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Cytotoxicity of semisynthetic acetal triterpenes from one-pot vicinal diol cleavage following by lactolization: Reaction promoted by $NaIO_4/SiO_2$ gel in THF

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Cytotoxicity of semisynthetic acetal triterpenes from one-pot vicinal diol cleavage following by lactolization: Reaction promoted by $NaIO_4/SiO_2$ gel in THF

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In situ C–C bond cleavage of vicinal diol following by the lactolisation resulted from separated treatment of Arjunolic acid (**1**), 24-hydroxytormentic acid (**2**) and 3-*O*-β-D-glucopyranosylsitosterol (**3**) with sodium periodate and silica gel in dried THF according to the strategic position of hydroxyl functions in the molecule. The reaction led to a lactol pentacyclic triterpenes **1A**, **2A** and a bicyclotriacetal of β-sitosterol **3A**. These products were further acetylated and the cytotoxicity of all molecules was evaluated against human fibrosarcoma HT1080 cancer cells lines.

Keywords: hemisynthesis; acetal triterpenoids; cytotoxicity

1. Introduction

Arjulonic acid (**1**), 24-hydroxyltormentic acid (**2**) and β-sitosterol glucopyranoside (**3**) were transformed into the acetal derivatives by using $NaIO_4$–SiO_2 in THF. Formerly, the bond between vicinal diol in triterpenoids was cleaved with the aim of structure elucidation and this was performed with sodium periodate in MeOH for 30 min (Bombardelli, Bonati, Gabetta, & Mustich, 1974) or in ethyl acetate : ethanol overnight (Bhagirath & Rastogi, 1969) to yield dialdehyde product (Kumaraswamy, Nivedita, Sastry, & Ramakrishna, 2005). In contrast, a seven-member ring lactol was formed if there was an OH function at C-23 or C-24 of this class of compounds (Bombardelli et al., 1974). The problem of weak solubility of the starting compounds (**1**–**3**) in MeOH prompted us to select THF as solvent for this reaction. But, the product was barely formed after 3 h and overnight, the transformation was not effective. To get around this obstacle, dried silica gel described as a solid phase catalyst in several kinds of reactions was added and a seven-member ring triterpenoid was obtained within 6 h. The same reaction was repeated successfully by

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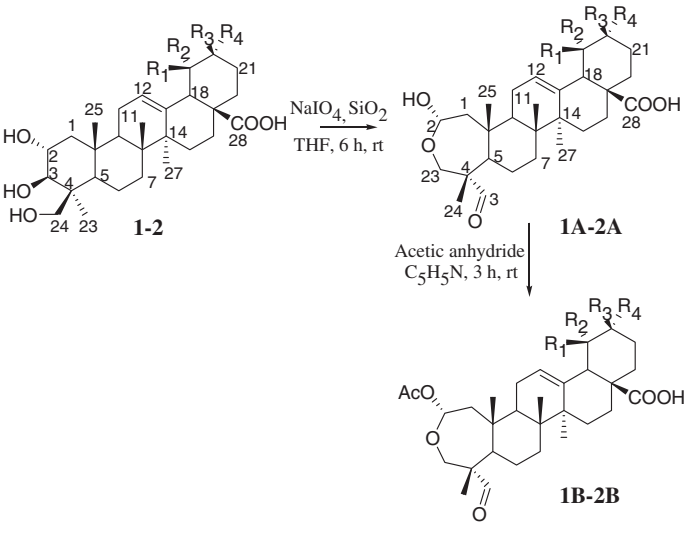

Compound	Product	R_1	R_2	R_3	R_4	Yield (%)
Arjulonic acid **1**	**1A**	H	H	Me	Me	97
	1B					98
24-hydroxytormentic acid **2**	**2A**	Me	OH	H	Me	95
	2B		OAc			98

Figure 1. Hemisynthesis route of **1A**, **2A**, **1B** and **2B**.

dissolving starting compounds in dried THF and adding an adequate quantity of sodium periodate and dried silica gel.

