A Review of Natural and Modified Betulinic, Ursolic and Echinocystic Acid Derivatives as Potential Antitumor and Anti-HIV Agents

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Abstract. The aim of this review is to update current knownledge on the betulinic, ursolic and echinocystic acids and their natural and semisynthetic analogs, focussing on their cytotoxic and anti-HIV activities. Then, the last results of the authors' team on unusual semisynthetic derivatives of these triterpenoids will be presented in order to establish structure/activity relationships.

INTRODUCTION

Cancer and AIDS are the two major pathologies besides the cardiovascular diseases which affect a hudge number of people in the world. The therapeutic benefit of plant or synthetic derivatives against AIDS is very poor. On the contrary, natural products such as Vinca-alkaloids, taxoids, camptothecin and podophyllotoxin represent some of the most important drugs currently used to decrease the progression of human cancer. The development of combinatorial chemistry, molecular biology, genomic, proteomic technologies stimulates the search for novel anticancer and anti AIDS chemotherapeutic drugs. Recent developments in the biological and pharmacological activities of saponins (triterpene and steroid glycosides) from natural sources revealed the important role of many acylated compounds having anticancer, immunoadjuvant, antiviral, antiinflammatory, hypocholesterolemic and hypoglycemic activities [1]. This has prompted us to develop methods in the acylation and glycosylation of triterpenic acids in order to establish structure/activity relationships.

Since natural derivatives of betulinic (**1**), ursolic (**2**) and echinocystic (**3**) acids were shown to present anticancer and anti HIV activities, we have therefore considered that synthesis of derivatives of these three triterpenic acids which are commercially available could be of great value in discovering potential bioactive semi-synthetic compounds with cancer and AIDS related activities [2].

Therefore, the current review will highlight the updated reports on bioactive natural and semi-synthetic analogs of the three above mentionated triterpenic acids. Then, we will summarize the latest research developments of our group in the synthesis and biological evaluation of various modified triterpenoids as potential cytotoxic and anti-HIV agents [2]. An approach of a structure/activity relationship study will be discussed.

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I. CANCER RELATED ACTIVITIES

A. Betulinic Acid and Derivatives

1. Betulinic Acid

Betulinic acid (3β-hydroxy-lup-20(29)-en-28-oic acid) (**1**) is a triterpene isolated from various plants from a screen of about 2500 extracts tested for cytotoxicity by the NCI [3]. The main species which are reported to contain **1** are *Tryphyllum peltatum, Ancistrocladus heyneus, Diospyros leucomelas, Tetracera boliviana, Zizyphus joazeiro, Z. mauritania, Syzygium formosanum,* etc....

The cytotoxicity of **1** was evaluated against a panel of human melanoma cancer lines, including MEL-1 (lymph nodes), -2 (pleural fluids), -3 (liver) and -4 (primary skin tumor) (ED_{50} = 1-5 μg/ml) [3]. Since **1** was not effective on the growth of other cancer cell types, it was concluded to be an inhibitor of growth of a number of different melanoma cells with unusual specificity in the contrary of various other antineoplasic agents such as for example ellipticine, vinblastine, vincristine, taxol which showed intense cytotoxicity with no discernable cell-type selectivity.

This activity was confirmed by experiments *in vivo* [3]. After subcutaneously injection of human MEL-2 cells to athymic (nude) mice, **1** demonstrated highly effective tumor growth inhibition ($P<0.001$) for each of the doses tested (50, 250 and 500 mg per kg body weight) and the response was durable after three weeks following the end of treatment. A highly significant decrease in tumor size ($P<0.0001$) that corresponded to over 80% regression was observed with MEL-1 treated animal, with a favorable therapeutic index.

Then the mechanism of action was investigated. Apoptosis is characterized by a series of distinct morphological and biochemical changes (DNA fragmentation, nuclear condensation, cell shrinkage and blebbing, caspase activation) which promote the physiologically silent removal of cells by phagocytic neighbors. These events are mediated by the activation of caspases, which orchestrate apoptosis via the cleavage of key substrates [42]. Treatment of MEL-2 cells with **1** led to apoptosis-related responses such as morphologic changes and formation of 50-kbp DNA fragments. These results showed **1** to be an attractive and promising new lead compound. A closely related compound betulin (**4**) can be isolated from

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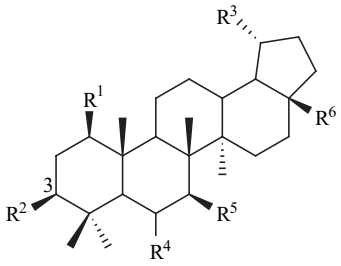
Betula species and smoothly converted to **1** in good yield. Thus the interesting bioactivity with lack of toxicity described above and the natural abundance of *Betula* species suggested the preclinical and clinical development of **1**. Another study showed that **1** was an interesting candidate, not only as a single agent but also in combination with radiotherapy [4]. It was also shown that human metastatic C8161 melanoma cells showed greater DNA fragmentation and growth arrest and earlier loss of viability in response to **1** than their non metastatic C8161/neo 6.3 counterpart [5]. Furthermore, **1** was shown to induce mitochondrial cytochrome c release and DNA fragmentation in some melanoma cell populations [6].

