliminary structure-activity relationship (SAR) analysis revealed that cardiac glycosides with *O*-linkage exhibited stronger cytotoxic activities and induction of Nur77 expression than MeO*N*-neoglycosides. Especially, 3*β*-*O*-neoglycosides exhibited the strongest cytotoxic activity; which is consistent with the literature [23]. Meanwhile, 3*β*-*O*-neoglycosides **3g**, **3h**, and **3i** could induce the Nur77 expression and its translocation from the nucleus to cytoplasm, leading the apoptosis of cancer cells.

Their potential anticancer effects deserve future study in vivo.

4. Experimental

4.1. Chemistry

4.1.1 General materials

All reagents were obtained from commercial suppliers as follows. All reducing sugars, *N*,*N*-dimethylformamide (DMF), pyridine (Pyr), 4-dimethylaminopyridine (DMAP), Dess-Martin periodinane, methoxylamine hydrochloride (CH_3ONH_2·HCl), Sodium cyanoborohydride ($NaCNBH_3$), Sodium borohydride ($NaBH_4$), 6-aminocaproic acid, Sodiumperiodate ($NaIO_4$), Silver oxide (Ag_2O), Silver carbonate (Ag_2CO_3), hydrogen bromide (33% in acetic acid) and sodium methoxide (CH_3ONa) were purchased from Shanghai Aladdin Bio-Chem Technology Co., LTD (Shanghai, China). Acetic anhydrides (Ac_2O), sulfuric acid (H_2SO_4), hydrochloric acid (HCl), acetic acid (AcOH), were purchased from Tianjin Tie Xiang Commerce Development Co., LTD (Tianjin, China). Digoxin was purchased from Shanghai Raise Chemical Technology Co., LTD (Shanghai, China).

4.1.2 General methods.

TLC analysis was performed on precoated silica gel GF254 plates (Qingdao Haiyang Chemical Group Corp., Qingdao). Reactions were monitored by UV and/or by treatment with 5% Vanillin-sulfuric acid and subsequent heating. Silica gel (100-200, 300-400 mesh) for column chromatography was from Qingdao Haiyang Chemical Group Corp., Qingdao, China. Optical rotations were measured on a P-1020 digital polarimeter (JASCO Corporation Tokyo, Japan). UV spectra were measured on a Jasco V-550 UV/vis spectrophotometer. NMR spectra were measured on a Bruker AV 300, 400, and 600 instruments. The chemical shifts are expressed in δ (ppm) relative to the chemical shifts of solvent resonances using $CDCl_3$, CD_3OD, and pyridine-d_5 with 0.05% v/v TMS (Cambridge Isotope Laboratories, MA, USA), and coupling constants (*J*) are given in hertz. All NMR spectra were recorded at ambient temperature. HRESIMS data was obtained on a Micromass Q-TOF mass spectrometer. Analytical HPLC was performed on a Shimadzu LC-20AB liquid chromatography

system equipped with an SPD-M20A diode array detector using a Luna C18column (4.6 × 250 mm, 5 μm, Phenomenex, Torrance, California, USA) by following methods at a wavelength of 220 nm, flow rate = 1 ml/min. Semi-preparative reverse-phase HPLC was conducted on a Gemini C18 column (5 μm, 10 × 250 mm, Phenomenex, Torrance, California, USA) using the following methods at a wavelength of 220 nm, flow rate =3 ml/min.

4.1.3. General procedure for preparation of 1-bromo-peracetylated glycosyl donors

To a solution of reducing sugars (4 mmol) in anhydrous pyridine (10mL) was added Ac_2O (20 mL) and AcOH (3 mL) at room temperature. After stirring at 90°C for 6h, the reaction was quenched with MeOH and the resulting mixture was concentrated under reduced pressure. The residue was diluted with EtOAc and was washed with 1*M* HCl, saturated $NaHCO_3$ and H_2O successively, dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure. The peracetylated sugars were obtained and used without further purification.

To a solution of peracetylated sugars (1.28 mmol) in CH_2Cl_2 (7 mL) was slowly added a 33% (w/w) solution of HBr in AcOH and stirred for 3 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 and washed with ice water, saturated $NaHCO_3$ and water successively, dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure. The crude 1-bromo-peracetylated glycosyl donors (**1a-1o**) was obtained and used without further purification.

4.1.4. General procedure for preparation of C_3-O-neoglycoside library

Glycosylation of digoxigenin aglycones with 1-bromo glycosyl donors was accomplished following the Koenigs-Knorr reaction method. To a solution of digoxigenin aglycones (**Dig-1a**, **Dig-1b**, and **Dig-3a**, 0.25 mmol) in CH_2Cl_2 was added a mixture solution of 1-bromo-peracetylated sugars (0.80 mmol) and Ag_2O/Ag_2CO_3 (1:1 w/w) (1.2 mmol) in anhydrous CH_2Cl_2 (8 mL). The reaction mixture was added molecular sieves (4Å) and stirred under N_2 atmosphere for 72 h at room temperature. The crude product was obtained by filtered and concentrated under reduced pressure. The residue was purified by semipreparative RP-HPLC (65% MeOH-H_2O) to obtain glycosylation products (**2a-2o** and **4a-4e**).

The NaOMe solution was prepared by dissolving 10 mg of NaOMe (0.18mmol) in 10 mL of MeOH until the *p*H value was 9. The glycosylation product (**2a**-**2o** and **4a**-**4e**) was dissolved in the preparative NaOMe solution (2mL) and the reaction mixture was stirred for 5 h at room temperature. The reaction solution was neutralized with AcOH (pH = 7) and then concentrated under reduced pressure. The residue was purified by semipreparative RP-HPLC (35%-40% MeOH-H_2O) to obtain 3*β*-*O*-neoglycosides **3a**-**3o** and 3*α*-*O*-neoglycosides **5a**-**5e**. All compounds were characterized by analyses of ^{1}H and ^{13}C NMR spectra as well as ESI-MS.

*4.1.4.1. 3β-O-(β-D-glucopyranosyl)-digoxigenin (**3a**)*. Obtained as a white solid (12.8 mg, 61%) from 3*β*-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-*D*-glucopyranosyl)-*12-O-acetyl -digoxigenin* (**2a**). $[\alpha]_D^{29}$ +19.6 (*c* 0.70, MeOH); ^{1}H NMR (400 MHz, Pyridine-d_5): δ_H 6.25 (1H, s, H-22) , 5.28 (1H, dd, *J* = 18.1, 1.8 Hz, H-21a), 5.13 (1H, dd, *J* = 18.0, 1.8 Hz, H-21b), 4.95 (1H, d, *J* = 8.0 Hz, H-1'), 4.57 (1H, d, *J* = 10.0 Hz), 4.40 (1H, br s, H-3), 4.26 (2H, d, *J* = 6.1 Hz), 4.01 (2H, m) 3.74 (1H, m, H-17), 3.72 (1H, m, H-12), 2.11-1.76 (15H, m), 1.76-1.36 (4H, m), 1.23 (3H, s, H-18), 0.83 (3H, s, H-19); ^{13}C NMR (100 MHz, Pyridine-d_5): δc 177.0 (C-20), 175.1 (C-23), 117.7 (C-22), 85.7 (C-14), 74.9 (C-12), 74.5 (C-3), 74.4 (C-21), 57.2 (C-13), 46.9 (C-17), 42.0 (C-8), 33.3 (C-9), 35.7 (C-10), 33.8 (C-15), 37.1 (C-5), 27.3 (C-6),31.2 (C-11), 30.8 (C-4), 28.2 (C-16), 27.5 (C-2), 31.2 (C-1), 24.1 (C-19), 22.8 (C-7), 10.5 (C-18), 103.3 (C-1'), 75.7 (C-2'), 78.8 (C-3'), 72.2 (C-4'), 78.8 (C-5'), 63.3 (C-6'); ESI-MS *m/z* 575.5 $[M + Na]^+$; 1127.5 $[2M + Na]^+$.

*4.1.4.2. 3β-O-(α-D-mannopyranosyl)-digoxigenin (**3b**)*. Obtained as a white solid (4.8 mg, 74%) from 3*β*-*O*-(2,3,4,6-tetra-*O*-acetyl-*α*-*D*-mannopyranosyl)-*12-O-acetyl-digoxigenin* (**2b**). $[\alpha]_D^{29}$ +13.2 (*c* 0.40, MeOH); ^{1}H NMR (600 MHz, Pyridine-d_5): δ_H 6.26 (1H, s, H-22) , 5.49 (1H, d, *J* = 1.2 Hz, H-1'), 5.28 (1H, dd, *J* = 18.1, 1.8 Hz, H-21a), 5.13 (1H, dd, *J* = 18.0, 1.8 Hz, H-21b), 4.66-4.60 (3H, m), 4.57 (1H, m), 4.44-4.39 (2H, m), 4.27 (1H, br s, H-3), 3.76 (1H, m, H-17), 3.72 (1H, m, H-12), 2.15-1.75 (11H, m), 1.68 (2H, m), 1.56 (1H, m), 1.46 (2H, m), 1.32 (3H, m), 1.25 (3H, s, H-18), 0.85 (3H, s, H-19); ^{13}C NMR (150 MHz, Pyridine-d_5): δc177.0 (C-20), 175.2 (C-23), 117.8 (C-22), 85.7 (C-14), 74.9 (C-12), 74.4 (C-21), 72.1 (C-3),

57.1 (C-13), 47.0 (C-17), 42.0 (C-8), 33.2 (C-9), 35.8 (C-10), 33.9 (C-15), 37.8 (C-5), 27.4 (C-6), 31.1 (C-11), 32.7 (C-4), 28.3 (C-16), 24.6 (C-2), 31.2 (C-1), 24.3 (C-19), 22.6 (C-7), 10.5 (C-18), 103.8 (C-1'), 73.1 (C-2'), 73.6 (C-3'), 69.8 (C-4'), 76.0 (C-5'), 63.7 (C-6'); ESI-MS *m/z* 553.3 $[M + H]^+$;1127.4 $[2M + Na]^+$.