Silica gel has been used to perform cyclisation of polyketone into the phenolic derivative and olefinic aldehyde into cyclo alcohol (Banerjee, Laya Mimó, & Vera Vegas, 2001); it was also used for acetal and ketal preparation (R. Kumar, D. Kumar, & Chakraborti, 2007); silica gel and sodium periodate under certain conditions constitute an efficient co-catalyst to cleave vicinal diol (Ravindranadh & Rodney, 2005).

We herein report the intramolecular lactolisation of Arjulonic acid (**1**), 24-hydroxyltormentic acid (**2**) and β-sitosterol glucopyranoside (**3**), catalysed by sodium periodate and dried silica gel in absolute THF. The cytotoxicities of phytocomponents (**1**–**3**) and their semi-synthetic products (**1A**–**3A** and **1B**–**3B**) will also be reported.

2. Results and discussion

Arjunolic acid (**1**) was dissolved in dried THF and treated with sodium periodate and silica gel as solid phase catalyst (Figure 1). The reaction was complete within 6 h and the structure of an acetal triterpene was characterised using NMR data. The adsorbent seemed to play a role of acid catalyst in the medium facilitating after the cleavage of the vicinal diol into dialdehyde, the ring A closing to form an acetal function. The second step required a free hydroxyl group at C-24 which was the β-position of the aldehyde function (C-3) suitable for the ring closing (Figure. 2). The *stereo*-selectivity of the reaction was confirmed by one of the 2D NMR spectra of the afforded compound. From NOESY correlations between the acetalic proton at δ_H 5.13 (dd, 5.0, 9.5) and those of CH_3 group (C-25) at δ_H 1.05 (s), the absolute configuration of the acetal carbon was found to be *S*.

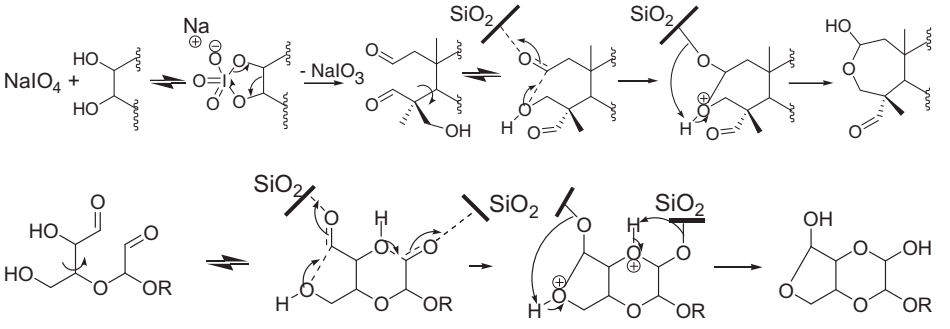

Figure 2. Vicinal diol cleavage and lactolization mechanism.