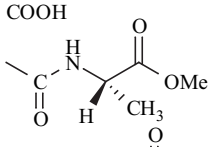
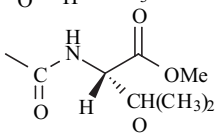
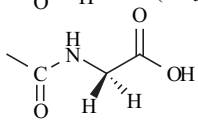
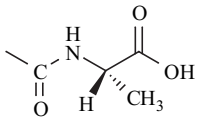
The antimelanoma activity was also assessed in experiments which also showed that **1** was an inhibitor of aminopeptidase N activity in a dose-dependent manner (IC_{50} = 7.3 ± 1.4 μM). This activity was higher than that of bestatin, a well known inhibitor of this enzyme (IC_{50} = 16.9 ± 4.1 μM) [7]. Aminopeptidase N is tightly associated with extracellular matrix components, and its inhibition seems to prevent the melanoma invasion into basement membranes and by that metastasis [8]. This assumption was supported by reports about the inhibition of tumor cell invasion by a variety of aminopeptidase inhibitors including bestatin, actinonin, leuhistin and matlystatin [7].

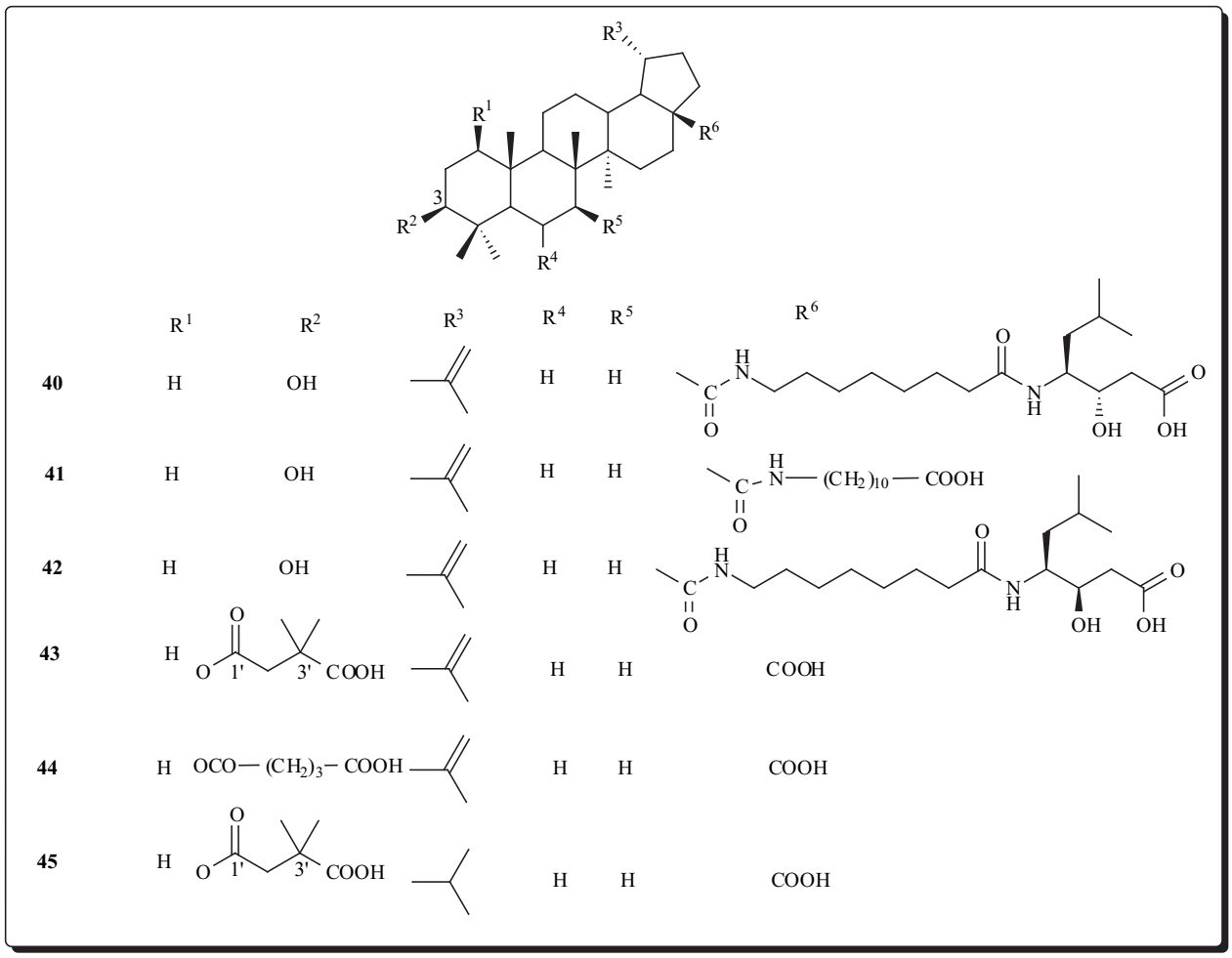
1 was shown to be cytotoxic in neuroectodermal tumors, such as neuroblastoma, medulloblastoma, and Ewing's sarcoma, representing the most common solid tumors of childhood [9-12]. Apoptosis by **1** was independent of CD95-ligand/receptor interaction and accumulation of wild-type p53 protein, but depended on activation of caspases and involves ROS formation [9]. CD 95 is a cell surface receptor of the TNF/nerve growth factor receptor superfamily expressed on a variety of normal and neoplastic cells. An analogous phenomenon was observed in apoptosis induced by **1** in glioma cells [13]. **1** triggers apoptosis via a direct effect on mitochondria inducing alteration of mitochondrial permeability transition resulting in the release of apoptogenic proteins such as cytochrome c into the cytosol, where they activate caspases, endonucleases and nuclear fragmentation [14,15]. **1** was shown to trigger apoptosis in medulloblastoma and glioblastoma cell lines in a dose-dependent manner, as determined by DNA fragmentation (ED_{50} = 3-15 μg/ml for medulloblastoma and ED_{50} = 5-16 μg/ml for glioblastoma cell lines) [16]. The absence of cytotoxicity against murine non malignant neuronal cells *in vitro*, indicates that **1** may be a selective cytotoxic compound for malignant brain-tumor cells that clearly warrants further preclinical and clinical evaluation [16]. Some findings suggested that KILLER/DR5 (a death receptor gene) might be an important target in melanoma and glioblastoma cells as it was induced independent of p53 in cells that are undergoing apoptosis [17]. Another study has shown that **1** causes a significant increase of intracellular-free levels of Ca^{2+} and a mild decrease in viability in MDCK renal tubular cells. These results may help to explain the diverse *in vitro* and *in vivo* effects of **1** [18]. DNA polymerase β, is believed to play an important role in repairing DNA damage after exposure to some anticancer drugs such as cisplatin and bleomycin [19]. Although several DNA β polymerase inhibitors including triterpenoids [20] have been reported, more potent agents are still needed, which may consequently be able to potentiate the activity of DNA-damaging antitumor agents. **1** was shown to inhibit DNA polymerase β (IC_{50} = 14 μM with bovin serum albumin (BSA) and 6.5 μM in the absence of BSA). Furthermore, **1** was shown to potentiate the bleomycin cytotoxicity in cultured P-388 D1 cells [19].

