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New Ursolic and Betulinic Derivatives as Potential Cytotoxic Agents

I. BAGLIN[a,*], A. POUMAROUX[a], M. NOUR[a], K. TAN[a], A.C. MITAINE-OFFER[a], M.A. LACAILLE-DUBOIS[a], B. CHAUFFERT[b] and C. CAVÉ[a]

[a]*Université de Bourgogne, Faculté de Pharmacie, Unité de Molécules d'Intérêt Biologique, JE 2244, BP 87900, 21079 Dijon Cedex, France;* [b]*Unité INSERM 517, BP 87900, 21079 Dijon Cedex, France*

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Fifteen new ursolic and betulinic triterpenoids, bearing various functionalities at C-3 and C-28 were synthesized as potential cytotoxic agents. All compounds were obtained by a hemisynthetic route via ursolic and betulinic acids. Preliminary screening of these compounds on human HT 29 colon cancer cells revealed inhibitory activity for three of them. β-D-Glucopyranosyl-3β-hydroxyurs-12(13)-en-28-oate 1c, 3β-3-(3-pyridyl)-prop-2-enoyloxyurs-12(13)-en-28-oic acid 1i and the potassium salt of 3β-cinnamoyloxylup-20(29)-en-28-oic acid 2d demonstrated cytotoxic activity in the micromolar range: 8.0, 45.0 and 8.0 μM, respectively.

Keywords: Ursolic acid, Betulinic acid, Triterpenoids, Hemisynthesis, Cytotoxicity, HT 29 cells

INTRODUCTION

Triterpenes are widely distributed in plants and have been shown to exhibit a variety of biological properties including antiinflammatory, antihyperlipemia, anti-ulcer, hepatoprotective, antifungal, antiviral activities.[1]

During a drug discovery from natural resources initiative for potential anticancer activity agents, ursolic and betulinic acids **1a** and **2a** (Table I) were isolated. They displayed cytotoxic activity[2–6] but, unfortunately, these acids suffer from a low water-solubility, resulting in a lack of biological efficacy. Thus, the literature reports only a few synthetic analogues of **1a** and **2a** exhibiting a significant cytotoxicity.[7–8] Saponins are steroids or triterpene glycosides widely distributed in the plant kingdom, and are known to show an amphiphilic character (lipophilic and hydrophilic moieties).[9] So, these amphiphilic properties of saponins possibly enable these molecules to penetrate into the lipid bilayer to form complexes with the cholesterol molecule.[10] This interaction may create pore-like structures visible in electron microscopy, leading eventually to the bursting of the membrane. According to the literature, it seems that the presence of acyl groups in many natural saponins may improve their biological activities.[9]

As part of our programme directed toward the synthesis of novel antitumour agents, we describe here the development of semi-synthetic compounds resulting from the chemical modulation at C-3 (acyl derivatives) and/or C-28 (glycoside derivatives) of ursolic and betulinic acids.

MATERIALS AND METHODS

Chemistry

Melting points were recorded on a Kofler bench. Infra-red spectra (IR) were taken as KBr pellets or neat films between NaCl plates on a Perkin Elmer 881 spectrometer. NMR spectra were recorded on a Bruker AC 200 P (200 MHz), or a Bruker ARX 400 (400 and 100 MHz for ^{1}H and ^{13}C, respectively) spectrometer. Silica gel flash column chromatography was done using SDS chromagel 60A (35–70 mesh). Thin layer chromatography was accomplished using SDS silica gel $60F_{254}$ and detection of compounds was achieved by spraying with a solution of

*Corresponding author. Tel.: +00 33 380393234. Fax: +00 33 380393432. E-mail: ibaglin@u-bourgogne.fr

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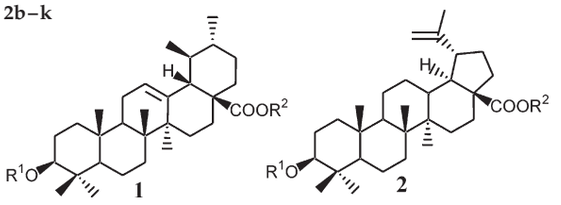
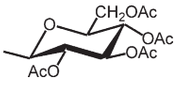
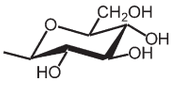
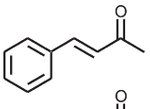
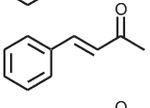
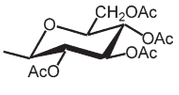
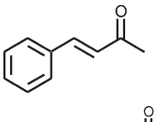
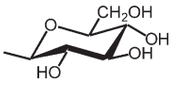
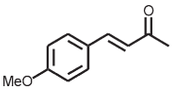
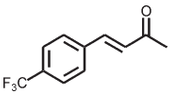
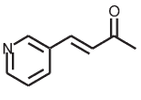
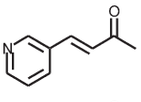
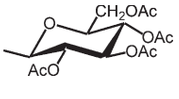
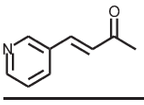
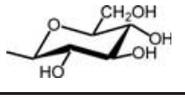
TABLE I Ursolic acid **1a**, betulinic acid *2a* and derivatives **1b–k**, **2b–k**



