



Novel semisynthetic derivatives of betulin and betulinic acid with cytotoxic activity

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ABSTRACT

A series of new imidazole carboxylic esters (carbamates) and *N*-acylimidazole derivatives of betulin and betulinic acid (**14**–**29**) have been synthesized. The new compounds were screened for *in vitro* cytotoxicity activity against human cancer cell lines HepG2, Jurkat and HeLa. A number of compounds have shown IC₅₀ values lower than 2 μM against the cancer cell lines tested and the vast majority has shown a better cytotoxicity profile than betulinic acid, including the betulin derivatives. *N*-Acylimidazole derivatives **26** and **27** (IC₅₀ 0.8 and 1.7 μM in HepG2 cells) and the C-3 carbamate derivative **16** (IC₅₀ 2.0 μM in HepG2 cells) were the most promising compounds. Based on the observed cytotoxicity, structure–activity relationships have been established.

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1. Introduction

Cancer is the second most important disease leading to death in both the developing and developed countries nowadays. Numerous experimental and epidemiological studies have shown that several plant derived natural products may serve as effective anti-cancer drugs, among which are plant triterpenes, for example betulin and betulinic acid.^{1,2} Betulin **1** is a main component of birch bark,³ and can be synthetically converted to betulinic acid **2** in a two-step procedure, in high yield.⁴ Both **1** and **2** (Fig. 1) were reported to display several biological effects including anti-inflammatory, antiviral, antimalarial and in particular anticancer.^{5,6} Previous reports revealed that compound **2** is a melanoma-specific cytotoxic agent,⁷ however recent evidence has indicated that **2** possesses a broader spectrum of cytotoxic activity against other cancer types.^{8–11} Moreover, compound **2** has been suggested to induce apoptosis via the activation of caspases, regardless of cellular p53 gene status and CD95 activation.^{12,13} This apoptosis inducing ability, the apparent lack of toxicity on normal cells,¹⁴ and the favourable therapeutic index have made **2** an attractive and a very promising anticancer agent.¹⁵

This lupane triterpene is currently undergoing clinical trials at the National Cancer Institute (NCI).¹⁶ Nevertheless, the poor aqueous solubility of lupane triterpenes such as **2**, which compromises blood serum solubilization, has limited the exploitation of their po-

tential in both the medical and pharmaceutical areas.¹⁷ For this reason a huge amount of effort has been directed towards performing modifications of **2** in order to increase and broaden its range of biological activities. In contrast to **2**, compound **1** has no significant cytotoxic activity.^{18,19} However, some derivatives were found to be active.^{19,20}

The structures of **1** and **2** consist of a 30-carbon skeleton which has three available sites for simple chemical modifications at C-3, C-20 and C-28. Modifications of the parent structure of these compounds at these positions can produce potentially important derivatives, found to be more effective than the starting ones, thus making them appealing for further development as antitumour drugs.^{18,20–36} Nitrogen-containing derivatives of **1** and **2**, such as

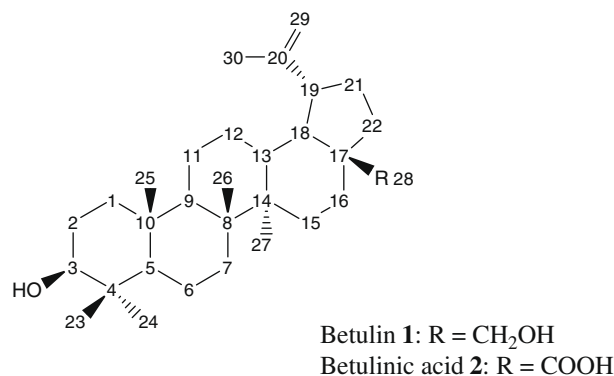


Figure 1. Chemical structure of betulin **1** and betulinic acid **2**.

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amine derivatives,^{21,23,35} oxime derivatives,^{21,23,30} amino acid conjugates,²² amide derivatives,^{20,32} hydrazine²⁹ and hydrazone derivatives,^{28–30,35} imidazolide derivatives,³⁷ and other *N*-heterocyclic derivatives^{24,31,34,36} have been reported to possess antiproliferative effect against tumour cell lines.

In the last few years several carbamates have been synthesized as anticancer agents either in the form of drugs per se or as prodrugs. Some carbamates inhibit endothelial cell proliferation in vitro and tumour induced angiogenesis in vivo as well as tumour growth in mice.³⁸ Recently, C17 steroidal methylimidazolyl carbamates have been reported to possess antiproliferative effects against prostate cancer cell lines.³⁹

Herein we describe the synthesis and in vitro biological evaluation of novel lupane imidazole carbamates and *N*-acylimidazole derivatives, based on the reaction of the commercially available 1,1'-carbonyldiimidazole (CDI) with the alcohol and carboxylic acid functions of compounds **1**, **2** and several derivatives. All new analogs were tested for cytotoxic activity against HepG2 (human hepatocellular carcinoma), Jurkat (human leukemia) and HeLa (human cervical adenocarcinoma) cancer cell lines in order to obtain a preliminary biological profile of this series of compounds that will be useful for future design and development of other derivatives.⁴⁰

2. Results and discussion

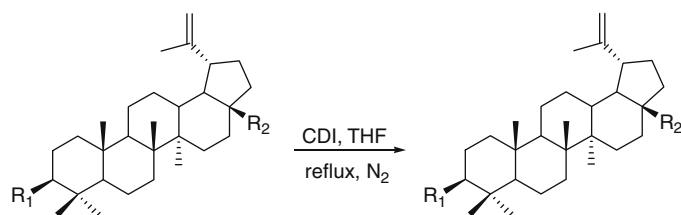
2.1. Chemistry

The general procedure for the synthesis of the 16 novel lupane derivatives **14–29** (Table 1, Schemes 1 and 2) involved dissolution of the corresponding lupanes and CDI, in THF at reflux, under N₂. The reaction of CDI with alcohols and phenols has been reported to afford either *N*-alkylimidazoles^{41–47} or imidazole carboxylic esters (carbamates),^{39,41,42,48–50} depending both on alcohol type and on the reaction conditions used. In our case the reaction afforded the lupane carbamates **14–25** and **29** in good yields. On the other hand the reaction of CDI with the carboxylic acid function of lupane substrates afforded the *N*-acylimidazoles **26–28** (Scheme 2) which was in accordance with previous results.^{51–53}

Due to the fact that the reactivity of the hydroxyl group at C-28 is much higher than that of C-3, the C-28 lupane carbamate **14** could be directly prepared by using mild reaction conditions. In order to synthesize the C-3 lupane carbamate **16**, compound **15** was previously prepared according the general procedure with CDI, and was then selective hydrolyzed at C-28 with silica gel (Table 1) and purified by flash column chromatography (FCC) to afford **16**, in 68% yield. For the synthesis of compounds **19** and **20**, the corresponding acetylated substrates were prepared (Table 1). Compound **1** was converted into the 28-monoacetate **3** in 78% yield by treatment with acetic anhydride and 2 equiv of imidazole, whereas the 3-monoacetate **4** was prepared by a selective hydrolysis of betulin diacetate **6** with methanolic potassium hydroxide, in 74% yield, according to a known procedure.⁵⁴ Derivative **21** was prepared in 88% yield from methyl ester **5** that was obtained from the reaction of compound **2** with methyl iodide in the presence of K₂CO₃ (Table 1).

The synthesis of derivatives **22** and **23** (Scheme 1) began with the methoxylation of the isopropenyl group of the commercially available compound **1** in two steps, as previously described.³¹ In the first step the reaction of betulin diacetate **6** with *N*-bromosuccinimide (NBS) in CCl₄ produced a 30-bromo derivative which was further hydrolyzed by NaOH (4 M) in a MeOH:THF mixture, at room temperature, to afford the 30-methoxy derivative **7** and the 30-bromo compound **8**. The ratio of compounds **7** and **8** was dependent on the reaction conditions used. An increase in the hydrolysis time caused an increase in the amount of the

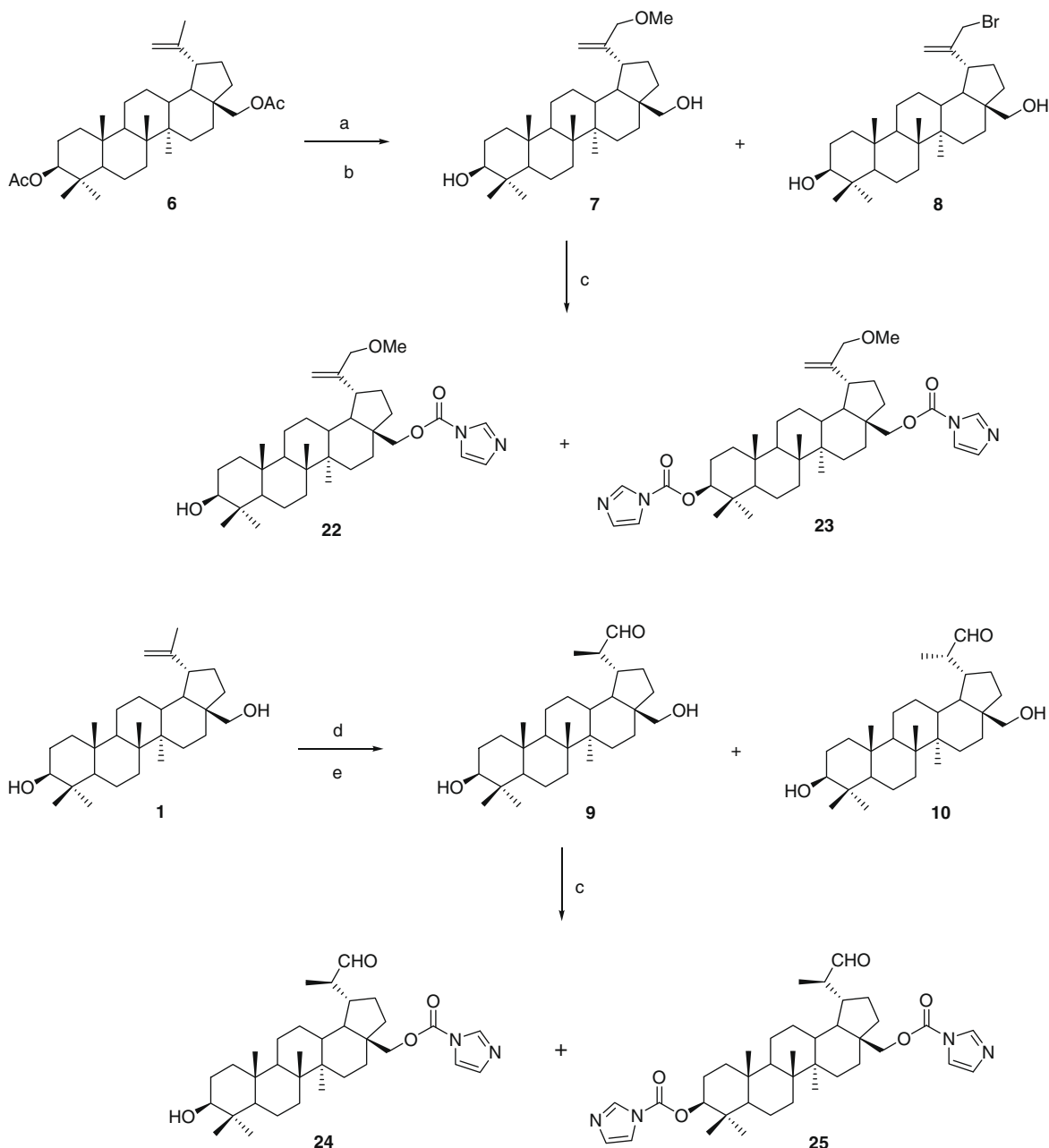
Table 1
Betulin **1** and betulonic acid **2** derivatives **14–21**