The *in vivo* studies showed that **1** might be valuable as anti-tumor-promoter in chemical carcinogenesis. **1** showed a good inhibitory effect on mouse skin tumor promotion on the basis of the two stage (7,12-dimethylbenz[*a*]anthracene/12-*O*-tetradecanoylphorbol-13-acetate) (DMBA/TPA) carcinogenesis *in vivo* [21]. When **1** at 5 μM was administered before each TPA treatment, it reduced the number of papillomas per mouse on mouse skin (60% reduction) as compared to the control.

Since **1** is undergoing preclinical development for treatment of malignant melanoma, an important factor is the evaluation of its mammalian metabolism. A prospective approach has been undertaken using microorganisms particularly fungi, as *in vitro* model systems [22]. The fungal metabolite of **1** from resting-cell suspensions of *Cunninghamella* species NRRL 5695 was identified as the 28-*O*-β-D-glucopyranosyl ester of **1** (**5**). Since this molecule was not active against the four melanoma cell lines (ED_{50} >20 μg/ml) when compared to **1**, the results suggested that the free COOH group in **1** is essential for cytotoxic activity. Namely, the *in vitro* antimelanoma activity of **1** was considerably attenuated when the carboxylic acid was converted to the methyl ester, the aldehyde group, or a methyl group [22]. Further bioconversions of **1** resulted in the production of the known betulonic acid (**6**) and two new metabolites, 3β,7β-dihydroxy-lup-20(29)-en-28-oic acid (**7**) and 3β,6α,7β-trihydroxy-lup-20(29)-en-28-oic acid (**8**) from cultures of *Bacillus megaterium* ATCC 14581. Another new metabolite characterized as 1β,3β,7β-trihydroxy-lup-20(29)-en-28-oic acid (**9**) was obtained from culture of *Cunninghamella elegans* ATCC 9244. The antimelanoma activity of **6**-**9** was evaluated against MEL-1 and -2 melanoma cell lines [23]. Compared to **1** (ED_{50} = 3.3, 1.0 μg/ml respectively), **9** showed no activity (ED_{50}>20μg/ml), whereas **7** and **8** were less active against both cell lines. **6** (ED_{50} = 0.1 μg/ml) was more active than **1** (ED_{50} = 1.0 μg/ml) against MEL-2 with a high degree of selectivity in comparison with **1** and the other tested metabolites. The cytotoxicity results indicate that the *in vitro* antimelanoma activity of **1** was significantly altered by oxidation at different sites of the molecules and that the structural requirements for cytotoxicity of the tested molecules differ from one melanoma cell line to the others [23]. A rapid and sensitive method of HPLC/MS was used to determine the tissue distribution of **1** in mice [24]. When athymic mice bearing human melanoma were treated with **1** (500 mg/kg), the tumor showed the highest concentration (452.2 ± 261.2 μg/g), which was nearly twice the level observed in the liver (233 ± 80.3 μg/g). This factor may help to explain the manner in which **1** can inhibit tumor growth without apparent toxicity. A pharmacokinetic study in mice showed that the distribution of **1** in tissues at 24h post IP-administration in a descending order was as follows: perirenal fat, ovary, spleen, mammary gland, uterus, bladder, lymph node, liver, small intestine, caecum, lung, thymus, colon, kidney, skin, heart and brain [25].



