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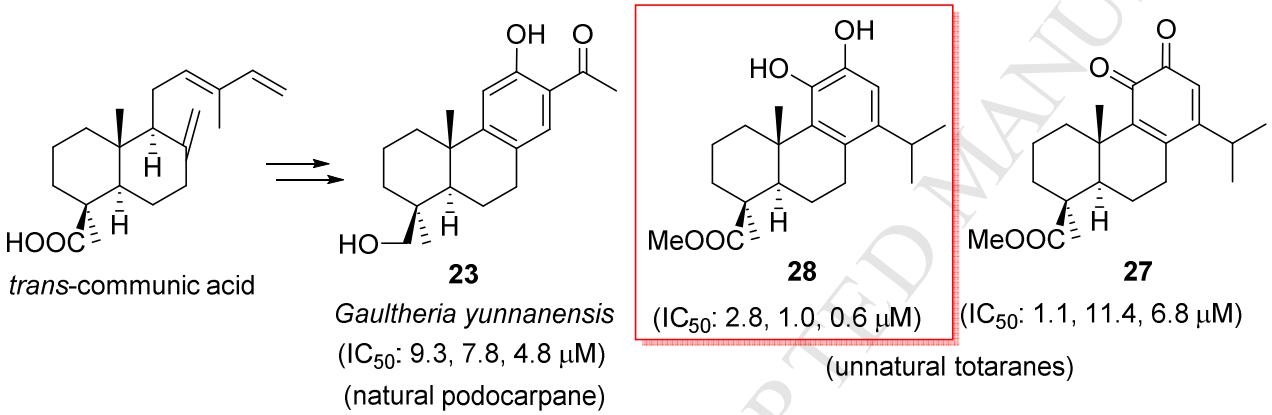
The synthesis of podocarpanes, including the acetoxy phenol **23**, isolated from *Gaultheria yunnanensis* and not previously synthesised, and totarane-type terpenoids, such as the catechol **28** and the *ortho*-quinone **27**, starting from the natural labdane *trans*-communic acid is described. Their antiproliferative activities against MCF-7, T-84 and A-549 human tumoural cell lines are studied. The totarane o-quinone **27**, and especially the catechol **28**, which is readily oxidisable to compound **27**, were the most active compounds. The results of the present study indicate that compound **28,** at least, might be useful as an antitumoural agent.



Synthesis and antiproliferative activity of podocarpane and totarane derivatives

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KEYWORDS Podocarpane terpenes; Totarane terpenes; Synthesis; Catechol; ortho-Quinone; Cancer

ABSTRACT

The synthesis of podocarpanes, including 12,19-dihydroxy-13-acetyl-8,11,13-podocarpatriene (**23**), isolated from *Gaultheria yunnanensis* and not previously synthesised, and totarane-type terpenoids, starting from the natural labdane *trans*-

communic acid (**15**), is described. Their antiproliferative activities against MCF-7, T-84 and A-549 human tumoural cell lines are studied. An antiproliferative effect was induced by compounds **23**, **27** and **28**, with IC_{50} <10 μM in two (**27**) or three cell lines (**23** and **28**). No correlation with log P values was observed. The totarane o-quinone **27**, and especially the catechol **28**, which is readily oxidisable to compound **27**, were the most active compounds, highlighting the functional groups present in C11 and C12. Compound **28** showed limited toxicity in normal peripheral blood mononuclear cells (78.5% cell viability versus non-treated control cultures at 10 μM), and appeared to exert an antiproliferative effect in A-549 cells (IC_{50} 0.6 μM) through a mechanism that involves the induction of apoptosis mediated by an increased Bax/Bcl-2 ratio. The results of the present study indicate that compound **28,** at least, might be useful as an antitumoral agent. Further studies are required to elucidate the cellular and molecular elements involved in its effect, and the activity/toxicity in preclinical models.

1. Introduction

Totarane-type diterpenes are a family of compounds with a tricyclic skeleton, isomer from that of abietane derivatives, which are isolated from a wide variety of vegetal species. The most representative example of these compounds is totarol (**1**), which was first isolated from *Podocarpus totara* [1]. The considerable resistance of this plant to different types of diseases has long encouraged the investigation of the components responsible for this behaviour. Totarol (1) protects mitochondrial respiratory enzyme activities against NADPH-dependent peroxidation injury [2]. Recent studies have shown that totarol (**1**) exhibits antiplasmodial, antifungal and antimicrobial properties [3]. Moreover, it presents a neuroprotective activity, as an activator of the

Akt/HO-1 pathway, protecting against ischaemic stroke by reducing oxidative stress [4]. Other totarane-type diterpenes are phenols **2-6**, with significant antibacterial activity against oral pathogenic microorganisms [5]. Podocarpane belongs to a group of tri-*nor*-abietane metabolites that are widely distributed, and most frequently found in Podocarpaceae species [6]. The most important member of this family is podocarpic acid (**7**), which was first isolated from *Podocarpus cupressinus*, *P. dacrydioides* and *Dacrydium cupressinum* [7] and presents a variety of significant biological activities. For example, it is a potent agonist of liver X receptor and a cytokine release inhibitor, being potentially useful for the treatment of atherosclerosis or inflammatory diseases [8]. Moreover, methyl podocarpate (**8**) and some *O*-acyl derivatives, such as compounds **9-11**, exert activity against Influenza type A viruses [9]. It has recently been reported that podocarpic acid (**7**) is a TRPA1 activator, and thus a potential therapeutic agent against pathologies related to diabetes and neurodegenerative diseases [10]. Other interesting podocarpane-type terpenes include nimbidiol (**12**), which inhibits intestinal carbohydrases, and is not only a potential antidiabetic drug [11], but also has an anti-angiogenesis effect [12], 7-deoxynimbidiol (**13**), with anti-tumoural [13], analgesic and anti-inflammatory activities [14], and the aminophenol **14**, which is a potent 5-lypoxygenase inhibitor [15] (Fig. 1).

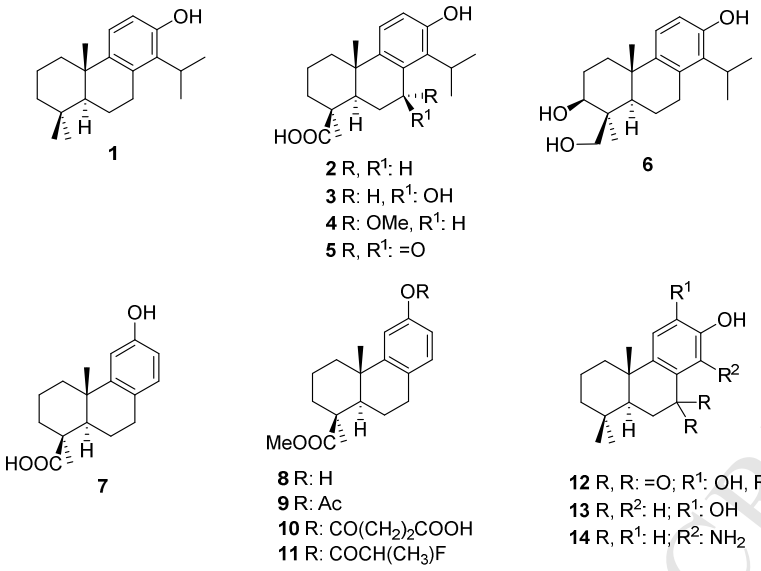


Fig. 1. Totarane and podocarpane-type diterpenes with biological properties.

