

# Ursolic Acid Derivatives Induced Apoptosis and Reduces the NF- $\kappa$ B in Human Lung Adenocarcinoma Cells

Elaine Carlos Scherrer<sup>1</sup>, Ydia Mariele Valadares<sup>1</sup>, Caio Cesar Souza Alves<sup>2</sup>, Gabriella Freitas Ferreira<sup>1</sup>, Marcela Pereira Leão<sup>1</sup>, Jessica Aline Soares<sup>1</sup>, Fernando Sa Silva<sup>1</sup>, Alessandra Paula Carli<sup>2</sup>, Oswaldo Cardoso Jr.<sup>2</sup>, Fabiana Simão Machado<sup>3</sup>, Sandra Bertelli Ribeiro Castro<sup>1\*</sup>

<sup>1</sup>Life Sciences Institute, Federal University of Juiz de Fora, Governador Valadares, MG, Brazil

<sup>2</sup>Faculty of Medicine, Federal University of the Valleys of Jequitinhonha and Mucuri, Teófilo Otoni, MG, Brazil

<sup>3</sup>Department of Biochemistry and Immunology, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil

Email: \*bertelli.ribeiro@ufjf.edu.br

**How to cite this paper:** Scherrer, E.C., Valadares, Y.M., Alves, C.C.S., Ferreira, G.F., Leão, M.P., Soares, J.A., Silva, F.S., Carli, A.P., Cardoso Jr., O., Machado, F.S. and Castro, S.B.R. (2019) Ursolic Acid Derivatives Induced Apoptosis and Reduces the NF- $\kappa$ B in Human Lung Adenocarcinoma Cells. *Journal of Cancer Therapy*, 10, 863-876.

<https://doi.org/10.4236/jct.2019.1010073>

**Received:** September 25, 2019

**Accepted:** October 20, 2019

**Published:** October 23, 2019

Copyright © 2019 by author(s) and Scientific Research Publishing Inc. This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).

<http://creativecommons.org/licenses/by/4.0/>



Open Access

## Abstract

Lung cancer is the major cause of death in the neoplastic diseases. In spite of the advances in the chemotherapy, the lung cancer treatments are still complex and costly, being necessary the seeking of new drugs. In this context, the ursolic acid (UA) becomes the target of studies that investigate its antitumor potential and, thus, structural modifications can enhance its biological activities. Eight UA semisynthetic derivative compounds (UAD1-8) were synthesized and evaluated their cytotoxic activity against human alveolar adenocarcinoma cells (A549). UAD1, UAD3, UAD5, UAD6 and UAD8 were able to reduce the viability of the A549 cells. Only UAD1 and UAD6 reduced the viability at 24 h, and only UAD3 didn't reduce the NF- $\kappa$ B expression. The compound UAD1 showed the greater apoptosis induction. Moreover, the compound UAD1 showed better results than UA in all assays. The present study shows, for the first time, the action of these compounds in the apoptotic effect, in the expression of NF- $\kappa$ B and in the A549 cell line. The ursolic acid derivatives showed substantial results in the apoptosis, cytotoxicity and NF- $\kappa$ B inhibition of A549 cells, and further studies are necessary for the development of possible new therapeutic drugs.

## Keywords

Ursolic Acid, Apoptosis, NF- $\kappa$ B, A549 Cells, Tumoral

## 1. Introduction

The lung cancer is the major cause of death in the neoplastic diseases that can

aggravate with bone metastasis, turning more expensive the treatment and reducing the life quality of the patient [1] [2]. It affects both men and women and there is a clear association with smoking in the development of the disease, although it is not the only risk factor [3] [4].

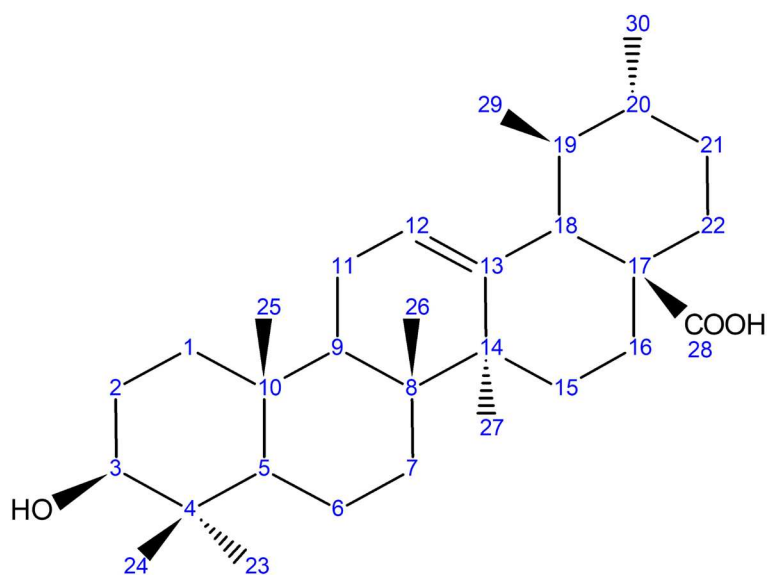
Despite the advances in the chemotherapy, the results of the lung cancer treatment are not good enough; being necessary the seeking of new therapeutic strategies and the development of new drugs [4]. In this context, the ursolic acid (UA), a triterpene widely distributed in plant species, becomes the target of studies that investigate its antitumor potential [5] [6] [7] [8]. Moreover, structural modifications in the carbons 3, 12 and 28, and the rings A, C and D (**Figure 1**) are the potential target to increase the antimicrobial, antioxidant, anti-inflammatory or antitumor activities of the ursolic acid [9] [10] [11].

Different studies already showed the action of the ursolic acid as an anti-tumor drug [6] [7] [9] [10], however, this is the first time that is shown the action of the derivatives on apoptotic effect, on NF- $\kappa$ B expression and upon A549 cells. In light of the mentioned, eight UA semisynthetic derivative compounds, commonly obtained in structural modification studies, were synthesized and evaluated their cytotoxic activity against human alveolar adenocarcinoma cells (A549).

## 2. Materials and Methods

### 2.1. Materials

The reagents and the solvents were used directly from the manufacturers. CH<sub>3</sub>I (Fluka, Sigma-Aldrich, St. Louis, MO, USA); Acetone, EtOAc, t-BuOOH, n-hexane, CH<sub>2</sub>Cl<sub>2</sub>, anhydrous diethyl ether and acetic anhydride (Merck, Darmstadt, Germany); K<sub>2</sub>CO<sub>3</sub>, NaHCO<sub>3</sub>, NaClO<sub>2</sub>, Na<sub>2</sub>SO<sub>4</sub>, LiAlH<sub>4</sub>, BF<sub>3</sub>-Et<sub>2</sub>O, NaCl and pyridine (Sigma-Aldrich, St. Louis, MO, USA) were employed to obtain ursolic acid derivatives. RPMI-1640 (Gibco, Grand Island, USA); RPMI-1640



**Figure 1.** Ursolic Acid structure.

(Lonza, Basel, Switzerland); L-glutamine, streptomycin-penicillin, dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT), fetal bovine serum (Sigma-Aldrich); NF-KPA-B (PS529)-PE (BD, Biosciences Pharmingen, San Diego, CA, USA); Annexin-V Apoptosis Detection Kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) were used for the biological assays.