R^1	R^2		
H	H	**1a**	**2a**
H		**1b**	**2b**
H		**1c**	**2c**
	H	**1d**	**2d**
		**1e**	**2e**
		**1f**	**2f**
	H	**1g**	**2g**
	H	**1h**	**2h**
	H	**1i**	**2i**
		**1j**	**2j**
		**1k**	**2k**

EtOH/*p*-anisaldehyde/sulfuric acid/acetic acid (9:0.5:0.5:0.1).

General Procedure for Triterpenic Acid Glycosylation

2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl-3β-hydroxyurs-12(13)-en-28-oate **(1b)**[11]

2, 3, 4, 6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (0.160 g, 0.389 mmol) was added to a suspension of ursolic acid **1a** (0.114 g, 0.25 mmol) and potassium carbonate (0.138 g, 1 mmol) in 35 mL of anhydrous acetone. This mixture was allowed to stand at room temperature with stirring overnight. The suspension was then filtered off and the solution was evaporated under reduced pressure. The crude product was purified by flash chromatography (hexane/AcOEt [3:1]) to afford **1b**. Yield 95%—mp 164°C (lit.[11,12] mp 153–157°C).

β-D-glucopyranosyl-3β-hydroxyurs-12(13)-en-28-oate **(1c)**

The tetra-*O*-acetyl-β-D-Glucopyranosyl compound **1b**, previously obtained, was deacylated in 40 mL of a mixture of NEt_3/MeOH/H_2O [8/1/1].[13] The solution was allowed to stand at room temperature for 6 h and then concentrated in vacuum. After evaporation of the solvent the resulting solid was recrystallised (chloroform) to afford **1c**. Yield 50%—mp 192°C (lit.[11] mp 197–203°C).

2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-3β-hydroxylup-20(29)-en-28-oate **(2b)**[12]

Yield 92%—mp 112°C.

β-D-glucopyranosyl-3β-hydroxylup-20(29)-en-28-oate **(2c)**

Yield 60%—mp 234°C (lit.[14,15] 213–216°C).

General Procedure for the Synthesis of the 3-O-β-Acyl triterpenic acids 1d, 1g–i and 2d, 2g–i

3β-cinnamoyloxyurs-12(13)-en-28-oic Acid **(1d)**[16]

To a solution of ursolic acid **1a** (100 mg, 0.219 mmol) in 20 mL of anhydrous THF was successively added cinnamic acid (65 mg, 0.439 mmol), DMAP (53 mg, 0.434 mmol) and DCC (90 mg, 0.437 mmol), under nitrogen. The reaction mixture was stirred at room temperature and followed by TLC (CH_2Cl_2/Hexane [2/1]). After 18 h, a second equivalent of each compound: DCC, DMAP and cinnamic acid, was added. This addition was repeated three times at 24, 34 and 45 h. After 48 h, the reaction mixture was filtered off and the organic layer was evaporated under reduced pressure. The residue obtained was dissolved in 25 mL of dichloromethane; a precipate of dicyclohexylurea appeared and was filtered off. The organic layer was successively washed with 30 mL of a solution of 0.5 M HCl and 3 × 10 mL of water. Drying over Na_2SO_4 and evaporation of the solvent under reduced pressure left a white solid which was purified by flash chromatography using CH_2Cl_2/Hexane (2/1) to afford **1d**.

Yield 94%—mp 258°C, $[\alpha]_D^{25} + 61°$ ($CHCl_3$; *c*1.42). (Found: C, 78.68; H, 9.48 $C_{39}H_{54}O_4$,1/2 H_2O requires C, 78.61; H, 9.30%). IR ν_{max} cm^{-1}: 2924, 1710, 1695, 1640, 1449, 1278, 1170; ^1H NMR ($CDCl_3$, TMS) δ ppm: 7.68 (d, 1H, *J* 16, H3′), 7.03–6.97 (m, 2H, H-Ar), 6.81–6.74 (m, 3H, H-Ar), 6.45 (d, 1H, *J* 16, H-2′), 5.23 (s, 1H, H12), 4.60 (t, 1H, *J* 7.5, H3) 2.30–0.70 (m, 45H, H-ursane).