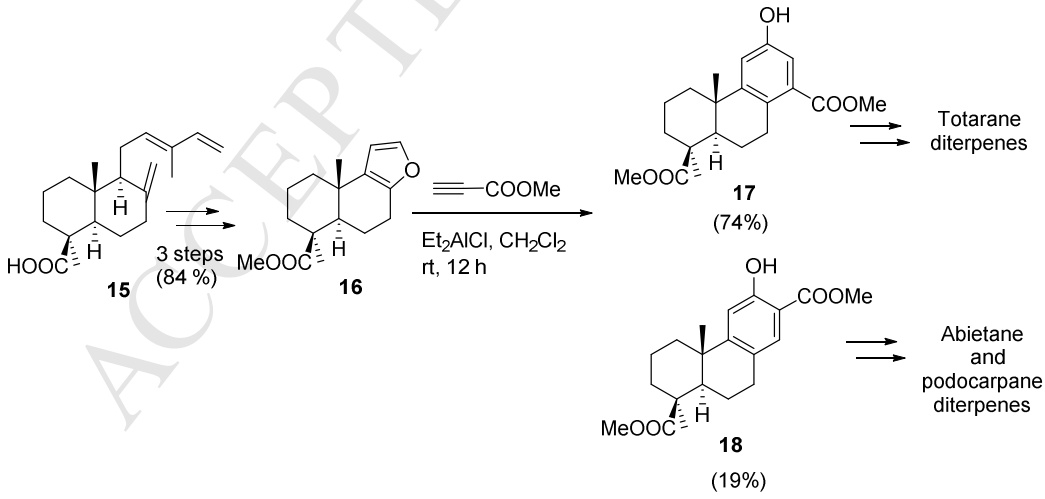
The diverse and important biological properties described above prompted us to undertake the synthesis of totarane and podocarpane-type terpenoids. In this respect, total syntheses of totarane derivatives based on the biomimetic cyclisation of aryl polyenes [16], and aryl epoxypolyenes [17], have been reported previously. Enantiospecific synthesis starting from totarol (**1**) has also been described [18], and more recently processes have been performed based on the α-alkylation of a podocarpane α,β-unsaturated ketone [19], or *via* a Pd-catalyzed Stille cross-coupling of the corresponding *o*-bromophenol podocarpane [20]. Cationic cyclizations of aryl polyprenes [21], including enantioselective processes [22], have also been described for synthesizing podocarpane-type terpenoids. Enantiospecific syntheses of these compounds have also been reported, utilizing as starting materials podocarpic acid [23] or abietic acid derivatives; the latter being deisopropylated after rearrangement of the corresponding 15-hydroperoxide [24]. Labdane diterpenes, such as manool [25] and sclareol [26], have also been utilised for synthesizing podocarpane terpenes.

This paper describes the syntheses of some podocarpanes, including 12,19-dihydroxy-13-acetyl-8,11,13-podocarpatriene (**23**), isolated from *Gaultheria yunnanensis* [27] and not previously synthesised, and totarane-type terpenoids. The antiproliferative activities of these and of previously-reported synthetic precursors, against MCF-7, T-84 and A-549 human tumoural cell lines are also discussed.

2. Results and discussion

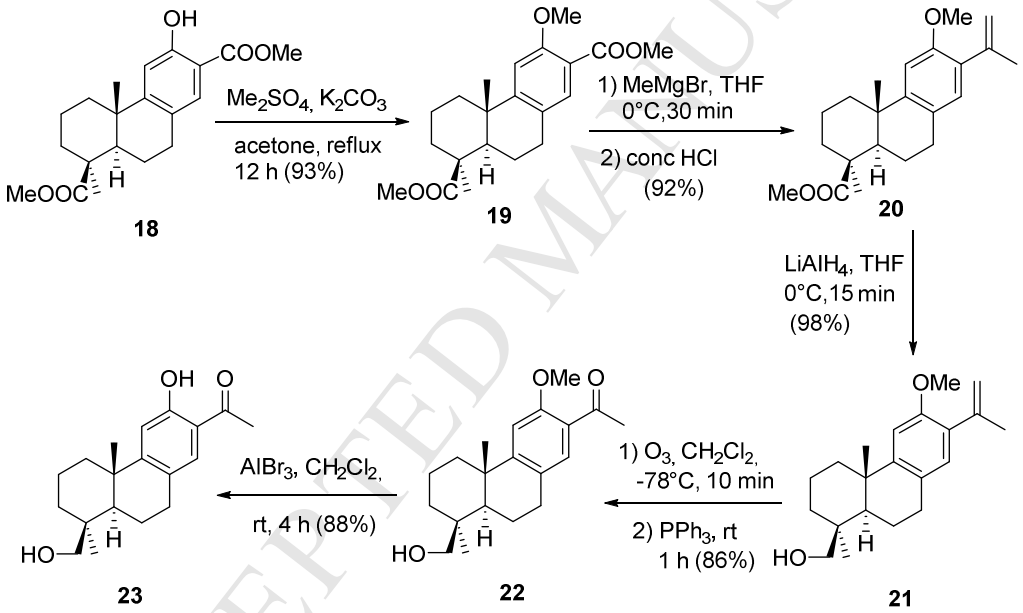
2.1. Chemistry

During our recent first synthesis of a cassane-type benzofurane diterpene [28], phenols **17** and **18** were synthesized from *trans*-communic acid (**15**), a labdane diterpene that is very abundant in some species of *Juniperus* and *Cupressus* [29] (Scheme 1). Compounds **17** and **18** are suitable intermediates for the synthesis of totarane, and of abietane or podocarpane diterpenes. Thus, the hydroxy ester **18** has been transformed into the abietanes sugikurojin A and 19-hydroxyferruginol [30].



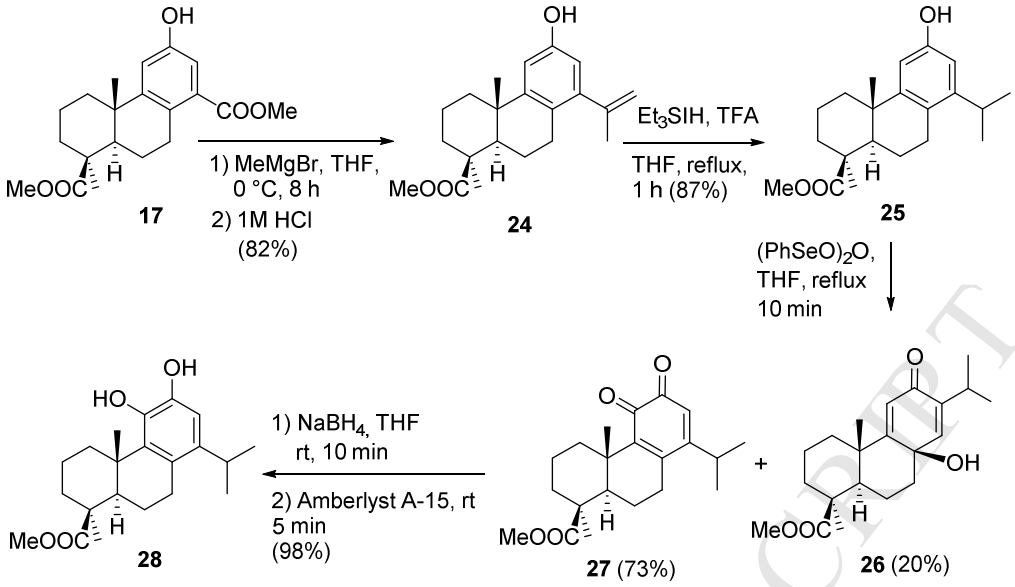
Scheme 1. Synthesis of phenols **17** and **18** from *trans*-communic acid (**15**).

In the present study, hydroxy ester **18** was transformed into the podocarpane **23**, a constituent from *Gaultheria yunnanensis* [27] that has not previously been synthesized (Scheme 2). The treatment of methoxy ester **19** [28] with MeMgBr and the further dehydration of the resulting alcohol gave the isopropylene derivative **20**. After reduction of the ester group and ozonolysis of the carbon-carbon double bond, the acetyl derivative **22** was obtained, which was reacted with $AlBr_3$ to give the natural hydroxy ketone **23**¸whose physical properties were found to be identical to those reported in the literature [27]**.**



Scheme 2. Synthesis of podocarpane **23** from hydroxy ester **18**.

In addition, hydroxy ester **17** was transformed into the totarane *o*-quinones **26** and **27**, and the catechol **28** (Scheme 3). The cationic reduction of the isopropylene group of intermediate **24** with Et_3SiH and trifluoroacetic acid gave the totarane phenol **25**. Treatment of this with $(PhSeO)_2O$ afforded the mixture of *o*-quinone **27** and hydroxy dienone **26**. Finally, the further reaction of quinone **27** with Raney Ni gave catechol **28**.



Scheme 3. Synthesis of totarane diterpenes from hydroxy ester **17**.