## 2.2. Spectral Data

NMR spectra were recorded in CDCl<sub>3</sub> on a Bruker AC200 at 200 MHz for <sup>1</sup>H and 50 MHz for <sup>13</sup>C, using TMS as internal reference for both nuclei. For each peak, chemical shift values are expressed in parts per million, followed by multiplicity, relative peak integration (when appropriate), and coupling constants (*J*) in Hertz. High-resolution mass spectra (HRMS) were obtained using a QSTAR XL spectrometer. The spectra in the IR were obtained in Spectrum One apparatus, Perkin-Elmer coupled to the diffuse reflectance accessory. The specific rotational power values [ $\alpha$ ] D were measured on a Perkin-Elmer 241 polarimeter at 20 °C. Column chromatography was performed on Silica Gel 60 (230 - 400 mesh, Merck), whereas thin-layer chromatography was carried out on Silica Gel 60 F254 plates (0.25 mm thick, Merck). Solvents and reagents were used directly from the manufacturer, or purified by standard procedures when required.

## 2.3. Cell Culture

A549 cell line was placed in 96-well plates containing RPMI-1640 (Gibco, Grand Island, USA) supplemented (2.0 mM L-glutamine, 100.0 µg/mL of streptomycin and penicillin, 5% fetal bovine serum) at  $2 \times 10^5$  cells/mL by 24 and 48 hours. Cells were incubated at 37 °C in 5% CO<sub>2</sub> atmosphere in the presence of the ursolic acid derivatives UAD1, UAD2, UAD3, UAD4, UAD5, UAD6, UAD7 or UAD8 at 30 µM, 60 µM or 90 µM. In the control experiments, cells were treated with ursolic acid at 30 µM, 60 µM or 90 µM. The compounds were solubilized in dimethyl sulfoxide (DMSO), never exceeding 0.1% (v/v), and diluted in RPMI-1640 (Lonza, Basel, Switzerland) before use. The DMSO concentration was determined to allow the solubilization of the UAD and UA but without affecting the A549 viability [12].

## 2.4. MTT Assay

Cellular viability was measured using the MTT [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide] assay. After 24 and 48 hours of culture the supernatants were removed and the cells were incubated with 100 µL of supplemented RPMI medium and 10 µL of MTT (5.0 mg/mL) during 4 h at 37 °C in 5% CO<sub>2</sub>. After purple formazan crystal formation, the supernatants were gently removed and crystal products were solubilized and incubated with DMSO. Complete solubilization was obtained by shaking the plates for 10 mins. The optical density (OD) values were determined in the microplate reader (Multiskan™ FC

Microplate Photometer, Thermo Scientific, MA, USA) at 560 nm wavelength. The cellular viability was calculated using the formula  $(X1/X2)*100$ , considering X1 the OD of treated cells and X2 the mean OD of untreated cells. Compounds were considered cytotoxic when the viability was lower than 70% [12].

### 2.5. NF- $\kappa$ B Concentration in A549 Cells

The cells were cultured in the presence of the ursolic acid or UAD1, UAD2, UAD3, UAD4, UAD5, UAD6, UAD7 or UAD8 at 30  $\mu$ M, 60  $\mu$ M or 90  $\mu$ M. A549 cells ( $1 \times 10^6$  cells/mL) were incubated by 24 h at 37°C in 5% CO<sub>2</sub> atmosphere. After this period, the cells were detached and stained for the analysis of the p65 expression (indirectly NF- $\kappa$ B), following the manufacturer's instructions (NF-KPA-B (PS529)-PE 558423, BD, Biosciences Pharmingen, San Diego, CA, USA). The cells were acquired in the FACSVerse (BD, Biosciences Pharmingen, San Diego, CA, USA) and analyzed in the FCS Express software (De Novo software).

### 2.6. Analysis of Apoptosis by Flow Cytometry

For apoptosis detection, the Annexin-V Apoptosis Detection Kit (Invitrogen, Thermo Fisher Scientific) was used. To perform the assay, A549 cells ( $1 \times 10^6$  cells/mL) were cultured in the presence or absence of the ursolic acid or UAD1, UAD5, UAD6 or UAD8 at 90  $\mu$ M, which had the best results in MTT assay. After 36 hours of culture, the cells were trypsinized and washed with phosphate buffer. After washing, the cells were labeled with Annexin-V FITC and propidium iodide, according to the manufacturer's instructions. The cells were incubated at room temperature for 15 mins in the dark, and then acquired in FACSVerse and analyzed in the FCS Express software [13].

### 2.7. Statistical Analysis

The results represent at least three independent experiments and are presented as the mean  $\pm$  SEM. All data were analyzed using two-way ANOVA followed by Bonferroni posttests (GraphPad Prism 5.00), and the differences were considered significant at  $p < 0.05$ .