3β-CINNAMOYLOXYLUP-20(29)-EN-28-OIC ACID **(2d)**

Yield 66%—mp 320°C, $[\alpha]_D^{25} + 42.4°$ ($CHCl_3$; *c*2.2). (Found: C, 77.81; H, 9.59.$C_{39}H_{54}O_4$,1 H_2O requires C, 77.44; H, 9.34%). IR $\nu_{max}\,cm^{-1}$: 2940, 1727, 1696, 1674, 1643, 1449, 1296, 1189; ^1H NMR ($CDCl_3$, TMS) δ ppm: 7.67 (d, 1H, *J* 16, H3′), 7.60–7.46 (m, 2H, H-Ar), 7.44–7.31 (m, 3H, H–Ar), 6.46 (d, 1H, *J* 16, H2′), 4.76 (s, 1H, H29b), 4.63 (m, 2H, H3, H29a), 3.05 (m, 1H, H19), 2.37–0.70 (m, 43H, H-lupane).

2,3,4,6-TETRA-*O*-ACETYL-β-D-GLUCOPYRANOSYL-3β-CINNAMOYLOXYURS-12(13)-EN-28-OATE **(1e)**

Yield 90%–mp 118°C, $[\alpha]_D^{25} + 25.64$ ($CHCl_3$; *c*2.34). (Found: C, 69.59; H, 8.05, $C_{53}H_{72}O_{13}$ requires C, 69.41; H, 7.91%). IR $\nu_{max}\,cm^{-1}$: 2946, 1764, 1760, 1756, 1745, 1720, 1637, 1449, 1366, 1248, 1169; ^1H NMR ($CDCl_3$, TMS, 200 MHz) δ ppm: 7.64 (d, 1H, *J* 16 H3′), 7.60–7.46 (m, 2H, H-Ar), 7.45–7.32 (m, 3H, H-Ar), 6.42 (d, 1H, *J* 16, H2′), 5.56 (d, 1H, *J* 7, H1-glc), 5.37–5.05 (m, 4H, H2,3,4-glc, H12), 4.64 (t, 1H, *J* 7.5, H3), 4.25 (dd, 1H, *J* 4.3, *J* 12.4, H6b-glc), 4.05 (dd, 1H, *J* 2, *J* 12.4, H6a-glc), 3.80 (m, 1H, H5glc), 2.30–0.70 (m, 56H, H-ursane, CH_3).

β-D-GLUCOPYRANOSYL-3β-CINNAMOYLOXYURS-12(13)-EN-28-OATE **(1f)**

Yield 87%—mp 185°C, $[\alpha]_D^{25} + 44.12$ ($CHCl_3$; *c*4.08). (Found: C, 72.06; H, 8.50. $C_{45}H_{64}O_9$ requires C, 72.16; H, 8.61%). IR $\nu_{max}\,cm^{-1}$: 3428, 2924, 1712, 1638, 1449, 1305, 1280, 1173; ^1H NMR ($CDCl_3$, TMS, 200 MHz) δ ppm: 7.65 (d, 1H, *J* 16, H3′), 7.53–7.48 (m, 2H, H-Ar), 7.34–7.37 (m, 3H, H-Ar), 6.42 (d, 1H, *J* 16, H2′), 5.46 (d, 1H, *J* 6.3, H1-glc), 5.27 (s, 1H, H12), 4.63 (t, 1H, *J* 7.5, H3), 3.80–3.43 (m, 10H, H2,3,4,5,6b,6a-glc, OH), 2.25–0.70 (m, 44H, H-ursane, CH_3).

2,3,4,6-TETRA-*O*-ACETYL-β-D-GLUCOPYRANOSYL-3β-CINNAMOYLOXYLUP-20(29)-EN-28-OATE **(2e)**

Yield 91%—mp 116°C, $[\alpha]_D^{25} + 10.4°$ ($CHCl_3$; *c*5.3). (Found: C, 69.52; H, 8.08. $C_{53}H_{72}O_{13}$ requires C, 69.41; H, 7.91%). IR $\nu_{max}\,cm^{-1}$: 2944, 1760, 1748, 1713, 1638, 1450, 1366, 1304, 1172; ^1H NMR ($CDCl_3$, TMS, 200 MHz) δ ppm: 7.64 (d, 1H, *J* 16, H3′), 7.54–7.49 (m, 2H, H–Ar), 7.38–7.35 (m, 3H, H-Ar), 6.44 (d, 1H, *J* 16, H2′), 5.69 (d, 1H, *J* 8, H1-glc), 5.38–5.09 (m, 3H, H2,3,4-glc), 4.73 (s, 1H, H29b), 4.60 (m, 2H, H3, H29a), 4.32 (dd, 1H, *J* 4.4, *J* 12.4, H6b-glc), 4.08 (dd, 1H, *J* 2.2, *J* 12.4, H6a-glc), 3.84 (m, 1H, H5-glc), 2.96 (m, 1H, H19), 2.30–0.70 (m, 54H, H-lupane, CH_3).