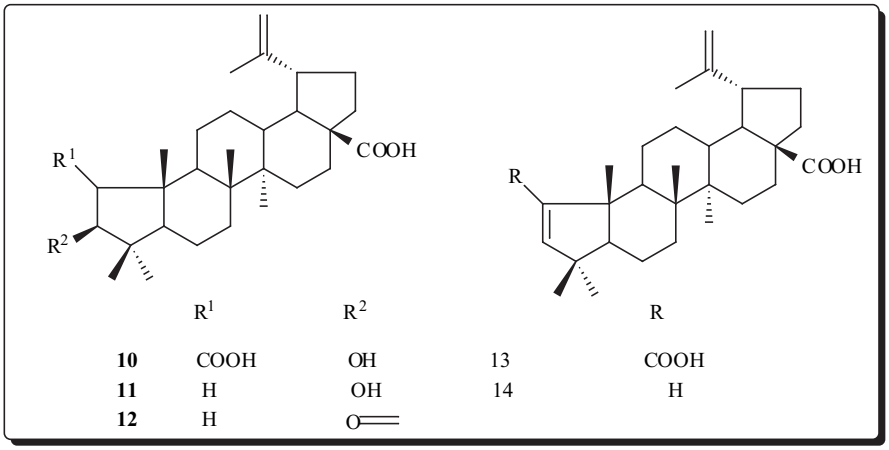
	R^1	R^2	R^3	R^4	R^5	R^6
1	H	OH		H	H	COOH
4	H	OH		H	H	CH_2OH
5	H	OH		H	H	COO-β-D-Glc
6	H	O		H	H	COOH
7	H	OH		H	OH	COOH
8	H	OH		OH	OH	COOH
9	OH	OH		H	OH	COOH
15	H	CH_3O-N		H	H	COOH
16	H	HO-N		H	H	COOH
17	H	H_2N		H	H	COOH
18	H	HO-N		H	H	CHNOH
19	H	O		H	H	COOH
20	H	HO		H	H	COOH
21	H	HO-N		H	H	CHNOH
22	H	CH_3O-N		H	H	COOH
23	H	OH		H	H	
24	H	OH		H	H	
25	H	OH		H	H	
26	H	OH		H	H	



2. Modified Betulinic Acid Derivatives

The ring-A homologue of **1** named ceanothic acid (**10**) was isolated from *Paliurus ramossisimus* (Rhamnaceae) and the cytotoxic activity of synthetic analogs of this acid was recently reported [26]. 1-norceanothane derivatives such as **11**, **12**, **14** are more potent than the other ceanothane analogs tested. It seems that the exo-ring C-1 substitution decreases cytotoxicity, especially polar groups such as in **10** and **13**. The most cytotoxic compound against OVCAR-3 and Hela cancer cell lines was **12** (1-decarboxy-3-oxo-ceanothic acid) (IC_{50} = 2.8 and 6.6. μg/ml, respectively, and 11.3 μg/ml against normal cell line FS-5 [26]). **14** showed about the same potency as **12** on tumor cells but it was two times less toxic to normal cells than was **12**. This observation is informative to develop a lead compound having selective cytotoxicity. Regarding some structure/activity relationships, the double bond between C-20 and C-30 and the 3-OH group are not essential to the cytotoxic activity of 1-norceanothane derivatives. However the 17-COOH group is required for this activity, as was reported in another biological activity of **1** [65].

Another study reported that modification of **1** at C-3, C-20, and C-28 positions (**4**, **6**, **15**-**22**) can produce a number of potentially important derivatives, which may improve the



selective cytotoxic activity after their evaluation against MEL-2 and epidermoid carcinoma of the mouth (KB) cell lines [27]. The introduction of the methoxy oxime (CH_3O-N=) at position C-3 resulted in a loss of cytotoxicity against MEL-2. The loss of cytotoxicity of a compound possessing a phenyl carboxylic group at C-3 suggested a size limitation at position C-3. The other C-3 modified compounds with the keto group, a hydroxy oxime (HO-N=) showed a good cytotoxic activity (IC_{50} = 0.9 - 5.8 μg/ml). **17** showed about the same cytotoxic effect (IC_{50} = 1.3 μg/ml) as **1** but the comparison with KB - cytotoxicity was not enough clear to establish structure requirements for a melanoma selective cytotoxicity. A comparison between hydrogenated compounds **19** and **21** and the non-hydrogenated **6** and **16** suggests that the hydrogenation of the C-17 side chain was not important for biological activity. However the loss of antimelanoma activity of **20** and **22** (IC_{50}> 20 μg/ml) when compared to **1** (IC_{50} = 1.2 μg/ml) and **16** (IC_{50} = 8.3 μg/ml), respectively, indicates that the functionality at this position should be taking into account in the synthesis of other analogs [27]. This study also showed the importance of the COOH free group at C-28 because **4** (CH_2OH-28), and other derivatives substituted by CHO, $COOCH_3$, CH_3 at C-28 showed a decreased cytotoxic activity with an IC_{50} ranging from 7.4 μg/ml to >20 μg/ml. **18** with a CHNOH group at C-28 improved general cytotoxic effects (IC_{50} = 2.2 μg/ml in MEL-2 cells; IC_{50} = 3.3 μg/ml in KB cells) but showed the loss of selectivity [27].