2.2. Biological assays

2.2.1. In Vitro Cytotoxicity Assay

Eight newly-synthesised diterpenoids were evaluated to determine their *in vitro* antiproliferative activity against selected human cancer cell lines of breast adenocarcinoma (MCF-7), lung adenocarcinoma (A-549) and colorectal carcinoma derived from lung metastasis (T-84), using the Sulforhodamine B colorimetric assay [31]. 5-fluorouracil (5-Fu), a commercial pharmaceutical product that is widely used to treat solid tumours [32] was used as the reference standard (Table 1). An antiproliferative effect was induced by compounds **23**, **27** and **28**, with an inhibitory concentration 50 (IC_{50}) <10 µM in two (**27**) or three cell lines (**23** and **28**). The most active compound was **28**. The mean IC_{50} values obtained for the three cell lines were 1.5 µM for compound **28** versus 7.3 µM and 6.4 µM for compounds **23** and **27**, respectively, and **28** was found to be four times more active than 5-Fu (mean IC_{50} 6.1 µM). The best result was observed for compound **28** against the A-549 cells with IC_{50} 0.6 µM, whereas the IC_{50} of the reference standard 5-Fu was 7.1 µM.

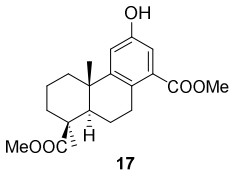
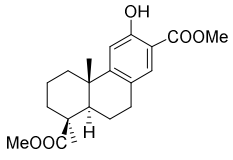
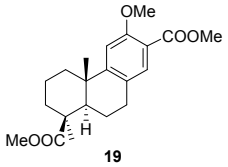
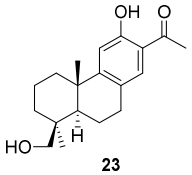
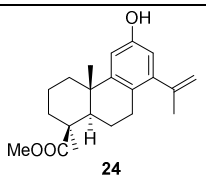
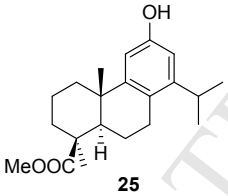
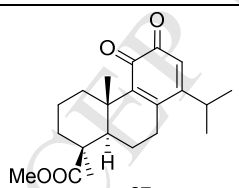
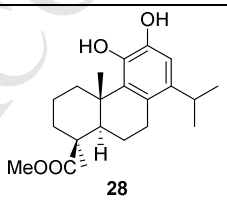
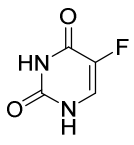
As observed for hydroxy esters **17** and **18**, the mean IC_{50} value was reduced by at least 50% when the ester group was changed from the *meta* (**17**) to the *ortho* position (**18**) (entries 1 and 2). Podocarpane **23**, a constituent of *Gaultheria yunnanensis*, showed no significant inhibitory activity against LPS-induced nitric oxide (NO), TNF-α, or IL-6 production in RAW 246.7 macrophages, as has been reported previously [27]. However,

it did exert an antiproliferative activity against the tumour cell lines used in our study. The totarane *o*-quinone **27** and especially the catechol **28**, which is readily oxidisable to compound **27**, were the most active compounds. The catechol moiety of compound **28**, compared to the *o*-quinone fragment in compound **27**, improved the mean level of activity in colon and lung tumour cells. However, phenol **25**, lacking the hydroxyl group in C-11, is not active (IC_{50} >10 μM). Interestingly, it has been found that natural catechol reduces lung cancer tumour growth *in vitro* and *in vivo* [33]. In general, our results indicate that the catechol or quinone C ring has the greatest influence on the activity of these compounds.

The different degree of oxidation of compounds **27** and **28** account for the greater activity of **28** in the T-84 and A-549 lines, for several reasons. The hydroxyl groups in C11 and C12 may enhance the interactive capacity of the skeleton with cellular elements that are responsible for the induction of the observed effect of **28** in A-549. Moreover, as in the case of caffeic or ferulic acid, hydroxyl groups could exert a cellular pro-oxidant activity mediated by reactions with transition metals [34]. As has been observed experimentally, catechol **28** undergoes rapid oxidation to give *o*-quinone **27**, which would lead to the transformation of **28** into **27** in a biological context. Therefore, the better activity of **28** could be derived from a higher level of intracellular incorporation. The correlation study between log P and IC_{50} revealed no significant association within the series of compounds. However, the log P of **28** was higher than that of compound **27**, which could affect its incorporation. At the cellular level, on the other hand, the redox cycling of catechol metabolites might contribute to cell death through the production of ROS and oxidative stress, and through the production of its *o*-quinone derivatives, possibly generating ROS once they are reduced by P450 reductases, thus altering the functioning of DNA and proteins [35], and even acting as Michael acceptors [36].

Table 1: *In vitro* antiproliferative activity of synthetic compounds against human cancer cell lines.

		IC_{50} (μM)[a]		
Entry	**Compound**	**MCF-7**	**T-84**	**A-549**

1	 **17**	> 40	> 40	> 40
2	 **18**	21.7 ± 1.3	20.7 ± 3.0	17.2 ± 1.9
3	 **19**	> 40	> 40	> 40
4	 **23**	9.3 ± 0.3	7.8 ± 0.4	4.8 ± 0.2
5	 **24**	26.1 ± 1.2	30.7 ± 1.0	23.7 ± 2.0
6	 **25**	28.8 ± 0.7	30.5 ± 2.4	18.6 ± 2.9
7	 **27**	1.1 ± 0.2	11.4 ±2.2	6.8 ± 0.8
8	 **28**	2.8 ± 0.2	1.0 ± 0.2	0.6 ± 0.02
9	 **5-Fu**	7.8±0.19	3.4±0.14	7.1±0.24

[a]IC_{50} values expressed as means ± SEM of seven biological replicates in the µM range.

The ability to inhibit the growth of cancer cells without affecting normal cells is a desirable characteristic of a chemotherapeutic drug candidate [37]. Accordingly, we evaluated the effect of the most active compounds, **27** and **28**, on normal cells, in order to assess nonspecific toxicity. Thus, peripheral blood mononuclear cells (PBMC) were isolated from healthy donor blood samples and induced with increasing concentrations of compounds. PBMC induced with **27** experienced a dose-dependent reduction of cell viability ranging from 25 % at 0.5 µM to 43.8 % at 10 µM, while cultures induced with **28** only reduced the viability by 21.5 % at 10 µM (Fig. 2).

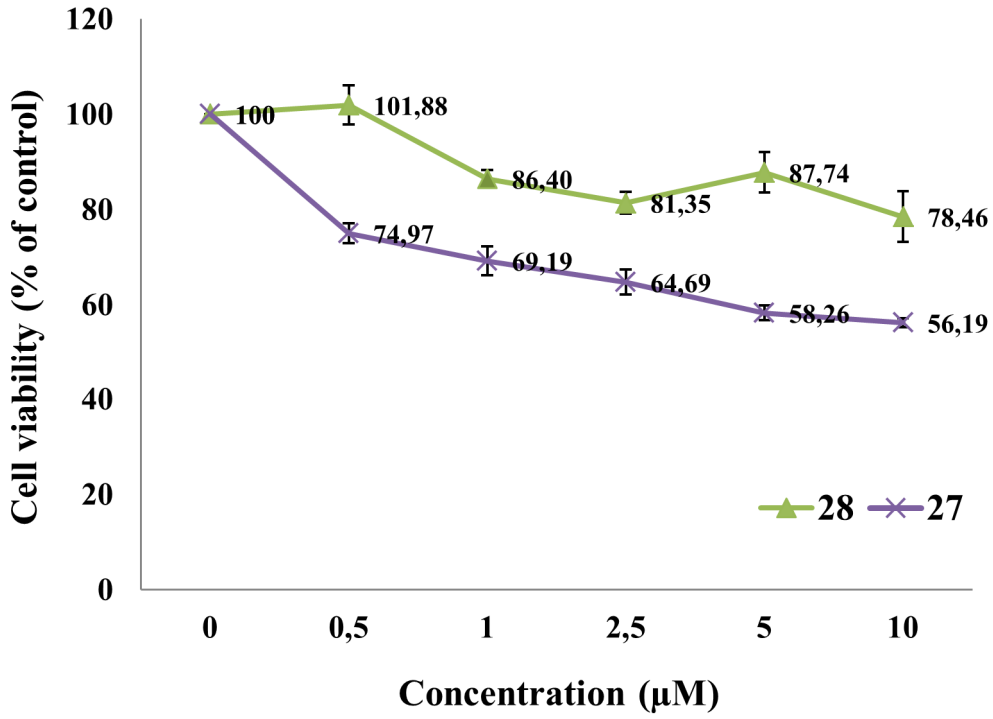


Fig. 2. Effect of compounds **27** and **28** on the viability of normal human PBMC.