	R ₁	R ₂		R ₁	R ₂
1	OH	CH ₂ OH	14	OH	
1	OH	CH ₂ OH	15		
1	OH	CH ₂ OH	16		CH ₂ OH
2	OH	COOH	17		COOH
2	OH	COOH	18		
3	OH	CH ₂ OAc	19		CH ₂ OAc
4	OAc	CH ₂ OH	20	OAc	
5	OH	COOMe	21		COOMe

30-methoxy derivative **7**. This compound was then treated with CDI in THF, at reflux, to afford **22** and **23** in 68% and 28% yield, respectively, after FCC. For the synthesis of compounds **24** and **25**, previous to the introduction of the carbamate moieties at C-3 and C-28, compound **1** was epoxidized with *m*-chloroperbenzoic acid (*m*-CPBA), followed by acid catalyzed epoxy ring opening to afford the epimeric isomers (20*R*-aldehyde) **9** (39%) and (20*S*-aldehyde) **10** (20%) as major products (Scheme 1). The downfield shift values of the H-20 and H-30 signals on the ¹H NMR spectra of compound **9** bearing the 20*R* configuration (δ 9.86 and δ 1.10 ppm) and compound **10** bearing the 20*S* configuration (δ 9.62 and δ 1.04 ppm) are characteristic and are consistent with data reported on the literature.⁵⁵

For the synthesis of derivative **27**, betulonic acid **11** was dehydrated with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) in dioxane according to the known method (Scheme 2).⁵⁶ Compound **12**



Scheme 1. Synthesis of derivatives 22–25. Reagents and conditions: (a) NBS, CCl₄, reflux, 3 h; (b) NaOH aq (4 N), MeOH, THF, rt, 29 h; (c) CDI, dry THF, N₂, reflux, 7–8 h; (d) *m*-CPBA, CH₂Cl₂, 0–5 °C, 5 h; (e) H₂SO₄ (2 M), 0–5 °C, 1 h.

was then treated with CDI to afford **27**, in 82% yield. A previously published procedure using oxygen in the presence of potassium *tert*-butoxide (*t*-BuOK) was used for the preparation of the diosphenol **13** as major product (72%).⁵⁷ Further reaction with CDI and purification by FCC led to the derivatives **28** and **29**, in 70% and 30% yield, respectively (Scheme 2).

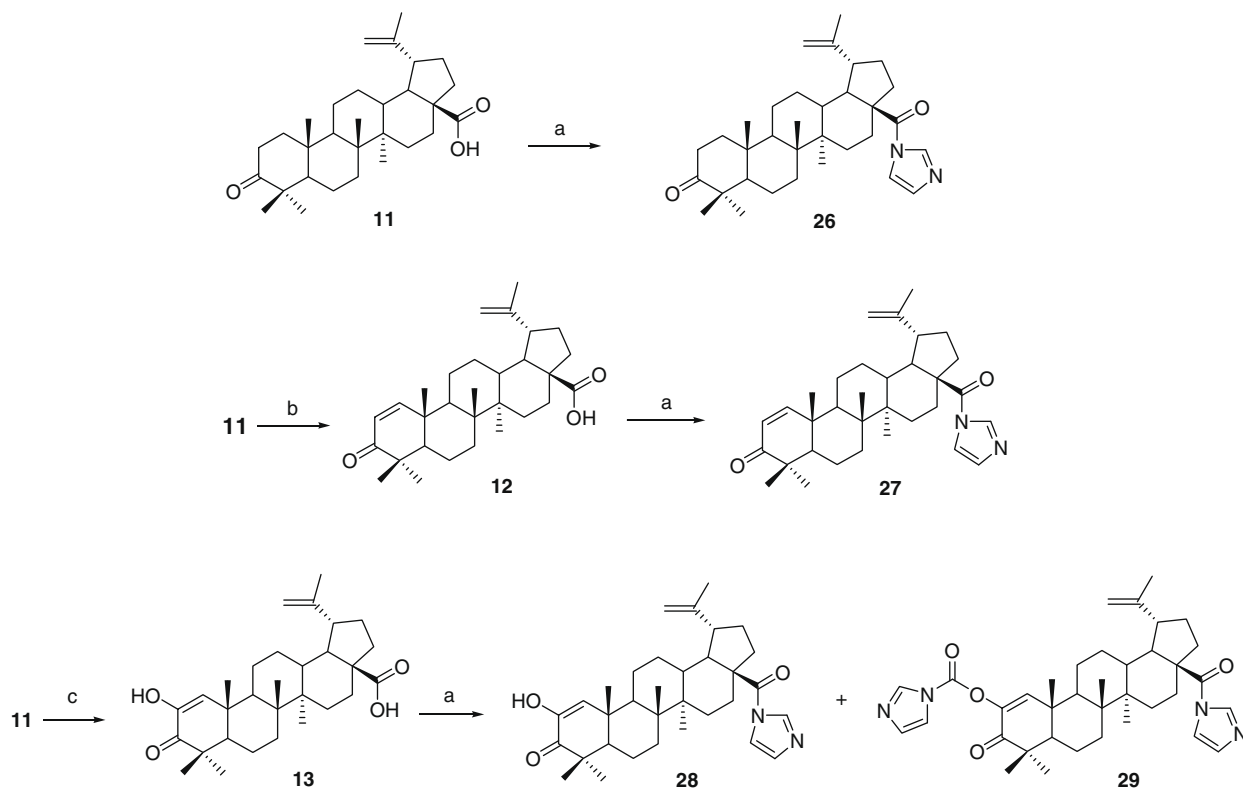
Full structural elucidation of the new lupane derivatives was made using IR, NMR and MS spectroscopy, and assignments were performed based on our analysis and related literature. The presence of a carbamate moiety on the new derivatives **14–25** and **29** was supported by a strong band at 1751–1762 cm⁻¹ on the IR spectrum that corresponded to the C=O stretching vibration. Three ¹H NMR peaks at δ 8.12–8.29 ppm, δ 7.41–7.53 ppm and δ 7.05–7.15 ppm, typical for the imidazole H-2, H-5 and H-4 on the imidazole carbamates were found as well as a quaternary ¹³C NMR

signal at δ 147.9–149.1 ppm that was assigned as the carbamate carbonyl carbon (OCO).⁴²

2.2. Antiproliferative activity

It was been reported that a carbonyl group at position C-17 was important for the cytotoxic activity of lupane derivatives.^{25,27} In the light of this, new lupane imidazole carbamates and *N*-acylimidazoles with one or two carbonyl groups were synthesized.

To evaluate the anticancer potency of these synthesized compounds, the cytotoxic activity of derivatives **14**, **17**, **22**, **24** and **28** was first screened at concentration of 20 μ M against eight human cancer cell lines of different origin: MCF-7 (breast adenocarcinoma), HepG2 (hepatocellular carcinoma), A-375 (melanoma), HT-29 (colon adenocarcinoma), HeLa (cervical adenocarcinoma),



Scheme 2. Synthesis of derivatives 26–29. Reagents and conditions: (a) CDI, dry THF, N₂, reflux, 8–9 h; (b) DDQ, dioxane, N₂, reflux, 15 h; (c) O₂, *t*-BuOK, *t*-BuOH, 40 °C, 2 h.

MIA PaCa-2 (pancreas adenocarcinoma), SH-SY5Y (neuroblastoma) and Jurkat (leukemia) (Fig. 2). Although compound 2 has been reported to be preferentially effective against melanoma and neuroectodermal tumours, the synthesized derivatives tested in this study have demonstrated a broad cytotoxicity activity. The percentages of cell proliferation as compared to control indicated that the different compounds were more potent against HepG2, HeLa

and Jurkat cell lines. These three cancer cell lines were further used for the determination of the potential of all the new derivatives to inhibit tumour cell proliferation (Table 2). This potential antitumour effect was tested estimating the concentration at which tumour cell proliferation was inhibited by 50%, after 72 h of treatment with the compounds. Compound 2 was used as positive control. As shown in Table 2, analysis of the IC₅₀ values revealed

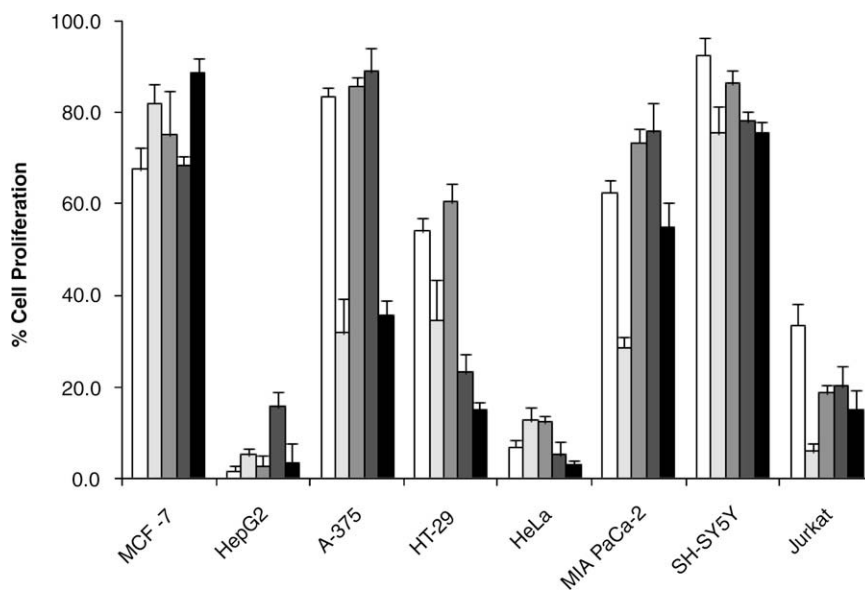


Figure 2. Screening of antiproliferative activity of synthesized lupane derivatives □ 14, ▤ 17, ▥ 22, ▧ 24 and ■ 28 on human cancer cell lines. Cells were treated with different compounds at concentration of 20 μM for 72 h, and cell proliferation was measured using MTT or XTT assay. The assay was done using three replicates and repeated three times.