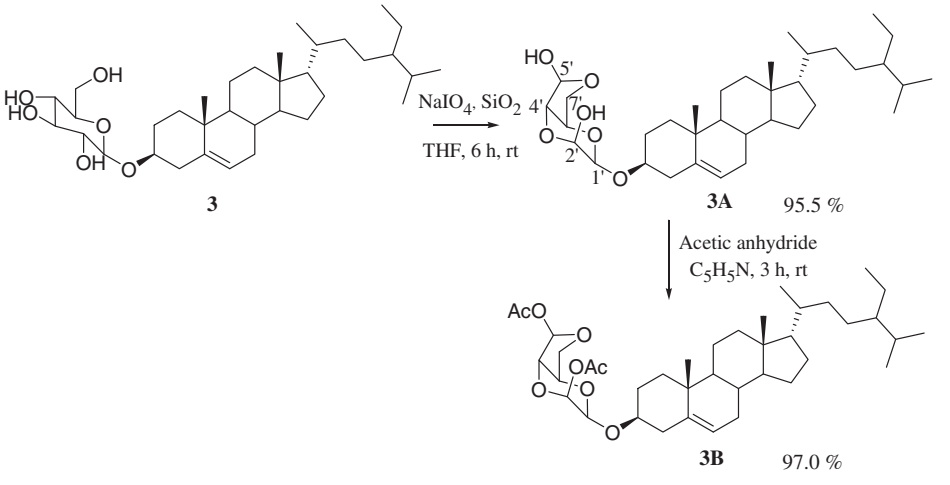

Figure 3. Hemisynthesis route of **3A** and **3B**.

Furthermore, same interactions were found between the aldehyde proton at δ_H 9.95 (s) and one of the oxymethylene hydrogens H-23 at δ_H 3.73 which correlated with the angular proton H-5 (δ_H 1.18) suggesting an absolute configuration *S* at C-4.

This reaction is close to Ferrier rearrangement of glucals (Misra, Tiwari, & Agnihotri, 2005) or to acetal preparation using acetic anhydride and silica supported perchloric acid (Khan, Choudhury, & Ghosh, 2006). Two additional secondary metabolites were further subjected to this couple of catalysts as **1** in order to have more bioactive compounds and to check the efficiency of this reaction according that it was so far catalysed by sulphuric acid (Hill, Alto, Calif, & Shipp, 1970) or zeolite coupled to a Lewis acid (Bejoy, Sreedharan, & Sankaran 2005). Thus, 24-hydroxytormentic acid **2** was subjected to the same reaction yielding like arjulonic acid an acetal seven-member ring A triterpene (Figure 1) with the same absolute configurations at C-2 and C-4 as in **1A**. 3-*O*-*β*-D-glucopyranosylsitosterol **3** was the best candidate to this one-pot preparation because of the presence of sugar containing many vicinal diol systems; obviously, the reaction afforded a bicyclotriacetal moiety with five- and six-member ring containing two additional acetal functions (Figure 3). The NOESY spectrum of **3A** (Figure 4) displayed correlation between the proton at δ_H 4.88 and the oxymethine proton H-3 at δ_H 3.56 in the steroid scaffold.

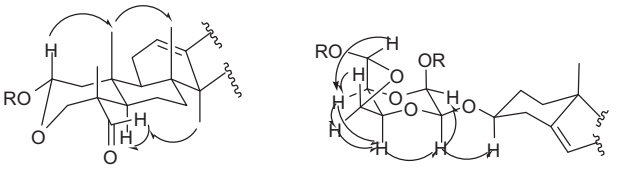

Figure 4. NOESY correlations of products **1A**, **2A** and **3A**.

Table 1. Cytotoxic activities of compounds (IC_{50}, 48 h) against HT1080.

Compound	**1**	**1A**	**1B**	**2**	**2A**	**2B**	**3**	**3A**	**3B**	**RD**
IC_{50} (μM)	210	400	110	10	135	10	> 500	350	460	1

Note: Reference drug: etoposide.

This observation suggested the acetal proton which was attached to anomeric carbon before, kept its configuration as in the case of β-D-glucopyranoside (Figure 4). The resonance at δ_H 4.88 (H-1′) further revealed correlations with those at δ_H 5.72 (H-2′) and 4.02 (H-8′). The proton H-8′ at δ_H 4.02 correlated with the methylene protons at δ_H 3.62 (H-7′) and oxymethine proton at δ_H 3.70 (H-4′). Both acetal proton at δ_H 5.49 (H-5′) and CH_2 protons at δ_H 3.64 (H-7′a) showed NOE interactions with the protons of oxymethine at δ_H 3.70 (H-4′). All the correlations led to determine the absolute configurations in the triacetal moeity to be 1′*S*, 2′*R*, 4′*S*, 5′*S* and 8′*S*.