β-D-GLUCOPYRANOSYL-3β-CINNAMOYLOXYLUP-20(29)-EN-28-OATE **(2f)**

Yield 88%—mp 184°C, $[\alpha]_D^{25} + 8.30°$ ($CHCl_3$; *c*4.82). (Found: C, 71.92; H, 8.30. $C_{45}H_{64}O_9$ requires C, 72.16; H, 8.61%). IR $\nu_{max}\,cm^{-1}$: 3424, 2943, 1749, 1713, 1638, 1449, 1278, 1173; ^1H NMR ($CDCl_3$, TMS, 200 MHz) δ ppm: 7.64 (d, 1H, *J* 16, H3′), 7.53–7.48 (m, 2H, H-Ar), 7.36–7.33 (m, 3H, H-Ar), 6.41 (d, 1H, *J* 16, H2′), 5.58 (d, 1H, *J* 6.8, H1-glc), 4.74 (s, 1H, H3), 4.60 (m, 2H, H29b, H29a), 3.86–3.46 (m, 10H, H2,3,4,5,6b,6a-glc, OH), 2.99 (m, 1H, H19), 2.40–0.70 (m, 42H, H-lupane, CH_3).

3β-*p*-methoxycinnamoyloxyurs-12(13)-en-28-oic Acid **(1g)**

Yield 93%—mp 267°C, $[\alpha]_D^{25} + 33°$ ($CHCl_3$; *c*3.12). (Found: C, 78.02; H, 8.88. $C_{40}H_{56}O_5$ requires C, 77.88; H, 9.15%). IR $\nu_{max}\,cm^{-1}$: 2921, 1710, 1697, 1634, 1605, 1513, 1251, 1169; ^1H NMR ($CDCl_3$, TMS, 200 MHz) δ ppm: 7.63 (d, 1H, *J* 16, H3′), 7.48 (d, 2H, *J* 8.8, H-Ar), 6.90 (d, 2H, *J* 8.8, H-Ar), 6.32 (d, 1H, *J* 16, H2′), 5.24 (s, 1H, H12), 4.64 (t, 1H, *J* 8.1, H3), 3.84 (s, 3H, OCH_3), 2.37–0.70 (m, 45H, H-ursane).

3β-*p*-methoxycinnamoyloxylup-20(29)-en-28-oic Acid **(2g)**

Yield 40%—mp 320°C, (lit.[16] 245°C) $[\alpha]_D^{25} + 29.4°$ ($CHCl_3$; *c*0.85).

3β-(*p*-trifluoromethyl)-cinnamoyloxyurs-12(13)-en-28-oic Acid **(1h)**

Yield 75%—mp 313°C, $[\alpha]_D^{25} + 74°$ ($CHCl_3$; *c*1.82). (Found: C, 73.10; H, 8.15. $C_{40}H_{53}F_3O_4$ requires C, 73.37; H, 8.16%). IR $\nu_{max}\,cm^{-1}$: 2941, 1715, 1695, 1645, 1456, 1322, 1274, 1165; ^1H NMR ($CDCl_3$, TMS, 200 MHz) δ ppm: 7.70 (d, 1H, *J* 16, H3′), 7.62 (s, 4H, H-Ar), 6.50 (d, 1H, *J* 16, H2′), 5.24 (s, 1H, H12), 4.63 (t, 1H, *J* 8, H3), 2.28–0.70 (m, 45H, H-ursane).

3β-(*p*-trifluoromethyl)-cinnamoyloxylup-20(29)-en-28-oic Acid **(2h)**

Yield 85%—mp 302°C, $[\alpha]_D^{25} + 13°$ ($CHCl_3$; *c*3.05) (Found: C, 71.01; H, 7.93. $C_{40}H_{53}F_3O_4$, H_2O requires C, 71.40; H, 8.23%). IR $\nu_{max}\,cm^{-1}$: 2941, 1724, 1696, 1644, 1323, 1166; ^1H NMR ($CDCl_3$, TMS, 200 MHz) δ ppm: 7.70 (d, 1H, *J* 16, H3′), 7.66 (s, 4H, H-Ar), 6.54 (d, 1H, *J* 16, H2′), 4.77 (s, 1H, H29b), 4.65 (m, 2H, H3, H29a), 3.06 (m, 1H, H19), 2.41–0.77 (m, 43H, H-lupane).