A series of free acid and methyl ester of amino acid conjugates of **1** have been synthesized and evaluated for their cytotoxic activity against MEL-2 and KB cell lines [28]. Methyl ester of alanine (**23**) and valine (**24**) conjugates and free acid of glycine (**25**) conjugate showed toxicity against MEL-2 comparable to **1** with ED_{50} of 3.5, 2.1, 4.2, and 4.2 μg/ml, respectively. Free acid of alanine (**26**) conjugate showed the best toxicity profile (ED_{50} = 1.5 μg/ml) against MEL-2 cells; however it showed toxicity against KB cells (ED_{50} = 4.6 μg/ml). **23**-**25** also demonstrated remarkably improved water solubility as well as selective cytotoxicity. However, a more extensive investigation using a greater number of derivatives is needed for SAR study in order to synthesise more effective derivatives of **1**.

Derivatives of **1** modified at C-20 were evaluated for their cytotoxicity potential using different cell lines than previously tested. Human colon carcinoma cell line (HCT 116) and human prostate adenocarcinoma cell line (PC3) were used as the non melanoma models, while M14-MEL, SK-MEL-2, and UACC-257 cells were used as the melanoma models, to evaluate the selective cytotoxicity. The C-20 keto and the corresponding oximes derivatives, the hydroxyl amine, methoxyl amine, the primary alcohol and the methoxyl ether modified compounds at C-20, and the previously synthesized derivatives **19**-**22** were evaluated in this study. Among them, **19** was the most active (IC_{50} = 1.3 μg/ml in PC3 cells and 1.6 μg/ml in SK-MEL-2 cells), followed by **20** and **21** [29]. The loss of cytotoxicity when the double bond at C-20 was functionalized with different groups suggested that this position was not a favorable place to derivatize in order to improve the cytotoxicity. Furthermore, the evaluation of these compounds using different cell lines did not show any selective cytotoxicity [29].

B. Ursolic Acid and Derivatives

1. Ursolic Acid

Ursolic acid, 3β-hydroxy-urs-12-en-28-oic acid (**2**) is a triterpenoid widely distributed in food, medicinal herbs and other plants as a free form or as glycosides or acylated analogs having various pharmacological properties, including cytotoxic and antitumor-promotor activities [30]. Some recent studies completed this survey. **2** was reported to have an antiproliferative effect in human gastric tumor cells (HGT) in a dose-dependent manner [31]. The C-28 COOH group seems to be implicated because the methylation at this position decreases the activity. The lipophilicity of the terpene is also implicated since uvaol (C-28 CH_2OH) is more inhibitory than methylursolate. However HGT cell proliferation is less sensitive to **2** (IC_{50} = 20 μM) than is human leukemia HL-60 cells proliferation [31]. It seems that **2** probably acts as a membrane agent that seeps into the lipidic layers and affects multiple signal transduction pathways in mammalian cells. In order to confirm this hypothesis, a study suggested that fluidity-modulating and condensing effects might have some implications in biological functions of liposomal membranes [32]. In order to improve the understanding of the mechanism of anti-proliferative action of **2**, its influence on the viability and tumor cell cycle was studied [33]. It was shown to induce cell-cycle arrest at G1 in MCF-7 human breast cancer [33] and in B-16 melanoma cells [34] as determinated by DNA flow cytometric technique (IC_{50} = 10-20 μM) [33]. **2** exerts both cytostatic and cytotoxic activity. It exerts an early cytostatic effect at G1 followed by cell death. To further investigate the possible mechanism of action, the effect of **2** on human epidermoid carcinoma A431 cell growth and the inhibitory activity against proteine-tyrosine-kinases (PTKs) were investigated [35]. These enzymes are critical components of signaling pathways that control cell proliferation and differentiation. Enhanced activity of PTKs due to activating mutations or overexpression has been implicated in many cancers and other proliferative diseases (atherosclerosis, psoriasis etc...). **2** markedly reduced A 431 cell growth in a time- and dose-dependent manner and inhibited PKC activity of A 431 cells *in vitro* in a biochemical assay in a dose-dependent manner with an IC_{50} of 24 μM. Furthermore **2** showed a high selective inhibition of cAK (cyclic AMP-dependent protein kinase) (IC_{50} = 9.0 μM) and is one of the most potent non-aromatic plant-derived inhibitors of cAK yet found [36]. Furthermore, **2** at a high concentration of 50 and 100 μM was shown to present an *in vivo* anti-tumor activity of implanted tumor by ascitic cells [37]. The inhibitory tendency of tumor growth by **2** appeared from the 19th day after treatment and lasted for at least 19-33 days. However, combination with irradiation, the administration of **2** showed less significant inhibition of tumor growth than after administration of **2** alone [37].

Angiogenesis inhibitors might be a novel strategy for tumor growth and metastasis inhibition. **2** was found to inhibit angiogenesis and capillary formation in a dose-dependent manner by using the chick embryo chorioallantoic membrane (CMA) assay. **2** was effective at doses as low as 4 nmol/egg, (ID_{50} = 10 nmol/egg). Since **2** also showed an antiproliferative effect on the bovine aortic endothelial cell

(IC_{50} = 5 μM), **2** was postulated to express the antiangiogenic activity by inhibiting the proliferation of vascular endothelial cells [38].

The anti-invasive activity of **2** was shown to correlate with the reduced expression of the proteolytic enzyme Matrix Metalloproteinase-9 (MMP-9) in the highly metastatic HT 1080 human fibrosarcoma cell line [39].