*4.1.4.3. 3β-O-(β-D-galactopyranosyl)-digoxigenin (**3c**)*. Obtained as a white solid (22.5 mg, 75%) from 3*β-O*-(2,3,4,6-tetra-*O*-acetyl-*β-D*-galactopyranosyl)-*12-O-acetyl-digoxigenin* (**2c**). $[\alpha]_D^{29}$ +5.2 (*c* 0.80, MeOH); ^{1}H NMR (400 MHz, Pyridine-d_5): δ_H 6.25 (1H, s, H-22) , 5.28 (1H, dd, *J* = 18.1, 1.8 Hz, H-21a), 5.13 (1H, dd, *J* = 18.0, 1.8 Hz, H-21b), 4.86 (1H, d, *J* = 7.8 Hz, H-1'), 4.60 (1H, d, *J* = 3.0 Hz), 4.50-4.45 (3H, m), 4.40 (1H, br s, H-3), 4.18 (1H, dd, *J* = 9.8, 3.6 Hz), 4.09 (1H, t, *J* = 5.8 Hz), 3.75(1H, m, H-17), 3.71(1H, m, H-12), 2.18-1.68 (15H, m), 1.64-1.28 (4H, m), 1.23 (3H, s, H-18), 0.81 (3H, s, H-19); ^{13}C NMR (100 MHz, Pyridine-d_5): δc 177.1 (C-20), 175.1 (C-23), 117.7 (C-22), 85.7 (C-14), 74.9 (C-12), 74.6 (C-3), 74.4 (C-21), 57.1 (C-13), 46.9 (C-17), 41.9 (C-8), 33.2 (C-9), 35.7 (C-10), 33.8 (C-15), 37.0 (C-5), 27.3 (C-6), 31.1 (C-11), 30.7 (C-4), 28.3 (C-16), 27.5 (C-2), 31.1 (C-1), 24.0 (C-19), 22.8 (C-7), 10.6 (C-18), 104.1 (C-1'), 73.0 (C-2'), 75.8 (C-3'), 70.6 (C-4'), 77.3 (C-5'), 63.3 (C-6'); ESI-MS *m/z* 575.7 $[M + Na]^+$; 1127.8 $[2M + Na]^+$.

*4.1.4.4. 3β-O-(α-D-arabinopyranosyl)-digoxigenin (**3d**)*. Obtained as a white solid (28.3 mg, 76%) from 3*β-O*-(2,3,4-tri-*O*-acetyl-*α-D*-arabinopyranosyl)-*12-O-acetyl-digoxigenin* (**2d**). $[\alpha]_D^{29}$ +24.5 (*c* 0.80, MeOH); ^{1}H NMR (400 MHz, Pyridine-d_5): δ_H 6.25 (1H, s, H-22) , 5.28 (1H, dd, *J* = 18.1, 1.8 Hz, H-21a), 5.13 (1H, dd, *J* = 18.0, 1.8 Hz, H-21b), 4.73 (1H, d, *J* = 7.2 Hz, H-1'), 4.34 (1H, br s, H-3), 4.32 (1H, m), 4.29 (1H, m), 4.16 (1H, m), 3.76 (1H, m, H-17), 3.72 (1H, m, H-12), 2.16-1.68 (15H, m), 1.61-1.31 (4H, m), 1.23 (3H, s, H-18), 0.80 (3H, s, H-19); ^{13}C NMR (100 MHz, Pyridine-d_5): δc 177.1 (C-20), 175.1 (C-23), 117.7 (C-22), 85.8 (C-14), 74.8 (C-12), 74.3 (C-3), 74.4 (C-21), 57.0 (C-13), 46.8 (C-17), 41.9 (C-8), 33.1 (C-9), 35.7 (C-10), 33.8 (C-15), 37.3 (C-5), 27.4 (C-6), 31.1 (C-11), 32.8 (C-4), 28.1 (C-16), 25.0 (C-2), 31.1 (C-1), 24.0 (C-19), 22.5 (C-7), 10.5 (C-18), 103.8 (C-1'), 75.0(C-2'), 72.8 (C-3'), 69.9 (C-4'), 67.3 (C-5') ;

ESI-MS *m/z* 523.6 $[M + H]^+$; 1067.6 $[2M + Na]^+$.

*4.1.4.5. 3β-O-(β-D-xylopyranosyl)-digoxigenin (**3e**).* Obtained as a white solid (18.8 mg, 67%) from 3*β*-*O*-(2,3,4-tri-*O*-acetyl-*β*-*D*-xylopyranosyl)-*12*-*O*-*acetyl*-*digoxigenin* (**2e**). $[\alpha]_D^{29}$ +20.3 (*c* 0.70, MeOH); ^{1}H NMR (400 MHz, Pyridine-d_5): δ_H 6.25 (1H, s, H-22) , 5.28 (1H, dd, *J* = 18.1, 1.8 Hz, H-21a), 5.13 (1H, dd, *J* = 18.0, 1.8 Hz, H-21b), 4.82 (1H, d, *J* = 7.4 Hz, H-1'), 4.34 (1H, br s, H-3), 4.33 (1H, m), 4.29-4.14 (2H, m), 4.03 (1H, t, *J* = 6.4 Hz), 3.74 (1H, m, H-17), 3.72 (1H, m, H-12), 2.16-1.68 (15H, m), 1.57-1.34 (4H, m), 1.24 (3H, s, H-18), 0.85 (3H, s, H-19); ^{13}C NMR (100 MHz, Pyridine-d_5): δc 177.1 (C-20), 175.2 (C-23), 117.7 (C-22), 85.7 (C-14), 75.0 (C-12), 74.9 (C-3), 74.4 (C-21), 57.1 (C-13), 46.9 (C-17), 42.0 (C-8), 33.3 (C-9), 35.7 (C-10), 33.8 (C-15), 37.1 (C-5), 27.3 (C-6), 31.1 (C-11), 31.0 (C-4), 28.2 (C-16), 27.5 (C-2), 31.0 (C-1), 24.1 (C-19), 22.6 (C-7), 10.5 (C-18), 100.2 (C-1'), 75.5 (C-2'), 78.9 (C-3'), 71.6 (C-4'), 67.5 (C-5'); ESI-MS *m/z* 523.4 $[M + H]^+$; 1067.6 $[2M + Na]^+$.

*4.1.4.6. 3β-O-(α-D-lyxopyranosyl)-digoxigenin (**3f**).* Obtained as a white solid (10.7 mg, 56%) from 3*β*-*O*-(2,3,4-tri-*O*-acetyl-*α*-*D*-lyxopyranosyl)-*12*-*O*-*acetyl*-*digoxigenin* (**2f**). $[\alpha]_D^{29}$ +17.8 (*c* 0.55, MeOH); ^{1}H NMR (400 MHz, Pyridine-d_5): δ_H 6.26 (1H, s, H-22) , 5.42 (1H, d, *J* = 1.2 Hz, H-1'), 5.28 (1H, dd, *J* = 18.1, 1.8 Hz, H-21a), 5.13 (1H, dd, *J* = 18.0, 1.8 Hz, H-21b), 4.67 (1H, s), 4.56 (1H, br s, H-3), 4.25 (2H, m), 4.14 (1H, m), 3.75 (1H, m, H-17), 3.73 (1H, m, H-12), 2.18-1.69 (13H, m), 1.64-1.35 (6H, m), 1.26 (3H, s, H-18), 0.86 (3H, s, H-19); ^{13}C NMR (100 MHz, Pyridine-d_5): δc 177.0 (C-20), 175.1 (C-23), 117.8 (C-22), 85.7 (C-14), 74.9 (C-12), 72.8 (C-3), 74.4 (C-21), 57.1 (C-13), 47.0 (C-17), 42.0 (C-8), 33.2 (C-9), 35.8 (C-10), 33.9 (C-15), 37.7 (C-5), 27.3 (C-6), 31.2 (C-11), 32.9 (C-4), 28.3 (C-16), 24.7 (C-2), 31.0 (C-1), 24.3 (C-19), 22.7 (C-7), 10.5 (C-18), 100.5 (C-1'), 72.5 (C-2'), 73.8 (C-3'), 69.3 (C-4'), 65.4 (C-5'); ESI-MS *m/z* 523.4 $[M + H]^+$; 1067.7 $[2M + Na]^+$.