3β-3-(3-PYRIDYL)-PROP-2-ENOYLOXYURS-12(13)-EN-28-OIC ACID **(1i)**

Yield 84%—mp 208°C, $[\alpha]_D^{25} + 55°$ ($CHCl_3$; *c*2). (Found: C, 77.80; H, 9.17; N, 2.30. $C_{38}H_{53}NO_4$ requires C, 77.64; H, 9.09; N, 2.38%). IR $\nu_{max}\,cm^{-1}$: 3324, 2927, 1712, 1696, 1628, 1574, 1310, 1268, 1183; ^1H NMR ($CDCl_3$, TMS, 200 MHz) δ ppm: 8.69 (s, 1H, H5′), 8.55 (d, 1H, *J* 3.3, H7′), 7.85 (d, 1H, *J* 7.9, H9′), 7.58 (d, 1H, *J* 16, H3′), 7.35 (dd, 1H, *J* 7.9, *J* 3.3, H8′), 6.47 (d, 1H, *J* 16, H2′), 5.19 (s, 1H, H12), 4.60 (t, 1H, *J* 8, H3), 2.25–0.67 (m, 45H, H-ursane).

3β-3-(3-PYRIDYL)-PROP-2-ENOYLOXYLUP-20(29)-EN-28-OIC ACID **(2i)**

Yield 98%—mp 321°C, $[\alpha]_D^{25} + 41.5°$ ($CHCl_3$; *c*1.9) (Found: C, 77.80; H, 9.00; N, 2.46. $C_{38}H_{53}NO_4$ C, 77.64; H, 9.09; N, 2.38%). IR $\nu_{max}\,cm^{-1}$: 3322, 2930, 1718, 1630, 1575, 1310; ^1H NMR ($CDCl_3$, TMS, 200 MHz) δ ppm: 8.75 (s, 1H, H5′), 8.62 (d, 1H, *J* 4, H7′), 7.94 (d, 1H, *J* 8.1, H9′), 7.64 (d, 1H, *J* 16, H3′), 7.40 (dd, 1H, *J* 8.1, *J* 4, H8′), 6.53 (d, 1H, *J* 16, H2′), 4.77

(s, 1H, H29b), 4.65 (m, 2H, H3, H29a), 3.06 (m, 1H, H19), 2.50–0.75 (m, 43H, H-lupane).

2,3,4,6-TETRA-O-ACETYL-β-D-GLUCOPYRANOSYL-3β-3-(3-PYRIDYL)-PROP-2-ENOYLOXY-URS-12(13)-EN-28-OATE **(1j)**

Yield 65%—mp 117°C (dec.), $[\alpha]_D^{25} + 30°$ (*c*0.05, CH_2Cl_2) (Found C, 67.88; H, 8.08; N, 1.29. $C_{52}H_{71}NO_{13}$ requires C, 68.03; H, 7.79; N, 1.53%). IR $\nu_{max}\,cm^{-1}$: 2925, 1762, 1714, 1640, 1454, 1369, 1220; ^1H NMR ($CDCl_3$, TMS, 200 MHz) δ ppm: 8.69 (s, 1H, H5′), 8.55 (s, 1H, H7′), 7.83 (d, 1H, *J* 8.0, H9′), 7.58 (d, 1H, *J* 16, H3′), 7.31 (dd, 1H, *J* 8.0, *J* 4.9, H8′), 6.47 (d, 1H, *J* 16, H2′), 5.48 (d, 1H, *J* 8, H1glc), 5.23–5.05 (m, 4H, H2,3,4-glc, H12), 4.58 (t, 1H, *J* 8.0, H3), 4.20 (dd, 1H, *J* 4.3, *J* 12.4, H6bglc), 4.05 (dd, 1H, *J* 2, *J* 12.4, H6a-glc), 3.80 (m, 1H, H5-glc), 2.18–0.70 (m, 56H, H-ursane, CH_3).