Table 2
Cytotoxic activity data for compounds **14–29** against HepG2, Jurkat and HeLa

Entry	Compound	IC ₅₀ ^a (μM ± SD)		
		HepG2	Jurkat	HeLa
1	2	36.4 ± 1.5	26.9 ± 2.2	26.0 ± 2.1
2	14	4.2 ± 0.3	16.3 ± 1.2	7.6 ± 0.6
3	15	>30	>30	>30
4	16	2.0 ± 0.4	11.1 ± 1.3	3.0 ± 0.2
5	17	6.2 ± 0.2	5.2 ± 0.7	5.1 ± 0.3
6	18	2.9 ± 0.4	6.0 ± 1.2	8.2 ± 0.4
7	19	>30	>30	>30
8	20	>30	>30	>30
9	21	4.3 ± 0.1	9.9 ± 0.6	8.2 ± 0.5
10	22	8.3 ± 0.4	12.2 ± 1.3	9.8 ± 0.6
11	23	19.6 ± 1.6	16.2 ± 1.0	>30
12	24	11.5 ± 1.1	13.7 ± 1.4	11.1 ± 1.4
13	25	26.1 ± 1.1	20.2 ± 1.6	19.2 ± 1.5
14	26	0.8 ± 0.05	1.4 ± 0.2	2.0 ± 0.3
15	27	1.7 ± 0.2	2.3 ± 0.3	3.0 ± 0.2
16	28	6.5 ± 0.4	7.0 ± 1.6	4.6 ± 0.7
17	29	4.0 ± 0.3	8.1 ± 0.6	3.9 ± 0.3

Exponentially growing cells were treated with the compounds at different concentrations for 72 h. Cell-growth inhibition was analysed by the MTT (HepG2 and HeLa) or XTT (Jurkat) assay. The assay was done using three replicates and repeated four times.

^a IC₅₀ is the concentration of compound that inhibits 50% of cell growth.

that the synthesized derivatives markedly inhibited the proliferation of all the tested cancer cell lines and were particularly effective against the hepatoma HepG2 cell line.

The majority of the novel compounds, **14**, **16–18** and **21–29**, (Table 2, entries 2, 4–6 and 9–17) showed improved cytotoxic activity against the three tested tumoural cell lines when compared to compound **2** (Table 2, entry 1), compound **26** being particularly active (Table 2, entry 14) and 45, 19 and 13-fold more potent than compound **2** (Table 2, entry 1) towards HepG2, Jurkat and HeLa cell lines, respectively. The acetyl derivatives **19** and **20** (Table 2, entries 7 and 8) and the disubstituted derivative **15** (Table 2, entry 3) showed higher IC₅₀ values than compound **2** (>30 μM), which is consistent with the reported data for the in vitro antitumour activity of acetyl derivatives of **1**.¹⁸ The fact that betulonic acid derivatives **26** and **27** (Table 2, entries 14 and 15) were the most active compounds showed that oxidation of the hydroxyl group to a ketone at C-3 markedly influenced the cytotoxic potential. The introduction of the carbamate moiety at position C-3 in compounds **15**, **23** and **25** (Table 2, entries 3, 11 and 13) resulted in a loss of cytotoxicity in all the tested cell lines when compared with compounds **14**, **22** and **24** (Table 2, entries 2, 10 and 12), suggesting a size limitation at position C-3. Moreover, the loss of antiproliferative activity of compounds **22** and **23** (Table 2, entries 10 and 11) modified at C-30, and **24** and **25** (Table 2, entries 12 and 13) modified at C-20, indicated that the cytotoxic profile of derivatives of compound **1** may be sensitive to the size and electronic density of the substituents at C-20, and supports the idea that the C-20 position of compounds **1** and **2** is not a favourable site to modify in order to improve antitumoural activity.²³

Compounds **18** and **21** (Table 2, entries 6 and 9) inhibited HepG2 cell proliferation at lower concentrations than compound **17** (Table 2, entry 5), which confirmed that the carbonyl group and not the carboxyl group was essential for the cytotoxic activity of the lupane derivatives.²⁵

3. Conclusions

A large number of structural modifications of compounds **1** and **2** as possible antitumour agents were studied. This investigation demonstrates that the incorporation of an imidazole scaffold at

the C-28 or C-3 position of compounds **1** and **2** to obtain carbamates or *N*-acylimidazoles can produce a number of potentially important derivatives with improved cytotoxic activity. Modifications of the less potent compound **1** can lead to derivatives with a better cytotoxicity profile as shown with derivatives **14**, **16**, **22** and **24**. Compounds **16**, **26** and **27** are potential lead compounds for new anti-tumour agent development and have been selected for further studies.

4. Experimental

4.1. Chemistry

4.1.1. General

Betulin **1**, betulonic acid **2**, CDI, NBS, NaOH, *m*-CPBA, H₂SO₄, DDQ, *t*-BuOK and *tert*-butyl alcohol (*t*-BuOH) were purchased from Sigma Aldrich Co., whereas solvents were obtained from VWR Portugal. For thin layer chromatography (TLC) analysis Kieselgel 60HF₂₅₄/Kieselgel 60G was used and FCC was performed using Kieselgel 60 (230–400 mesh, Merck). Melting points were determined using a BUCHI melting point B-540 apparatus and are uncorrected. IR spectra were obtained using a JASCO FT/IR-420 spectrophotometer. NMR spectra were recorded on a Bruker Digital NMR-Avance 300 apparatus and on a Bruker Digital NMR-Avance 400 apparatus in CDCl₃ with Me₄Si as the internal standard. Elucidation of the chemical structures was based on ¹H, ¹³C, DEPT135, COSY, HMQC and HMBC NMR experiments. Chemical shifts values (δ) are given in ppm and the coupling constants (*J*) are presented in hertz. Mass spectra were obtained using a Finnigan Polaris QGC/MS Benchtop Ion Trap mass spectrometer with a direct insertion probe.

4.1.1.1. 3β-Hydroxylup-20(29)-en-28-yl acetate (3). Details of the synthesis of this compound were reported previously.⁵⁴ Compound **3** (323 mg, 84%): IR (film) ν_{max} 3471, 3070, 1734, 1642, 1244, 1102, 880 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 4.69 (s, 1H, H-29_a), 4.59 (s, 1H, H-29_b), 4.24 (d, *J* = 10.9 Hz, 1H, H-28_a), 3.86 (d, *J* = 10.9 Hz, 1H, H-28_b), 3.18 (dd, *J* = 11.0 Hz, *J* = 4.7 Hz, 1H, H-3α), 2.45 (dt, *J* = 10.9 Hz, *J* = 5.8 Hz, 1H, H-19), 2.07 (s, 3H, OCOCH₃), 1.68 (s, 3H, H-30), 1.03 (s, 3H), 0.97 (s, 3H), 0.96 (s, 3H), 0.82 (s, 3H), 0.76 (s, 3H); ¹³C NMR (CDCl₃, 75 MHz) δ 171.6 (OCOCH₃), 150.2 (C20), 109.9 (C29), 78.9 (C3), 62.8 (C28); EI-MS *m/z* (% rel. intensity): 484 (12) M⁺, 203 (54), 189 (100), 187 (71), 147 (46), 145 (49), 133 (67), 119 (61), 105 (69), 91 (56).

4.1.1.2. 28-Hydroxylup-20(29)-en-3β-yl acetate (4). Details of the synthesis of this compound were reported previously.⁵⁴ Compound **4** (259 mg, 89%): IR (film) ν_{max} 3440, 3070, 1729, 1642, 1246, 978, 882 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 4.68 (s, 1H, H-29_a), 4.58 (s, 1H, H-29_b), 4.47 (dd, *J* = 10.3 Hz, *J* = 5.7 Hz, 1H, H-3α), 3.79 (d, *J* = 10.6 Hz, 1H, H-28_a), 3.33 (d, *J* = 10.7 Hz, 1H, H-28_b), 2.39 (dt, *J* = 10.7 Hz, *J* = 5.9 Hz, 1H, H-19), 2.04 (s, 3H, OCOCH₃), 1.69 (s, 3H, H-30), 1.02 (s, 3H), 0.97 (s, 3H), 0.85 (s, 6H), 0.84 (s, 3H); ¹³C NMR (CDCl₃, 75 MHz) δ 171.1 (OCOCH₃), 150.5 (C20), 109.7 (C29), 80.9 (C3), 60.5 (C28); EI-MS *m/z* (% rel. intensity): 484 (9) M⁺, 203 (71), 189 (100), 187 (55), 107 (61), 105 (52), 95 (77), 91 (67), 81 (60), 79 (85).