The cytotoxicity against tumor cells is dependent on the activation of macrophages, which is strongly correlated with the expression pattern of several cytokines. As mechanism of antitumorigenic activity, it was demonstrated that **2** stimulates NO and TNF α release via NF-kB activation in the resting macrophages [40]. NO is known to act as a powerful inducer of apoptosis through upregulation of proapoptotic proteins such as p53 and Bax [40].

Furthermore **2** was shown to induce tumor cell differentiation by regulation of the expression of differentiation-specific genes in mouse F9 tetracarcinoma cells [41].

2 was shown to induce a concentration-dependent decreased cell viability in HL-60 promyelocytic leukemia cells. Furthermore the DNA fragmentation induced by **2** indicated a mechanism of cell death by apoptosis in which the enhanced intracellular Ca^{++} signals might be involved [43]. **2** was also shown to trigger calcium-dependent apoptosis in human lymphoma Daudi cells [44]. A study showed that **2** induces apoptosis in HepG2 cells which might be mediated by activation of caspase-3 and release of cytochrome c from mitochondria [45]. The induction of apoptosis by **2** was also evoked through activation of caspases and down regulation of c-IAPs (inhibitor of apoptosis) family proteins in human prostate epithelial cells [46]. The cAMP plays an important role in the differentiation of many cell types during development and often contributes to the modulation of programmed cell death. cAMP may protect HL-60 cells from apoptosis induced by **2** via activation of proteine kinase A in HL-60 cells [47].

Among the enzymes that are involed in DNA replication and repair, DNA ligases have recently retained the attention as possible targets for antitumor agents [48]. **2** was isolated from *Tricalysia niamniamensis* as the human DNA ligase I-inhibitory constituent [IC_{50} = 100 μg/ml (216 μM)]. At present, it is unclear whether the observed DNA ligase I inhibitory property of such compound is relevant to the modulation of their cytotoxic effect in cell culture [48]. **2** was also shown to be a potent inhibitor of calf DNA polymerase α, rat polymerase β and human topoisomerase I and II [49].

2. Natural Analogs of Ursolic Acid

If **2** is a well known biologically active triterpenoid, little is known about the pharmacological activities of its natural and semi synthetic analogs, which will be summarized in the following.

Corosolic acid (2α-hydroxy-ursolic acid) (**27**) isolated from the fruits of *Crataegus pinnatifida* var. *pilosa* was tested for anticancer activity and displayed about the same potent cytotoxicity as **2** against several human cancer cell lines (Hep G2, A 549, SNU-C4, HeLa S3, K-562) with ED_{50} of 0.4-5.0 μg/ml depending on the cell lines [50]. Solid cancer cells (HeLa S3 and SNU C4) have strong sensitivity to both compounds. **27** was shown to inhibit PKC activity with a dose-dependent pattern indicating that the cytotoxic activity was related to PKC inhibition. In another study, **27** isolated from *Physocarpus intermedius* showed about the same cytotoxicity as **2** against five cultured human tumor cell lines such as A 549 (non small cell lung), SK-OV-3 (ovary), SK-MEL-2 (melanoma), XF-498 (central nerve system) and HTC-15 (colon) with ED_{50} of 3.9-5.5 μg/ml depending on the cell line [51]. **2** and its hydroxy-19-derivatives were isolated from *Polylepis racemosa* by cytotoxicity-guided fractionation [52]. Among them, 19α-hydroxyursolic acid named pomolic acid (**28**) was the most cytotoxic component having a specificity for M-14 melanoma and ME180 cervical carcinoma, with GI_{50} of 6.9 and 8.3 μg/ml respectively.

Phospholipase C (PLC) plays a key role in intracellular signal transduction which leads to a series of events involved in the DNA synthesis and in the regulation of cell proliferation. Many reports have suggested that an inhibitor of PLC especially the γ isoform would be a useful tool for development of anticancer agents [53]. Pentacyclic triterpene esters (**29**-**32**) isolated from *Uncaria rhynchophylla* showed dose-dependent inhibitory activities against phospholipase Cγl *in vitro* with IC_{50} values of 9.5-44.1 μM and inhibited the cell proliferation of human cancer cells (A-549, HTC 15, MCF7, HT-1197) (IC_{50} = 0.5-6.5 μg/mL) [53]. From these results, the compounds seem to be worthy candidates for further development as potential anticancer agents.

3. Semi Synthetic Derivatives of Ursolic Acid

3-oxo-ursolic acid (**33**) was synthesized from **2** isolated from *Crataegus pinnatifida* by Jones method. It showed potent cytotoxic activitiy both in murine L1210 and in human cancer cell lines (A 549, SK-OV-3, SK-MEL-2, XF498, HCT15) [54].

A series of novel olean- and urs-12-ene triterpenoids with 1-en-3-one functionalities (**34**-**36**) having a substituent at C-2 in ring A have been synthesized and tested in a preliminary screening assay system in order to find potent anticarcinogenic compounds [55]. This assay consists in the inhibition of nitric oxide (NO) induced by interferon-γ (INF-γ) in mouse macrophages. The NO produced by inducible nitric oxide synthase (iNOS) which is expressed in activated macrophages, plays an important role in host defense. However, excessive production of NO also can destroy functional normal tissues during acute and chronic inflammation. This phenomenon is also closely related mechanistically to carcinogenesis. Thus, inhibitors of NO production in macrophages are potential anti-inflammatory and cancer chemopreventive drugs. Even if **34**-**36** were shown to be less active than oleanane analogs, they showed a significant inhibitory activity of 5.1-6.2 μM. In a structure/activity relationship study, the 1-en-3-one functionalities were shown to be important for activity [55].