2,3,4,6-TETRA-O-ACETYL-β-D-GLUCOPYRANOSYL-3β-3-(3-PYRIDYL)-PROP-2-ENOYLOXY-LUP-20(29)-EN-28-OATE **(2j)**

Yield 62%—mp 129°C (dec.), $[\alpha]_D^{25} + 11.4°$ (*c*0.3, MeOH), (Found: C, 67.90; H, 7.77; N, 1.68. $C_{52}H_{71}NO_{13}$ requires C, 68.03; H, 7.79; N, 1.53%). IR $\nu_{max}\,cm^{-1}$: 2951, 1761, 1715, 1644, 1452, 1365, 1218; ^1H NMR ($CDCl_3$, TMS, 300 MHz) δ ppm: 9.09–8.70 (m, 2H, H5′,H7′), 8.06 (d, 1H, *J* 8.8, H9′), 7.87 (d, 1H, *J* 16, H3′), 7.56 (m, 1H, H8′), 6.75 (d, 1H, *J* 16, H2′), 5.93 (d, 1H, *J* 8, H1-glc), 5.58-5.27 (m, 3H, H2,3,4-glc), 4.99 (s, 1H, H29b), 4.84 (m, 2H, H3, H29a), 4.53 (dd, 1H, *J* 4.5, *J* 12.4, H6b-glc), 4.30 (dd, 1H, *J* 2.2, *J* 12.4, H6a-glc), 4.06 (m, 1H, H5-glc), 3.18 (m, 1H, H19), 2.50–0.75 (m, 54H, H-lupane, CH_3).

β-D-GLUCOPYRANOSYL-3β-3-(3-PYRIDYL)-PROP-2-ENOYLOXY-URS-12(13)-EN-28-OATE **(1k)**

Yield 80%—mp 179°C (dec.), $[\alpha]_D^{25} + 30°$ (*c*0.2, MeOH) (Found: C, 70.68; H, 8.36; N, 2.01. $C_{44}H_{63}NO_9$ requires C, 70.47; H, 8.47; N, 1.87%. IR $\nu_{max}\,cm^{-1}$: 3400, 2923, 1713, 1639, 1457, 1365, 1221, 1176, 1071; ^1H NMR ($CDCl_3$, TMS, 200 MHz) δ ppm: 8.70 (s, 1H, H5′), 8.55 (s, 1H, H7′), 7.84 (d, 1H, *J* 8.0, H9′), 7.57 (d, 1H, *J* 16, H3′), 7.31 (m, 1H, H8′), 6.45 (d, 1H, *J* 16, H2′), 5.40 (d, 1H, *J* 7.0, H1-glc), 5.23 (s, 1H, H12), 4.58 (m, 1H, H3), 3.74–3.07 (m, 10H, H2,3,4,5,6b,6a-glc, OH), 2.19–0.70 (m, 44H, H-ursane, CH_3).

β-D-GLUCOPYRANOSYL-3β-3-(3-PYRIDYL)-PROP-2-ENOYLOXY-LUP-20(29)-EN-28-OATE **(2k)**

Yield 82%—mp 178°C (dec.), $[\alpha]_D^{25} + 13°$ (*c*0.35, MeOH), (Found: C, 70.62; H, 8.38; N, 2.02. $C_{44}H_{63}NO_9$ requires C, 70.47; H, 8.47; N, 1.87%). IR $\nu_{max}\,cm^{-1}$: 3405, 2942, 1716, 1644, 1453, 1318, 1172, 1070; ^1H NMR ($CDCl_3$, TMS, 300 MHz) δ ppm: 8.72–8.56 (m, 2H, H5′,H7′), 7.90 (d, 1H, *J* 8.0, H9′), 7.57 (d, 1H, *J* 16, H3′), 7.39 (m, 1H, H8′), 6.48 (d, 1H, *J* 16, H2′), 5.49 (d, 1H, *J* 8, H1-glc), 4.66 (s, 1H, H3), 4.56 (m, 2H, H29b, H29a), 3.78–3.39 (m, 10H, H2,3,4,5,6b,6a-glc, OH), 2.89 (m, 1H, H19), 2.30–0.70 (m, 42H, H-lupane, CH_3).

Cell Lines and Biological Screening Procedures

The human HT 29 cancer cell line originating from a colic adenocarcinoma in a non-treated patient was obtained from the American Tissue Culture Collection (ATCC, Rockville, MD, USA). The culture medium was a mixture of Ham F10 medium (Bio Whittaker, Ververs, Belgium) and 10% fetal calf serum (Boerhinger Ingelheim, Gagny, France). Cells were grown as monolayers in a controlled atmosphere (37°C, 5% CO_2) in Ham's F-10 medium supplemented with 10% fetal calf serum.[17]

All test compounds were solubilized in dimethylsulfoxyde (DMSO) and were tested twice at different concentrations.