4.1.1.3. Methyl 3β-hydroxylup-20(29)-en-28-oate (5). To a solution of compound **2** (150 mg, 0.33 mmol) and anhydrous K₂CO₃ (115 mg, 0.83 mmol) in dry DMF (2 ml) was added methyl iodide (41 μl, 0.66 mmol). The mixture was stirred at room temperature for 1 h. After reaction completion the reaction mixture was diluted in ethyl acetate (30 ml) and washed with water (3 × 20 ml) and brine (20 ml). The organic phase was dried with anhydrous Na₂SO₄, filtered and concentrated under reduced

pressure to give a yellowish solid. This solid was crystallized from methanol to yield compound **5** (129 mg, 83%); IR (film) ν_{\max} 3320, 3070, 1720, 1643 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 4.71 (br s, 1H, H-29_a), 4.58 (br s, 1H, H-29_b), 3.67 (s, 3H, COOCH_3), 3.18 (dd, 1H, $J = 10.9$ Hz, $J = 4.5$ Hz, H-3 α), 2.43 (m, 1H, H-19), 1.69 (s, 3H, H-30), 0.96 (s, 3H), 0.94 (3H), 0.92 (s, 3H), 0.82 (s, 3H), 0.75 (s, 3H); ^{13}C NMR (CDCl_3 , 75 MHz) δ 177.2 (C28), 149.7 (C20), 110.1 (C29), 80.6 (C3); EI-MS m/z (% rel. intensity): 470 (25) M^+ , 286 (26), 253 (52), 247 (29), 203 (36), 192 (100), 189 (100), 175 (64), 119 (47), 105 (51).

4.1.1.4. Lup-20(29)-en-3 β ,28-di-yl acetate (6). Details of the synthesis of this compound were reported previously.⁵⁴ Compound **6** (373 mg, 89%); IR (film) ν_{\max} 3073, 1735, 1642, 1241, 880 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 4.69 (br s, 1H, H-29_a), 4.59 (br s, 1H, H-29_b), 4.47 (dd, $J = 10.3$ Hz, $J = 5.8$ Hz, 1H, H-3 α), 4.25 (d, $J = 11.1$ Hz, 1H, H-28_a), 3.85 (d, $J = 11.1$ Hz, 1H, H-28_b), 2.45 (dt, $J = 10.9$ Hz, $J = 5.8$ Hz, 1H, H-19), 2.07 (s, 3H, OCOCH_3), 2.04 (s, 3H, OCOCH_3), 1.68 (s, 3H, H-30), 1.03 (s, 3H), 0.97 (s, 3H), 0.84 (s, 6H), 0.83 (s, 3H); ^{13}C NMR (CDCl_3 , 75 MHz) δ 171.6 (OCOCH_3), 171.0 (OCOCH_3), 150.1 (C20), 109.9 (C29), 80.9 (C3), 62.8 (C28); EI-MS m/z (% rel. intensity): 526 (5) M^+ , 466 (72), 216 (46), 203 (47), 202 (44), 190 (53), 189 (100), 187 (68), 119 (47), 91 (51).

4.1.1.5. 30-Methoxylup-20(29)-en-3 β ,28-diol (7) and 30-bromolup-20(29)-en-3 β ,28-diol (8). Details of the synthesis of these compounds were reported previously.³¹ Compound **7** (1.4 g, 64%); IR (film) ν_{\max} 3347, 3073, 1645 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 4.92 (s, 1H, H-29_a), 4.91 (s, 1H, H-29_b), 3.86 (br s, 2H, H-30), 3.78 (d, $J = 10.5$ Hz, 1H, H-28_a), 3.35 (s, 3H, OCH_3), 3.31 (d, $J = 10.5$ Hz, 1H, H-28_b), 3.18 (dd, $J = 10.8$ Hz, $J = 5.2$ Hz, 1H, H-3 α), 2.28 (dt, $J = 10.8$ Hz, $J = 5.4$ Hz, 1H, H-19), 1.02 (s, 3H), 0.98 (s, 3H), 0.97 (s, 3H), 0.82 (s, 3H), 0.76 (s, 3H); ^{13}C NMR (CDCl_3 , 75 MHz) δ 150.9 (C20), 109.0 (C29), 78.9 (C3), 74.8 (C30), 60.2 (C28), 58.3 (OCH_3); EI-MS m/z (% rel. intensity): 473 (25) M^+ , 201 (93), 189 (86), 187 (100), 145 (75), 131 (66), 121 (71), 119 (73), 95 (66), 81 (69). Compound **8** (451 mg, 18%); IR (film) ν_{\max} 3371, 3075, 1642 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 5.12 (s, 1H, H-29_a), 5.03 (s, 1H, H-29_b), 3.99 (s, 2H, H-30), 3.81 (d, $J = 10.8$ Hz, 1H, H-28_a), 3.33 (d, $J = 10.8$ Hz, 1H, H-28_b), 3.19 (dd, $J = 10.9$ Hz, $J = 5.1$ Hz, 1H, H-3 α), 2.39 (dt, $J = 11.0$ Hz, $J = 5.3$ Hz, 1H, H-19), 1.03 (s, 3H), 0.99 (s, 3H), 0.97 (s, 3H), 0.82 (s, 3H), 0.76 (s, 3H); ^{13}C NMR (CDCl_3 , 75 MHz) δ 151.0 (C20), 109.8 (C29), 78.9 (C3), 60.3 (C28). EI-MS m/z (% rel. intensity): 522 (3) M^+ , 121 (74), 119 (91), 107 (85), 105 (82), 93 (84), 91 (91), 81 (84), 79 (100), 67 (82).

4.1.1.6. 3 β ,28-Dihydroxy-(20R)-lupan-29-al (9) and 3 β ,28-dihydroxy-(20S)-lupan-29-al (10). Details of the synthesis of these compounds were reported previously.^{55,58} Compound **9** (354 mg, 39%); IR (film) ν_{\max} 3393, 1714 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 9.86 (d, $J = 2.0$ Hz, 1H, H-29), 3.77 (d, $J = 10.8$ Hz, 1H, H-28_a), 3.26 (d, $J = 10.8$ Hz, 1H, H-28_b), 3.20 (dd, $J = 10.9$ Hz, $J = 5.1$ Hz, 1H, H-3 α), 2.60 (m, 1H, H-20), 1.10 (d, $J = 6.9$ Hz, 3H, H-30), 1.03 (s, 3H), 0.98 (s, 3H), 0.95 (s, 3H), 0.84 (s, 3H), 0.77 (s, 3H); ^{13}C NMR (CDCl_3 , 75 MHz) δ 206.8 (CHO), 78.9 (C3), 60.2 (C28); EI-MS m/z (% rel. intensity): 458 (2) M^+ , 369 (100), 207 (43), 192 (51), 189 (72), 161 (67), 133 (31), 121 (31), 107 (36), 95 (33). Compound **10** (179 mg, 20%); IR (film) ν_{\max} 3340, 1716 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 9.62 (s, 1H, H-29), 3.80 (d, $J = 10.8$ Hz, H-28_a), 3.33 (d, $J = 10.8$ Hz, 1H, H-28_b), 3.20 (dd, $J = 10.9$ Hz, $J = 5.1$ Hz, 1H, H-3 α), 2.65 (m, 1H, H-20), 1.06 (s, 3H), 1.04 (d, $J = 7.0$ Hz, 3H, H-30), 0.98 (s, 6H), 0.84 (s, 3H), 0.77 (s, 3H); ^{13}C NMR (CDCl_3 , 75 MHz) δ 204.8 (CHO), 78.9 (C3), 60.2 (C28); EI-MS m/z (% rel. intensity): 458 (4) M^+ , 369 (76), 207 (48), 190 (46), 189 (100), 161 (70), 119 (51), 105 (46), 95 (47), 91 (59).

4.1.1.7. 3-Oxolup-20(29)-en-28-oic acid (11). Details of the synthesis of this compound were reported previously.⁴ Compound **11** (685 mg, 67%); IR (film) ν_{\max} 3070, 1703, 1686, 1642 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 4.77 (br s, 1H, H-29_a), 4.64 (br s, 1H, H-29_b), 3.04 (dt, $J = 10.7$ Hz, $J = 4.3$ Hz, 1H, H-19), 1.72 (s, 3H, H-30), 1.09 (s, 3H), 1.04 (s, 3H), 1.02 (s, 3H), 0.99 (s, 3H), 0.95 (s, 3H); ^{13}C NMR (CDCl_3 , 75 MHz) δ 218.2 (C3), 182.2 (C28), 150.3 (C20), 109.7 (C29); EI-MS m/z (% rel. intensity): 454 (19) M^+ , 408 (24), 393 (20), 248 (85), 189 (100), 175 (62), 133 (55), 119 (76), 105 (69), 79 (52).

4.1.1.8. 3-Oxolup-1,20(29)-dien-28-oic acid (12). A solution of compound **11** (400 mg, 0.88 mmol) and DDQ (597 mg, 2.64 mmol) in anhydrous dioxane (18 ml) was heated under reflux and N_2 atmosphere for 15 h. After the reaction mixture was diluted in ethyl acetate (60 ml) and the insoluble matter was removed by filtration. The filtrate was washed with saturated solution of Na_2CO_3 (3×30 ml), and then with water (30 ml) and brine (30 ml), dried with anhydrous Na_2SO_4 , filtered and concentrated under reduced pressure to give a yellowish solid. This solid was submitted to FCC with petroleum ether 40–60 °C/ethyl acetate (4:1) to afford compound **12** (179 mg, 45%); IR (film) ν_{\max} 3070, 1730, 1689, 1645 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) δ 7.11 (d, $J = 10.3$ Hz, 1H, H-1), 5.80 (d, $J = 10.3$ Hz, 1H, H-2), 4.76 (s, 1H, H-29_a), 4.63 (s, 1H, H-29_b), 3.03 (m, 1H, H-19), 1.70 (s, 3H, H-30), 1.13 (s, 3H), 1.07 (s, 3H), 1.06 (s, 3H), 1.02 (s, 3H), 0.99 (s, 3H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 205.9 (C3), 181.7 (C28), 160.1 (C1), 150.2 (C20), 125.1 (C2), 109.9 (C29); EI-MS m/z (% rel. intensity): 452 (17) M^+ , 213 (100), 150 (39), 137 (34), 95 (31), 91 (42), 81 (36), 79 (41), 77 (29), 67 (34).

4.1.1.9. 2-Hydroxy-3-oxolup-1,20(29)-dien-28-oic acid (13). Details of the synthesis of these compounds were reported previously.⁵⁷ Compound **13** (273 mg, 73%); ν_{\max} 3389, 3073, 1730, 1698, 1669, 1645 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 6.45 (s, 1H, H-1), 4.75 (s, 1H, H-29_a), 4.64 (s, 1H, H-29_b), 3.02 (m, 1H, H-19), 1.70 (s, 3H, H-30), 1.20 (s, 3H), 1.13 (s, 3H), 1.10 (s, 3H), 1.00 (s, 3H), 0.98 (s, 3H); ^{13}C NMR (CDCl_3 , 75 MHz) δ 201.2 (C3), 182.4 (C28), 150.1 (C20), 143.9 (C2), 128.9 (C1), 109.9 (C29); EI-MS m/z (% rel. intensity): 469 (11) M^+ , 321 (43), 213 (100), 189 (32), 150 (45), 136 (29), 91 (63), 80 (34), 75 (54), 69 (65).