Compounds **1A–3A** were further subjected to the acetylation reaction and the products along with the starting materials were tested against fibrosarcoma HT1080 a cancer cell line. The cytotoxicity was carried out by flow cytometry and most of the products showed moderate activities with the IC_{50} at 10 and 460 μM (Table 1). The acetal **1A** was less cytotoxic than arjulonic acid but after its acetylation the activity of the product **1B** was improved and was better than those of **1** and **1A**. Furthermore, the same feature was observed with **2** which give IC_{50} at 10 μM but its acetal **2A** was cytotoxic at 135 μM as IC_{50} and the activity became interesting with the acetylated product **2B**. In the pentacyclic triterpenes, the acetal function seems to reduce the cytotoxicity against HT1080 but its protection improves the activity. Nevertheless, this function provides the cytotoxic property to compound **3** although reducing the activity in some cases.

3. Experimental

3.1. *General*

1D and 2D NMR spectra were carried out on a Brucker DRX-400 MHz. Optical rotation was measured by Perkin-Elmer polarimeter model 341 at 589 nm. SiO_2 gel GF254 was used to perform thin layer chromatography. Iodine–silica gel was employed to visualise the spots on the TLC plates. HRESIMS and ESI–MS were carried out using MicroTOF-Q 98 (Bruker-Daltonics, Germany).

3.2. *General procedure for the preparation of lactol and acetylation*

Seven milligrams (0.0144 mmol) of arjulonic acid (**1**), 7 mg (0.014 mmol) of 24-hydroxytormentic acid (**2**) and 9 mg (0.016 mmol) of β-sitosterol glucopyranoside (**3**) were separately dissolved in 20 mL of THF. Each solution was separately treated with

15.4 mg (0.072 mmol, 5 eq) of $NaIO_4$ and 50 mg of SiO_2. The reaction mixture was vigorously stirred for 6 h and monitored with TLC. The mixture was dried and poured onto water. The products were extracted with $CHCl_3$ and concentrated under vacuum yielding 6.76 mg (97%) of **1A**, 6.63 mg (95%) of **2A** and 8.77 mg (95.5%) of **3A,** respectively. Acetylation of **1A** (4 mg), **2A** (4 mg) and **3A** (5 mg) were performed separately in 2 mL of pyridine with 3 mL of acetic anhydride stirred at room temperature for 3 h affording **1B** (4.25 mg, 98.0%), **2B** (4.57 mg, 98.0%) and **3B** (5.56 mg, 97.0%).

3.2.1. *Compound **1A***

Colorless amorphous solid, Rf = 0.6 (CH_2Cl_2–MeOH 37:3); [α] + 50.5 (c 1, CH_2Cl_2); IR; HR-ESI-MS *m/z* 509 $[C_{30}H_{46}O_5 + Na]^+$; ^{1}H-NMR (400 MHz, $CDCl_3$) 0.83 (s, Me-26), 0.89 (s, Me-29), 0.92 (s, Me-30), 1.00 (s, Me-24), 1.05 (s, Me-25), 1.09 (m, H-7a), 1.13 (s, Me-27), 1.15 (m, H-19a), 1.18 (m, H-5), 1.22 (m, H-15a), 1.26 (m, H-21), 1.34 (m, H-15b), 1.36 (m, H-6a), 1.45 (m, H-1a), 1.58 (m, H-22a), 1.59 (m, H-9), 1.62 (m, H-19b), 1.62 (m, H-16a), 1.66 (m, H-7b), 1.71 (m, H-6b), 1.76 (m, H-22b), 1.93 (m, H-11a), 1.97 (m, H-16b), 2.04 (m, H-11b), 2.17 (dd, 5.1, 15.5, H-1b), 2.83 (dd, 4.2, 13.8, H-18), 3.73 (d, 13.3, H-23a),3.94 (d, 13.3, H-23b), 5.13 (dd, 5.0, 9.5, H-2), 5.32 (br t, 3.3, H-12), 9.95 (s, H-3); ^{13}C-NMR (100 MHz, $CDCl_3$) 14.5 (C-25), 17.8 (C-26), 20.5 (C-6), 20.7 (C-24), 23.0 (C-16), 23.6 (C-29), 24.8 (C-11), 25.6 (C-27), 27.9 (C-7), 30.8 (C-21), 32.4 (C-22), 33.2 (C-30), 33.4 (C-20), 34.0 (C-15), 40.0 (C-10), 40.3 (C-8), 41.3 (C-18), 42.3 (C-14), 43.8 (C-9), 44.8 (C-1), 45.7 (C-19), 46.8 (C-17), 53.8 (C-4), 61.2 (C-5), 65.5 (C-23), 93.9 (C-2), 122.8 (C-12), 143.6 (C-13), 183.2 (C-28), 206.2 (C-3);