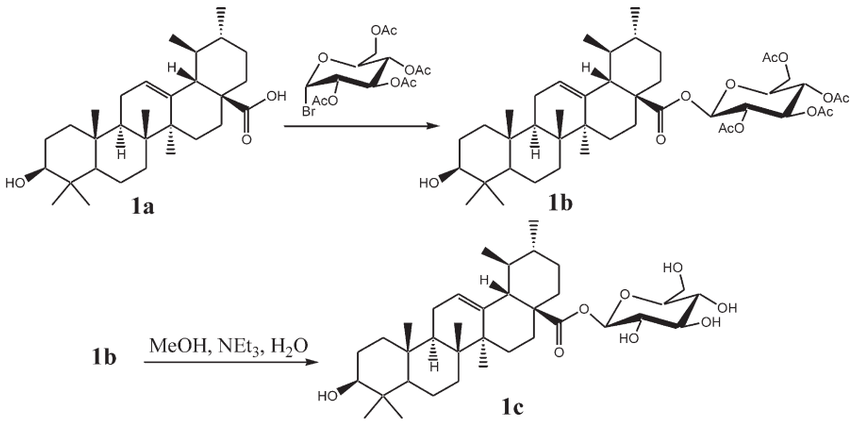
Cytotoxicity Assay

HT-29 cells (2×10^4 per well) were seeded in 96-well culture plates and cultured for two days before treatment.[17] Triterpenes were dissolved immediately before use in a mixture of DMSO and absolute EtOH (1:1, V/V), then diluted in serum-free Ham's F-10 medium. Final concentration of DMSO and EtOH, which did not exceed 1%, did not affect cell survival. Cells were treated for 3 h with triterpenes alone. After treatment, cells were washed twice with Ham's F-10 and cultured again for 7 days in drug-free culture medium. Cell survival was measured by the crystal violet colorimetric assay. In brief, cells were rinsed with phosphate buffered saline (PSB), and then surviving adherent cells were fixed for 5 min by pure ethanol. After drying, cells were stained by crystal violet (5 g/L in distilled water). Dye in excess was flushed off using tap water. Cell-fixed dye was eluted by 33% acetic acid.

RESULTS AND DISCUSSION

Chemistry

The glucopyranosyl compounds **1c** and **2c** were synthesized in two steps as illustrated in Scheme 1 for the ursolic acid series. Condensation of triterpenic acids **1a** and **2a** with the commercially available 2, 3, 4, 6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide afforded 28-*O*-β-D-tetra-*O*-acetyl glucopyranosides **1b**[11,12] and **2b**[12] which were deacetylated according to the method described by Lubineau *et al.*,[13] with a mixture of MeOH/$N(Et)_3$/H_2O (8:1:1) to give the required products **1c**[11,12] and **2c**.[14,15] (Scheme 1)



SCHEME 1 Synthesis of C-28-glycopyranyl derivatives of ursolic acid **1a**: **1b** and **1c**.

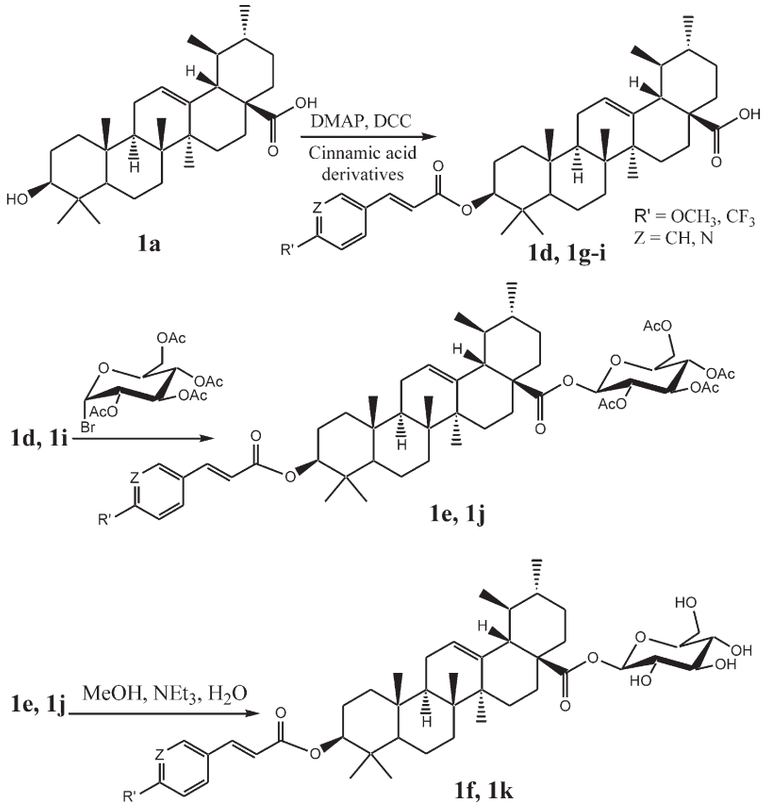
The hydroxyl group at C-3 position could be acylated by 3-arylpropenoic acids, such as *p*-methoxycinnamic acid, *p*-trifluoromethylcinnamic acid and 3-(3-pyridyl)prop-2-enoic acid. The C-3 acylation of triterpenic acids **1a** and **2a** was carried out in the presence of a coupling agent, dicyclohexylcarbodiimide (DCC), and a catalyst, 4-dimethylaminopyridine (DMAP) leading to **1d**, **1g–i** and **2d**, **2g–i**.