4.1.1.10. 3 β -Hydroxy-lup-20(29)-en-28-yl-1H-imidazole-1-carboxylate (14) and lup-20(29)-en-3 β ,28-di-yl-(1H-imidazole-1-carboxylate) (15). To a solution of compound **1** (200 mg, 0.45 mmol) in anhydrous THF (8 ml), CDI (219 mg, 1.35 mmol) was added. After 7 h under magnetic stirring at reflux temperature and N_2 atmosphere, the reaction was completed as verified by TLC control. The reaction mixture was poured onto water (30 ml) and extracted with diethyl ether (3×30 ml). The combined organic extract was then washed with water (30 ml), and brine (30 ml), dried with anhydrous Na_2SO_4 , filtered and concentrated under reduced pressure to give a yellowish solid. This solid was submitted to FCC with petroleum ether 40–60 °C/ethyl acetate (3:2) and afforded compound **14** (152 mg, 63%); mp (acetone) 202–204 °C; IR (film) ν_{\max} 3570, 3078, 1751, 1645, 880 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 8.26 (s, 1H, H-2'), 7.46 (br s, 1H, H-5'), 7.13 (br s, 1H, H-4'), 4.72 (d, $J = 1.4$ Hz, 1H, H-29_a), 4.67–4.63 (m, 2H, H-28_a and H-29_b), 4.21 (d, $J = 10.7$ Hz, 1H, H-28_b), 3.19 (dd, $J = 10.8$ Hz, $J = 5.2$ Hz, 1H, H-3 α), 2.47 (dt, $J = 10.7$ Hz, $J = 5.6$ Hz, 1H, H-19), 1.70 (s, 3H, H-30), 1.06 (s, 3H), 1.01 (s, 3H), 0.97 (s, 3H), 0.83 (s, 3H), 0.76 (s, 3H); ^{13}C NMR (CDCl_3 , 75 MHz) δ 149.5 (C20), 148.7 (OCO), 136.8 (C2'), 129.6 (C4'), 117.2 (C5'), 110.3 (C29), 78.9 (C3), 67.5 (C28); EI-MS m/z (% rel. intensity): 536 (12) M^+ , 207 (36), 189 (39), 187 (54), 119 (44), 107 (34), 105 (34), 91 (46), 79 (38), 69 (100) and compound **15** (56 mg, 20%); mp (acetone/*n*-hexane) 161–163 °C; IR (film) ν_{\max} 3070, 1757, 1642, 880 cm^{-1} ; ^1H NMR

(CDCl₃, 300 MHz): δ 8.23 and 8.22 (both s, each 1H, H-2', H-2''), 7.45 (s, 2H, H-5' and H-5''), 7.12 (s, 2H, H-4' and H-4''), 4.73–4.64 (m, 4H, H-3 α , H-28_a, H-29_a and H-29_b), 4.20 (d, J = 10.7 Hz, 1H, H-28_b), 2.48 (m, 1H, H-19), 1.71 (s, 3H, H-30), 1.08 (s, 3H), 1.02 (s, 3H), 0.96 (s, 6H), 0.91 (s, 3H); ¹³C NMR (CDCl₃, 75 MHz): δ 149.4 (C20), 148.9 (OCO), 148.2 (OCO), 136.8 and 136.7 (C2', C2''), 130.0 and 129.5 (C4', C4''), 117.2 and 117.1 (C5', C5''), 110.3 (C29), 86.8 (C3), 67.3 (C28); EI-MS m/z (% rel. intensity): 630 (2) M⁺, 189 (67), 187 (73), 119 (72), 105 (80), 95 (65), 93 (56), 91 (84), 79 (60), 69 (100).

4.1.1.11. 28-Hydroxy-lup-20(29)-en-3 β -yl-1H-imidazole-1-carboxylate (16). A solution of compound **1** (200 mg, 0.45 mmol) and CDI (365 mg, 2.25 mmol) in anhydrous THF (8 ml) was refluxed for 9 h to afford compound **15**. Silica gel (200 mg) was added and this mixture was stirred at room temperature for 18 h. The solid was filtered off and the filtrate was poured onto water (30 ml) and extracted with diethyl ether (3 \times 30 ml). The organic phase was then washed with water (30 ml), and brine (30 ml), dried with Na₂SO₄, filtered and evaporated to dryness. The crude product was purified by FCC eluting with petroleum ether 40–60 °C/ethyl acetate (3:2) to yield compound **16** (166 mg, 68%): mp (acetone/*n*-hexane) 198–199 °C; IR (film) ν_{\max} 3327, 3070, 1758, 1645, 882 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 8.29 (s, 1H, H-2'), 7.47 (s, 1H, H-5'), 7.15 (s, 1H, H-4'), 4.73–4.69 (m, 2H, H-3 α and H-29_a), 4.59 (m, 1H, H-29_b), 3.80 (d, J = 10.7 Hz, 1H, H-28_a), 3.34 (d, J = 10.7 Hz, 1H, H-28_b), 2.40 (dt, J = 10.7 Hz, J = 5.7 Hz, 1H, H-19), 1.69 (s, 3H, H-30), 1.04 (s, 3H), 0.99 (s, 3H), 0.96 (s, 3H), 0.95 (s, 3H), 0.89 (s, 3H); ¹³C NMR (CDCl₃, 75 MHz) δ 150.4 (C20), 147.9 (OCO), 136.6 (C2'), 128.9 (C4'), 117.4 (C5'), 109.7 (C29), 87.1 (C3), 60.4 (C28); EI-MS m/z (% rel. intensity): 536 (6) M⁺, 203 (37), 189 (35), 119 (41), 105 (37), 95 (35), 91 (30), 81 (29), 79 (32), 69 (100).

4.1.1.12. 3 β -(1H-imidazole-1-carboxyloxy)-lup-20(29)-en-28-oxic acid (17) and 28-(1H-imidazol-1-yl)-28-oxo-lup-20(29)-en-3 β -yl-1H-imidazole-1-carboxylate (18). The method followed that described for compound **14** but using compound **2** (297 mg, 0.65 mmol) and CDI (527 mg, 3.25 mmol) in anhydrous THF (12 ml) at reflux for 7 h. The resulting yellowish solid was purified by FCC using petroleum ether 40–60 °C/ethyl acetate (2:3) to afford compound **17** (249 mg, 70%): mp (acetone/*n*-hexane) 228–230 °C; IR (film) ν_{\max} 3070, 1762, 1699, 1642 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 8.21 (s, 1H, H-2'), 7.43 (s, 1H, H-5'), 7.12 (s, 1H, H-4'), 4.75 (br s, 1H, H-29_a), 4.69 (dd, J = 10.1 Hz, J = 6.2 Hz, 1H, H-3 α), 4.62 (br s, 1H, H-29_b), 3.04 (dt, J = 10.7 Hz, J = 4.1 Hz, 1H, H-19), 1.70 (s, 3H, H-30), 0.99 (s, 3H), 0.96–0.95 (m, 9H), 0.89 (s, 3H); ¹³C NMR (CDCl₃, 75 MHz) δ 181.5 (C28), 150.5 (C20), 148.3 (OCO), 136.9 (C2'), 129.8 (C4'), 117.2 (C5'), 109.7 (C29), 86.6 (C3); EI-MS m/z (% rel. intensity): 550 (4) M⁺, 203 (41), 189 (50), 187 (40), 175 (42), 159 (37), 119 (47), 91 (41), 79 (36), 69 (100) and compound **18** (88 mg, 23%): mp (acetone/*n*-hexane) 150–151 °C; IR (film) ν_{\max} 3073, 1757, 1722, 1642 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ 8.28 (s, 1H, H2'), 8.13 (s, 1H, H2''), 7.53 (s, 1H, H-5'), 7.41 (s, 1H, H-5''), 7.07 (s, 1H, H-4'') 7.05 (s, 1H, H-4'), 4.78 (br s, 1H, H-29_a), 4.69–4.66 (m, 2H, H-3 α , H-29_b), 2.97 (dt, J = 10.9 Hz, J = 4.5 Hz, 1H, H-19), 1.72 (s, 3H, H-30), 1.01 (s, 3H), 0.95 (s, 9H), 0.91 (s, 3H); ¹³C NMR (CDCl₃, 100 MHz) δ 172.9 (C28) 149.7 (C20), 148.5 (OCO), 137.3 (C2'), 137.0 (C2''), 130.5 (C4''), 129.6 (C4'), 117.4 (C5'), 117.0 (C5''), 110.2 (C29), 86.3 (C3); EI-MS m/z (% rel. intensity): 600 (4) M⁺, 393 (33), 203 (22), 189 (31), 107 (27), 105 (27), 95 (38), 91 (28), 81 (24), 69 (100).

4.1.1.13. 28-Acetoxy-lup-20(29)-en-3 β -yl-1H-imidazole-1-carboxylate (19). The method followed that described for compound **14** but using compound **3** (242 mg, 0.5 mmol) and CDI (162 mg,

1 mmol) in anhydrous THF (10 ml) at reflux for 7 h. The resulting white solid was purified by FCC using petroleum ether 40–60 °C/ethyl acetate (4:1) to afford compound **19** (235 mg, 81%): mp (acetone/*n*-hexane) 194–195 °C; IR (film) ν_{\max} 3070, 1756, 1733, 1642, 1239, 882 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ 8.12 (s, 1H, H-2'), 7.41 (s, 1H, H-5'), 7.07 (s, 1H, H-4'), 4.70–4.66 (m, 2H, H-3 α and H-29_a) 4.60 (s, 1H, H-29_b), 4.26 (d, J = 11.0 Hz, 1H, H-28_a), 3.85 (d, J = 11.0 Hz, 1H, H-28_b), 2.45 (dt, J = 10.8 Hz, J = 5.9 Hz, 1H, H-19), 2.07 (s, 3H, OCOCH₃), 1.69 (s, 3H, H-30), 1.05 (s, 3H), 0.99 (s, 3H), 0.95 (s, 6H), 0.89 (s, 3H); ¹³C NMR (CDCl₃, 100 MHz) δ 171.6 (OCOCH₃), 150.0 (C20), 148.5 (OCO), 137.0 (C2'), 130.5 (C4'), 117.0 (C5'), 109.9 (C29), 86.3 (C3), 62.8 (C28); EI-MS m/z (% rel. intensity): 578 (5), M⁺, 189 (64), 187 (56), 145 (44), 133 (43), 119 (51), 105 (66), 95 (50), 91 (71), 69 (100).