*4.1.4.7. 3β-O-(β-D-ribopyranosyl)-digoxigenin (**3g**).* Obtained as a white solid (8.6 mg, 57%) from 3*β*-*O*-(2,3,4-tri-*O*-acetyl-*β*-*D*-ribopyranosyl)-*12*-*O*-*acetyl*-*digoxigenin* (**2g**). $[\alpha]_D^{29}$ +7.8 (*c* 0.60, MeOH); ^{1}H NMR (400 MHz, Pyridine-d_5): δ_H 6.26 (1H, s, H-22), 5.39 (1H, d, *J* = 4.2, H-1'), 5.27 (1H, dd, *J* = 18.0, 1.8 Hz, H-21a), 5.13 (1H, dd, *J* = 18.0, 1.8 Hz, H-21b), 4.47 (1H, m), 4.28 (1H, m), 4.21 (1H, m), 4.21 (1H, br s,

H-3, overlap), 4.16 (2H, s), 3.76 (1H, m, H-17), 3.74 (1H, m, H-12), 2.16-2.09 (3H, m), 2.02 (1H, m), 1.94 (2H, m), 1.89-1.76 (6H, m), 1.66 (2H, m), 1.59 (3H, m), 1.35 (2H, m), 1.25 (3H, s, H-18), 1.18 (1H, m), 0.89 (3H, s, H-19); ^{13}C NMR (100 MHz, Pyridine-d_5): δc 177.1 (C-20), 175.2 (C-23), 117.7 (C-22), 85.7 (C-14), 74.9 (C-12), 73.5 (C-3), 74.4 (C-21), 57.2 (C-13), 46.9 (C-17), 42.0 (C-8), 33.3 (C-9), 35.8 (C-10), 33.9 (C-15), 37.4 (C-5), 27.3 (C-6), 31.2 (C-11), 30.6 (C-4), 28.3 (C-16), 27.5 (C-2), 31.4 (C-1), 24.3 (C-19), 22.7 (C-7), 10.5 (C-18), 100.9 (C-1'), 73.3 (C-2'), 69.0 (C-3'), 70.9 (C-4'), 65.6 (C-5'); ESI-MS *m/z* 523.5 $[M + Na]^+$;1067.4 $[2M + Na]^+$.

*4.1.4.8. 3β-O-(α-L-rhamnopyranosyl)-digoxigenin (**3h**).* Obtained as a white solid (20.4 mg, 60%) from 3*β-O*-(2,3,4-tri-*O*-acetyl-*α-L*-rhamnopyranosyl)-*12-O-acetyl-digoxigenin* (**2h**). $[\alpha]_D^{29}$ +11.6 (*c* 0.70, MeOH); ^{1}H NMR (400 MHz, Pyridine-d_5): δ_H 6.26 (1H, s, H-22) , 5.41 (1H, br s, H-1'), 5.28 (1H, dd, *J* = 18.1, 1.8 Hz, H-21a), 5.13 (1H, dd, *J* = 18.0, 1.8 Hz, H-21b), 4.54 (1H, br s, H-3), 4.53 (1H, m), 4.28 (2H, m), 4.16 (1H, s), 3.76 (1H, m, H-17), 3.73 (1H, m, H-12), 2.16-1.73 (13H, m), 1.67 (3H, d, *J* = 5.2 Hz), 1.58 (4H, m), 1.36 (1H, m), 1.25 (3H, s, H-18), 1.18 (1H, m), 0.85 (3H, s, H-19); ^{13}C NMR (100 MHz, Pyridine-d_5): δc 177.1 (C-20), 175.2 (C-23), 117.7 (C-22), 85.7 (C-14), 74.8 (C-12), 73.3 (C-3), 74.4 (C-21), 57.1 (C-13), 46.9 (C-17), 41.9 (C-8), 33.2 (C-9), 35.7 (C-10), 33.8 (C-15), 37.4 (C-5), 27.1 (C-6), 31.2 (C-11), 30.5 (C-4), 28.2 (C-16), 27.5 (C-2), 31.4 (C-1), 24.3 (C-19), 22.6 (C-7), 10.5 (C-18), 100.2 (C-1'), 72.5 (C-2'), 73.3 (C-3'), 74.4 (C-4'), 70.4 (C-5'), 19.0 (C-6'); ESI-MS *m/z* 537.3 $[M + H]^+$; 1095.7 $[2M + Na]^+$.

*4.1.4.9. 3β-O-(β-L-fucopyranosyl)-digoxigenin (**3i**).* Obtained as a white solid (26.6 mg, 65%) from 3*β-O*-(2,3,4-tri-*O*-acetyl-*β-L*-fucopyranosyl)-*12-O-acetyl-digoxigenin* (**2i**). $[\alpha]_D^{29}$ +16.2 (*c* 0.70, MeOH); ^{1}H NMR (400 MHz, Pyridine-d_5): δ_H 6.26 (1H, s, H-22) , 5.28 (1H, dd, *J* = 18.1, 1.8 Hz, H-21a), 5.13 (1H, dd, *J* = 18.0, 1.8 Hz, H-21b), 4.72 (1H, d, *J* = 7.8, H-1'), 4.36 (2H, m,), 4.11 (1H, m), 4.08 (1H, br s, H-3), 3.81 (1H, m, H-17), 3.74 (1H, m, H-12), 2.16-1.71 (15H, m), 1.56 (1H, m), 1.55 (3H, d, *J* = 6.4 Hz), 1.43 (3H, m), 1.28 (1H, m), 1.23 (3H, s, H-18), 0.80 (3H, s, H-19); ^{13}C NMR (100 MHz, Pyridine-d_5): δc 177.1 (C-20), 175.1 (C-23), 117.7 (C-22), 85.7 (C-14),

74.9 (C-12), 74.4 (C-3), 74.4 (C-21), 57.1 (C-13), 46.9 (C-17), 42.0 (C-8), 33.2 (C-9), 35.7 (C-10), 33.8 (C-15), 37.2 (C-5), 27.4 (C-6), 31.1 (C-11), 32.9 (C-4), 28.2 (C-16), 25.2 (C-2), 30.9 (C-1), 24.0 (C-19), 22.6 (C-7), 10.5 (C-18), 103.7 (C-1'), 73.1 (C-2'), 75.9 (C-3'), 72.8 (C-4'), 71.6 (C-5'), 17.8 (C-6'); ESI-MS *m/z* 537.5 $[M + Na]^+$; 1095.4 $[2M + Na]^+$.

*4.1.4.10. 3β-O-(β-D-cellobiosyl)-digoxigenin (**3j**).* Obtained as a white solid (8.0 mg, 51%) from 3*β*-*O*-(2,3,6,2',3',4',6'-hepta-*O*-acetyl-*β*-*D*-cellobiosyl)-*12-O-acetyl-digoxigenin* (**2j**). $[\alpha]_D^{29}$ +7.2 (*c* 0.50, MeOH); ^{1}H NMR (400 MHz, Pyridine-d_5): δ_H 6.25 (1H, s, H-22) , 5.28 (1H, dd, *J* = 18.1, 1.8 Hz, H-21a), 5.13 (1H, dd, *J* = 18.0, 1.8 Hz, H-21b), 5.43 (1H, br s), 5.22 (1H, d, *J* = 7.8, H-1'), 4.90 (1H, d, *J* = 7.8, H-1''), 4.53 (3H, m), 4.40-4.20 (5H, m), 4.15-3.99 (3H, m), 3.93 (1H, m), 3.76 (1H, m, H-17), 3.72 (1H, m, H-12), 2.16-1.65 (13H, m), 1.55 (2H, m), 1.45-1.27 (2H, m), 1.24 (3H, s, H-18), 0.84 (3H, s, H-19); ^{13}C NMR (100 MHz, Pyridine-d_5): δc 177.0 (C-20), 175.1 (C-23), 117.8 (C-22), 85.7 (C-14), 75.0 (C-12), 74.8 (C-3), 74.4 (C-21), 57.2 (C-13), 46.9 (C-17), 42.0 (C-8), 33.3 (C-9), 35.8 (C-10), 33.9 (C-15), 37.1 (C-5), 27.3 (C-6), 31.2 (C-11), 30.7 (C-4), 28.2 (C-16), 27.5 (C-2), 31.2 (C-1), 24.1 (C-19), 22.7 (C-7), 10.6 (C-18), 105.3 (C-1'),75.2 (C-2'), 76.8 (C-3'), 81.8 (C-4'), 77.3 (C-5'), 62.3 (C-6'), 103.2 (C-1''), 75.2 (C-2''), 78.6 (C-3''), 71.9 (C-4''), 78.8 (C-5''), 62.8 (C-6''); ESI-MS *m/z* 737.5$[M + Na]^+$;1451.5 $[2M + Na]^+$.