C. Echinocystic Acid Glycosides and Derivatives

Although echinocystic acid glycosides have been isolated from several sources such as *Aster scaber* [56], *Albizia lucida* [57], *Cucurbita foetidissima* [58], *Calliandra*

anomala [59], *Gleditsia sinensis*, [60], *Quillaja saponaria* [61], there are relatively few reports on their biological activities. The new echinocystic acid 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranoside (**37**) isolated from *Ixeris sonchifolia* was shown to present a moderate cytotoxic activity against the L929 (murine pneumoepithelia carcinoma) cell line (IC_{50} = 43.2 μg/ml) and was inactive against the tumor cell lines A375 (human melanoma) and Hela (human carcinoma) (IC_{50} > 100 μg/ml) [62]. Among several echinocystic acid derivatives which have been tested in the YAC-1 and P-815 tumor cell model, the bidesmosidic chrysantellin A (**38**) isolated from *Chrysantellum procumbens* (Asteraceae) showed the best cytotoxicity with IC_{50} of 1.68 μM and 8.40 μM for YAC-1 and P-815 tumor cells respectively [63]. The bidesmoside of echinocystic acid was more active than the corresponding prosapogenin. The unusual saponin C from *Gleditsia japonica* (**39**), an octaglycoside of echinocystic acid having two monoterpenyl ester groups was shown to be a potential anti-tumor promoter. Namely it showed a moderate inhibitory effect on Epstein Barr Virus Early Antigen (EBV-EA) activation induced by TPA, a stronger promoter, in Raji cells [64]. This activity was confirmed in the *in vivo* two stage carcinogenesis assay. It appeared that the glycosylation at C-3 and the unusual acylation at C-28 were important for inhibiting activity.

II. ANTI-HIV ACTIVITIES

A. Betulinic Acid and Derivatives

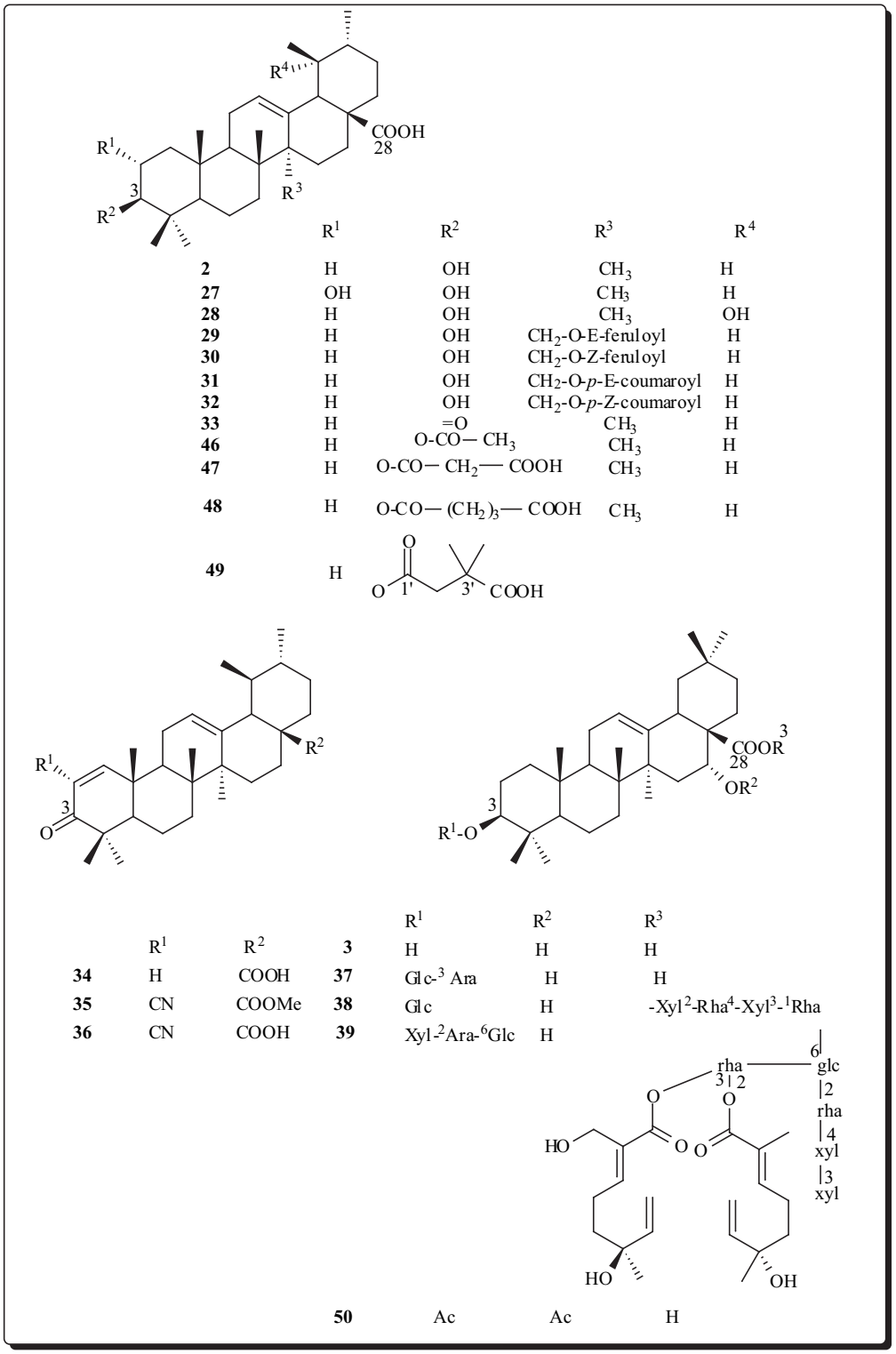
Three major different approaches have been used to find **1** and **1** derivatives as potent anti-HIV agents. On the one hand, a bioassay-linked extraction of the leaves of *Syzigium claviflorum* (Myrtaceae) led to the isolation and estimation of the anti-HIV-1 activity of **1**. The latter inhibited HIV replication in H9 lymphocytes with an EC_{50} value of 1.4 μM and inhibited uninfected H9 cell growth with an IC_{50} value of 13 μM (Therapeutic index (TI): ratio IC_{50}/EC_{50} = 9.3) [65]. Hemisynthetic derivatives were thus prepared and tested, revealing dihydrobetulinic acid (**20**) as potent as **1** (EC_{50} = 0.9 μM, IC_{50} = 13.0 μM, H9 cells, TI = 14.0) [65]. To extend this study, other derivatives of **1** and **20** were synthetized to examine structure-activity relationships [66,67]. 3-*O*-(3',3'-dimethylsuccinyl)-betulinic acid (**43**) and 3-*O*-(3',3'-dimethylsuccinyl)-dihydrobetulinic acid (**45**) demonstrated potent anti-HIV activity (EC_{50} <$3.5.10^{-4}$ μM, IC_{50} = 7 μM, and EC_{50} <$3.5.10^{-4}$ μM, IC_{50} = 4.9 μM, H9 cells, respectively) and exhibited remarkable TI (>2.10^4 and 14.10^3, respectively). Preparation of 3-alkylamido-3-deoxy-betulinic acid derivatives that were much less potent than their ester analogs or inactive, indicated that the presence of the ester group at C-3 is essential for potent anti-HIV activity [68]. Further investigations on the mechanism of action of **43**, named YK-FH312 [69], revealed that it might interfere with viral maturation.