3.2.2. *Compound **1B***

Yellow amorphous solid, Rf = 0.7 (CH_2Cl_2); [α] + 41.8 (c 1, CH_2Cl_2); HR-ESI-MS: *m/z* 551.3342 (Calcd 551.3349) $[C_{32}H_{48}O_6 + Na]^+$; Some characteristic ^{1}H-NMR data (400 MHz, $CDCl_3$) 0.83 (s, Me-26), 0.90 (s, Me-29), 0.92 (s, Me-30), 1.00 (s, Me-24), 1.08 (s, Me-25), 9.96 (s, H-3), 1.12 (s, Me-27), 1.18 (m, H-5), 2.04 (s, MeCOO), 3.33 (d, 13.4, H-23a), 3.93 (d, 13.4, H-23b), 5.31 (br t, 3.3, H-12), 6.01 (dd, 5.0, 10.0, H-2); ^{13}C-NMR (100 MHz, $CDCl_3$) 14.7 (C-25), 17.8 (C-26), 20.5 (C-6), 20.6 (C-24), 23.1 (C-16), 23.7 (C-29), 24.9 (C-11), 25.6 (C-27), 27.7 (C-7), 30.8 (C-21), 32.4 (C-22), 33.2 (C-20), 33.2 (C-30), 34.0 (C-15), 40.0 (C-10), 40.5 (C-8), 41.2 (C-18), 42.3 (C-14), 42.7 (C-1), 43.9 (C-9), 45.8 (C-19), 46.7 (C-17), 53.5 (C-4), 61.4 (C-5), 67.8 (C-23), 94.5 (C-2), 122.7 (C-12), 143.8 (C-13), 183.2 (C-28), 205.4 (C-3), (CH_3COO) 21.5, 170.2;

3.2.3. *Compound **2A***

Brown amorphous gum; Rf = 0.6 (CH_2Cl_2–MeOH 9:1); [α] + 16.52 (c 0.14, MeOH); HR-ESI-MS *m/z* 525.3190 $[C_{30}H_{46}O_6 + H]^+$ (Calcd 527.3187); ESI-MS: *m/z* 503.3 $[C_{30}H_{46}O_6 + H]^+$; ^{1}H-NMR (400 MHz, $CDCl_3$); 0.86 (s, Me-26), 0.93 (d, 6.7 Me-30), 0.98 (m, H-6a), 1.02 (m, H-15a), 1.17 (s, Me-25), 1.20 (s, Me-29), 1.20 (s, Me-24), 1.25 (m, H-16a), 1.30 (m, H-7a), 1.37 (m, H-20), 1.38 (s, Me-27), 1.52 (m, H-6b), 1.53 (m, H-1a), 1.53 (m, H-21a),1.64 (m, H-7b), 1.64 (m, H-22a), 1.73 (m, H-5), 1.74 (m, H-22b), 1.75 (m, H-16b), 1.80 (m, H-15b),1.83 (m, H-9), 2.05 (m, H-11a), 2.15 (m, H-1b), 2.16 (m, H-11b), 2.52 (dd, 4.2, 13.8, H-18), 2.57 (m, H-21b), 3.05 (d, 12.7, H-23a), 4.09 (d, 12.7, H-23b), 5.05 (dd, 4.9, 9.6, H-2), 5.34 (br t, 3.6, H-12), 9.40 (s, H-3); ^{13}C-NMR (100 MHz, $CDCl_3$) 14.6 (C-24), 15.1 (C-25), 16.5 (C-30), 18.0 (C-26), 23.1 (C-6), 24.5 (C-27), 25.6 (C-11), 26.6 (C-21), 27.3 (C-16), 27.3 (C-29), 30.1 (C-15), 33.6 (C-7), 39.3 (C-22), 41.3 (C-8), 41.3 (C-10), 43.1 (C-20), 43.2 (C-14), 45.4 (C-9), 46.4 (C-1), 49.1 (C-17), 54.9 (C-5), 55.2 (C-18),