The glycosylation of 3-*O*-β-cinnamoylursolic acid **1d**, and of 3-*O*-β-cinnamoyl betulinic acid **2d** was achieved under the same conditions as those described for **1a** leading to **1e**, **2e** and **1j**, **2j**, which were deacylated to afford **1f**, **2f** and **1k**, **2k** (Scheme 2).

Antitumour Activity

Our investigations confirmed that ursolic acid **1a** and betulinic acid **2a** exerted moderate cytotoxic activity against human colon adenocarcinoma cells HT 29: $IC_{50} = 30$ and 26 μM, respectively (Table II).

The esterification of the two acids **1a** and **2a** by acetylglycopyranosylation, leading to **1b** and **2b**,



SCHEME 2 Synthesis of C-3-monoesters and C-3, C-28-diesters of ursolic acid **1a**: **1d**, **1g–i** and **1e**, **1f**, **1j**, **1k**.

TABLE II Cytotoxic activity on HT 29 colon cancer cells

Ursane serie		Lupane serie	
Compounds	Cytotoxic activity IC_{50} μM	Compounds	Cytotoxic activity IC_{50} μM
1a	30	**2a**	26
1a K salt	60	**2a K salt**	10
1b	nc*	**2b**	nc
1c	8	**2c**	nc
1d	nc	**2d**	nc
1d K salt	nc	**2d K salt**	8
1e	nc	**2e**	nc
1f	nc	**2f**	nc
1g	350	**2g**	nc
1h	nc	**2h**	nc
1i	45	**2i**	nc
1j	nc	**2j**	nc
1k	nc	**2k**	nc

*nc: no cytotoxicity at the highest experimented concentration (500 μM).

suppressed their cytotoxic activity and unexpectedly, only the free pyranosyl ester **1c**, (contrary to **2c**) exhibited antitumour activity: $IC_{50} = 8\,\mu M$. These results suggested the importance of the hydrogen bonding capability and/or acidity in the expression of the cytotoxic effect. These observations are consistent with the conclusions of previously reported studies dealing with the antitumour activities of betulinic and ursolic acids analogs.[2,8,15]

The influence of the substitution at the C-3 position was also investigated. Irrespective of the nature of the arylpropenoyl moiety introduced in both series (**1d**, **1g–i** and **2d**, **2g–i**), we observed a detrimental effect on the cytotoxicity against HT 29 cell lines. Nevertheless as previously observed in the betulinic acids series with **2a**, the potassium salt of **2d** was as efficient as the corresponding salt of **2a**: $IC_{50} = 8$ and $10\,\mu M$, respectively. So, in accordance with Kim *et al.*,[8] the loss of toxic effect suggested a size limitation at the C-3 position. Surprisingly, C-3 substitution with the (pyridin-2-yl)propenoyl moiety in the ursolic series, produced a compound, **1i,** that exhibited a moderate activity: $IC_{50} = 45\,\mu M$ versus $30\,\mu M$ for **1a**. This result suggested more than a size limitation at C-3 position and, the possible influence of the electronic density of the introduced moiety.

Finally the glycosylation of these C-3 substituted acids **1i** and **2d** induced, as previously observed, a deleterious effect; none of the four tested compounds **2e**, **2f**, **1j** and **1k** exhibited cytotoxic activity at the highest experimented concentration (500 μM).

The above investigations suggest that simple modifications of the parent structure either in the ursane or lupane series could produce new potentially interesting derivatives which may modify the cytotoxic profile. However, this study brings to the fore that no correlation could be established between ursane and lupane substituted compounds even though they are close in structure. These observations support Kashiwada suggestion,[18] that the structure of the E ring, which differs in the ursane and lupane series, could play an important role in cytotoxic potency.

In conclusion, taken together, these results show that the incorporation of a glycosyl or arylpropenoyl scaffold at the C-28 or C-3 position induced suppression of cytotoxic activity or at the best maintained it. The inconsistent variable activities observed in the two series of ursolic and betulinic acids derivatives, emphasize the difficulty of working out a rationale. Finally, a more extensive investigation including a greater number of compounds is currently being carried out, so as to bring out a more broadly based structure–activity relationship in the two series studied.

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