Lipophilicity (log P) plays a significant role in drug discovery and compound design, because it can determine pharmacokinetic processes such as drug absorption, distribution and excretion [38], [39]. The log P of the molecules studies was determined by using Marvin 18.3.8 software (ChemAxon Ltd., 2013) (Table 2). We found no correlation between the IC_{50} in the three tumour lines and the log P values of the compounds (Pearson's correlation, $p > 0.05$).

Table 2: Log P values of synthetic compounds

Compounds	**17**	**18**	**19**	**23**	**24**	**25**	**27**	**28**
Log P	4.5	5.2	3.9	3.8	3.8	5.6	4.8	5.3

2.2.2. Cell Cycle and Apoptosis Analysis

Many cytotoxic compounds exert an inhibitory effect on growth by arresting the cell cycle at a particular checkpoint of the cell cycle [40], by the induction of apoptosis [41] or by a combined effect on cell cycle and apoptosis [42]. In consequence, the blockage of cell cycle progression by compounds is an interesting area for study [43]. In the present case, the best antiproliferative result was observed for compound **28** against A-549 cells (IC_{50} 0.6 µM), and as this compound was less toxic in normal cells, we analysed the effect of **28** on the A-549 cell cycle (Figure 3). After 24h of induction with **28** at IC_{50}, we observed a significant increase in cells in the G2/M fraction (14.5 % vs 8.8 % in the control group) and a reduction in the G0/G1 fraction (53.7% vs 60.6 % in the controls), but in both fractions the changes were of low magnitude. However, at 48h, compound **28** induced a large increase in the sub-G1 phase (70.1 % vs 2.6% in the controls), associated with a reduction in the other phases, which may reflect the induction of apoptosis [44].

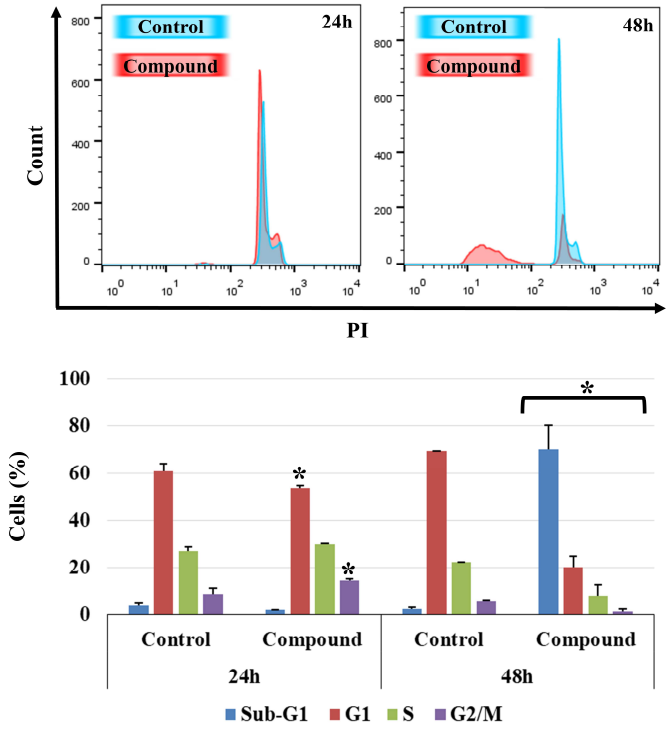


Fig. 3. Flow cytometric analysis of A-549 human lung cancer cell cycle after the treatment with or without (control) compound **28** for 24 and 48h. * Significant difference with control group, $p < 0.05$.

Through various molecular effectors, cytotoxic agents may influence cell cycle progression and apoptosis [45]. Apoptosis is one of the major processes in cell death, and among its typical characteristics are chromatin condensation and fragmentation [46, 47]. We evaluated A-549 cell viability after induction or not (control) for 24h with **28** using an annexin V-FITC/PI kit for flow cytometry [48] and found that **28** induces a significant reduction in cell viability (54.2% vs 83.6% of the controls) with an increase in total apoptosis (44.0% vs 13.2%) (Figure 4). The expression of the proteins Bax and Bcl-2 plays a critical role in the apoptotic process [49, 50]. A-549 cells were induced with 0x (control), 0.5x or 1x IC_{50} of compound **28** for 24h. Western blot analysis showed a decrease and an increase in Bcl-2 and Bax proteins, respectively, which is a molecular hallmark of apoptosis (Figure 5). In general, members of the Bcl-2 family, which includes Bcl-2, Bad, and Bax, are important regulators of various apoptotic pathways [51]. Bax exerts pro-apoptotic effects whereas Bcl-2 presents anti-apoptotic activities [52, 53]. Collectively, these results suggest that compound **28** promotes

apoptosis. This mechanism of action is similar to that reported for various other compounds, including: 1,8-Cineole, a predominant compound present in the hexane extract of *Callistemon citrinus* in A431 human skin carcinoma cells [54]; ovatodiolide, isolated from the herb *Anisomeles indica*, in human lung cancer A549 and H1299 cell lines [55]; [1–9-NαC]-crourorb A1, a cyclic peptide isolated from *Croton urucurana*, in Huh-7 human hepatocarcinoma cells [56]; and Glaucocalyxin A (GLA), a major component isolated from *Rabdosia japonica*, in the UMUC3 human bladder cancer cell line [57].

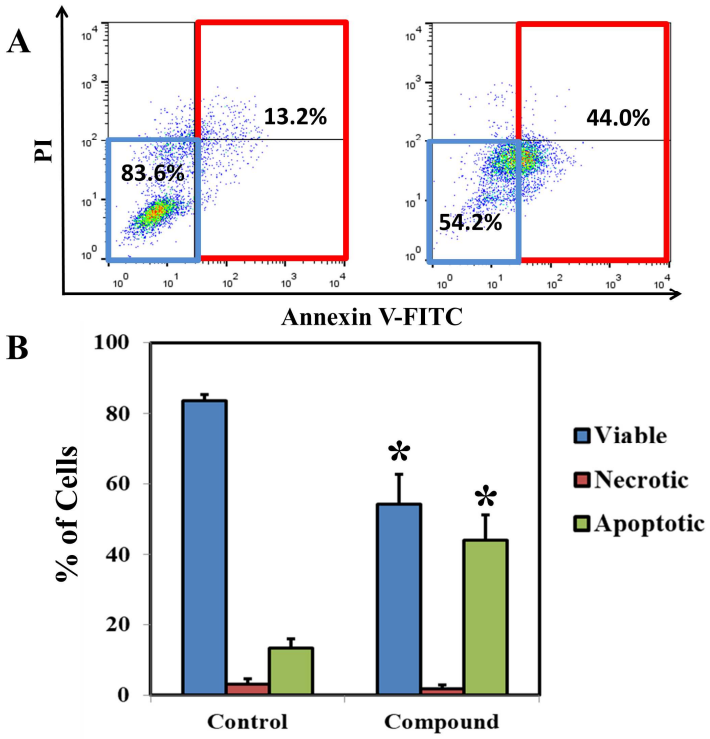


Fig. 4. Flow cytometric analysis of A-549 viability after treatment with or without (control) compound **28** for 24 h, revealed by annexin V-FITC/PI staining. A) Selected cytograms of control (left) and treated (right) cultures. B) Histograms representing the percentages of viable, necrotic and apoptotic cells. * Significant difference with control group, $p < 0.05$.

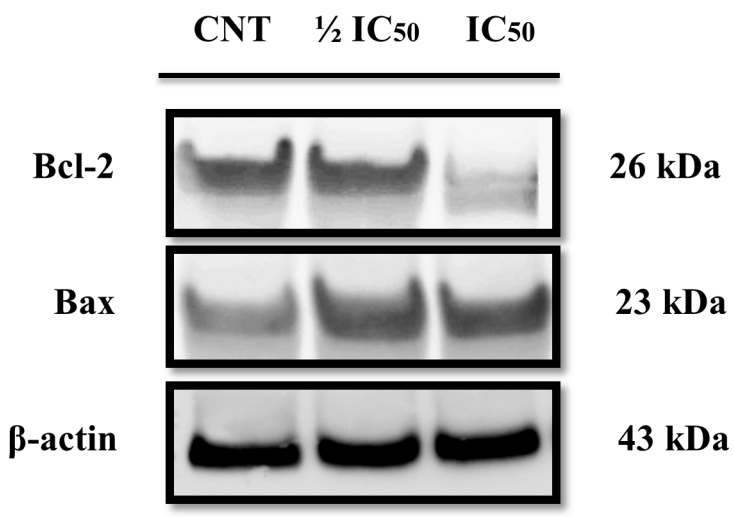


Fig. 5. Western blot analysis of Bcl-2 and Bax expression in A-549 cultures induced with 0x (CNT), 0.5x (1/2IC_{50}) or 1x IC_{50} (IC_{50}) of compound 28 for 24h.