57.3 (C-4), 64.4 (C-23), 73.5 (C-19), 129.6 (C-12), 140.0 (C-13), 95.2 (C-2), 182.3 (C-28), 207.7 (C-3);

3.2.4. *Compound **2B***

Brown dark amorphous gum; Rf = 0.55 (CH_2Cl_2); HR-ESI-MS: *m/z* 587.3575 $[C_{34}H_{50}O_8 + H]^+$ (Calcd 587.3578); ESI-MS: *m/z* 587.4 $[C_{34}H_{50}O_8 + H]^+$; some characteristic ^{1}H-NMR data (400 MHz, $CDCl_3$) 0.86 (s, Me-26), 0.94 (d, 6.5, Me-30), 1.18 (s, Me-25), 1.20 (s, Me-24), 1.20 (s, Me-29), 1.28 (s, Me-27), 2.54 (d, 9.8, H-18), 3.25 (d, 12.7, H-23a), 4.05 (d, 12.7, H-23b), 5.36 (br s, H-12), 6.04 (dd, 5.2, 10.0, H-2), 9.44 (s, H-3), C-2 (CH_3COO) 2.04 (s), C-19 (CH_3COO) 2.20 (s); ^{13}C-NMR (100 MHz, $CDCl_3$) 14.2 (C-24), 14.7 (C-25), 16.2 (C-30), 17.6 (C-26), 22.8 (C-6), 24.0 (C-27), 24.7 (C-11), 26.1 (C-21), 27.5 (C-16), 27.5 (C-29), 29.5 (C-15), 32.1 (C-7), 36.3 (C-22), 40.3 (C-8), 40.4 (C-10), 41.1 (C-20), 42.5 (C-1), 42.5 (C-14), 53.1 (C-18), 44.1 (C-9), 49.3 (C-17), 54.3 (C-5), 56.0 (C-4), 65.5 (C-23), 73.1 (C-19), 94.9 (C-2), 129.7 (C-12), 137.4 (C-13), 182.2 (C-28), 206.1 (C-3), C-2 (CH_3COO) 21.5, 170.4, C-19 (CH_3COO) 22.7, 167.3