*4.1.4.11. 3β-O-(β-D-lactobiosyl)-digoxigenin (**3k**).* Obtained as a white solid (3.5 mg, 27%) from 3*β*-*O*-(2,3,6,2',3',4',6'-hepta-*O*-acetyl-*β*-*D*-lactobiosyl)-*12-O-acetyl-digoxigenin* (**2k**). $[\alpha]_D^{29}$ +5.2 (*c* 0.50, MeOH); ^{1}H NMR (400 MHz, Pyridine-d_5): δ_H 6.25 (1H, s, H-22) , 5.44 (1H, s), 5.28 (1H, dd, *J* = 18.1, 1.8 Hz, H-21a), 5.13 (1H, dd, *J* = 18.0, 1.8 Hz, H-21b), 5.13 (1H, d, *J* = 8.2, H-1'), 4.89 (1H, d, *J* = 8.0, H-1''), 4.58-4.45 (5H, m), 4.42-4.29 (4H, m), 4.15 (2H, m), 4.07 (1H, m), 3.90 (1H, m), 3.76 (1H, m, H-17), 3.72 (1H, m, H-12), 2.14-1.51 (15H, m), 1.45-1.26 (3H, m), 1.24 (3H, s, H-18), 0.84 (3H, s, H-19); ^{13}C NMR (100 MHz, Pyridine-d_5): δc 177.1 (C-20), 175.2 (C-23), 117.8 (C-22), 85.7 (C-14), 75.0 (C-12), 74.8 (C-3), 74.4 (C-21), 57.2 (C-13), 47.0

(C-17), 42.0 (C-8), 33.3 (C-9), 35.8 (C-10), 33.9 (C-15), 37.1 (C-5), 27.3 (C-6), 31.2 (C-11), 30.7 (C-4), 28.3 (C-16), 27.5 (C-2), 31.2 (C-1), 24.1 (C-19), 22.7 (C-7), 10.8 (C-18), 106.2 (C-1'), 75.2 (C-2'), 76.8 (C-3'), 82.7 (C-4'), 77.2 (C-5'), 62.4 (C-6'), 103.2 (C-1"), 72.9 (C-2"), 75.6 (C-3"), 70.5 (C-4"), 77.8 (C-5"), 62.8 (C-6"); ESI-MS *m/z* 737.7 $[M + Na]^+$.

*4.1.4.12. 3β-O-(2,3-dideoxy-β-D-glucal)-digoxigenin (**3l**).* Obtained as a white solid (5.2 mg, 53%) from 3*β-O*-(4,6-di-*O*-acetyl-2,3-dideoxy-*β-D*-glucal)-*12-O-acetyl-digoxigenin* (**2l**). $[\alpha]_D^{29}$ +32.8 (*c* 0.65, MeOH); ^{1}H NMR (400 MHz, Pyridine-d_5): δ_H 6.26 (1H, s, H-22) , 5.28 (1H, dd, *J* = 18.1, 1.8 Hz, H-21a), 5.13 (1H, dd, *J* = 18.0, 1.8 Hz, H-21b), 4.71 (1H, d, *J* = 7.8, H-1'), 6.33 (1H, d, *J* = 9.2, H-3'), 5.97 (1H, d, *J* = 9.2, H-2'), 5.47 (1H, s), 5.38 (1H, s), 4.73 (1H, m), 4.40 (3H, m), 4.27 (1H, br s, H-3), 3.78 (1H, m, H-17), 3.76 (1H, m, H-12), 2.15-1.72 (13H, m), 1.57 (3H, m), 1.42-1.30 (3H, m), 1.26 (3H, s, H-18), 0.92 (3H, s, H-19); ^{13}C NMR (100 MHz, Pyridine-d_5): δc 177.1 (C-20), 175.2 (C-23), 117.7 (C-22), 85.7 (C-14), 75.0 (C-12), 74.6 (C-3), 74.4 (C-21), 57.2 (C-13), 47.0 (C-17), 42.0 (C-8), 37.8 (C-9), 35.8 (C-10), 33.2 (C-15), 33.9 (C-5), 27.5 (C-6), 31.2 (C-11), 32.9 (C-4), 28.2 (C-16), 25.7 (C-2), 31.2 (C-1), 24.3 (C-19), 22.6 (C-7), 10.6 (C-18), 94.0 (C-1'), 127.5 (C-2'), 135.5 (C-3'), 73.6 (C-4'), 64.4 (C-5'), 63.1 (C-6') ; ESI-MS *m/z* 519.5 $[M + H]^+$; 1059.7 $[2M + Na]^+$.

*4.1.4.13. 3β-O-(β-L-glucopyranosyl)-digoxigenin (**3m**).* Obtained as a white solid (12.6 mg, 63%) from 3*β-O*-(2,3,4,6-tetra-*O*-acetyl-*β-L*-glucopyranosyl)-*digoxigenin* (**2m**). $[\alpha]_D^{29}$ +7.4 (*c* 0.60, MeOH); ^{1}H NMR (600 MHz, Pyridine-d_5): δ_H 6.25 (1H, s, H-22) , 5.43 (1H, s), 5.27 (1H, dd, *J* = 18.0, 1.8 Hz, H-21a), 5.13 (1H, dd, *J* = 18.0, 1.8 Hz, H-21b), 4.93 (1H, d, *J* = 7.8 Hz, H-1'), 4.55 (1H, m), 4.41(1H, m), 4.39 (1H, br s, H-3), 4.28 (2H, m), 4.06 (1H, m) 3.95 (1H, m), 3.74 (2H, m), 2.16-1.96 (9H, m), 1.84 (2H, m), 1.76 (2H, m), 1.55 (3H, m), 1.24 (3H, s, H-18), 0.83 (3H, s, H-19); ^{13}C NMR (150 MHz, Pyridine-d_5): δc 177.1 (C-20), 175.1 (C-23), 117.8 (C-22), 85.7 (C-14), 75.0 (C-12), 74.4 (C-3), 74.3 (C-21), 57.1 (C-13), 47.0 (C-17), 42.0 (C-8), 33.3 (C-9), 35.8 (C-10), 33.9 (C-15), 37.3 (C-5), 27.5 (C-6),31.2 (C-11), 32.9 (C-4), 28.3 (C-16), 25.1 (C-2), 30.9 (C-1), 24.1 (C-19), 22.6 (C-7), 10.6 (C-18), 103.3 (C-1'),

75.7 (C-2'), 79.1 (C-3'), 72.2 (C-4'), 78.8 (C-5'), 63.3 (C-6'); ESI-MS *m/z* 575.5 [M + Na]$^+$; 1127.5 [2M + Na]$^+$.

*4.1.4.14. 3β-O-(α-L-arabinopyranosyl)-digoxigenin (**3n**).* Obtained as a white solid (7.0 mg, 56%) from 3*β*-*O*-(2,3,4-tri-*O*-acetyl-*α*-*L*-arabinopyranosyl)-*digoxigenin* (**2n**). $[\alpha]_D^{29}$ +16.2 (*c* 0.60, MeOH); ^{1}H NMR (600 MHz, Pyridine-d_5): δ_H 6.25 (1H, s, H-22) , 5.40 (1H, s), 5.27 (1H, dd, *J* = 18.0, 1.8 Hz, H-21a), 5.13 (1H, dd, *J* = 18.0, 1.8 Hz, H-21b), 4.76 (1H, d, *J* = 7.0 Hz, H-1'), 4.44 (1H, t, d = 7.6 Hz), 4.34 (1H, br s, H-3), 4.32 (2H, m), 4.17 (1H, m), 3.75 (1H, m, H-17), 3.73 (1H, m, H-12), 2.16-1.68 (13H, m), 1.56 (2H, m), 1.40 (1H, m), 1.24 (3H, s, H-18), 0.84 (3H, s, H-19); ^{13}C NMR (150 MHz, Pyridine-d_5): δc 177.1 (C-20), 175.1 (C-23), 117.8 (C-22), 85.8 (C-14), 75.1 (C-12), 75.0 (C-3), 74.3 (C-21), 57.2 (C-13), 47.0 (C-17), 42.1 (C-8), 33.3 (C-9), 35.8 (C-10), 33.9 (C-15), 37.2 (C-5), 27.6 (C-6), 31.1 (C-11), 31.3 (C-4), 28.3 (C-16), 27.4 (C-2), 31.0 (C-1), 24.2 (C-19), 22.7 (C-7), 10.6 (C-18), 104.4 (C-1'), 73.0 (C-2'), 75.0(C-3'), 70.0 (C-4'), 67.4 (C-5'); ESI-MS *m/z* 523.6 [M + H]$^+$; 1067.6 [2M + Na]$^+$.

*4.1.4.15. 3β-O-(β-L-xylopyranosyl)-digoxigenin (**3o**).* Obtained as a white solid (6.0 mg, 72%) from 3*β*-*O*-(2,3,4-tri-*O*-acetyl-*β*-*L*-xylopyranosyl)-*digoxigenin* (**2o**). $[\alpha]_D^{29}$ +9.2 (*c* 0.74, MeOH); ^{1}H NMR (600 MHz, Pyridine-d_5): δ_H 6.25 (1H, s, H-22) , 5.43 (1H, s), 5.27 (1H, dd, *J* = 18.0, 1.8 Hz, H-21a), 5.13 (1H, dd, *J* = 18.0, 1.8 Hz, H-21b), 4.82 (1H, d, *J* = 7.5 Hz, H-1'), 4.37 (1H, br s, H-3), 4.36 (1H, m), 4.27 (1H, m), 4.17 (1H, t, d = 8.5 Hz), 4.04 (1H, t, *J* = 8.2 Hz), 3.75 (1H, m, H-17), 3.73 (1H, m, H-12), 2.18-1.77 (15H, m), 1.62-1.53 (3H, m), 1.41 (1H, m), 1.24 (3H, s, H-18), 0.85 (3H, s, H-19); ^{13}C NMR (150 MHz, Pyridine-d_5): δc 177.1 (C-20), 175.1 (C-23), 117.8 (C-22), 85.8 (C-14), 75.0 (C-12), 74.5 (C-3), 74.4 (C-21), 57.2 (C-13), 47.0 (C-17), 42.1 (C-8), 33.3 (C-9), 35.8 (C-10), 33.9 (C-15), 37.4 (C-5), 27.5 (C-6), 31.2 (C-11), 33.0 (C-4), 28.3 (C-16), 25.2 (C-2), 31.0 (C-1), 24.2 (C-19), 22.7 (C-7), 10.6 (C-18), 104.0 (C-1'), 75.5(C-2'), 79.0 (C-3'), 71.7 (C-4'), 67.7 (C-5'); ESI-MS *m/z* 523.4 [M + H]$^+$; 1067.6 [2M + Na]$^+$.