3. Conclusions

A new series of podocarpane and totarane derivatives, including the natural podocarpane **23**, has been synthesized and evaluated for *in vitro* antiproliferative potential against different human cancer cell lines (MCF-7, T-84 and A-549). An antiproliferative effect was induced by compounds **23**, **27** and **28**, with IC_{50} <10 μM in two (**27**) or three cell lines (**23** and **28**). No correlation with log P values was observed. The totarane *o*-quinone **27**, and especially the catechol **28**, which is readily oxidisable to compound **27**, were the most active compounds, highlighting the functional groups present in C11 and C12. Compound **28** showed limited toxicity in normal PBMC, and appeared to exert an antiproliferative effect in A-549 cells through a mechanism that involves the induction of apoptosis mediated by an increased Bax/Bcl-2 ratio. The results of the present study indicate that compound **28** might be useful as antitumoural agent. Further studies are required to elucidate the cellular and molecular elements involved in its effect, and the levels of activity/toxicity in preclinical models.

4. Experimental

4.1 General

Unless stated otherwise, reactions were performed in oven-dried glassware under an argon atmosphere using dry solvents. Solvents were dried as follows: benzene over Na–benzophenone, dichloromethane (DCM) over CaH_2. Thin-layer chromatography (TLC) was performed using F254 precoated plates (0.25 mm) and visualized by UV fluorescence quenching and phosphomolybdic acid solution staining. Flash chromatography was performed on silica gel (230-400 mesh). Chromatography separations were carried out by conventional column on silica gel 60 (230-400 Mesh), using hexanes-AcOEt (AcOEt-hexane) mixtures of increasing polarity. ^{1}H and ^{13}C NMR spectra were recorded at 500 and 400 MHz, and at 150, 125 and 100 MHz, respectively. Chemical shifts (δ H) are quoted in parts per million (ppm) referenced to the appropriate residual solvent peak and tetramethylsilane. Data for ^{1}H NMR spectra are reported as follows: chemical shift (δ ppm) (multiplicity, coupling constant (Hz), integration), with the abbreviations s, br s, d, br d, t. dd, ddd, sext and m denoting singlet, broad singlet, doublet, broad doublet, triplet, double doublet, double double doublet, sextet and multiplet, respectively. *J* = coupling constant in Hertz (Hz). Data for ^{13}C NMR spectra are reported in terms of chemical shift relative to Me_4Si (δ 0.0) and the signals were assigned utilizing DEPT experiments and on the basis of heteronuclear correlations. Infrared spectra (IR) were recorded as thin films or as solids on a FTIR spectrophotometer with samples between sodium chloride plates and are reported in frequency of absorption (cm^{-1}). Only selected absorbances (ν_{max}) are reported. ($[\alpha]^{D}$) measurements were carried out in a polarimeter; utilizing a 1dm length cell and $CHCl_3$ as a solvent. Concentration is expressed in mg/mL. HRMS were recorded on a spectrometer, utilizing a Q-TOF analyzer, and ESI^{+} ionization.

4.2. Synthesis

*4.2.1. (1S,4aS,10aR)-dimethyl 6-methoxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1,7-dicarboxylate (**19**)[28]*

K_2CO_3 (125 mg, 0.905 mmol) was added to a solution of **18** (190 mg, 0.548 mmol) in acetone (8 mL) and the reaction mixture was kept stirring at room temperature for 15 min. Then, dimethyl sulfate (150 mg, 1.19 mmol) was added and the reaction mixture was stirred at reflux for 12 h. The solvent was evaporated and the crude product was poured into AcOEt – water (30 : 10 mL). The phases were shaken and separated, and the organic phase was washed with brine (2 x 10 mL), dried over anh Na_2SO_4, filtered and the solvent evaporated to give a crude product. Flash chromatograpy on silica gel using 10% AcOEt/ hexane gave **19** (184 mg, 93%) as a colourless syrup.

*4.2.2 (1S,4aS,10aR)-dimethyl 6-methoxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1,7-dicarboxylate (**20**)*

To a solution of **19** (161 mg, 0.446 mmol) in dry THF (5 mL) was slowly added 1M methylmagnesium bromide solution in THF (1.2 mL, 1.2 mmol) at 0°C and under argon atmosphere. The reaction mixture was stirred at 0 °C for 30 min, at which time TLC showed no starting material. The reaction was quenched with conc. HCl (2 mL), and the reaction mixture was stirred at room temperature for an additional 10 min. Then, the solvent was removed under vacuum and the mixture was diluted with AcOEt - Water (20 : 5 mL). The phases were shaken and separated. The organic extract was washed with water (5 mL) and brine (5 mL), dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated to give a crude product which was purified by flash chromatography on silica gel (5% AcOEt/hexane), affording **20** (140 mg, 92%) as a colorless syrup.

$[\alpha]_D^{25}$ = + 108.2 (c = 1.3, $CHCl_3$). IR (film) ν_{max}: 2929, 2849, 1725, 1609, 1498, 1463, 1403, 1378, 1328, 1305, 1243, 1213, 1190, 1171, 1141, 1111, 1097, 1057, 1033, 973, 892, 848, 773 cm^{-1}. ^{1}H RMN ($CDCl_3$, 500 MHz) δ (ppm): 1.07 (s, 3 H), 1.13 (ddd, *J* = 13.5, 13.5, 4.3 Hz, 1 H), 1.29 (s, 3 H), 1.44 (ddd, *J* = 13.3, 13.3, 7.1 Hz, 1H), 1.55 (d, *J* = 10.49 Hz, 1 H), 1.63 – 1.67 (m, 1 H), 1.93 – 2.04 (m, 1 H), 2.11 (s, 3H), 2.16 – 2.32 (m, 3 H), 2.73 (ddd, *J* = 14.8, 14.8, 6.3 Hz, 1 H), 2.84 (dd, *J* = 15.2, 3.6 Hz, 1 H), 3.68 (s, 3 H), 3.80 (s, 3 H), 5.07 (s, 1 H), 5.12 (s, 1 H), 6.76 (s, 1 H), 6.88 (s, 1 H). ^{13}C RMN ($CDCl_3$, 125 MHz) δ (ppm): 19.9 (CH_2), 21.1 (CH_2), 22.8 (CH_3), 23.3 (CH_3), 28.5 (CH_3), 31.1 (CH_2), 37.7 (CH_2), 38.6 (C), 39.5 (CH_2), 44.0 (C), 51.2 (CH), 52.9 (CH_3), 55.6 (CH_3), 108.1 (CH), 114.7 (CH_2), 127.3 (C), 129.7 (CH), 130.3 (C), 143.9 (C),

147.9 (C), 154.9 (C), 177.9 (C). HRMS (ESI) m/z: calcd for $C_{22}H_{31}O_3$ ($M+H^+$) 343.2273, found: 343.2276.

*4.2.3 (1S,4aS,10aR)-dimethyl 6-methoxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1,7-dicarboxylate (**21**)*

$LiAlH_4$ (60 mg, 1.57 mmol) was added to a stirred solution of **20** (103 mg, 0.3 mmol) in dry Et_2O (5 mL) cooled to 0°C and the reaction mixture was kept stirred at room temperature under an argon atmosphere for 15 min, at which time TLC showed no remaining starting material. Then, 2 N HCl (0.5 mL) was slowly added at 0°C, and the mixture was diluted with Et_2O – H_2O (20 : 5 mL). The phases were shaken and separated, and the organic phase was washed with brine (2 x 5 mL), dried over anh Na_2SO_4 and filtered. Removal of the solvent under vacuum afforded pure **21** (92 mg, 98%) as a colourless syrup.