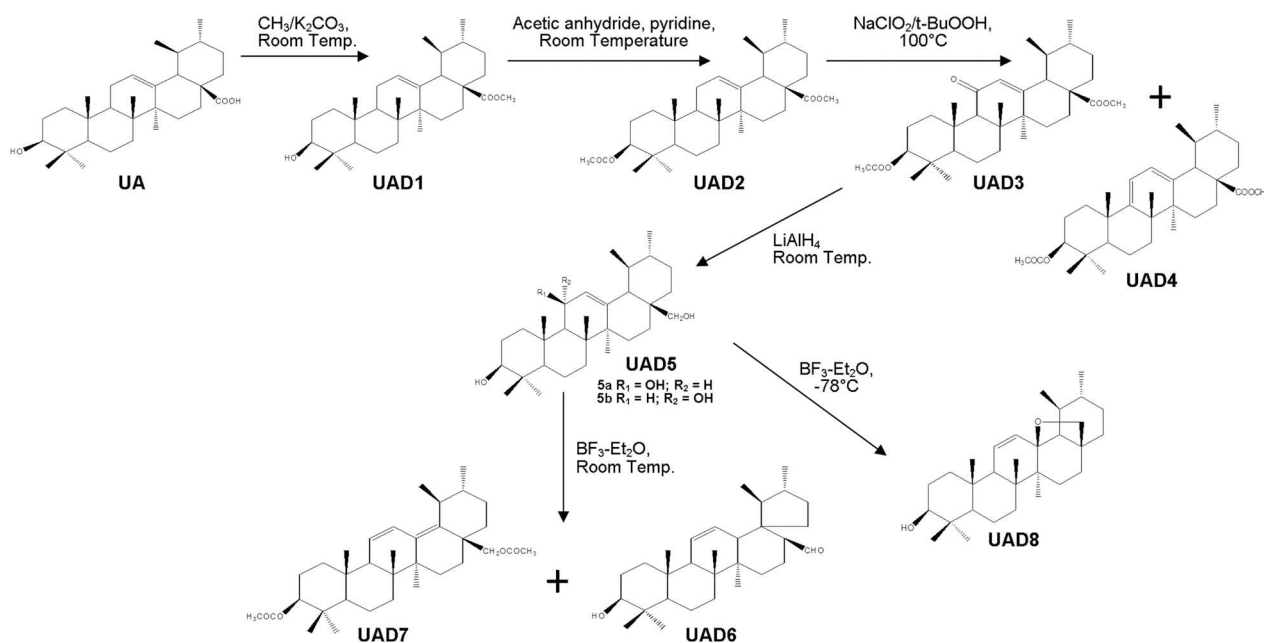
## 3. Results and Discussion

### 3.1. Spectral Data

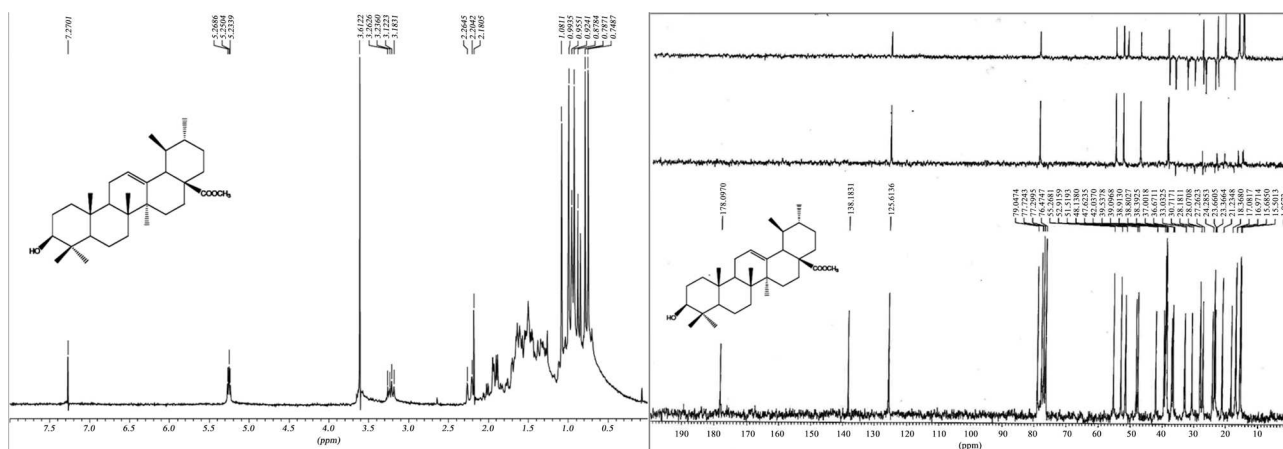
The eight UA semisynthetic derivative compounds were synthesized (Figure 2) with previously described structural modifications. The structures were elucidated by NMR spectra recorded in CDCl<sub>3</sub> on a Bruker AC200 at 200 MHz for <sup>1</sup>H and 50 MHz for <sup>13</sup>C. The spectra of the compounds UAD1-UAD8 are described below. The obtained spectra of the compounds correspond to the data in the literature [14] [15].

*Methyl 3 $\beta$ -hydroxyurs-12-en-28-oate (UAD1)*: To a solution of UA (100 mg, 0.22 mmol) in dry acetone (5.0 mL), K<sub>2</sub>CO<sub>3</sub> (122.0 mg, 0.88 mmol) and CH<sub>3</sub>I (1.0 mL, 0.07 mmol) were added and the reaction mixture was stirred at room

temperature for 4 h. Usual work up of the reaction mixture afforded **UAD1** as white crystals (92.0 mg, 89.3% yield).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  5.25 (t, 1H,  $J = 3.5$  Hz, H12); 3.61 (s, 1H,  $\text{COOCH}_3$ ); 3.22 (m, 1H, H3); 2.23 (d, 1H, H18); 1.08 (s, 3H,  $\text{CH}_3$ ); 0.99 (s, 3H,  $\text{CH}_3$ ); 0.93 (d, 3H,  $J = 6.3$  Hz,  $\text{CH}_3$ ); 0.92 (s, 3H,  $\text{CH}_3$ ); 0.86 (d, 3H,  $J = 6.3$  Hz,  $\text{CH}_3$ ); 0.78 (s, 3H,  $\text{CH}_3$ ); 0.74 (s, 3H,  $\text{CH}_3$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  178.09 (C28); 138.18 (C13); 125.61 (C12); 79.04 (C3); 55.26 (C18); 52.91 (C5); 51.51 ( $\text{COOCH}_3$ ); 48.13 (C17); 47.62 (C9); 42.03 (C14); 39.53 (C8); 39.09 (C19); 38.91 (C20); 38.80 (C4); 38.69 (C1); 37.00 (C10); 36.67 (C22); 33.03 (C7); 30.71 (C21); 28.18 (C23); 28.07 (C15); 27.26 (C2); 24.28 (C16); 23.66 (C27); 23.36 (C11); 21.23 (C30); 18.36 (C6); 17.08 (C26); 16.97 (C29); 15.68 (C25); 15.50 (C24). HRMS calcd for  $\text{C}_{31}\text{H}_{50}\text{O}_3\text{Na}$ : 493.7240, found:  $m/z$  493.3610.  $[\alpha]_D^{25}$ : +41.16 (MeOH,  $c$  1.0). IR ( $\nu_{\text{max}}$ ,  $\text{cm}^{-1}$ ): 3525 (O-H); 2969 (C-H); 1718 (C=O); 1384 (C-O-H); 1230 (C-O) (**Figure 3**).



**Figure 2.** Synthesis of ursolic acids derivatives.



*Methyl 3 $\beta$ -acetoxyurs-12-en-28-oate (UAD2)*: Compound **UAD2** was obtained as white crystals (236.1 mg, 86% yield) after acetylation of derivative **UAD1** (252 mg, 0.54 mmol) with acetic anhydride in pyridine.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  5.18 (t, 1H,  $J = 3.6$  Hz, H12); 4.43 (m, 1H, H3); 3.54 (s, 3H,  $\text{COOCH}_3$ ); 2.17 (d, 1H,  $J = 11$  Hz, H18); 1.98 (s, 3H,  $\text{OCOCH}_3$ ); 1.01 (s, 3H,  $\text{CH}_3$ ); 0.88 (s, 3H,  $\text{CH}_3$ ); 0.81 (s, 3H,  $\text{CH}_3$ ); 0.79 (s, 3H,  $\text{CH}_3$ ); 0.68 (s, 3H,  $\text{CH}_3$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  177.38 (C28); 170.89 ( $\text{OCOCH}_3$ ); 138.14 (C13); 125.43 (C12); 55.26 (C5); 52.84 (C18); 51.47 ( $\text{COOCH}_3$ ); 48.04 (C17); 47.49 (C9); 41.97 (C14); 39.47 (C8); 39.03 (C19); 38.88 (C20); 38.26 (C1); 37.67 (C4); 36.86 (C10); 36.64 (C22); 32.89 (C7); 30.65 (C21); 28.00 (C15); 28.00 (C23); 24.18 (C16); 23.56 (C27); 23.30 (C2); 21.20 (C30); 21.20 ( $\text{OCOCH}_3$ ); 18.19 (C6); 17.09 (C29); 16.90 (C26); 16.75 (C25); 15.50 (C24). HRMS calcd for  $\text{C}_{33}\text{H}_{52}\text{O}_4\text{Na}$ : 535.7612; found:  $m/z$  535.3686. IR ( $\nu_{\text{max}}$ , cm): 2978 (C-H); 1726 (C=O); 1706 (C=O); 1229 (C-O).  $[\alpha]_D$ : +57.2 (c 1,  $\text{CHCl}_3$ ).

*Methyl 3 $\beta$ -acetoxy-11-oxours-12-en-28-oate (UAD3) and methyl 3 $\beta$ -acetoxyursa-9(11),12-dien-28-oate (UAD4)*: Derivative **UAD2** (288.0 mg, 0.56 mmol) was dissolved in EtOAc (10 mL) and *t*-BuOOH (0.35 mL of a 6 M solution, 2.10 mmol) was added, followed by the slow addition of  $\text{NaClO}_2$  (16.27 mg, 1.80 mmol). After 20 h under magnetic stirring at 100°C - 110°C, the reaction was complete (TLC control). The reaction mixture was poured into 10% aqueous sodium sulfite solution and extracted with EtOAc. The extract was successively partitioned with satd. aqueous  $\text{NaHCO}_3$  solution and water, dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and the EtOAc was removed completely under reduced pressure. The residue was chromatographed over silica gel, employing *n*-hexane/EtOAc (9:1) as eluent to afford **UAD3** and **UAD4** as white crystals (163.2 mg, 56.7% yield and 26.1 mg, 9.1% yield, respectively).