4.1.1.14. 3 β -Acetoxy-lup-20(29)-en-28-yl-1H-imidazole-1-carboxylate (20). The method followed that described for compound **14** but using compound **4** (242 mg, 0.5 mmol) and CDI (162 mg, 1 mmol) in anhydrous THF (10 ml) at reflux for 5 h. The resulting white solid was purified by FCC using petroleum ether 40–60 °C/ethyl acetate (4:1) to afford compound **20** (247 mg, 85%): mp (acetone/*n*-hexane) 101–103 °C; IR (film) ν_{\max} 3073, 1760, 1730, 1642, 1240, 882 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 8.22 (s, 1H, H-2'), 7.45 (s, 1H, H-5'), 7.12 (s, 1H, H-4'), 4.72 (br s, 1H, H-29_a), 4.67–4.63 (m, 2H, H-28_a and H-29_b), 4.47 (dd, J = 10.2 Hz, J = 5.8 Hz, 1H, H-3 α), 4.20 (d, J = 10.6 Hz, 1H, H-28_b), 2.48 (m, 1H, H-19), 2.05 (s, 3H, OCOCH₃), 1.71 (s, 3H, H-30), 1.06 (s, 3H), 1.00 (s, 3H), 0.86 (s, 3H), 0.85 (s, 3H), 0.84 (s, 3H); ¹³C NMR (CDCl₃, 75 MHz) δ 170.9 (OCOCH₃), 149.5 (C20), 148.8 (OCO), 136.8 (C2'), 129.9 (C4'), 117.2 (C5'), 110.3 (C29), 80.8 (C3), 67.4 (C28); EI-MS m/z (% rel. intensity): 578 (19) M⁺, 189 (24), 187 (26), 133 (20), 119 (35), 105 (36), 93 (19), 91 (42), 79 (26), 69 (100).

4.1.1.15. Methyl 3 β -(1H-imidazole-1-carboxyloxy)-lup-20(29)-en-28-oate (21). The method followed that described for compound **14** but using compound **5** (100 mg, 0.2 mmol) and CDI (65 mg, 0.4 mmol) in anhydrous THF (4 ml), at reflux for 6 h. The crude product was purified by FCC eluting with petroleum ether 40–60 °C/ethyl acetate (3:2) to yield compound **21** (105 mg, 88%): mp (acetone/*n*-hexane) 220–224 °C; IR (film) ν_{\max} 3070, 1758, 1725, 1642, 1240 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 8.24 (s, 1H, H2'), 7.45 (s, 1H, H-5'), 7.13 (s, 1H, H-4'), 4.74–4.67 (m, 2H, H-3 α , H-29_a), 4.61 (br s, 1H, H-29_b), 3.67 (s, 3H, COOCH₃), 3.00 (dt, J = 10.3 Hz, J = 3.7 Hz, 1H, H-19), 1.69 (s, 3H, H-30), 0.97 (s, 3H), 0.95 (s, 6H), 0.93 (s, 3H), 0.89 (s, 3H); ¹³C NMR (CDCl₃, 75 MHz) δ 176.6 (C28), 150.5 (C20), 148.1 (OCO), 136.6 (C2'), 129.3 (C4'), 117.2 (C5'), 109.6 (C29), 86.9 (C3); EI-MS m/z (% rel. intensity): 564 (4) M⁺, 203 (35), 190 (28), 189 (71), 187 (38), 175 (35), 119 (43), 105 (33), 91 (37), 79 (33), 69 (100).

4.1.1.16. 3 β -Hydroxy-30-methoxylup-20(29)-en-28-yl-1H-imidazole-1-carboxylate (22) and 30-methoxylup-20(29)-en-3 β ,28-di-yl-(1H-imidazole-1-carboxylate) (23). The method followed that described for compound **14** but using compound **7** (213 mg, 0.45 mmol) and CDI (219 mg, 1.35 mmol) in anhydrous THF (8 ml), at reflux for 8 h. The resulting white solid was purified by FCC eluting with petroleum ether 40–60 °C/ethyl acetate (1:1) to afford compound **22** (174 mg, 68%): mp (acetone/*n*-hexane) 136–138 °C; IR (film) ν_{\max} 3406, 3078, 1760, 1645, 1239 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 8.23 (s, 1H, H-2'), 7.47 (s, 1H, H-5'), 7.13 (s, 1H, H-4'), 4.98 (s, 1H, H-29_a), 4.95 (s, 1H, H-29_b), 4.65 (d, J = 10.8 Hz, 1H, H-28_a), 4.21 (d, J = 10.8 Hz, 1H, H-28_b), 3.89 (s, 2H, H-30), 3.38 (s, 3H, OCH₃), 3.20 (dd, J = 10.8 Hz, J = 5.1 Hz, 1H, H-3 α), 2.39 (dt, J = 11.0 Hz, J = 5.3 Hz, 1H, H-19), 1.07 (s, 3H), 1.02 (s, 3H), 0.98 (s, 3H), 0.84 (s, 3H), 0.78 (s, 3H); ¹³C NMR (CDCl₃, 75 MHz) δ 150.3 (C20), 148.8 (OCO), 136.8 (C2'), 129.9 (C4'), 117.2 (C5'), 109.7

(C29), 78.8 (C3), 74.9 (C30), 67.2 (C28), 58.3 (OCH₃); EI-MS *m/z* (% rel. intensity): 566 (7) M⁺, 187 (30), 119 (34), 105 (32), 93 (32), 91 (46), 81 (33), 79 (39), 69 (100), 67 (29) and compound **23** (83 mg, 28%): mp (acetone/*n*-hexane) 174–176 °C; IR (film) ν_{\max} 3078, 1757, 1645, 1239 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ 8.15 (s, 1H, H-2'), 8.13 (s, 1H, H-2''), 7.43 (s, 1H, H-5'), 7.41 (s, 1H, H-5''), 7.08 (s, 1H, H-4'), 7.07 (s, 1H, H-4''), 4.98 (s, 1H, H-29_a), 4.95 (s, 1H, H-29_b), 4.70–4.62 (m, 2H, H-3 α , H-28_a), 4.18 (d, *J* = 10.7 Hz, 1H, H-28_b), 3.89 (s, 2H, H-30), 3.37 (s, 3H, OCH₃), 2.40 (dt, *J* = 11.0 Hz, *J* = 5.3 Hz, 1H, H-19), 1.09 (s, 3H), 1.03 (s, 3H), 0.96 (s, 6H), 0.91 (s, 3H); ¹³C NMR (CDCl₃, 100 MHz) δ 150.4 (C20), 149.1 (OCO), 148.5 (OCO), 137.0 (C2', C2''), 130.7 and 130.6 (C4', C4''), 117.0 (C5', C5''), 109.9 (C29), 86.2 (C3), 75.1 (C30), 66.9 (C28), 58.4 (OCH₃); EI-MS *m/z* (% rel. intensity): 660 (11) M⁺, 199 (23), 189 (25), 187 (30), 145 (28), 143 (20), 119 (22), 105 (21), 91 (34), 69 (100).

4.1.1.17. 3 β -Hydroxy-(20R)-lupan-29-oxo-28-yl-1H-imidazole-1-carboxylate (24) and (20R)-lupan-29-oxo-3 β ,28-di-yl-(1H-imidazole-1-carboxylate) (25). The method followed that described for compound **14** but using compound **9** (298 mg, 0.65 mmol) and CDI (316 mg, 1.95 mmol) in anhydrous THF (12 ml), at reflux for 7 h. The resulting white solid was purified by FCC eluting with petroleum ether 40–60 °C/ethyl acetate (2:3) to afford compound **24** (239 mg, 67%): mp (acetone/*n*-hexane) 175–177 °C; IR (film) ν_{\max} 3414, 1762, 1722, 1239 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ 9.85 (d, *J* = 1.9 Hz, 1H, H-29), 8.15 (br s, 1H, H-2'), 7.42 (br s, 1H, H-5'), 7.09 (br s, 1H, H-4'), 4.60 (d, *J* = 10.9 Hz, 1H, H-28_a), 4.13 (d, *J* = 10.9 Hz, 1H, H-28_b), 3.21 (dd, *J* = 10.8 Hz, *J* = 5.1 Hz, 1H, H-3 α), 2.64 (m, 1H, H-20), 1.15 (d, *J* = 7.0 Hz, 3H, H-30), 1.07 (s, 3H), 0.98 (s, 6H), 0.85 (s, 3H), 0.77 (s, 3H); ¹³C NMR (CDCl₃, 100 MHz) δ 206.3 (C29), 149.1 (OCO), 137.0 (C2'), 130.6 (C4'), 117.1 (C5'), 78.8 (C3), 66.4 (C28), 48.8 (C20), 14.4 (C30); EI-MS *m/z* (% rel. intensity): 552 (18) M⁺, 207 (46), 189 (58), 187 (47), 145 (49), 119 (45), 105 (51), 91 (58), 79 (48), 69 (100) and compound **25** (128 mg, 30%): mp (acetone/*n*-hexane) 145–146 °C; IR (film) ν_{\max} 1758, 1716, 1239 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 9.85 (d, *J* = 1.9 Hz, 1H, H-29), 8.15 (br s, 2H, H-2', H-2''), 7.43 (br s, 2H, H-5', H-5''), 7.09 (br s, 2H, H-4', H-4''), 4.69 (dd, *J* = 10.9 Hz, *J* = 5.4 Hz, 1H, H-3 α), 4.61 (d, *J* = 10.9 Hz, 1H, H-28_a), 4.13 (d, *J* = 10.9 Hz, 1H, H-28_b), 2.64 (m, 1H, H-20), 1.16 (d, *J* = 7.0 Hz, 3H, H-30), 1.09 (s, 3H), 0.99 (s, 3H), 0.97 (s, 6H), 0.92 (s, 3H); ¹³C NMR (CDCl₃, 75 MHz) δ 206.1 (C29), 148.9 (OCO), 148.4 (OCO), 136.9 (C2', C2''), 130.6 (C4', C4''), 117.0 (C5', C5''), 86.2 (C3), 66.3 (C28); EI-MS *m/z* (% rel. intensity): 646 (6) M⁺, 187 (23), 159 (23), 119 (23), 105 (29), 93 (20), 91 (34), 81 (19), 79 (22), 69 (100).