*4.1.4.16. 3α-O-(β-D-glucopyranosyl)-digoxigenin (**5a**).* Obtained as a white solid (14.0 mg, 70.2%) from 3*α*-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-*D*-glucopyranosyl)-*digoxigenin* (**4a**). $[\alpha]_D^{29}$ +19.5 (*c*

0.70, MeOH); ^{1}H NMR (600 MHz, Pyridine-d_5): δ_H 6.25 (1H, s, H-22) , 5.26 (1H, dd, *J* = 18.0, 1.8 Hz, H-21a), 5.13 (1H, dd, *J* = 18.0, 1.8 Hz, H-21b), 5.05 (1H, d, *J* = 7.6 Hz, H-1'), 4.60 (1H, d, *J* = 10.0 Hz), 4.43 (1H, m), 4.30 (2H, m), 4.07 (1H, m), 4.02 (1H, qd, H-3), 3.72 (1H, m, H-17), 3.59 (1H, m, H-12), 1.22 (3H, s, H-18), 0.83 (3H, s, H-19); ^{13}C NMR (150 MHz, Pyridine-d_5): δc 177.0 (C-20), 175.1 (C-23), 117.8 (C-22), 85.6 (C-14), 74.9 (C-12), 78.3 (C-3), 74.4 (C-21), 57.1 (C-13), 46.9 (C-17), 42.1 (C-8), 33.7 (C-9), 35.5 (C-10), 33.9 (C-15), 42.4 (C-5), 27.9 (C-6), 30.9 (C-11), 35.1 (C-4), 28.2 (C-16), 35.6 (C-2), 27.9 (C-1), 23.8 (C-19), 22.8 (C-7), 10.5 (C-18), 102.7 (C-1'), 75.8 (C-2'), 79.1 (C-3'), 72.2 (C-4'), 78.9 (C-5'), 63.3 (C-6'); ESI-MS *m/z* 553.3 $[M + H]^+$; 1127.3 $[2M + Na]^+$.

*4.1.4.17. 3a-O-(β-D-galactopyranosyl)-digoxigenin (**5b**).* Obtained as a white solid (35.3 mg, 70.1%) from 3*a-O*-(2,3,4,6-tetra-*O*-acetyl-*β-D*-galactopyranosyl)-*digoxigenin* (**4b**). $[\alpha]_D^{29}$ +30.2 (*c* 0.85, MeOH); ^{1}H NMR (300 MHz, Pyridine-d_5): δ_H 6.24 (1H, s, H-22) , 5.25 (1H, dd, *J* = 18.0, 1.8 Hz, H-21a), 5.11 (1H, dd, *J* = 18.0, 1.8 Hz, H-21b), 4.94 (1H, d, *J* = 7.5 Hz, H-1'), 4.58 (1H, d, *J* = 10.0 Hz), 4.46 (2H, m), 4.22 (1H, m), 4.11 (1H, m), 3.98 (1H, qd, H-3), 3.70 (1H, m, H-17), 3.54 (1H, m, H-12), 1.20 (3H, s, H-18), 0.80 (3H, s, H-19); ^{13}C NMR (75 MHz, Pyridine-d_5): δc 177.0 (C-20), 175.1 (C-23), 117.6 (C-22), 85.5 (C-14), 74.7 (C-12), 78.2 (C-3), 74.3 (C-21), 56.9 (C-13), 46.7 (C-17), 41.9 (C-8), 33.6 (C-9), 35.3 (C-10), 33.7 (C-15), 42.2 (C-5), 27.7 (C-6), 30.8 (C-11), 35.0 (C-4), 28.0 (C-16), 35.5 (C-2), 27.7 (C-1), 23.6 (C-19), 22.6 (C-7), 10.4 (C-18), 103.2 (C-1'), 73.0 (C-2'), 75.7 (C-3'), 70.5 (C-4'), 77.2 (C-5'), 62.8 (C-6'); ESI-MS *m/z* 575.7 $[M + Na]^+$; 1127.8 $[2M + Na]^+$.

*4.1.4.18. 3α-O-(α-D-arabinopyranosyl)-digoxigenin (**5c**).* Obtained as a white solid (11.5 mg, 62.7%) from 3*α-O*-(2,3,4-tri-*O*-acetyl-*α-D*-arabinopyranosyl)-*digoxigenin* (**4c**). $[\alpha]_D^{29}$ +15.2 (*c* 0.70, MeOH); ^{1}H NMR (300 MHz, Pyridine-d_5): δ_H 6.25 (1H, s, H-22), 5.28 (1H, dd, *J* = 18.1, 1.8 Hz, H-21a), 5.13 (1H, dd, *J* = 18.0, 1.8 Hz, H-21b), 4.85 (1H, d, *J* = 7.4 Hz, H-1'), 4.46 (1H, m), 4.35 (2H, m), 4.23 (1H, m), 3.95 (1H, qd, H-3), 3.87 (1H, m), 3.74 (1H, m, H-17), 3.65 (1H, m, H-12), 2.16-1.96 (4H, m), 1.94-1.64 (10H, m), 1.53 (1H, m), 1.29 (1H, m), 1.23 (3H, s, H-18), 0.98 (1H, m),

0.85 (3H, s, H-19); ^{13}C NMR (75 MHz, Pyridine-d_5): δc 177.0 (C-20), 175.1 (C-23), 117.8 (C-22), 85.6 (C-14), 74.9 (C-12), 78.4 (C-3), 74.4 (C-21), 57.1 (C-13), 47.0 (C-17), 42.1 (C-8), 33.7 (C-9), 35.5 (C-10), 33.8 (C-15), 42.3 (C-5), 27.9 (C-6), 31.0 (C-11), 33.5 (C-4), 28.2 (C-16), 36.0 (C-2), 29.7 (C-1), 23.8 (C-19), 22.8 (C-7), 10.6 (C-18), 103.6 (C-1'), 73.0 (C-2'), 75.1(C-3'), 70.0 (C-4'), 67.3 (C-5'); ESI-MS *m/z* 523.6 $[M + H]^+$; 1067.6 $[2M + Na]^+$.

*4.1.4.19. 3α-O-(β-D-xylopyranosyl)-digoxigenin (**5d**).* Obtained as a white solid (11.0 mg, 68.3%) from 3*α-O*-(2,3,4-tri-*O*-acetyl-*β-D*-xylopyranosyl)-*digoxigenin* (**4d**). $[\alpha]_D^{29}$ +20.2 (*c* 0.80, MeOH); ^{1}H NMR (600 MHz, Pyridine-d_5): δ_H 6.25 (1H, s, H-22), 5.27 (1H, dd, *J* = 18.0, 1.8 Hz, H-21a), 5.12 (1H, dd, *J* = 18.0, 1.8 Hz, H-21b), 4.91 (1H, d, *J* = 7.4 Hz, H-1'), 4.42 (1H, dd, *J* = 11.1, 5.2 Hz), 4.28 (1H, m), 4.19 (1H, t, *J* = 8.5 Hz), 4.03 (1H, t, *J* = 8.2 Hz), 3.96 (1H, qd, H-3), 3.78 (1H, t, *J* = 11.1 Hz, H-17), 3.73 (1H, m, H-12), 3.59 (1H, m), 2.13-1.98 (5H, m), 1.87-1.73 (8H, m), 1.60 (1H,m), 1.52 (1H, m), 1.39-1.29 (3H, m), 1.23 (3H, s, H-18), 0.96 (1H,m), 0.86 (3H, s, H-19); ^{13}C NMR (175 MHz, Pyridine-d_5): δc 177.0 (C-20), 175.1 (C-23), 117.8 (C-22), 85.6 (C-14), 74.9 (C-12), 78.9 (C-3), 74.4 (C-21), 57.1 (C-13), 47.0 (C-17), 42.1 (C-8), 33.8 (C-9), 35.5 (C-10), 33.9 (C-15), 42.5 (C-5), 28.0 (C-6), 31.0 (C-11), 35.4 (C-4), 28.2 (C-16), 35.7 (C-2), 27.9 (C-1), 23.8 (C-19), 22.8 (C-7), 10.6 (C-18), 103.8 (C-1'), 75.6 (C-2'), 79.0 (C-3'), 71.6 (C-4'), 67.7 (C-5'); ESI-MS *m/z* 523.4 $[M + H]^+$; 1067.6 $[2M + Na]^+$.