**UAD3**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  5.46 (s, 1H, H12); 4.37 (dd, 1H,  $J = 5.3, 10$  Hz, H3); 3.48 (s, 3H,  $\text{COOCH}_3$ ); 2.29 (d, 1H,  $J = 10.8$ , H18); 1.91 (s, 3H,  $\text{OCOCH}_3$ ); 1.18 (s, 3H,  $\text{CH}_3$ ); 1.13 (s, 3H,  $\text{CH}_3$ ); 1.01 (s, 3H,  $\text{CH}_3$ ); 0.85 (s, 3H,  $\text{CH}_3$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  199.75 (C11); 177.22 (C28); 171.01 ( $\text{OCOCH}_3$ ); 162.96 (C13); 130.65 (C12); 80.60 (C3); 61.37 (C9); 55.05 (C5); 52.74 (C18); 51.89 ( $\text{COOCH}_3$ ); 47.67 (C14); 44.69 (C14); 43.74 (C8); 38.81 (C1); 38.59 (C19); 38.59 (C20); 38.04 (C4); 37.01 (C10); 35.98 (C22); 32.97 (C21); 28.37 (15); 28.11 (C23); 23.93 (C16); 23.56 (C2); 21.35 (C27); 21.02 (C30); 18.85 (C26); 17.34 (C6); 17.16 (C29); 16.76 (C25); 16.28 (C24). HRMS calcd for  $\text{C}_{33}\text{H}_{50}\text{O}_5\text{Na}$ : 549.7448; found:  $m/z$  549.3541. IR ( $\nu_{\text{max}}$ , cm): 2971 (C-H); 1726 (C=O); 1654 (C=O); 1620 (C=O); 1233 (C-O).  $[\alpha]_D$ : +45.15 (c 2,  $\text{CHCl}_3$ ).

**UAD4**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  5.58 (d, 1H,  $J = 6.1$  Hz, H11); 5.51 (d, 1H,  $J = 6.1$  Hz; H12); 4.51 (dd, 1H,  $J = 5.7, 10.4$  Hz, H3); 3.62 (s, 3H,  $\text{COOCH}_3$ ); 2.35 (d, 1H,  $J = 11.2$  Hz, H18); 2.04 (s, 3H,  $\text{OCOCH}_3$ ); 1.20 (s, 3H,  $\text{CH}_3$ ); 0.97 (s, 3H,  $\text{CH}_3$ ); 0.95 (s, 3H,  $\text{CH}_3$ ); 0.92 (s, 3H,  $\text{CH}_3$ ); 0.90 (s, 3H,  $\text{CH}_3$ ); 0.88 (s, 3H,  $\text{CH}_3$ ); 0.86 (s, 3H,  $\text{CH}_3$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  178.09 (C28); 171.11 ( $\text{OCOCH}_3$ ); 154.53 (C9); 139.43 (C13); 123.29 (C12); 115.54 (C11); 80.62 (C3); 51.67 ( $\text{COOCH}_3$ ); 51.23 (C18); 51.23 (C5); 47.52 (C17); 42.67 (C8); 40.68 (C14); 38.73 (C10); 38.62

(C19); 38.33 (C20); 37.89 (C4); 36.90 (C1); 36.49 (C22); 31.97 (C7); 30.57 (C21); 28.15 (C23); 27.08 (C15); 25.21 (C25); 24.62 (C16); 21.35 (C26); 21.35 (OCOCH<sub>3</sub>); 20.94 (C27); 20.94 (C30); 18.15 (C6); 17.01 (C29); 16.79 (C24). HRMS calcd for C<sub>33</sub>H<sub>51</sub>O<sub>4</sub>Na: 533.7454; found: *m/z* 533.3603. IR ( $\nu_{\max}$ , cm): 2924 (C-H); 1732 (C=O); 1723 (C=O); 1026 (C-H). [ $\alpha$ ]D: +158.60 (c 0.5, CHCl<sub>3</sub>).

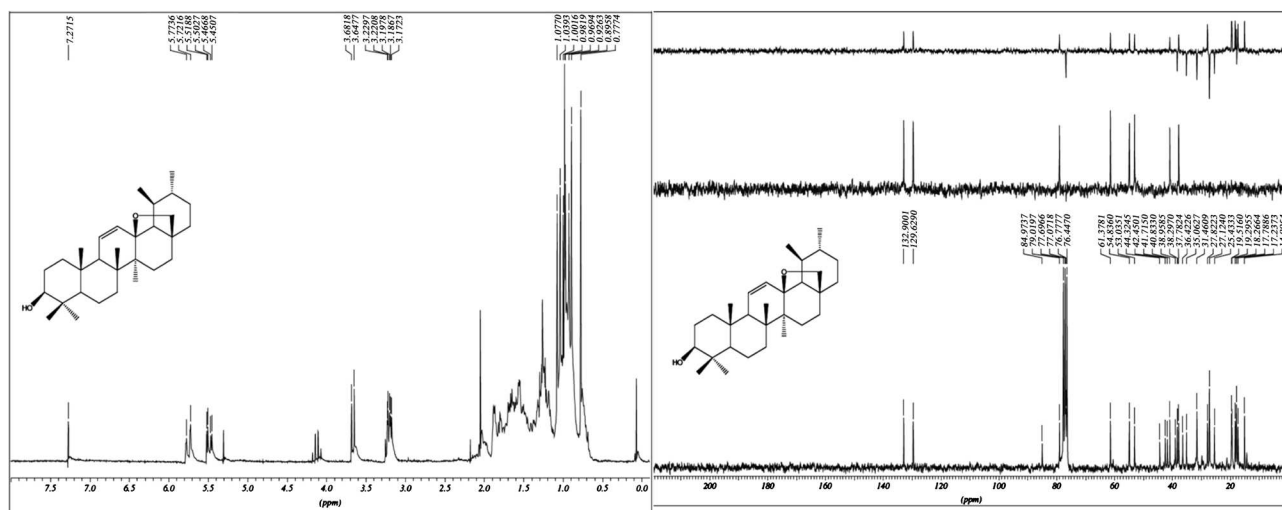
*Urs-12-ene-3 $\beta$ ,11,28-triol* (UAD5): The product was synthesized by reduction of UAD3 (230 mg, 0.45 mmol) with LiAlH<sub>4</sub> (345 mg, 9.08 mmol) in anhydrous diethyl ether, stirred at room temperature for 20 h. Excess LiAlH<sub>4</sub> was quenched by the addition of wet diethyl ether and the reaction mixture was worked up as usual to give a white solid UAD5 (179 mg, 87.4% yield). <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  5.22 (d, 1H, *J* = 4.0 Hz, H12); 4.35 (br d, 1H, *J* = 4.3 Hz, H11); 3.36 (d, 1H, *J* = 10.0 Hz, H28a); 3.19 (m, 1H, H3); 3.17 (d, 1H, *J* = 10.0 Hz, H28b); 2.00 (m, 1H, H18); 1.37 (s, 3H, CH<sub>3</sub>); 1.22 (s, 3H, CH<sub>3</sub>); 1.01 (s, 3H, CH<sub>3</sub>); 0.95 (s, 3H, CH<sub>3</sub>); 0.93 (s, 3H, CH<sub>3</sub>); 0.78 (s, 3H, CH<sub>3</sub>); 0.76 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  132.89 (C13); 129.62 (C12); 79.01 (C3); 61.37 (C11); 54.83 (C9); 53.03 (C5); 44.29 (C8); 42.45 (C14); 40.83 (C19); 40.83 (C20); 38.95 (C10); 38.29 (C1); 37.81 (C4); 36.42 (C17); 35.09 (C7); 35.09 (C22); 31.46 (C21); 27.82 (C23); 27.12 (C15); 27.12 (C2); 25.43 (C16); 19.51 (C27); 19.29 (C30); 18.26 (C29); 17.82 (C6); 17.82 (C26); 17.27 (C25); 14.99 (C24). HRMS calcd for C<sub>33</sub>H<sub>50</sub>O<sub>3</sub>Na: 481.7130; found: *m/z* 481.3651. IR ( $\nu_{\max}$ , cm): 3676 (O-H); 2921 (C-H); 1045 (C-O).