3.2.5. *Compound **3A***

Yellow bright amorphous solid, Rf = 0.5 (CH_2Cl_2–MeOH 37 : 3); HR-ESI-MS: *m/z* 575.4315 $[C_{35}H_{58}O_6 + H]^+$ (Calcd 575.4306), 597.4135 $[C_{35}H_{58}O_6 + Na]^+$ (Calcd 597.4126); ESI-MS: *m/z* 575.4 $[C_{35}H_{58}O_6 + H]^+$; $[\alpha] - 6.2$ (c 0.22, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$) 0.68 (s, CH_3-18), 0.81 (d, 6.9, CH_3-26), 0.83 (d, 7.2, CH_3-27), 0.85 (m, CH_3-29), 0.91 (overlapped with C-21, H-9), 0.92 (overlapped, H-24), 0.92 (d, 6.5, CH_3-21), 0.98 (m, H-14), 1.00 (s, CH_3-19), 1.00 (m, H-22a), 1.06 (m, H-1a), 1.06 (m, H-15a), 1.10 (m, H-17), 1.15 (H-12a), 1.25 (m, H-16a), 1.26 (m, H-23a), 1.28 (m, H-23b), 1.28 (m, H-28), 1.32 (m, H-22b), 1.35 (m, H-20), 1.42 (br s, H-8), 1.43 (m, H-11a), 1.48 (m, H-11b), 1.53 (m, H-7a), 1.57 (m, H-15b), 1.58 (m, H-2a), 1.65 (m, H-25), 1.83 (m, H-16b), 1.85 (m, H-1b), 1.90 (m, H-2b), 1.97 (m, H-7b), 2.00 (m, H-12b), 2.23 (m, H-4a), 2.32 (ddd, 1.9, 4.7, 13.0, H-4b), 3.57 (m, H-3), 5.35 (d, 5.1, H-6); *Triacetal moiety* 3.64 (br d, 5.7, H-7′a), 3.67 (d, 15.4, H-7′b), 4.02 (br d, 1.0, H-8′), 3.72 (dd, 5.7, 8.2, H-4′), 4.88 (br d, 1.3, H-1′), 5.72 (br s, H-2′), 5.49 (br s, H-5′). ^{13}C-NMR (100 MHz, $CDCl_3$) 12.0 (C-18), 12.1 (C-29), 18.9 (C-26), 19.2 (C-21), 19.5 (C-19), 20.0 (C-27), 21.2 (C-11), 23.1 (C-23), 24.4 (C-15), 28.3 (C-16), 29.2 (C-28), 29.3 (C-25), 29.9 (C-2), 32.0 (C-8), 32.1 (C-7), 34.0 (C-22), 36.3 (C-20), 36.2 (C-10), 37.2 (C-1), 38.9 (C-4), 39.8 (C-12), 42.4 (C-13), 46.0 (C-24), 50.3 (C-9), 56.1 (C-17), 56.8 (C-14), 78.5 (C-3), 122.4 (C-6), 140.2 (C-5); *Triacetal part* 61.7 (C-7′), 75.9 (C-4′), 80.4 (C-8′), 94.4 (C-2′), 94.5 (C-1′), 100.6 (C-5′).

3.2.6. *Compound **3B***

Brown amorphous solid, Rf = 0.69 (CH_2Cl_2); $[\alpha] - 4.2$ (c 0.18, CH_2Cl_2); HR-ESI-MS: *m/z* 681.4331 $[C_{39}H_{62}O_8 + Na]^+$ (Calcd 681.4337); ESI-MS: *m/z* 659.4 $[C_{39}H_{62}O_8 + H]^+$; some characteristic ^{1}H-NMR signals (400 MHz, $CDCl_3$): 0.68 (s, Me-18), 0.82 (d, 6.9, Me-26), 0.84 (d, 7.2, Me-27), 0.92 (H-24), 0.85 (m, Me-29), 0.91 (overlapped with C-21, H-9), 0.92 (d, 6.5, CH_3-21), 0.98 (m, H-14), 1.00 (s, CH_3-19), 1.10 (m, H-17), 1.06 (m, H-15a), 1.15 (H-12a), 1.25 (m, H-16a), 1.26 (m, H-23a), 1.28 (m, H-23b), 1.28 (m, H-28), 1.42 (br s, H-8), 1.43 (m, H-11a), 1.48 (m, H-11b), 1.53 (m, H-7a), 1.57 (m, H-15b), 1.65 (m, H-25), 1.83 (m, H-16b), 1.97 (m, H-7b), 2.00 (m, H-12b), 5.36 (d, 5.2, H-6); *Triacetal moiety* 3.81 (t, 6.9, H-4′), 4.02 (dd, 7.2, 11.4, H-7′b), 4.13 (dd, 6.0, 11.6, H-7′a), 4.19 (br d, 1.5, H-8′) 4.91 (br d, 1.5, H-1′), 5.55 (br s, H-5′), 6.53 (br s, H-2′); *Acetyl part* 2.06 ($C\underline{H}_3$), 2.11 ($C\underline{H}_3$) ^{13}C-NMR (100 MHz, $CDCl_3$) 12.0 (C-18), 12.1 (C-29), 18.9 (C-26), 19.2 (C-21), 19.5 (C-19), 20.0 (C-27), 21.2 (C-11), 23.2 (C-23), 24.4 (C-15), 28.4 (C-16), 29.3 (C-28), 29.5