4.1.1.18. 28-(1H-imidazol-1-yl)-lup-20(29)-en-3,28-dione (26).

The method followed that described for compound **14** but using compound **11** (205 mg, 0.45 mmol) and CDI (219 mg, 1.35 mmol) in anhydrous THF (8 ml), at reflux for 9 h. The crude product was purified by FCC eluting with petroleum ether 40–60 °C/ethyl acetate (3:2) to yield compound **26** (203 mg, 89%): mp (benzene) 189–190 °C; IR (film) ν_{\max} 3073, 1721, 1703, 1642 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ 8.28 (s, 1H, H-2'), 7.54 (s, 1H, H-5'), 7.05 (s, 1H, H-4'), 4.78 (s, 1H, H-29_a), 4.65 (s, 1H, H-29_b), 2.97 (dt, *J* = 10.7 Hz, *J* = 4.2 Hz, 1H, H-19), 1.71 (s, 3H, H-30), 1.06 (s, 3H), 1.01 (br s, 6H), 0.96 (s, 3H), 0.94 (s, 3H); ¹³C NMR (CDCl₃, 100 MHz) δ 217.9 (C3), 172.9 (C28), 149.7 (C20), 137.3 (C2'), 129.6 (C4'), 117.4 (C5'), 110.2 (C29); EI-MS *m/z* (% rel. intensity): 504 (4) M⁺, 410 (30), 409 (100), 245 (46), 203 (49), 189 (58), 147 (25), 107 (31), 105 (27), 91 (24).

4.1.1.19. 28-(1H-imidazol-1-yl)-lup-1,20(29)-dien-3,28-dione (27). The method followed that described for compound **14** but using compound **12** (204 mg, 0.45 mmol) and CDI (219 mg,

1.35 mmol) in anhydrous THF (8 ml), at reflux for 9 h. The crude product was purified by FCC eluting with petroleum ether 40–60 °C/ethyl acetate (3:2) to yield compound **27** (186 mg, 82%): mp (acetone/*n*-hexane) 95–96 °C; IR (film) ν_{\max} 3070, 1762, 1720, 1668, 1645 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ 8.29 (s, 1H, H-2'), 7.54 (s, 1H, H-5'), 7.12 (d, *J* = 10.1 Hz, 1H, H-1), 7.05 (s, 1H, H-4'), 5.80 (d, *J* = 10.1 Hz, 1H, H-2), 4.79 (s, 1H, H-29_a), 4.67 (s, 1H, H-29_b), 2.98 (dt, *J* = 11.0 Hz, *J* = 4.6 Hz, 1H, H-19), 1.72 (s, 3H, H-30), 1.12 (s, 3H), 1.07 (s, 6H), 1.01 (s, 3H), 1.00 (s, 3H); ¹³C NMR (CDCl₃, 100 MHz) δ 205.4 (C3), 172.6 (C28), 159.6 (C1), 149.5 (C20), 137.1 (C2'), 128.8 (C4'), 125.2 (C2), 117.5 (C5'), 110.3 (C29); EI-MS *m/z* (% rel. intensity): 502 (4) M⁺, 408 (32), 407 (100), 243 (43), 205 (30), 203 (26), 189 (34), 135 (25), 105 (28), 91 (29).

4.1.1.20. 2-Hydroxy-28-(1H-imidazol-1-yl)-lup-1,20(29)-dien-3,28-dione (28) and 28-(1H-imidazole-1-yl)-3,28-dioxo-lup-1,20(29)-dien-2-yl-1H-imidazole-1-carboxylate (29).

The method followed that described for compound **14** but using compound **13** (304 mg, 0.65 mmol) and CDI (524 mg, 3.25 mmol) in anhydrous THF (12 ml), at reflux for 8 h. The resulting white solid was purified by FCC eluting with petroleum ether 40–60 °C/ethyl acetate (1:4) to afford compound **28** (237 mg, 70%): mp (acetone/*n*-hexane) 145–148 °C; IR (film) ν_{\max} 3448, 3078, 1762, 1724, 1667, 1645 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ 8.43 (s, 1H, H-2'), 7.56 (s, 1H, H-5'), 7.09 (s, 1H, H-4'), 6.45 (s, 1H, H-1), 4.79 (s, 1H, H-29_a), 4.67 (s, 1H, H-29_b), 2.97 (dt, *J* = 10.6 Hz, *J* = 4.0 Hz, 1H, H-19), 1.72 (s, 1H, H-30), 1.19 (s, 3H), 1.14 (s, 3H), 1.10 (s, 3H), 0.99 (br s, 6H); ¹³C NMR (CDCl₃, 100 MHz) δ 201.1 (C3), 172.9 (C28), 149.5 (C20), 143.9 (C2), 137.3 (C2'), 129.6 (C4'), 128.8 (C1), 117.4 (C5'), 110.4 (C29); EI-MS *m/z* (% rel. intensity): 518 (22) M⁺, 423 (100), 215 (94), 213 (65), 189 (57), 119 (55), 105 (54), 91 (89), 79 (54), 69 (55) and compound **29** (109 mg, 27%): mp (acetone/*n*-hexane) 211–213 °C; IR (film) ν_{\max} 3070, 1826, 1757, 1721, 1642 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 8.42 and 8.27 (both s, each 1H, H-2', H-2''), 7.79 and 7.51 (both s, each 1H, H-5', H-5''), 7.21 and 7.04 (both s, each 1H, H-4', H-4''), 6.97 (s, 1H, H-1), 4.74 (br s, 1H, H-29_a), 4.67 (br s, 1H, H-29_b), 2.92 (dt, *J* = 10.9 Hz, *J* = 4.6 Hz, 1H, H-19), 1.73 (s, 3H, H-30), 1.36 (s, 3H), 1.17 (s, 3H), 1.16 (s, 3H), 1.00 (s, 3H), 0.89 (s, 3H); ¹³C NMR (CDCl₃, 75 MHz) δ 202.3 (C3), 172.8 (C28), 152.2 (C20), 149.1 (OCO), 146.5 (C2), 137.2 (C2' and C2''), 132.6 (C1), 129.6 (C4' and C4''), 117.3 (C5' and C5''), 110.6 (C29); EI-MS *m/z* (% rel. intensity): 612 (9) M⁺, 518 (35), 517 (100), 449 (19), 405 (14), 295 (15), 189 (27), 105 (18), 91 (21), 69 (16).

4.2. Antiproliferative activity

4.2.1. General

All cell lines were purchased from the American Type Culture Collection (ATCC, Rockville, MD). RPMI 1640 medium, Dulbecco's Modified Eagle Medium (DMEM–D5796), Dulbecco's Phosphate Buffered Saline (DPBS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) powder and dimethylsulfoxide (DMSO) were obtained from Sigma–Aldrich Co. Penicillin/streptomycin (P/S) and L-glutamine were obtained from Gibco-BRL. Sodium pyruvate and trypsin/EDTA (0.05%/0.02%) were obtained from Biological Industries. Fetal Bovine Serum (FBS) were purchased from PAA Laboratories and XTT kit was obtained from Roche.

A-375, HT-29, HeLa, MIA PaCa-2 and SH-SY5Y cells were routinely maintained in DMEM supplemented with 10% heat-inactivated FBS and 1% P/S solution. HepG2 cells were maintained in DMEM supplemented with 10% heat-inactivated FBS, 1% P/S and 1 mM of sodium pyruvate. Jurkat cells were grown in RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 1% P/S

and 2 mM L-glutamine. MCF-7 were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 0.2% P/S, 2 mM L-glutamine, 1 mM of sodium pyruvate and 0.1 mg/ml of insulin.

All cell cultures were incubated at 37 °C in 5% CO₂ saturated atmosphere and 99% humidity.

4.2.2. Viability assay

The cell viability of HeLa and HepG2 cells in the presence of the synthesized compounds was determined by the MTT assay. Briefly, exponentially growing cells were plated in 96-well plates at a density of 1×10^3 and 8×10^3 cells/well respectively and incubated for 24 h before treatment. The growth medium was replaced with one containing either the test compounds dissolved in DMSO (final DMSO concentration <0.1%) at different concentrations or only DMSO, in triplicate wells, and cells were incubated for 72 h. After incubation with the compounds, the medium was removed and MTT solution (0.5 mg/ml, 100 µl) was added to each well and the plates were incubated again for 1 h. MTT solution was removed and DMSO (100 µl) was then added to dissolve the formazan crystals. The plates were immediately read at 550 nm on an ELISA plate reader (Tecan Sunrise MR20-301, TECAN, Austria).

For Jurkat cells, the cell viability was determined by XTT assay. Briefly, exponentially growing cells were plated in 96-well plates at a density of 5.5×10^3 cells/well, treated with different concentrations of compounds or vehicle (medium with DMSO) in triplicate and incubated for 72 h. After incubation with the compounds the XTT labeling mixture (100 µl) was added to each of the wells and after a 4 h incubation period the plates were read at 450 nm on an ELISA plate reader.

Concentrations that inhibit cell growth by 50% (IC₅₀) compared to non-treated cells were determined by non-linear regression with Graphpad Prism software version 4.0 (GraphPad Software, Inc., San Diego, CA). All the IC₅₀ results represent an average of a minimum of three experiments and were expressed as means ± standard deviation (SD). Compound **2** was used as a positive control.

For the screening of antiproliferative activity, cell suspensions were prepared and diluted according to the particular cell type and the expected target cell density (1×10^3 – 30×10^3 cells/well based on the cell growth characteristics). Cells were plated in 96-well plates and cell viability was determined by the MTT assay (MCF-7, HepG2, A-375, HT-29, HeLa and MIA PaCa-2) or XTT assay (Jurkat and SH-SY5Y) after 72 h of incubation with the different derivatives at the concentration of 20 µM, in triplicate.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.07.050.