$[\alpha]_D^{25}$ = + 55.1 (c = 0.9, $CHCl_3$). IR (film) ν_{max}: 3375, 2924, 2851, 1609, 1563, 1497, 1463, 1402, 1375, 1327, 1260, 1241, 1207, 1104, 1066, 1027, 987, 970, 891, 848, 802, 756, 666, 477 cm^{-1}. ^1H RMN ($CDCl_3$, 500 MHz) δ (ppm): 1.04 (ddd, *J* = 13.6, 13.6, 4.2 Hz, 1 H), 1.07 (s, 3 H), 1.22 (s, 3 H), 1.46 – 1.53 (m, 2 H), 1.58 (d, *J* =4.9 Hz, 1 H), 1.63-1.76 (m 3 H), 1.91 (d, *J* = 13.7 Hz, 1 H), 1.97 – 2.01 (m, 1 H), 2.11 (s, 3H), 2.31 (d, *J* = 14.7 Hz, 1 H), 2.77 (ddd, *J* = 10.7, 10.7, 7.1 Hz, 1 H), 2.87 (dd, *J* = 15.1, 6.7 Hz, 1 H), 3.57 (d, *J* = 10.9, 1 H), 3.80 (s, 3 H), 3.88 (d, *J* = 10.9, 1 H), 5.06 (s, 1 H), 5.11 (s, 1 H), 6.77 (s, 1 H), 6.86 (s, 1 H). ^{13}C RMN ($CDCl_3$, 125 MHz) δ (ppm): 19.0 (CH_2), 19.3 (CH_2), 22.3 (CH_3), 25.6 (CH_3), 26.8 (CH_3), 30.1 (CH_2), 35.2 (CH_2), 37.9 (C), 38.7 (C), 38.9 (CH_2), 51.3 (CH), 55.7 (CH_3), 65.3 (CH_2), 107.3 (CH), 114.7 (CH_2), 126.7 (C), 129.7 (CH), 130.2 (C), 143.9 (C), 149.6 (C), 154.9 (C). HRMS (ESI) m/z: calcd for $C_{21}H_{31}O_2$ ($M+H^+$) 315.2324, found: 315.2326.

*4.2.4 1-((4bS,8S,8aR)-8-(hydroxymethyl)-3-methoxy-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-2-yl)ethanone (**22**)*

An ozone stream was bubbled into a solution of **21** (81 mg, 0.257 mmol) in CH_2Cl_2 (10 mL) cooled at -78 °C for 10 min, at which time TLC showed no starting material. Then

an argon stream was bubbled for eliminating the ozone excess, and PPh_3 was added to the cooled solution, and then stirred for 1 h at room temperature. Solvent was evaporated to afford a crude product that was purified by flash chromatography on silica gel (30% AcOEt/hexane) to yield **22** (70 mg, 86%) as a colourless syrup

$[\alpha]_D^{25}$ = + 49.3 (c = 0.7, $CHCl_3$). IR (film) ν_{max}: 3447, 2924, 2850, 1666, 1604, 1494, 1463, 1402, 1356, 1328, 1264, 1235, 1184, 1034, 989, 969, 850, 752, 612 cm^{-1}. ^1H RMN ($CDCl_3$, 400 MHz) δ (ppm): 1.03 (ddd, *J* = 13.6, 13.6, 4.2 Hz, 1 H), 1.06 (s, 3 H), 1.19 (s, 3 H), 1.22 – 1.27 (m, 3 H), 1.43-1.51 (m 3 H), 1.61 – 1.76 (m, 2 H), 2.30 (d, *J* = 14.7 Hz, 1 H), 2.58 (s, 3H), 2.71 – 2.81 (m, 1 H), 2.89 (dd, *J* = 17.1, 6.8 Hz, 1 H), 3.55 (d, *J* = 10.9, 1 H), 3.85 (d, *J* = 10.9, 1 H), 3.87 (s, 3 H), 6.83 (s, 1 H), 7.43 (s, 1 H). ^{13}C RMN ($CDCl_3$, 100 MHz) δ (ppm): 18.9 (CH_2), 19.1 (CH_2), 25.5 (CH_3), 26.8 (CH_3), 29.8 (CH_2), 35.0 (CH_2), 38.4 (C), 38.8 (C), 38.9 (CH_2), 50.9 (CH), 55.5 (CH_3), 65.2 (CH_2), 107.6 (CH), 125.7 (C), 127.2 (C), 130.9 (CH), 155.9 (C), 157.3 (C), 199.5 (C).). HRMS (ESI) m/z: calcd for $C_{20}H_{29}O_3$ ($M+H^+$) 317.2117, found: 317.2117.

*4.2.5 1-((4bS,8S,8aR)-3-hydroxy-8-(hydroxymethyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-2-yl)ethanone (**23**)*

To a solution of **22** (50 mg, 0.158 mmol) in dry CH_2Cl_2 (5 mL) was added $AlBr_3$ (121 mg, 0.454 mmol), and the reaction mixture was stirred at room temperature for 4 h, at which time TLC showed no starting material. Then, the mixture was poured into ice and diluted with AcOEt – Water (15 : 5 mL). The phases were shaken and separated. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4 and filtered. Removal of the solvent under vacuum afforded a crude product which was purified by flash silicagel column chromatography using 40% AcOEt/hexanes to give **23** (42 mg, 88%) as a white solid.

$[\alpha]_D^{25}$ = + 57.4 (c = 0.8, $CHCl_3$). IR (film) ν_{max}: 3429, 2925, 2851, 1640, 1489, 1373, 1333, 1308, 1266, 1220, 1003, 772 cm^{-1}. ^1H RMN ($CDCl_3$, 400 MHz) δ (ppm): 1.02 (ddd, *J* = 13.6, 13.6, 4.2 Hz, 1 H), 1.06 (s, 3 H), 1.17 (s, 3 H), 1.39 – 1.47 (m, 3 H), 1.62-1.76 (m, 3 H), 1.89 (d, *J* = 14.3 Hz, 1 H), 1.98 – 2.03 (m, 1 H), 2.27 (d, *J* = 12.5 Hz, 1 H), 2.58 (s, 3H), 2.74 – 2.82 (m, 1 H), 2.91 (dd, *J* = 17.7, 6.4 Hz, 1 H), 3.56 (d, *J* = 10.9, 1 H), 3.84 (d, *J* = 10.9, 1 H), 6.87 (s, 1 H), 7.38 (s, 1 H), 11.89 (s, 1 H). ^{13}C

RMN ($CDCl_3$, 100 MHz) δ (ppm): 18.8 (CH_2), 19.1 (CH_2), 25.2 (CH_3), 26.5 (CH_3), 26.8 (CH_3), 29.6 (CH_2), 35.1 (CH), 38.5 (C), 38.6 (CH_2), 38.8 (C), 50.6 (CH), 65.3 (CH_2), 113.7 (CH), 117.9 (C), 125.8 (C), 130.9 (CH), 159.6 (C), 160.1 (C), 203.8 (C). HRMS (ESI) m/z: calcd for $C_{19}H_{27}O_3$ ($M+H^+$) 303.1960, found: 303.1952.

*4.2.6 (1S,4aS,10aR)-methyl 6-hydroxy-1,4a-dimethyl-8-(prop-1-en-2-yl)-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylate (**24**)*

To a solution of **17** (279 mg, 0.805 mmol) in dry THF (10 mL) was slowly added at 0 °C 1M methylmagnesium bromide solution in THF (3 ml, 3 mmol) and under argon atmosphere and the reaction mixture was stirred for 8 h. Then, it was quenched at 0 °C with conc HCl (3 mL), and the reaction mixture was stirred at room temperature for an additional 10 min. Then, the solvent was removed under vacuum and the mixture was diluted with AcOEt - Water (30 : 10 mL). The phases were shaken and separated. The organic extract was washed with water (10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated to give **24** (217 mg, 82%) as a yellow syrup. $[\alpha]_D^{25}$ = + 157.1 (c = 1.1, $CHCl_3$). IR (film) ν_{max}: 3417, 2933, 1723, 1588, 1448, 1378, 1305, 1219, 1192, 114, 1089, 1033, 1219, 1192, 1140, 981, 772, 667 cm^{-1}. ^1H RMN ($CDCl_3$, 500 MHz) δ (ppm): 1.03 (s, 3 H), 1.08 (ddd, *J* = 13.6, 13.6, 4.3 Hz, 1 H), 1.27 (s, 3 H), 1.40 (ddd, *J* = 13.2, 13.2, 4.2 Hz, 1 H), 1.52 (d, *J* = 10.6, Hz, 1 H), 1.59 – 1.63 (m, 1 H), 1.86 (dddd, *J* = 12.9, 12.9, 12.9, 5.2 Hz, 1 H), 1.98 (s, 3 H), 2.16 (d, *J* = 13.3, Hz, 3 H), 2.27 (d, *J* = 13.5, Hz, 2 H), 2.57 (ddd, *J* = 14.7, 14.7, 6.1 Hz, 1 H), 2.81 (dd, *J* = 16.6, 7.0 Hz, 1 H), 3.66 (s, 3 H), 4.79 (s, 1 H), 5.12 (s, 1 H), 6.45 (s, 1 H), 6.69 (s, 1 H). ^{13}C RMN ($CDCl_3$, 125 MHz) δ (ppm): 20.0 (CH_2), 21.1 (CH_2), 22.9 (CH_3), 24.2 (CH_3), 28.5 (CH_3), 29.2 (CH_2), 37.5 (CH_2), 39.0 (C), 39.7 (CH_2), 43.9 (C), 51.3 (CH), 52.7 (CH_3), 110.9 (CH), 112.8 (CH), 114.4 (CH_2), 124.2 (C), 145.2 (C), 146.1 (C), 149.8 (C), 153.3 (C), 178.2 (C). HRMS (ESI) m/z: calcd for $C_{21}H_{29}O_3$ ($M+H^+$) 329.2093, found: 329.2104.