*4.1.4.20. 3α-O-(α-L-rhamnopyranosyl)-digoxigenin (**5e**).* Obtained as a white solid (10.4 mg, 51.2%) from 3*α-O*-(2,3,4-tri-*O*-acetyl-*α-L*-rhamnopyranosyl)-*digoxigenin* (**4e**). $[\alpha]_D^{29}$ +8.2 (*c* 0.60, MeOH); ^{1}H NMR (600 MHz, Pyridine-d_5): δ_H 6.25 (1H, s, H-22), 5.53 (1H, br s, H-1'), 5.28 (1H, dd, *J* = 18.0, 1.8 Hz, H-21a), 5.13 (1H, dd, *J* = 18.0, 1.8 Hz, H-21b), 4.57 (2H, m), 4.35 (2H, m), 3.87 (1H, qd, H-3), 3.75 (1H, m, H-17), 3.70 (1H, m, H-12), 2.09 (3H, m), 1.95-1.76 (6H, m), 1.60-1.27 (6H, m), 1.25 (3H, s, H-18), 0.91 (1H, m), 0.86 (3H, s, H-19); ^{13}C NMR (150 MHz, Pyridine-d_5): δc 177.1 (C-20), 175.1 (C-23), 117.8 (C-22), 85.6 (C-14), 74.8 (C-12), 76.1 (C-3), 74.4 (C-21), 57.1 (C-13), 47.0 (C-17), 42.1 (C-8), 33.7 (C-9), 35.5 (C-10), 33.8 (C-15), 42.5 (C-5), 27.9 (C-6), 31.0 (C-11), 34.9 (C-4), 28.3 (C-16), 35.6 (C-2), 27.2 (C-1),

23.8 (C-19), 22.8 (C-7), 10.6 (C-18), 99.5 (C-1'), 73.2 (C-2'), 73.3 (C-3'), 74.6 (C-4'), 70.3 (C-5'), 19.1 (C-6'); ESI-MS *m/z* 537.3 $[M + H]^+$; 1095.7 $[2M + Na]^+$.

4.1.5. General procedure for preparation of C_3-MeON-neoglycosideslibrary

3β-MeO*N*-digoxigenin (**Dig-4**/β) or 3α-MeO*N*-digoxigenin (**Dig-5**/α) (0.07-0.14 mmol) and free reducing sugar (2.0-2.5 eq) were dissolved in DMF/AcOH (3: 1, 600–1500 μL) and stirred at 50 °C for 48 h, and then was neutralized with AcOH until pH = 7. The crude product was obtained by filtered and concentrated under reduced pressure. The residue was purified by semipreparative RP-HPLC (45% MeOH-H_2O) to obtain C_3-MeO*N*-neoglycosides of digoxigenin (**6a**-**6d** and **7a**-**7d**). All compounds were characterized by analyses of ^{1}H and ^{13}C NMR spectra as well as ESI-MS.

*4.1.5.1. 3β-N-(β-D-glucopyranosyl)-N-(O-methyl)-digoxigenin (**6a**).* Obtained as a white solid (6.2 mg, 17.9%) from 3β-*N-(O-methyl)-digoxigenin* (**Dig-4**). $[\alpha]_D^{29}$ +8.2 (*c* 0.70, MeOH); ^{1}H NMR (300 MHz, CD_3OD): δ_H 5.91 (1H, s, H-22) , 4.99 (1H, dd, *J* = 18.0, 1.8 Hz, H-21a), 4.91 (1H, dd, *J* = 18.0, 1.8 Hz, H-21b), 4.10 (1H, d, *J* = 8.6 Hz, H-1'), 3.80 (1H, m), 3.73 (3H, s), 3.70-3.57 (3H, m), 3.16 (1H, m), 2.17-1.52 (15H, m), 1.29 (6H, m) 1.00 (3H, s, H-18), 0.79 (3H, s, H-19); ^{13}C NMR (75 MHz, CD_3OD) δ_c 178.6 (C-20), 177.4 (C-23), 117.7 (C-22), 86.8 (C-14), 75.5 (C-12), 71.7 (C-21), 62.7 (OMe), 58.5 (C-13), 57.3 (C-3), 47.0 (C-17), 42.3 (C-8), 37.8 (C-5), 36.7 (C-10), 34.0 (C-15), 33.5 (C-9), 31.7 (C-11), 30.9 (C-1), 30.7 (C-6), 28.4 (C-16), 28.2 (C-4), 24.5 (C-19), 24.1 (C-2), 22.5 (C-7), 9.9 (C-18), 91.0 (C-1'),75.7 (C-2'), 79.6 (C-3'), 71.1 (C-4'), 79.8 (C-5'), 64.5 (C-6'); ESI-MS *m/z* 582.6 $[M + H]^+$; 1185.7 $[2M + Na]^+$.

*4.1.5.2. 3β-N-(β-D-allopyranosyl)-N-(O-methyl)-digoxigenin (**6b**).* Obtained as a white solid (8.0 mg, 23.1%) from 3β-*N-(O-methyl)-digoxigenin* (**Dig-4**). $[\alpha]_D^{29}$ +12.2 (*c* 0.73, MeOH); ^{1}H NMR (400 MHz, Pyridine-d_5): δ_H 6.26 (1H, s, H-22), 5.30 (1H, dd, *J* = 18.0, 1.8 Hz, H-21a), 5.15 (1H, dd, *J* = 18.0, 1.8 Hz, H-21b), 5.15 (1H, d, *J* = 8.5 Hz, H-1′), 4.82 (2H, m), 4.44 (2H, m), 3.92 (1H, br s, H-3), 3.87 (1H, br s, H-3), 2.17-1.77 (10H, m), 1.68-1.53 (5H, m) 1.25 (3H, s, H-18), 0.79 (3H, s, H-19); ^{13}C NMR (100 MHz, Pyridine-d_5): δ 177.1 (C-20), 175.2 (C-23), 117.8 (C-22), 86.7 (C-14), 75.0 (C-12), 74.4 (C-21), 63.8 (OMe), 57.6 (C-3), 57.2 (C-13), 47.0 (C-17), 42.2 (C-8), 37.3 (C-5), 36.3 (C-10), 34.0 (C-15), 33.6 (C-9), 31.7 (C-11), 31.3 (C-1),

30.5 (C-6), 28.3 (C-16), 27.8 (C-4), 24.4 (C-19), 24.4 (C-2), 22.4 (C-7), 10.6 (C-18), 88.1 (C-1'),74.0 (C-2'), 69.8 (C-3'), 69.7 (C-4'), 77.4 (C-5'), 64.0 (C-6'); ESI-MS *m/z* 582.5 $[M + H]^+$; 1185.5 $[2M + Na]^+$.

*4.1.5.3. 3β-N-(β-D-xylofuranosyl)-N-(O-methyl)-digoxigenin (**6c**).* Obtained as a white solid (6.1 mg, 18.6%) from 3*β-N-(O-methyl)-digoxigenin* (**Dig-4**). $[\alpha]_D^{29}$ +15.6 (*c* 0.48, MeOH); ^{1}H NMR (400 MHz, Pyridine-d_5): δ_H 6.27 (1H, s, H-22) , 5.30 (1H, dd, *J* = 18.0, 1.8 Hz, H-21a), 5.15 (1H, dd, *J* = 18.0, 1.8 Hz, H-21b), 4.62 (1H, d, *J* = 8.5 Hz, H-1'), 4.46 (2H, m), 4.28 (1H, m), 4.20 (1H, m), 3.98 (3H, s), 3.87 (1H, br s, H-3), 3.78-3.73 (2H, m), 2.17-1.94 (10H, m), 1.86 (2H, m), 1.73-1.36 (6H, m) 1.27 (3H, s, H-18), 1.14 (1H, m), 0.87 (3H, s, H-19); ^{13}C NMR (100 MHz, Pyridine-d_5): δc 177.1 (C-20), 175.2 (C-23), 117.8 (C-22), 85.7 (C-14), 75.0 (C-12), 74.4 (C-21), 64.1 (OMe), 57.9 (C-3), 57.2 (C-13), 47.0 (C-17), 42.2 (C-8), 37.7 (C-5), 36.3 (C-10), 33.9 (C-15), 33.7 (C-9), 31.6 (C-11), 31.3 (C-1), 30.6 (C-6), 28.3 (C-16), 27.9 (C-4), 24.5 (C-19), 24.4 (C-2), 22.5 (C-7), 10.6 (C-18), 92.5 (C-1'),71.5 (C-2'), 72.2 (C-3'), 81.0 (C-4'), 69.6 (C-5'); ESI-MS *m/z* 574.5 $[M + Na]^+$.