(17S)-3 $\beta$ -hydroxy-22 (17  $\rightarrow$  18)-abeours-11-en-28-al (UAD6): Compound UAD5 (458 mg, 0.99 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (previously dried in CaCl<sub>2</sub> and filtered over NaHCO<sub>3</sub>) and kept under inert atmosphere (Ar), following the addition of BF<sub>3</sub>-Et<sub>2</sub>O (54  $\mu$ L, 0.42 mmol). The mixture was stirred at room temperature for 2 h. EtOAc was added and the solution was washed with satd. aqueous NaCl solution, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and evaporated to dryness. The residue (398 mg) was chromatographed on silica gel (eluent *n*-hexane/EtOAc 8:2) to afford the aldehyde UAD6 (42.9 mg; 10.8% yield) along with a mixture of dienes (122.5 mg). <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  9.29 (s, H28); 5.60 (br d, 1H, *J* = 10.4 Hz, H12); 5.42 (br d, 1H, *J* = 10.4, H11); 3.21 (dd, 1H, *J* = 5.8, 10.4 Hz, H3); 1.03 (d, 3H, *J* = 6.3 Hz, C<sub>30</sub>H<sub>3</sub>); 0.99 (d, 3H, *J* = 5.2 Hz, C<sub>29</sub>H<sub>3</sub>); 0.96 (s, 3H, C<sub>23</sub>H<sub>3</sub>); 0.91 (s, 3H, C<sub>25</sub>H<sub>3</sub> or C<sub>26</sub>H<sub>3</sub>); 0.82 (s, 3H, C<sub>26</sub>H<sub>3</sub> or C<sub>25</sub>H<sub>3</sub>); 0.75 (s, 3H, C<sub>24</sub>H<sub>3</sub>); 0.71 (s, 3H, C<sub>27</sub>H<sub>3</sub>). <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  206.35 (C28); 133.81 (C12); 123.54 (C11); 78.93 (C3); 54.60 (C5); 52.61 (C13); 552.18 (C18); 47.28 (C17); 44.27 (C9); 41.04 (C10); 39.87 (C14); 39.46 (C19); 339.35 (C20); 338.82 (C8); 37.99 (C1); 336.38 (C4); 32.19 (C7); 30.33 (C21); 30.10 (C15); 27.74 (C23); 27.02 (C2); 25.72 (C16); 20.45 (C25); 18.55 (C30); 18.26 (C6); 17.00 (C26); 16.38 (C29); 16.06 (C27); 14.96 (C24). HRMS calcd for C<sub>30</sub>H<sub>44</sub>O<sub>2</sub>Na: 444.7057; found: *m/z* 443.3633. IR ( $\nu_{\max}$ , cm): 3413 (O-H); 2928 (C-H); 1716 (C=O). [ $\alpha$ ]D: +23.40 (c 1.5, MeOH).

(*ursa*-11,13(18)-diene-3 $\beta$ ,28-diyl diacetate) (UAD7): The diene mixture (122.5 mg) was acetylated with pyridine and acetic anhydride. The product was chromatographed over silica gel (eluent *n*-hexane/EtOAc 19:1) to afford the diene

**UAD7** (15 mg; 11.8%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  6.41 (dd, 1H,  $J = 3.1, 10.6$  Hz, H11); 5.60 (d, 1H,  $J = 10.6$  Hz, H12); 4.51 (dd, 1H,  $J = 6.2, 10.0$  Hz, H3); 4.28 (d, 1H,  $J = 11.2$  Hz, H28a); 3.87 (d, 1H,  $J = 11.2$  Hz, H28b); 2.06 (s, 3H,  $\text{OCOCH}_3$ ); 2.05 (s, 3H,  $\text{OCOCH}_3$ ); 1.02 (s, 3H,  $\text{CH}_3$ ); 0.94 (s, 3H,  $\text{CH}_3$ ); 0.92 (s, 3H,  $\text{CH}_3$ ); 0.89 (s, 3H,  $\text{CH}_3$ ); 0.86 (s, 3H,  $\text{CH}_3$ ); 0.85 (s, 3H,  $\text{CH}_3$ ); 0.76 (s, 3H,  $\text{CH}_3$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  171.18 ( $\text{OCOCH}_3$ ); 171.07 ( $\text{OCOCH}_3$ ); 138.36 (C18); 136.86 (C13); 126.97 (C12); 125.24 (C11); 80.88 (C3); 66.25 (C28); 54.97 (C5); 54.20 (C9); 42.74 (C17); 40.68 (C14); 38.48 (C10); 37.89 (C1); 37.89 (C22); 37.78 (C8); 36.56 (C19); 36.49 (C4); 34.62 (C20); 32.12 (C21); 29.95 (C16); 29.95 (C7); 27.82 (C23); 24.36 (C15); 23.48 (C2); 22.67 (C27); 21.39 (C30); 21.35 ( $\text{OCOCH}_3$ ); 21.35 ( $\text{OCOCH}_3$ ); 20.65 (C26); 18.15 (C29); 16.72 (C25); 16.20 (C24). IR ( $\nu_{\text{max}}$ ,  $\text{cm}^{-1}$ ): 2940 (C-H); 1728 (C=O); 1237 (C-O).  $[\alpha]_D$ :  $-25.00$  (c 1,  $\text{CHCl}_3$ ).

(13*S*)-13,28-epoxyurs-11-en-3 $\beta$ -ol (**UAD8**): The derivative **UAD5** (213 mg, 0.46 mmol) was dissolved in  $\text{CH}_2\text{Cl}_2$  and treated with  $\text{BF}_3\cdot\text{Et}_2\text{O}$  (24  $\mu\text{L}$ , 0.19 mmol) at  $-78^\circ\text{C}$  for 2 h, a similar procedure to that described for preparing compound **UAD6**, except that the reaction was carried out at low temperature. The reaction product (172.4 mg) was chromatographed on silica gel (eluent n-hexane/EtOAc 19:1) to afford the cyclic ether **UAD8** (79.8 mg; 39% yield).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  5.73 (d, 1H,  $J = 10.3$  Hz, H12); 5.48 (dd, 1H,  $J = 3.2, 10.3$  Hz, H11); 3.66 (d, 1H,  $J = 6.7$  Hz, H28a); 3.21 (d, 1H,  $J = 6.7$ , H28b); 3.20 (dd, 1H,  $J = 4.9, 11.3$  Hz, H3); 1.26 (s, 3H,  $\text{CH}_3$ ); 1.00 (s, 3H,  $\text{CH}_3$ ); 0.98 (s, 3H,  $\text{CH}_3$ ); 0.96 (s, 3H,  $\text{CH}_3$ ); 0.96 (s, 3H,  $\text{CH}_3$ ); 0.89 (s, 3H,  $\text{CH}_3$ ); 0.77 (s, 3H,  $\text{CH}_3$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  132.89 (C12); 129.62 (C11); 84.96 (C13); 79.01 (C3); 77.06 (C28); 61.36 (C5); 54.82 (C18); 53.02 (C9); 44.32 (C17); 42.45 (C10); 41.71 (C14); 40.83 (C19); 38.95 (C8); 38.29 (C1); 37.78 (C20); 36.42 (C4); 35.06 (C22); 31.46 (C21); 31.46 (C7); 27.82 (C23); 27.12 (C15); 25.43 (C16); 25.43 (C2); 19.51 (C27); 19.29 (C30); 18.26 (C29); 17.78 (C6); 17.23 (C26); 14.99 (C25); 14.21 (C24). HRMS calcd for  $\text{C}_{30}\text{H}_{48}\text{O}_2\text{H}$ : 441.7159; found:  $m/z$  441.3716. IR ( $\nu_{\text{max}}$ ,  $\text{cm}^{-1}$ ): 3306 (O-H); 2920 (C-H); 1022 (C-O-C); 989 (C-O-C).  $[\alpha]_D$ :  $+94.6$  (c 1,  $\text{CHCl}_3$ ) (**Figure 4**).