References and notes

- Setzer, W. N.; Setzer, M. C. *Mini-Rev. Med. Chem.* **2003**, *3*, 540.
- Kinghorn, A. D.; Su, B. N.; Jang, D. S.; Chang, L. C.; Lee, D.; Gu, J. Q.; Carcache-Blanco, E. J.; Powlus, A. D.; Lee, S. K.; Park, E. J.; Cuendet, M.; Gills, J. J.; Bhat, K.; Park, H. S.; Mata-Greenwood, E.; Song, L. L.; Jong, M. H.; Pezzuto, J. M. *Planta Med.* **2004**, *70*, 691.
- Krasutsky, P. A. *Nat. Prod. Rep.* **2006**, *23*, 919.
- Kim, D. S. H. L.; Chen, Z.; Nguyen, T.; Pezzuto, J. M.; Qiu, S.; Lu, Z.-Z. *Synth. Commun.* **1997**, *27*, 1607.
- Dzubak, P.; Hajdich, M.; Vydra, D.; Hustova, A.; Kvasnica, M.; Biedermann, D.; Markova, L.; Urban, M.; Sarek, J. *Nat. Prod. Rep.* **2006**, *23*, 294.
- Tolstikova, T. G.; Sorokina, I. V.; Tolstikov, G. A.; Tolstikov, A. G.; Flekhter, O. B. *Russ. J. Bioorg. Chem.* **2006**, *32*, 37.
- Pisha, E.; Chai, H.; Lee, I. S.; Chagwedera, T. E.; Farnsworth, N. R.; Cordell, G. A.; Beecher, C. W. W.; Fong, H. H. S.; Kinghorn, A. D.; Brown, D. M.; Wani, M. C.; Wall, M. E.; Hieken, T. J.; Dasgupta, T. K.; Pezzuto, J. M. *Nat. Med.* **1995**, *1*, 1046.
- Schmidt, M. L.; Kuzmanoff, K. L.; Indeck, L. L.; Pezzuto, J. M. *Eur. J. Cancer* **1997**, *33*, 2007.
- Fulda, S.; Jeremias, I.; Steiner, H. H.; Pietsch, T.; Debatin, K.-M. *Int. J. Cancer* **1999**, *82*, 435.
- Thurnher, D.; Turhani, D.; Pelzmann, M.; Wannemacher, B.; Knerer, B.; Formanek, M.; Wachek, V.; Selzer, E. *Head Neck* **2003**, *25*, 732.
- Kessler, J. H.; Mullauer, F. B.; de Roo, G. M.; Medema, J. P. *Cancer Lett.* **2007**, *251*, 132.
- Fulda, S.; Friesen, C.; Los, M.; Scalfidi, C.; Mier, W.; Benedict, M.; Nunez, G.; Krammer, P. H.; Peter, M. E.; Peter, M. E.; Debatin, K. M. *Cancer Res.* **1997**, *57*, 4956.
- Wick, W.; Grimmel, C.; Wagenknecht, B.; Dichgans, J.; Weller, M. *J. Pharmacol. Exp. Ther.* **1999**, *289*, 1306.
- Zuco, V.; Supino, R.; Righetti, S. C.; Cleris, L.; Marchesi, E.; Passerini, C. G.; Formelli, F. *Cancer Lett.* **2002**, *175*, 17.
- Eiznhamer, D. A.; Xu, Z.-Q. *IDrugs* **2004**, *7*, 359.
- Development therapeutics program national cancer institute/national institutes of health, B., MD, USA. <http://dtp.nci.nih.gov/dtpstandards/cancerscreeningdata/index.jsp>.
- Cichewicz, R. H.; Kouzi, S. A. *Med. Res. Rev.* **2004**, *24*, 90.
- Kvasnica, M.; Sarek, J.; Klinotova, E.; Dzubak, P.; Hajdich, M. *Bioorg. Med. Chem.* **2005**, *13*, 3447.
- Sarek, J.; Kvasnica, M.; Urban, M.; Klinot, J.; Hajdich, M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4196.
- Shintyapina, A. B.; Shults, E. E.; Petrenko, N. I.; Uzenkova, N. V.; Tolstikov, G. A.; Pronkina, N. V.; Kozhevnikov, V. S.; Pokrovsky, A. G. *Russ. J. Bioorg. Chem.* **2007**, *33*, 579.
- Kim, D. S. H. L.; Pezzuto, J. M.; Pisha, E. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1707.
- Jeong, H. J.; Chai, H. B.; Park, S. Y.; Kim, D. S. H. L. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1201.
- Kim, J. Y.; Koo, H.-M.; Kim, D. S. H. L. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2405.
- Flekhter, O. B.; Karachurina, L. T.; Nigmatullina, L. R.; Sapozhnikova, T. A.; Baltina, L. A.; Zrudii, F. S.; Galin, F. Z.; Spirikhin, L. V.; Tolstikov, G. A.; Plyasunova, O. A.; Pokrovskii, A. G. *Russ. J. Bioorg. Chem.* **2002**, *28*, 494.
- Hata, K.; Hori, K.; Takahashi, S. *J. Nat. Prod.* **2002**, *65*, 645.
- Baglin, I.; Poumaroux, A.; Nour, M.; Tan, K.; Offer, A. C. M.; Dubois, M. A. L.; Chauffert, B.; Cavé, C. *J. Enzyme Inhib. Med. Chem.* **2003**, *18*, 111.
- Hata, K.; Hori, K.; Ogasawara, H.; Takahashi, S. *Toxicol. Lett.* **2003**, *143*, 1.
- Mukherjee, R.; Jaggi, M.; Siddiqui, M. J. A.; Srivastava, S. K.; Rajendran, P.; Vardhan, A.; Burman, A. C. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4087.
- Mukherjee, R.; Jaggi, M.; Rajendran, P.; Srivastava, S. K.; Siddiqui, M. J. A.; Vardhan, A.; Burman, A. C. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3169.
- Mukherjee, R.; Jaggi, M.; Rajendran, P.; Siddiqui, M. J. A.; Srivastava, S. K.; Vardhan, A.; Burman, A. C. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2181.
- Uzenkova, N. V.; Petrenko, N. I.; Shakirov, M. M.; Shul'ts, E. E.; Tolstikov, G. A. *Chem. Nat. Compd.* **2005**, *41*, 692.
- Sorokina, I. V.; Tolstikova, T. G.; Zhukova, N. A.; Petrenko, N. I.; Uzenkova, N. V.; Shul'ts, E. E.; Popova, N. A. *Bull. Exp. Biol. Med.* **2006**, *142*, 69.
- Gauthier, C.; Legault, J.; Lebrun, M.; Dufour, P.; Pichette, A. *Bioorg. Med. Chem.* **2006**, *14*, 6713.
- Urban, M.; Sarek, J.; Kvasnica, M.; Tislerova, I.; Hajdich, M. *J. Nat. Prod.* **2007**, *70*, 526.
- Rajendran, P.; Jaggi, M.; Singh, M. K.; Mukherjee, R.; Burman, A. C. *Invest. New Drugs* **2008**, *26*, 25.
- Kumar, V.; Rani, N.; Aggarwal, P.; Sanna, V. K.; Singh, A. T.; Jaggi, M.; Joshi, N.; Sharma, P. K.; Irchhaiya, R.; Burman, A. C. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5058.
- Liby, K.; Honda, T.; Williams, C. R.; Risingsong, R.; Royce, D. B.; Suh, N.; Dinkova-Kostova, A. T.; Stephenson, K. K.; Talalay, P.; Sundararajan, C.; Gribble, G. W.; Sporn, M. B. *Mol. Cancer Ther.* **2007**, *6*, 2113.
- Ray, S.; Chaturvedi, D. *Drugs Future* **2004**, *29*, 343.
- Moreira, V. M. A.; Vasaitis, T. S.; Guo, Z. Y.; Njar, V. C. O.; Salvador, J. A. R. *Steroids* **2008**, *73*, 1217.
- Salvador, J. A. R.; Santos, R. C.; Cascante, M. P.T. Patent App. 20091000049475, 2009.
- Totleben, M. J.; Freeman, J. P.; Szmuszkovicz, J. *J. Org. Chem.* **1997**, *62*, 7319.
- Tang, Y. Q.; Dong, Y. X.; Vennerstrom, J. L. *Synthesis* **2004**, 2540.
- Freyne, E.; Raeymaekers, A.; Venet, M.; Sanz, G.; Wouters, W.; De Coster, R.; Van Wauwe, J. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 267.
- Pouget, C.; Fagnere, C.; Basly, J. P.; Habrioux, G.; Chulia, A. J. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2859.
- Tafi, A.; Costi, R.; Botta, M.; Di Santo, R.; Corelli, F.; Massa, S.; Ciacci, A.; Manetti, F.; Artico, M. *J. Med. Chem.* **2002**, *45*, 2720.
- Njar, V. C. O.; Nnane, I. P.; Brodie, A. M. H. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1905.

47. Mulvihill, M. J.; Cesario, C.; Smith, V.; Beck, P.; Nigro, A. *J. Org. Chem.* **2004**, *69*, 5124.
48. Silva, M. R.; Beja, A. M.; Moreira, V. M.; Santos, R. C.; Salvador, J. A. R. *Acta Crystallogr., Sect. E* **2007**, *63*, O4824.
49. D'Addona, D.; Bochet, C. G. *Tetrahedron Lett.* **2001**, *42*, 5227.
50. Herbez, V.; Fischer, F. *Tetrahedron Lett.* **2005**, *46*, 6797.
51. Paul, R.; Anderson, G. W. *J. Am. Chem. Soc.* **1960**, *82*, 4596.
52. Anderson, G. W.; Paul, R. *J. Am. Chem. Soc.* **1958**, *80*, 4423.
53. Rannard, S. P.; Davis, N. J. *Org. Lett.* **2000**, *2*, 2117.
54. Tietze, L. F.; Heinzen, H.; Moyna, P.; Rischer, M.; Neunaber, H. *Liebigs Ann. Chem.* **1991**, *12*, 1245.
55. Okamoto, I.; Takeya, T.; Kagawa, Y.; Kotani, E. *Chem. Pharm. Bull.* **2000**, *48*, 120.
56. Walker, D.; Hiebert, J. D. *Chem. Rev.* **1967**, *67*, 153.
57. Urban, M.; Sarek, J.; Klinot, J.; Korinkova, G.; Hajduch, M. *J. Nat. Prod.* **2004**, *67*, 1100.
58. Pramanick, S.; Mandal, S.; Mukhopadhyay, S.; Jha, S. *Synth. Commun.* **2005**, *35*, 2143.