*4.1.5.4. 3β-N-(D-ribofuranosyl)-N-(O-methyl)-digoxigenin (α/β, 1:1) (**6d**).* Obtained as a white solid (7.3 mg, 22.2%) from 3*β-N-(O-methyl)-digoxigenin* (**Dig-4**). $[\alpha]_D^{29}$ +6.2 (*c* 0.52, MeOH); ^{1}H NMR (600 MHz, Pyridine-d_5): δ_H 6.26 (2H, s, H-22) , 5.28 (2H, dd, *J* = 18.0, 1.8 Hz, H-21a), 5.14 (2H, dd, *J* = 18.0, 1.8 Hz, H-21b), 5.42 (1H, d, *J* = 4.0 Hz, H-1'), 5.39 (1H, s, H-1'), 4.46 (2H, m), 4.88 (2H, m), 4.79 (2H, m), 4.71 (1H, m), 4.68 (1H, m), 4.03 (3H, s), 3.86 (1H, br s, H-3), 3.74 (3H, s), 1.26 (6H, s, H-18), 0.90 (3H, s, H-19), 0.80 (3H, s, H-19); ^{13}C NMR (150 MHz, Pyridine-d_5): δ 177.1 (C-20), 177.1 (C-20), 175.2 (C-23), 175.1 (C-23), 117.8 (C-22), 117.8 (C-22), 85.7 (C-14), 85.7 (C-14), 74.9 (C-12), 74.4 (C-21), 64.1 (OMe), 64.0 (OMe), 58.6 (C-3), 57.9 (C-3), 57.2 (C-13), 57.1 (C-13), 47.0 (C-17), 47.0 (C-17), 42.2 (C-8), 42.1 (C-8), 37.7 (C-5), 37.4 (C-5), 36.3 (C-10), 36.3 (C-10), 33.9 (C-15), 33.9 (C-15), 33.7 (C-9), 33.6 (C-9), 31.7 (C-11), 31.6 (C-11), 31.3 (C-1), 31.3 (C-1), 30.6 (C-6), 30.5 (C-6), 28.3 (C-16), 28.3 (C-16), 27.9 (C-4), 27.7 (C-4), 24.5 (C-19), 24.4 (C-19), 24.4 (C-2), 24.4 (C-2), 22.4 (C-7), 22.4 (C-7), 10.6 (C-18), 10.5 (C-18), 97.4 (C-1'), 88.1 (C-1'), 69.4 (C-2'), 69.0 (C-2'), 73.6 (C-3'), 73.1 (C-3'), 85.8 (C-4'), 85.0 (C-4'), 66.4

(C-5'), 64.6 (C-5'); ESI-MS *m/z* 574.5 $[M + Na]^+$; 1125.5 $[2M + Na]^+$.

*4.1.5.5. 3α-N-(β-D-glucopyranosyl)-N-(O-methyl)-digoxigenin (**7a**).* Obtained as a white solid (12.0 mg, 28.8%) from 3α-*N*-(*O*-methyl)-digoxigenin (**Dig-5**). $[\alpha]_D^{29}$ +18.5 (*c* 0.60, MeOH); ^{1}H NMR (300 MHz, CD_3OD): δ_H 5.91 (1H, s, H-22) , 5.00 (1H, dd, *J* = 18.0, 1.8 Hz, H-21a), 4.91 (1H, dd, *J* = 18.0, 1.8 Hz, H-21b), 4.17 (1H, d, *J* = 8.6 Hz, H-1'), 3.81 (1H, m), 3.70 (3H, s), 3.66 (1H, m), 3.57 (1H, m), 3.36 (3H, m), 3.18 (2H, m), 2.17-1.52 (15H, m), 1.44-1.24 (6H, m), 1.10 (2H, m), 0.96 (3H, s, H-18), 0.79 (3H, s, H-19); ^{13}C NMR (75 MHz, CD_3OD): δ 178.5 (C-20), 177.3 (C-23), 117.7 (C-22), 86.8 (C-14), 75.5 (C-12), 71.3 (C-21), 63.2 (C-3), 62.7 (OMe), 57.3 (C-13), 47.1 (C-17), 43.1 (C-8), 36.7 (C-5), 35.9 (C-10), 35.9 (C-15), 34.0 (C-9), 33.5 (C-11), 32.6 (C-1), 30.6 (C-6), 28.4 (C-16), 28.3 (C-4), 25.5 (C-19), 23.8 (C-2), 23.0 (C-7), 9.9 (C-18), 91.0 (C-1'),75.7 (C-2'), 79.7 (C-3'), 71.1 (C-4'), 79.8 (C-5'), 64.6 (C-6'); ESI-MS *m/z* 582.6 $[M + H]^+$; 1185.7 $[2M + Na]^+$.

*4.1.5.6. 3α-N-(β-D-allopyranosyl)-N-(O-methyl)-digoxigenin (**7b**).* Obtained as a white solid (19.5 mg, 28.1%) from 3α-*N*-(*O*-methyl)-digoxigenin (**Dig-5**). $[\alpha]_D^{29}$ +17.2 (*c* 0.75, MeOH); ^{1}H NMR (400 MHz, Pyridine-d_5): δ_H 6.26 (1H, s, H-22), 5.28 (1H, dd, *J* = 18.0, 1.8 Hz, H-21a), 5.14 (1H, dd, *J* = 18.0, 1.8 Hz, H-21b), 5.24 (1H, d, *J* = 8.5 Hz, H-1′), 4.83 (1H, br s), 4.53-4.40 (3H, m), 4.04 (1H, br s, H-3), 3.80-3.69 (3H, m), 2.15-1.70 (15H, m) 1.25 (3H, s, H-18), 0.83 (3H, s, H-19); ^{13}C NMR (100 MHz, Pyridine-d_5): δ 177.1 (C-20), 175.1 (C-23), 117.8 (C-22), 85.7 (C-14), 75.0 (C-12), 74.4 (C-21), 62.7 (OMe), 63.5 (C-3), 57.2 (C-13), 47.0 (C-17), 42.4 (C-8), 42.2 (C-5), 36.4 (C-10), 35.6 (C-15), 34.0 (C-9), 33.8 (C-11), 32.7 (C-1), 30.9 (C-6), 30.4 (C-16), 28.3 (C-4), 25.6 (C-19), 24.0 (C-2), 23.0 (C-7), 10.6 (C-18), 88.3 (C-1'), 73.9 (C-2'), 69.5 (C-3'), 69.3 (C-4'), 77.4 (C-5'), 64.4 (C-6'); ESI-MS *m/z* 604.7 $[2M + Na]^+$; 1185.7 $[2M + Na]^+$.

4.1.5.7. 3α-*N*-(β-*D*-xylofuranosyl)-*N*-(*O*-methyl)-digoxigenin *(**7c**).* Obtained as a white solid (12.7 mg, 38.6%) from 3α-*N*-(*O*-methyl)-digoxigenin (**Dig-5**). $[\alpha]_D^{29}$ +16.1 (*c* 0.65, MeOH); ^{1}H NMR (400 MHz, Pyridine-d_5): δ_H 6.26 (1H, s, H-22) , 5.28 (1H, dd, *J* = 18.0, 1.8 Hz, H-21a), 5.14 (1H, dd, *J* = 18.0, 1.8 Hz, H-21b), 4.68 (1H, d, *J* = 8.8 Hz, H-1'), 4.45 (1H, m), 4.39 (1H, m), 4.24 (1H, m), 4.18 (1H, m), 3.96 (3H, s),

3.80-3.56 (5H, m), 2.16-2.05 (4H, m), 1.98-1.73 (8H, m), 1.62-1.51 (3H, m), 1.39 (2H, m), 1.26 (3H, s, H-18), 1.04 (1H, m), 0.89 (3H, s, H-19); ^{13}C NMR (100 MHz, Pyridine-d_5): δc 177.1 (C-20), 175.1 (C-23), 117.8 (C-22), 86.6 (C-14), 74.9 (C-12), 74.4 (C-21), 62.8 (OMe), 64.4 (C-3), 57.2 (C-13), 47.0 (C-17), 42.6 (C-8), 42.2 (C-5), 36.5 (C-10), 35.6 (C-15), 34.0 (C-9), 33.9 (C-11), 32.8 (C-1), 31.0 (C-6), 28.3 (C-16), 28.3 (C-4), 25.6 (C-19), 24.1 (C-2), 23.0 (C-7), 10.6 (C-18), 92.6 (C-1'), 71.4 (C-2'), 71.7 (C-3'), 81.0 (C-4'), 69.5 (C-5'); ESI-MS *m/z* 552.3 $[M + H]^+$; 1125.5 $[2M + Na]^+$.

*4.1.5.8. 3α-N-(β-D-*lactosyl)*-N-(O-methyl)-digoxigenin (**7d**).* Obtained as a white solid (3.0 mg, 6.8%) from *3α-N-(O-methyl)-digoxigenin* (**Dig-5**). $[\alpha]_D^{29}$ +28.2 (*c* 0.40, MeOH); ^{1}H NMR (600 MHz, Pyridine-d_5): δ_H 6.26 (1H, s, H-22) , 5.28 (1H, dd, *J* = 18.0, 1.8 Hz, H-21a), 5.14 (2H, dd, *J* = 18.0, 1.8 Hz, H-21b), 5.12 (1H, d, *J* = 6.9 Hz, H-1"), 4.73 (1H, d, *J* = 8.3 Hz, H-1'), 4.57-4.46 (6H, m), 4.42 (1H, m), 4.31 (2H, m), 4.17 (2H, m), 3.97 (3H, s), 3.83 (1H, m), 3.76 (2H, m), 3.51 (1H, m), 2.18-2.08 (4H, m), 2.01-1.75 (8H, m), 1.67 (1H, m), 1.56-1.48 (3H, m), 1.41-1.30 (3H, m), 1.26 (3H, s, H-18), 0.93 (1H, m), 0.85 (3H, s, H-19); ^{13}C NMR (150 MHz, Pyridine-d_5): δc 177.1 (C-20), 175.2 (C-23), 117.8 (C-22), 85.7 (C-14), 74.9 (C-12), 74.4 (C-21), 62.7 (OMe), 64.3 (C-3), 57.2 (C-13), 47.0 (C-17), 42.6 (C-8), 42.2 (C-5), 36.5 (C-10), 35.6 (C-15), 34.0 (C-9), 33.8 (C-11), 32.6 (C-1), 31.0 (C-6), 28.3 (C-16), 28.3 (C-4), 25.6 (C-19), 24.0 (C-2), 23.0 (C-7), 10.6 (C-18), 91.6 (C-1'), 71.5 (C-2'), 78.7 (C-3'), 82.9 (C-4'), 78.9 (C-5'), 62.5 (C-6'), 106.3 (C-1"), 72.9 (C-2"), 75.6 (C-3"), 70.5 (C-4"), 77.7 (C-5"), 62.9 (C-6"); ESI-MS *m/z* 766.7 $[2M + Na]^+$.