**Figure 4.**  $^1\text{H}$  NMR spectrum of **UAD8** (200 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C}$  NMR spectrum of **UAD8** (50 MHz,  $\text{CDCl}_3$ ).

### 3.2. Effect of the Ursolic Acid Derivatives on Cell Viability, NF- $\kappa$ B Expression and Apoptosis

Ursolic acid is a pentacyclic triterpene extracted and purified from many plant species that presents considerable pharmacological effects [5]-[11] [16]. In the present study, some of the UA derivative compounds were able to reduce the viability of A549 cells and, among these, the UAD1 showed better results. The improvement in the efficacy of UAD1 could be related to the esterification at C-28 in this compound. In HT-29, HepG2 and BGC-823 (human cancer cell lines) were already observed that UA derivatives obtained by esterification in C-3 or C-28 had increased cytotoxicity against these cells, due to the enhance in the lipophilicity and better permeability through cell membranes [17].

In **Table 1**, it is possible to observe that the compounds UAD1, UAD3, UAD5, UAD6 and UAD8 were able to reduce the viability of the A549 cells in at least one of the tested concentrations, when compared to the control (not treated A549 cells). The UAD1 was more efficient than UA to reduce the cellular viability in all concentrations tested. In the 24 h of culture (**Table 2**) only the UA (90  $\mu$ M), UAD1 (60  $\mu$ M; 90  $\mu$ M), UAD6 (90  $\mu$ M) were capable to reduce the cellular viability in relation to the control. No differences were observed between the control and DMSO treated cells.

The derivatives UAD5, UAD6 and UAD8, which have a hydroxyl in C-3, showed cytotoxicity against A549 cells at 60  $\mu$ M and 90  $\mu$ M. The maintenance of the hydroxyl in the C-3 and/or C-28 shows to be essential to the cytotoxicity of the derivatives since, compounds that not display this hydroxyl have reduced cytotoxic capacity [18].

The NF- $\kappa$ B expression was evaluated after 24 h of culture (**Table 3**). The compounds UA, UAD1, UAD2, UAD4, UAD5, UAD6, UAD7 and UAD8 showed

**Table 1.** Viability of A549 cells after 48 hours of treatment with ursolic acid (UA) or ursolic acid derivatives (UAD1-UAD8) in the concentrations of 30, 60 and 90  $\mu$ M.

Compounds	Cellular viability $\pm$ SEM (%)		
	30 $\mu$ M	60 $\mu$ M	90 $\mu$ M
UA	99.66 $\pm$ 2.54	*23.45 $\pm$ 1.48	*2.88 $\pm$ 0.19
UAD1	*3.67 $\pm$ 0.41	**0.12 $\pm$ 0.05	*0.06 $\pm$ 0.02
UAD2	95.67 $\pm$ 1.17	96.25 $\pm$ 2.93	92.39 $\pm$ 2.93
UAD3	100	100	*90.31 $\pm$ 1.24
UAD4	100	100	100
UAD5	93.75 $\pm$ 1.21	*74.52 $\pm$ 2.28	*33.36 $\pm$ 1.24
UAD6	100	*74.2 $\pm$ 1.31	*33.75 $\pm$ 2.84
UAD7	99.41 $\pm$ 5.79	100	100
UAD8	98.83 $\pm$ 2.36	*75.25 $\pm$ 4.39	*46.38 $\pm$ 2.01

UA = Ursolic acid; UAD = Ursolic acid derivative; \*p < 0.05 *versus* untreated cells (100% viability); #p < 0.05 *versus* UA.

**Table 2.** Viability of A549 cells after 24 hours of treatment with ursolic acid (UA) or ursolic acid derivatives (UAD1-UAD8) in the concentrations of 30, 60 and 90  $\mu$ M.

Compounds	Cellular viability $\pm$ SEM (%)		
	30 $\mu$ M	60 $\mu$ M	90 $\mu$ M
UA	100	91.47 $\pm$ 3.74	*33.91 $\pm$ 1.67
UAD1	100	**28.59 $\pm$ 4.78	**5.36 $\pm$ 0.21
UAD2	100	100	100
UAD3	100	100	100
UAD4	100	100	99.54 $\pm$ 4.66
UAD5	100	100	100
UAD6	100	100	*55.24 $\pm$ 2.03
UAD7	100	100	100
UAD8	100	100	100

UA = Ursolic acid; UAD = Ursolic acid derivative; \*p < 0.05 versus untreated cells (100% viability); #p < 0.05 versus UA.

**Table 3.** NF- $\kappa$ B expression percentage in A549 cells after 24 hours of treatment with ursolic acid (UA) or ursolic acid derivatives (UAD1-UAD8) in the concentrations of 30, 60 and 90  $\mu$ M.

Compounds	NF- $\kappa$ B expression $\pm$ SEM (%)		
	30 $\mu$ M	60 $\mu$ M	90 $\mu$ M
UA	*83.12 $\pm$ 0.04	*65.74 $\pm$ 0.94	*41.96 $\pm$ 0.45
UAD1	*84.06 $\pm$ 0.03	**39.63 $\pm$ 0.29	**30.55 $\pm$ 0.32
UAD2	90.51 $\pm$ 0.04	91.41 $\pm$ 0.22	*83.66 $\pm$ 0.12
UAD3	93.83 $\pm$ 0.02	91.34 $\pm$ 0.14	90.72 $\pm$ 0.15
UAD4	89.92 $\pm$ 0.27	92.24 $\pm$ 0.05	*83.06 $\pm$ 0.02
UAD5	89.38 $\pm$ 0.22	91.41 $\pm$ 0.24	*86.44 $\pm$ 0.23
UAD6	*94.5 $\pm$ 0.21	92.42 $\pm$ 0.16	*81.46 $\pm$ 0.25
UAD7	90.37 $\pm$ 0.13	89.51 $\pm$ 0.22	*85.36 $\pm$ 0.02
UAD8	93.59 $\pm$ 0.22	89.15 $\pm$ 0.09	*81.82 $\pm$ 0.19

UA = Ursolic acid; UAD = Ursolic acid derivative; \*p < 0.05 versus untreated cells (91.50  $\pm$  0.57 NF- $\kappa$ B expression percentage); #p < 0.05 versus UA.

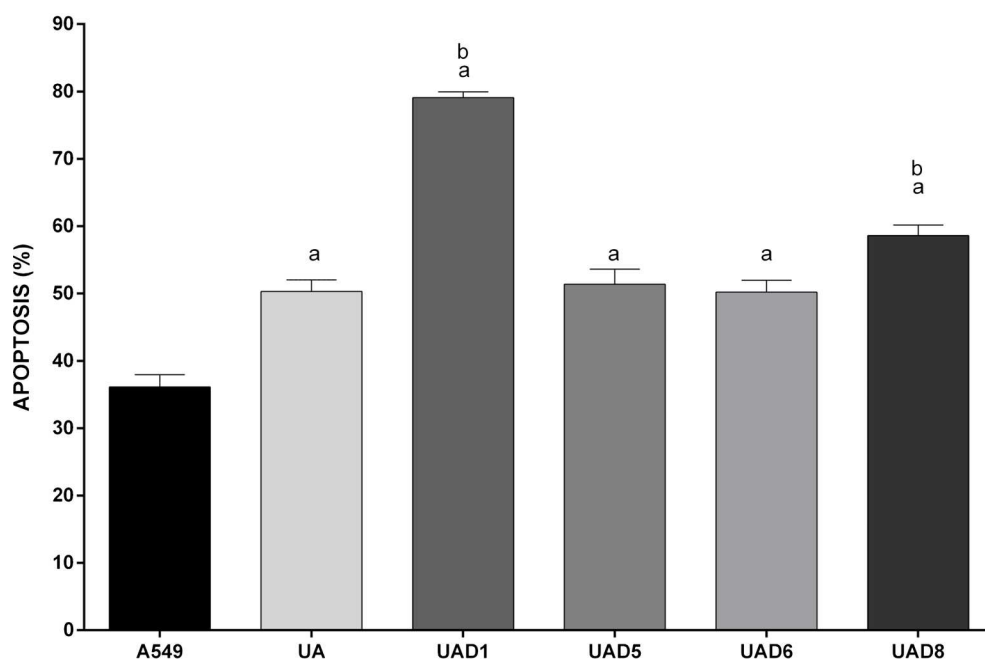
reduction of the NF- $\kappa$ B expression. The UAD1 showed the reduction in all concentrations. Interestingly, the UAD6 showed enhanced of NF- $\kappa$ B expression at 30  $\mu$ M and the reduction in the highest concentration. This increase could be related to its inability to reduce the cell viability in this concentration probably due to its different ring conformation. Studies already showed that unprecedented conformations could change the biological activity of the synthesized compounds [19] [20] [21] [22].