(C-25), 29.8 (C-2), 32.1 (C-8), 32.1 (C-7), 34.1 (C-22), 36.3 (C-20), 36.9 (C-10), 37.3 (C-1), 38.9 (C-4), 39.9 (C-12), 42.5 (C-13), 45.9 (C-24), 50.3 (C-9), 56.2 (C-17), 56.9 (C-14), 78.5 (C-3), 122.4 (C-6), 140.2 (C-5) *Triacetal part* 62.3 (C-7′), 73.4 (C-4′), 79.2 (C-8′), 92.8 (C-2′), 94.0 (C-1′), 101.1 (C-5′). *Acetyl part* 20.9, 170.2 ($\underline{C}H_3\underline{C}OOH$), 22.8, 170.6 ($\underline{C}H_3\underline{C}OOH$)

3.3. *Cellular viability*

The human HT1080 fibrosarcoma adherent cell line was cultured at 37°C in a humidified atmosphere containing 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM/F12) supplemented with 10% fetal bovine serum together with penicillin (100 µg mL^{-1}), streptomycin (100 U mL^{-1}) and glutamax (1% v/v) from Invitrogen. The cells were seeded in 12 well plates (5.10^4 cells well^{-1}). After 24 h, the medium was replaced in each well by 1 mL of complete medium with the appropriate concentrations of the tested drugs in DMSO and stored at −20°C. Cells were then incubated for 48 h and cellular viability was determined by flow cytometric analysis. Global cell death was then determined with the cationic lipophilic $DiOC_6$ (3) dye (Invitrogen) which specifically probes mitochondrial membrane potential ($\Delta\Psi m$) (Bras, Queenan, & Susin, 2005). After drug treatment, the media from each well were kept in centrifuge tubes. The adherent cells were detached using trypsine, pooled with the corresponding media, centrifuged and resuspended in complete medium. Cells were then loaded with 100 nM $DiOC_6$ (3) and incubated for 30 min at 37°C. Flow cytometric measurements were performed using a XL3C flow cytometer (Beckman–Coulter). Fluorescence was induced by the blue line of an argon ion laser (488 nm) at 15 mW. Green fluorescence of $DiOC_6$ (3) was collected with a 525 nm band pass filter. The percentage of dead cells was determined by measuring the percentage of cells harboring low $DiOC_6$ (3) fluorescence. Analyses were performed on 10^4 cells.

4. Conclusions

Many methods have been reported for the preparation of acetal or ketal but the solubility of starting material always remained a difficulty to be surmounted. Thus, THF has a facility to dissolve many natural products which are pyridine or DMSO soluble. From this advantage, some acetal triterpenes were prepared and most of the products showed a moderated cytotoxicity activity against HT1080 cancer cell lines. The obtained products showed that intra-molecular acetalisation required systems of 1,2,3-triol and 1,2,4-triol (Figure 2). Although, the aldehyde function is unsuitable as drug candidate, it could be useful in further synthesis reactions such as Claisen–Schmidt condensation, Grignard or Wittig reactions in order to develop more bioactive compounds. Besides, this lactol can also be oxidised into lactone triterpene.

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