*4.2.7 (1S,4aS,10aR)-methyl 6-hydroxy-8-isopropyl-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylate (**25**)*

To a solution of **24** (233 mg, 0.709 mmol) in CH_2Cl_2 (10 mL) were successively added Et_3SiH (0.5 mL) and trifluoroacetic acid (0.2 mL) and the reaction mixture was refluxed for 1 h. Then CH_2Cl_2, CF_3CO_2H and excess Et_3SiH were evaporated and the resulting crude product, was purified by column chromatography using 20% AcOEt/hexanes to give **25** (204 mg, 87%) as colourless syrup. $[\alpha]_D^{25}$ = + 127.1 (c = 1.6, $CHCl_3$). IR (film) ν_{max}: 3417, 2933, 1723, 1588, 1448, 1378, 1306, 1219, 1192, 1140, 1089, 981, 898, 862, 773 cm^{-1}. ^1H RMN ($CDCl_3$, 500 MHz) δ (ppm): 1.04 (s, 3 H), 1.07 (ddd, *J* = 13.6, 13.6, 4.3 Hz, 1 H), 1.16 (d, *J* = 6.8 Hz, 3 H), 1.19 (d, *J* = 6.8 Hz, 3 H), 1.28 (s, 3 H), 1.36 (ddd, *J* = 13.5, 13.5, 4.3 Hz, 1 H), 1.50 (dd, *J* = 12.5, 1.8 Hz, 2 H), 1.59 – 1.63 (m, 1 H), 1.90 (ddd, *J* = 12.9, 12.9, 6.4 Hz, 1 H), 1.94 – 2.02 (m, 1 H), 2.16 (d, *J* = 12.7, Hz, 1 H), 2.21 – 2.28 (m, 2 H), 2.51 – 2.60 (m, 1 H), 2.91 (dd, *J* = 16.5, 5.5 Hz, 1 H) 3.09 (h, *J* = 6.8 Hz, 1 H), 3.67 (s, 3 H), 6.61 (d, *J* = 2.7 Hz, 1 H), 6.63 (d, *J* = 2.6 Hz, 1 H). ^{13}C RMN ($CDCl_3$, 125 MHz) δ (ppm): 20.0 (CH_2), 21.1 (CH_2), 22.9 (CH_3), 23.1 (CH_3), 23.6 (CH_3), 28.0 (CH_2), 28.4 (CH_3), 37.5 (CH_2), 38.9 (C), 39.9 (CH_2), 43.9 (C), 51.3 (CH), 52.3 (CH_3), 109.76 (CH), 109.83 (CH), 124.5 (C), 148.3 (C), 149.8 (C), 153.7 (C), 178.1 (C).). HRMS (ESI) m/z: calcd for $C_{21}H_{31}O_3$ ($M+H^+$) 331.2273, found: 331.2260.

*4.2.8 Treatment of phenol **25** with $(PhSeO)_2O$*

$(PhSeO)_2O$ (720 mg,1.99 mmol) was added to a solution of phenol **25** (630 mg, 1.9 mmol) in anhydrous THF (10 mL) under an argon atmosphere, and the reaction was kept stirring at reflux for 10 min. The solvent was evaporated under vacuum to give a crude product, which after column chromatography on silica gel using 10% EtOAc/hexane, afforded *ortho*-quinone **27** (477 mg, 73%) and hydroxy dienone **26** (131 mg, 20%).

*(1S,4aS,8aR)-methyl 8a-hydroxy-7-isopropyl-1,4a-dimethyl-6-oxo-1,2,3,4,4a,6,8a,9,10,10a-decahydrophenanthrene-1-carboxylate (**26**)*

$[\alpha]_D^{25}$ = + 12.3 (c = 1.8, $CHCl_3$). IR (film) ν_{max}: 3403, 2928, 1726, 1661, 1619, 1463, 1387, 1219, 1151, 1044, 773 cm^{-1}. ^1H RMN ($CDCl_3$, 400 MHz) δ (ppm): 1.02 (ddd, *J* = 13.2, 13.2, 3.9J Hz, 1 H), 1.13 (d, *J* = 6.9 Hz, 3 H), 1.19 (d, *J* = 6.9 Hz, 3 H), 1.21 (s, 3 H), 1.22 (s, 3 H), 1.50 (ddd, *J* = 13.0, 13.0, 4.3 Hz, 1 H), 1.61 – 1.67 (m, 1 H), 1.75 (d, *J* = 12.0 Hz, 1 H), 1.91 – 2.03 (m, 2 H), 2.22 (d, *J* = 13.7 Hz, 1 H), 2.41 – 2.52 (m, 2

H), 2.87 (h, *J* = 6.9 Hz, 1 H), 6.05 (d, *J* = 1.8 Hz, 1 H), 6.09 (d, *J* = 1.8 Hz, 1 H). ^{13}C RMN ($CDCl_3$, 100 MHz) δ (ppm): 18.6 (CH_3), 18.9 (CH_2), 19.3 (CH_2), 24.1 (CH_3), 24.7 (CH_3), 27.8 (CH), 28.7 (CH_3), 37.2 (CH_2), 37.7 (CH_2), 38.3 (CH_2), 41.9 (C), 44.4 (C), 51.4 (CH), 55.8 (CH_3), 72.1 (C), 122.35 (CH), 122.57 (CH), 169.1 (C), 173.9 (C), 177.3 (C), 187.4 (C).

*(1S,4aS,10aR)-methyl 8-isopropyl-1,4a-dimethyl-5,6-dioxo-1,2,3,4,4a,5,6,9,10,10a-decahydrophenanthrene-1-carboxylate (**27**)*

$[\alpha]_D^{25}$ = + 24.3 (c = 0.5, $CHCl_3$). IR (film) ν_{max}: 2928, 2854, 1725, 1679, 1656, 1465, 1383, 1308, 1232, 1192, 1159, 1140, 1099, 980, 773 cm^{-1}. ^{1}H RMN ($CDCl_3$, 400 MHz) δ (ppm): 1.02 (ddd, *J* = 13.2, 13.2, 3.9J Hz, 1 H), 1.09 (s, 3 H), 1.15 (d, *J* = 6.7 Hz, 3 H), 1.16 (d, *J* = 6.7 Hz, 3 H), 1.23 (s, 3 H), 1.49 – 1.56 (m, 3 H), 1.75 – 1.92 (m, 2 H), 2.17 – 2.22 (m, 2 H), 2.34 – 2.44 (m, 1 H), 2.62 – 2.68 (m, 2 H), 2.82 (h, *J* = 6.7 Hz, 1 H), 3.64 (s, 3 H), 6.21 (s, 1 H). ^{13}C RMN ($CDCl_3$, 100 MHz) δ (ppm): 17.6 (CH_3), 19.5 (CH_2), 19.9 (CH_2), 22.6 (CH_3), 22.7 (CH_3), 28.6 (CH), 29.0 (CH_3), 30.4 (CH_2), 36.7 (CH_2), 37.4 (CH_2), 39.5 (C), 43.5 (C), 51.4 (CH), 52.9 (CH_3), 72.1 (C), 122.7 (CH), 146.4 (C), 147.7 (C), 164.7 (C), 177.3 (C), 180.4 (C), 180.9 (C).). HRMS (ESI) m/z: calcd for $C_{21}H_{29}O_4$ ($M+H^+$) 345.2066, found: 345.2055.