In the present study, the cytotoxicity and the NF- $\kappa$ B expression were evaluated in A549 cells treated with semisynthetic UA derivatives, which showed a

reduction in the NF- $\kappa$ B expression. Different studies showed that NF- $\kappa$ B promotes the cell survivability, the positive expression of proliferation and metastasis genes and the expression of anti-apoptotic factors being its inhibition a potential therapeutic target in cancer treatment [23] [24]. Moreover, was already shown that the inhibition of NF- $\kappa$ B expression promotes cancer cells death without effects on healthy cells, reinforcing the induction of cell death potential and the selectivity of inhibitors of this signalling pathway [25].

The apoptosis induction was evaluated in the compounds with better results in MTT assay at 90  $\mu$ M. The compound UAD1 showed the greater apoptosis induction. The UAD1 and UAD8 were significantly different from UA and A549 not treated. On the other hand, UA, UAD5 and UAD6 were different only from A549 not treated (Figure 5). The ursolic acid has been demonstrated a potential apoptosis induction activity in tumor cells [26] [27]. The treatment with ursolic acid was able to restrain the invasive capacity of Gallbladder carcinoma cells and to induce apoptosis, being these effects associated with the inhibition of NF- $\kappa$ B [26]. In oral squamous cell carcinoma, the treatment with ursolic acid acted as a potent apoptosis inducer dependent of the caspase, being this mechanism regulated by the inhibition of the Akt/mTor/NF- $\kappa$ B signalling [27].

Among the compounds were selected the UAD1, UAD5, UAD6 and UAD8 derivatives, which showed better results on cytotoxicity and NF- $\kappa$ B inhibition, for the apoptosis analysis. All these derivatives showed an increase in apoptosis in relation to A549 not treated cells. Moreover, the UAD1 and UAD8 showed



**Figure 5.** Flow cytometry apoptosis analysis of A549 cells. The A549 cells were treated or not with ursolic acid (UA) or ursolic acid derivatives (UAD1, UAD5, UAD6 or UAD8) at 90  $\mu$ M during 36 hours. The apoptosis was determined by flow cytometry analysis of annexin-V staining. a =  $p < 0.05$  when related to A549 not treated (control). b =  $p < 0.05$  when related to UA treated cells. Bars represent the mean  $\pm$  SEM of apoptosis percentage.

also more apoptosis than UA treatment. Thus, can be highlighted the UAD1 derivative that showed better results than UA in all assays. In previous studies, this derivative already showed cancer cell cytotoxicity; however, not in the A549 cell line and neither the expression of NF- $\kappa$ B or apoptotic effect of this compound were evaluated [18] [28]. In spite of the promising results of the present study, is relevant to state that this is a preliminary study, being necessary further tests to clear the mechanism of action of these compounds. Moreover, *in vivo* studies need to be done to prove the same effects observed.

#### 4. Conclusion

The ursolic acid derivatives showed that substantial results in the apoptosis, cytotoxicity and NF- $\kappa$ B inhibition of A549 cells, and further studies on the signaling pathway, also the mechanisms of action of these derivatives, are necessary for the development of possible new therapeutic drugs.

#### Acknowledgements

This work was supported in part by Grants from National Council for Scientific and Technological Development-CNPq (460184/2014-8), CT-INFRA 2013/FINEP (FINEP 0633/13); Fundação de Amparo à Pesquisa do Estado de Minas Gerais (APQ 02423-18); Federal University of Juiz de Fora.

#### Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

#### References

- [1] Siegel, R., Naishadham, D. and Jemal, A. (2013) Cancer Statistics. *CA: A Cancer Journal for Clinicians*, **63**, 11-30. <https://doi.org/10.3322/caac.21166>
- [2] Chen, W., Zheng, R., Baade, P.D., Zhang, S., Zeng, H., Bray, F., Jemal, A., Yu, X.Q. and He, J. (2015) Cancer Statistics in China. *CA: A Cancer Journal for Clinicians*, **66**, 115-132. <https://doi.org/10.3322/caac.21338>
- [3] Gandara, D.R., Hammerman, P.S., Sos, M.L., Lara Jr., P.N. and Hirsch, F.R. (2015) Squamous Cell Lung Cancer: From Tumor Genomics to Cancer Therapeutics. *Clinical Cancer Research*, **21**, 2236-2243. <https://doi.org/10.1158/1078-0432.CCR-14-3039>
- [4] Kleczko, E.K., Kwak, J.W., Schenk, E.L. and Nemenoff, R.A. (2019) Targeting the Complement Pathway as a Therapeutic Strategy in Lung Cancer. *Frontiers in Immunology*, **10**, 954. <https://doi.org/10.3389/fimmu.2019.00954>
- [5] Abdel-Monem, A.R., Kandil, Z.A., Abdel-Naim, A.B. and Abdel-Sattar, E. (2015) A New Triterpene and Protective Effect of *Periploca somaliensis* Browicz Fruits against CCl<sub>4</sub>-Induced Injury on Human Hepatoma Cell Line (Huh7). *Natural Product Research*, **29**, 423-429. <https://doi.org/10.1080/14786419.2014.950960>
- [6] Xu, J., Wang, X., Zhang, H., Yue, J., Sun, Y., Zhang, X. and Zhao, Y. (2018) Synthesis of Triterpenoid Derivatives and Their Anti-Tumor and Anti-Hepatic Fibrosis Activities. *Natural Product Research*, **16**, 1-7.