*4.1.6. Synthesis of digoxigenin bisdigitoxoside (**8a**) and digoxigenin monodigitoxoside (**9b**)*

To a solution of digoxin (2.0g, 2.56 mmol) in MeOH (360 mL) was added a solution of $NaIO_4$ (2.0g, 9.39 mmol) in H_2O (20 mL). The reaction mixture was stirred at room temperature for 3 h and was filtered to remove $NaIO_3$ precipitation. The filtrate was concentrated under reduced pressure and was diluted with EtOAc followed by washing with H_2O. The resulting solution was dried over anhydrous Na_2SO_4 and then concentrated to give dialdehyde intermediate as a white amorphous powder (1.95 g, 97.5%), which was subjected to next reaction without further purification. The

dialdehyde intermediate (1.95 g, 2.51 mmol) was dissolved in MeOH (60mL) and was added aminocaproic acid (420 mg, 3.21 mmol) in small portions. The reaction mixture was stirred for 4 h and then quenched with $NaHCO_3$. The mixture solution was concentrated at 40 °C under reduced pressure and was extracted with EtOAc followed by washing with H_2O. The resulting solution was dried over anhydrous Na_2SO_4 and was evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography (Petroleum ether/EtOAc, 1:3) to give 3*β*-*O*-(2,6-dideoxy-*β*-D-ribopyranosyl-(1→4)-*O*-2,6-dideoxy-*β*-D-ribopyranosyl)-digoxigenin (digoxigenin bisdigitoxoside, **8a**) as white solids (1.20 g, 74%). ^{1}H NMR (400 MHz, Pyridine-d_5): δ_H 6.24 (1H, s, H-22), 5.27 (1H, dd, *J* = 17.8, 1.8 Hz, H-21a), 5.12 (1H, dd, *J* = 17.8, 1.8 Hz, H-21b), 4.41 (1H, dd, *J* = 7.1, 2.0 Hz, H-1'), 4.69 (1H, dd, *J* = 7.1, 2.0 Hz, H-1"), 4.26 (1H, br s), 4.27 (2H, m), 3.75 (1H, m, H-17), 3.72 (1H, m, H-12), 3.54 (2H, m), 2.40 (2H, m), 2.11 (4H, m), 1.97-1.51 (15H, m), 1.49 (3H, d, *J* = 6.1 Hz), 1.43 (3H, d, *J* = 6.1 Hz), 1.23 (3H, s, H-18), 0.89 (3H, s, H-19); ^{13}C NMR (100 MHz, Pyridine-d_5): δc 177.0 (C-20), 175.1 (C-23), 117.8 (C-22), 85.8 (C-14), 74.9 (C-12), 74.4 (C-3), 74.3 (C-21), 57.1 (C-13), 46.9 (C-17), 42.0 (C-8), 33.3 (C-9), 35.8 (C-10), 33.9 (C-15), 37.4 (C-5), 27.5 (C-6), 31.2 (C-11), 31.2 (C-4), 28.2 (C-16), 27.3 (C-2), 30.8 (C-1), 24.3 (C-19), 22.7 (C-7), 10.6 (C-18), 96.9 (C-1'),39.5 (C-2'), 67.9 (C-3'), 83.8 (C-4'), 69.0 (C-5'), 19.1 (C-6'), 100.3 (C-1"), 39.8 (C-2"), 68.9 (C-3"), 73.6 (C-4"), 70.8 (C-5"), 19.3 (C-6"); ESI-MS *m/z* 673.7 $[M + Na]^+$; 1323.5 $[2M + Na]^+$.

Following the procedure for preparation of compound **8a**, 3*β*-*O*-(2,6-dideoxy-*β*-D-ribopyranosyl)-digoxigenin (digoxigenin monodigitoxoside, **9b**) was obtained as white solid (690 mg, 71.9 %) by silica gel column chromatography eluted with Petroleum ether/EtOAc (1:3). ^{1}H NMR (300 MHz, Pyridine-d_5): δ_H 6.24 (1H, s, H-22), 5.27 (1H, dd, *J* = 17.8, 1.8 Hz, H-21a), 5.12 (1H, dd, *J* = 17.8, 1.8 Hz, H-21b), 4.45 (1H, dd, *J* = 7.1, 2.0 Hz, H-1'), 4.30 (1H, br s, H-3), 4.31 (1H, m), 3.73 (1H, m, H-17), 3.70 (1H, m, H-12), 3.65 (1H, m), 2.45 (1H, m), 2.13 (4H, m), 1.98-1.66 (10H, m), 1.59 (3H, d, *J* = 6.1 Hz), 1.39 (2H, m), 1.23 (3H, s, H-18), 0.90 (3H, s, H-19); ^{13}C NMR (75 MHz, Pyridine-d_5): δc 177.0 (C-20), 175.1

(C-23), 117.7 (C-22), 85.7 (C-14), 74.9 (C-12), 74.6 (C-3), 74.4 (C-21), 57.1 (C-13), 46.9 (C-17), 42.0 (C-8), 33.3 (C-9), 35.8 (C-10), 33.8 (C-15), 37.4 (C-5), 27.5 (C-6), 31.2 (C-11), 31.2 (C-4), 28.2 (C-16), 27.4 (C-2), 30.8 (C-1), 24.3 (C-19), 22.7 (C-7), 10.6 (C-18), 97.0 (C-1'),40.4 (C-2'), 69.1 (C-3'), 73.4 (C-4'), 70.7 (C-5'), 19.5 (C-6'); ESI-MS *m/z* 521.4 $[M + H]^+$; 1063.5 $[2M + Na]^+$.

4.2. Biology assay

4.2.1 Cell Culture.

The NIH-H460 cell line was purchased from the American Type Culture Collection and maintained in RPMI 1640 medium containing 10% FBS and 1% L-glutamine, 100 units/mL penicillin, and 100 mg/mL streptomycin.

4.2.2 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

MTT assay was performed according to the method reported previously [24]. The cells were seeded in 96-well plates at 3×10^3/well in the complete medium and then substituted by the medium containing 50 nM glycosides for 48 h incubation. Next, 10 µL of 5 µg/mL MTT was directly added to each well while the cells continue to incubate at 37 °C for 4 hr. At the end of experiments, 100 µL of DMSO was used to dissolve formazan and measured at 560 nm on Victor 3V which is a multiwall plate reader from Perkin Elmer.

4.2.3. Western blotting analysis for Nur77 expression

Equal amounts of the lysates were electrophoresed on an 8% SDS-PAGE gel and transferred onto polyvinylidene difluoride (PVDF) membranes, which were then blocked with5% nonfat milk in TBST [50 mmol/L Tris-HCl (pH 7.4), 150 mmol/L NaCl, and 0.1% Tween 20] for 1 h, incubated with various primary antibodies overnight at 4 °C, and incubated with secondary antibodies for 1 h. Immunoreactive products were detected by using chemiluminescence with an enhanced chemiluminescence system (ECL, Amersham Biosciences). The dilution of the primary antibody was anti-Nur77 (Cell Signal, 3960) in 1:1000. The blots were reprobed with anti-*β*-actin antibody (Sigma, A3854) in 1:10000 for loading control.

4.2.4. Immunofluorescence microscopy

Cells mounted on glass slides were permeabilized with PBS containing 0.1% Triton

X-100 for 15 min, and blocked with 1% bovine serum albumin (BSA) in PBS for 30 min at room temperature, followed with incubation with Nur77 antibodies at 37°C for 1 h and detected by Cy3-labeled anti-rabbit IgG (Chemicon International) at room temperature for 30 min. Cells were co-stained with 4',6'-diamidino-2-phenylindole (DAPI) (Sigma) to visualize nuclei. The images were taken under an LSM-510 confocal laser scanning microscope system (Carl Zeiss, Oberkochen, Germany).

4.2.5 Flow cytometry assay

Apoptosis was determined by dual staining using Annexin V: FITC and propidium iodide (Invitrogen). Briefly, cells were seeded into 24-well cell culture plates (1×10^5 cells/well) and treated with glycosides. Cells were dissociated from wells with 0.25% trypsin, spun at 1,500 rpm for 5 min, resuspended in Annexin V binding buffer, and stained with Annexin V: FITC for 15 min and propidium iodide for 5 min. Cells were analyzed using the FACS Calibur System (BD Biosciences, San Jose, CA, USA). The relative proportion of Annexin V-positive cells, representing apoptotic cells, was determined using FlowJo software (FlowJo LLC, Ashland, OR, USA).

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1. A series of C_3-*O*-neoglycosides and C_3-MeO*N*-neoglycosides of digoxigenin were prepared.
2. SRA indicated that C_3-*O*-neoglycosides of digoxigenin exhibited stronger cytotoxicity than C_3-MeO*N*-neoglycosides.
3. 3*β*-*O*-(*β*-L-fucopyranosyl)-digoxigenin showed the highest activity of induction of cancer cell apoptosis.