*4.2.9 (1S,4aS,10aR)-methyl 5,6-dihydroxy-8-isopropyl-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylate (**28**).*

Sodium borohydride (60 mg, 1.58 mmol) was added to a solución of **27** (181 mg, 0.525 mmol) in THF (5 mL) and the yellow reaction mixture was stirred at room temperature for 10 min, at which time the solution became colourless. Then, Amberlyst A-15 (200 mg) was added carefully and the reaction was stirred for an additional 5 min. Then, the mixture was filtered and the solvent was removed under vacuum, affording **28** (178 mg, 98%) as a colourless syrup. $[\alpha]_D^{25}$ = + 21.5 (c = 0.9, $CHCl_3$). IR (film) ν_{max}: 3419, 1610, 1588, 1452, 1434, 1379, 1305, 1231, 1206, 1161, 1J142, 1087, 1007, 976, 773 cm^{-1}. ^{1}H RMN ($CDCl_3$, 400 MHz) δ (ppm): 1.09 (ddd, *J* = 13.6, 13.6, 4.3 Hz, 1 H), 1.14 (d, *J* = 6.8 Hz, 3 H), 1.15 (d, *J* = 6.8 Hz, 3 H), 1.21 (s, 3 H), 1.28 (s, 3 H), 1.76 (ddd, *J* = 13.0, 13.0, 4.7 Hz, 2 H), 1.86 – 2.04 (m, 2 H), 2.20 (dd, *J* = 13.9, 6.4 Hz, 2 H), 2.27 (d, *J* = 13.8, Hz, 2 H), 2.57 (ddd, *J* = 14.6, 14.6, 5.3 Hz, 1 H), 2.87 (d, *J* = 13.8, Hz, 1 H), 3.06 (h, *J* = 6.8 Hz, 1 H), 3.66 (s, 3 H), 5.30 (s, 1 H), 5.54 (s, 1 H), 6.64 (s, 1 H). ^{13}C

RMN ($CDCl_3$, 100 MHz) δ (ppm): 16.6 (CH_3), 19.9 (CH_2), 21.0 (CH_2), 23.4 (CH_3), 23.8 (CH_3), 27.9 (CH), 28.9 (CH_3), 29.8 (CH_2), 36.1 (CH_2), 37.7 (CH_2), 40.1 (C), 43.9 (C), 51.2 (CH), 54.7 (CH_3), 109.8 (CH), 127.6 (CH), 134.2 (C), 138.0 (C), 140.5 (C), 141.2 (C), 178.2 (C).). HRMS (ESI) m/z: calcd for $C_{21}H_{31}O_4$ ($M+H^+$) 347.2222, found: 347.2220.

4.3. Biological Assays

4.3.1. Cells and culture

Human breast tumor line MCF-7, human colon tumor line T-84 and human lung tumor line A-549 were cultured at 37°C in 5% CO_2 and 90% humidity with Dulbecco's modified Eagle medium (DMEM), supplemented with 10% heat inactivated fetal bovine serum, 10 ml/L penicillin-streptomycin 100x and 2 mM L-glutamine. All cell lines were provided by the Department of Cell Cultures of the Granada University Scientific Instrumentation Center. Peripheral blood mononuclear cells (PBMC) were isolated by a standard method of density gradient centrifugation using Histopaque-1077, and cultured with RPMI-1640 medium supplemented with 20% fetal bovine serum, 10 ml/L penicillin-streptomycin 100x and 2mM glutamine at 37°C in 5% CO_2 and 90% humidity. The protocol for the obtention and use of human blood samples had been approved by the local ethical committee. Culture media and supplements were supplied by Sigma-Aldrich (St. Louis, MO).

4.3.2. In vitro antiproliferative assay

In order to quantify the IC_{50} of compounds, cells were seeded in 96 well plates at a density of 5 x 10^3 cells/cm^2. At 24h, cells were induced with increasing compound concentrations for 3 days. Subsequently, cells were fixed with 10% cold trichloroacetic acid (4°C) and stained with 0.4% sulforhodamine B (SRB) in 1% acetic acid. The colorant was solubilized with 10 mM Tris-base pH 10.5, and optical density values were determined by colorimeter at 492 nm (Multiskan EX, Thermo Electron Corporation, Milford, MA, USA). The inhibitory concentration 50 (IC50), considered as the concentration of a drug that causes 50% reduction of proliferation and/or cell density versus control cultures, was calculated from the semilogarithmic dose-response curve

by linear interpolation. Alternatively, non-adherent PBMC cultures were quantified using MTT assay at 570 and 595nm [58,59].

4.3.3. Cell cycle and Sub-G1 analysis

A-549 cells were seeded in 6 well plates at a density of 4 x 10^4 cells/cm^2, and after 24h were induced or not (control) with IC_{50} of compound **28** for 24 or 48h. Then the cells were collected, fixed with 70% cold ethanol, incubated with a DNA extraction solution (0.2 M Na_2HPO_4, 0.1 M Citric Acid, pH 7.8) for 15 min at 37°C, and stained with a solution of propidium iodide/ RNAse for 30 min at 37°C in the dark. Finally, samples were analyzed using a flow cytometer (BD FACSCalibur) and the FlowJo software (v 7.6.5, Tree Star, Inc.) [60].

4.3.4. Apoptosis assays with annexin-V

A-549 cells were seeded in 6 well plates at a density of 4 x 10^4 cells/cm^2, and after 24h were induced or not (control) with IC_{50} of compound **28** for 24h. Cell viability was determined by flow cytometry using the TACS® Annexin V-FITC kit (Trevigen, Gaithersburg, MD, USA) following the manufacturer's instruction. Samples were analyzed using a flow cytometer (BD FACSCalibur) and the FlowJo software (v 7.6.5, Tree Star, Inc.).

4.3.5. Western blot analysis

A-549 cells were seeded in 6 well plates at a density of 4 x 10^4 cells/cm^2, and after 24h were induced with 0x (control), 0.5x or 1x IC_{50} of compound **28** for 24h. The medium was then removed, and cells were lysed with sample buffer (62.76 mM Trise HCl pH 6.8, 5% β-mercaptoethanol, 2% SDS, 10% glycerol, 0.5% bromophenol blue). Proteins were separated by electrophoresis in 12% acrylamide gels in an Mini Protean Tetracell (Bio-Rad, CA, USA), transferred to nitrocellulose membranes, and blocked with a buffer containing 3% non-fat milk powder, 1% bovine serum albumin (Sigma-Aldrich), and 0.5% Tween-20 in PBS for 1 h. Subsequently, the membranes were incubated overnight with mouse monoclonal anti-Bax (Santa Cruz Biotechnology, sc-7480) or anti-Bcl-2 (Santa Cruz Biotechnology, sc-56015), followed by incubation with HRP-conjugated anti-mouse IgG. β-actin was detected using a HRP-conjugated mouse monoclonal anti-β-actin (Sigma, A-3854). All antibodies were purchased from Santa Cruz Biotechnology (Texas, USA). Protein-antibody complexes were detected by

chemoluminiscence using the ECL prime immunodetection reagent (GE Healthcare, Little Chalfont, UK).

4.3.6. Statistical analysis

SPSS 24 for Windows (SPSS, Chicago, IL, USA) was used for the statistical analysis. Results were compared with the Student's test, one-way ANOVA or Pearson's test. $p < 0.05$ was considered significant. Data were graphically represented by using Microsoft Excel 2013 software (Microsoft Corporation).

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Appendix A. Supplementary data

Supplementary data related to this article (^{1}H and ^{13}C NMR spectra of all new compounds), can be found at https://doi.org/

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Several podocarpane and totarane terpenoids have synthesized from natural *trans*-communic acid.

The natural podocarpane hydroxy ketone **23**, isolated from *Gaultheria yunnanensis*, has been synthesized for the first time.

The unnantural totarane catechol **28** and the related *ortho*-quinone **27** have been synthesized.

The best activity was found for compound **28** in A549 cells (IC_{50} 0.6 µM).

Compound **28** induces apoptosis mediated by an increased Bax/Bcl-2 ratio in A549 cells.