- <https://doi.org/10.1080/14786419.2018.1499642>
- [7] Yin, R., Li, T., Tian, J.X., Xi, P. and Liu, R.H. (2018) Ursolic Acid, a Potential Anti-cancer Compound for Breast Cancer Therapy. *Critical Reviews in Food Science and Nutrition*, **58**, 568-574. <https://doi.org/10.1080/10408398.2016.1203755>
- [8] Mina, S.A., Mohamed, S.A., Melek, F.R. and El-Khalik, S.M.A. (2019) LC-ESI-MS/MS Profiling of Alkaloids and Antiproliferative Activity of *Pachypodium lamerei* Drake Leaves. *Natural Product Research*, **24**, 1-5. <https://doi.org/10.1080/14786419.2018.1556264>
- [9] Yang, X., Li, Y., Jiang, W., Ou, M., Chen, Y., Xu, Y., Wu, Q., Zheng, Q., Wu, F., Wang, L., Zou, W., Zhang, Y.J. and Shao, J. (2015) Synthesis and Biological Evaluation of Novel Ursolic acid Derivatives as Potential Anticancer Prodrugs. *Chemical Biology & Drug Design*, **86**, 1397-1404. <https://doi.org/10.1111/cbdd.12608>
- [10] Dar, B.A., Lone, A.M., Shah, W.A. and Qurishi, M.A. (2016) Synthesis and Screening of Ursolic Acid-Benzylidene Derivatives as Potential Anti-Cancer Agents. *European Journal of Medicinal Chemistry*, **111**, 26-32. <https://doi.org/10.1016/j.ejmech.2016.01.026>
- [11] Alves Monteath, S.A.F., Maciel, M.A.M., Vega, R.G., De Mello, H., De Araújo Martins, C., Esteves-Souza, A., Gattass, C.R. and Echevarria, A. (2017) Ultrasound-Assisted Extraction of Ursolic Acid from the Flowers of *Ixora coccinea* Linn (Rubiaceae) and Antiproliferative Activity of Ursolic Acid and Synthesized Derivatives. *Pharmacognosy Magazine*, **13**, 265-269. <https://doi.org/10.4103/0973-1296.204557>
- [12] Castro, S.B., Junior, C.O., Alves, C.C., Dias, A.T., Alves, L.L., Mazzoccoli, L., Zoet, M.T., Fernandes, S.A., Teixeira, H.C., Almeida, M.V. and Ferreira, A.P. (2012) Synthesis of Lipophilic Genistein Derivatives and Their Regulation of IL-12 and TNF- $\alpha$  in Activated J774A.1 Cells. *Chemical Biology & Drug Design*, **79**, 347-352. <https://doi.org/10.1111/j.1747-0285.2011.01296.x>
- [13] Riccardi, C. and Nicoletti, I. (2006) Analysis of Apoptosis by Propidium Iodide Staining and Flow Cytometry. *Nature Protocols*, **1**, 1458-1461. <https://doi.org/10.1038/nprot.2006.238>
- [14] Tkachev, A.V., Denisov, A.Y., Gatilov, Y.V., Bagryanskaya, I.Y., Shevtsov, S.A. and Rybalova, T.V. (1994) Stereochemistry of Hydrogen Peroxide Acetic Acid Oxidation of Ursolic Acid and Related Compounds. *Tetrahedron*, **50**, 11459-11488. [https://doi.org/10.1016/S0040-4020\(01\)89285-1](https://doi.org/10.1016/S0040-4020(01)89285-1)
- [15] Takeoka, G., Dao, L., Teranishi, R., Wong, R., Flessa, S., Harden, L. and Edwards, R. 2000. Identification of Three Triterpenoids in Almond Hulls. *Journal of Agricultural and Food Chemistry*, **48**, 3437-3439. <https://doi.org/10.1021/jf9908289>
- [16] Li, Q., Dong, D.D., Huang, Q.P., Li, J., Du, Y.Y., Li, B., Li, H.Q. and Huyan, T. (2017) The Anti-Inflammatory Effect of *Sonchus oleraceus* Aqueous Extract on Lipopolysaccharide Stimulated RAW 264.7 Cells and Mice. *Pharmaceutical Biology*, **55**, 799-809. <https://doi.org/10.1080/13880209.2017.1280514>
- [17] Bai, K.K., Chen, F.L., Yu, Z., Zheng, Y.Q., Li, Y.N. and Guo, Y.H. (2011) Synthesis of [ $3\beta$ -Acetoxy-Urs-12-En-28-Oyl]-1-Monoglyceride and Investigation on Its Anti Tumor Effects against BGC-823. *Bioorganic & Medicinal Chemistry*, **19**, 4043-4050. <https://doi.org/10.1016/j.bmc.2011.05.017>
- [18] Ma, J.Q., Ding, J., Xiao, Z.H. and Liu, C.M. (2014) Ursolic Acid Ameliorates Carbon Tetrachloride-Induced Oxidative DNA Damage and Inflammation in Mouse Kidney by Inhibiting the STAT3 and NF- $\kappa$ B Activities. *International Immunopharmacology*, **21**, 389-395. <https://doi.org/10.1016/j.intimp.2014.05.022>
- [19] Tolmacheva, I.A., Nazarov, A.V., Eroshenko, D.V. and Grishko, V.V. (2018) Syn-

- thesis, Cytotoxic Evaluation, and Molecular Docking Studies of the Semi-Synthetic “Triterpenoid-Steroid” Hybrids. *Steroids*, **140**, 131-143. <https://doi.org/10.1016/j.steroids.2018.10.005>
- [20] Song, Y.Y., Miao, J.H., Qin, F.Y., Yan, Y.M., Yang, J., Qin, D.P., Hou, F.F., Zhou, L.L. and Cheng, Y.X. (2018) Belamchinanes A-D from *Belamcanda chinensis*: Triterpenoids with an Unprecedented Carbon Skeleton and Their Activity against Age-Related Renal Fibrosis. *Organic Letters*, **20**, 5506-5509. <https://doi.org/10.1021/acs.orglett.8b02490>
- [21] Fu, L., Lin, Q.X., Onyango, E.O., Liby, K.T., Sporn, M.B. and Gribble, G.W. (2017) Design, Synthesis, and Biological Activity of Second-Generation Synthetic Oleanane Triterpenoids. *Organic & Biomolecular Chemistry*, **15**, 6001-6005. <https://doi.org/10.1039/C7OB01420A>
- [22] Siewert, B., Wiemann, J., Köwitsch, A. and Csuk, R. (2014) The Chemical and Biological Potential of C Ring Modified Triterpenoids. *European Journal of Medicinal Chemistry*, **72**, 84-101. <https://doi.org/10.1016/j.ejmech.2013.11.025>
- [23] Perkins, N.D. (2006) Post-Translational Modifications Regulating the Activity and Function of the Nuclear Factor Kappa B Pathway. *Oncogene*, **25**, 6717-6730. <https://doi.org/10.1038/sj.onc.1209937>
- [24] Sorriento, D., Illario, M., Finelli, R. and Iaccarino, G. (2012) To NF $\kappa$ B or Not to NF $\kappa$ B: The Dilemma on How to Inhibit a Cancer Cell Fate Regulator. *Translational Medicine UniSa*, **4**, 73-85.
- [25] Zanotto-Filho, A., Gelain, D.P., Schröder, R., Souza, L.F., Pasquali, M.A.B., Klamt, F. and Moreira, J.C.F. (2009) The NF $\kappa$ B-Mediated Control of RS and JNK Signaling in Vitamin A-Treated Cells: Duration of JNK-AP-1 Pathway Activation May Determine Cell Death or Proliferation. *Biochemical Pharmacology*, **77**, 1291-1301. <https://doi.org/10.1016/j.bcp.2008.12.010>
- [26] Chen, H, Wu, X., Duan, Y., Zhi, D., Zou, M., Zhao, Z., Zhang, X., Yang, X. and Zhang, J. (2019) Ursolic Acid Isolated from *Isodon Excisoides* Induces Apoptosis and Inhibits Invasion of GBC-SD Gallbladder Carcinoma Cells. *Oncology Letters*, **18**, 1467-1474. <https://doi.org/10.3892/ol.2019.10397>
- [27] Lin, C.W., Chin, H.K., Lee, S.L., Chiu, C.F., Chung, J.G., Lin, Z.Y., Wu, C.Y., Liu, Y.C., Hsiao, Y.T., Feng, C.H., Bai, L.Y. and Weng, J.R. (2019) Ursolic Acid Induces Apoptosis and Autophagy in Oral Cancer Cells. *Environmental Toxicology*, **34**, 983-991. <https://doi.org/10.1002/tox.22769>
- [28] Leal, A.S., Wang, R., Salvador, J.A.R. and Jing, Y. (2012) Synthesis of Novel Ursolic Acid Heterocyclic Derivatives with Improved Abilities of Antiproliferation and Induction of p53, p21waf1 and NOXA in Pancreatic Cancer Cells. *Bioorganic & Medicinal Chemistry*, **20**, 5774-5786. <https://doi.org/10.1016/j.bmc.2012.08.010>