On the other hand, a second approach in the research of anti-HIV derivatives of **1** began with Mayaux *et al*. [70] who estimated the inhibitory activity of hemisynthetic derivatives on different strains of HIV-1 and HIV-2 in several cellular infection assays. Chemical optimization of the lateral chain of **1** resulted in potent compounds that are represented by a statine derivative, (3*S*,4*S*)-*N'*-[*N*-[3β-hydroxylup-20(29)-en-28-oyl]-8-aminooctanoyl]-4-amino-3-hydroxy-6-methylheptanoic acid (**40**), named RPR 103611, with IC_{50} values (50% inhibitory concentration for the cytopathogenicity of HIV) between 40 and 100 nM in most cell systems. **40** presented a good selectivity index (SI>100), which corresponds to the ratio CC_{50}/IC_{50}, where CC_{50} is the 50% cytotoxic concentration for mock-infected cells. The activity is restricted to HIV-1, while no significant activity could be found against HIV-2 (ROD and EHO isolates, MT-4 cells, IC_{50}>10 μM, SI<1). Regarding the mechanism of action of these derivatives, they might block virus infection at a postbinding step necessary for virus-membrane fusion and might interact with the HIV-1 envelope glycoproteins gp120/gp41 [70]. In this field of investigations, a series of ω-undecanoic amides of **1** with systematic alterations of its back-bone were synthesized [71]. According to the results of the structure-activity relationships, it appeared that most of the modifications of the triterpene skeleton led to the loss of activity except for the lateral chain. Some of these compounds were potent HIV-1 inhibitors, such as *N*-[3β-hydroxylup-20(29)-en-28-oyl]-11-aminoundecanoic acid (**41**) (IC_{50} = 0.23 and 0.44 μM, CEM and MT-4 cells, respectively) presenting no inhibition on HIV-2 strain ROD. Further ω-aminoalkanoic acid derivatives of **1** were synthesized and tested as HIV-1 inhibitors [72]. Compound **40** was once more the most active one (IC_{50} = 50 and 40 nM, CEM and MT-4 cells, respectively) with a good SI (>100), and was used for further mechanistic studies, in order to determine its precise target within the viral envelope. Labrosse *et al.* [73] showed that genetic differences in gp41, and not in gp120, were associated with sensitivity or resistance of HIV-1 strains to **40**. Unfortunately, the frequency of spontaneous resistance to this compound among HIV-1 and HIV-2 strains limited the interest in it. Other assays [74] led to the conclusion that the antiviral efficacy of **40** depends on the sequence of the gp41 « loop region » and the stability of the gp120-gp41 complex, which could limit the accessibility of this target. Recently, a stereoisomer of **40** named IC9564 (**42**) was studied [75] and the two stereoisomers were shown to be equally potent in their anti-HIV-1 and antifusion activity. It was reported that mutations in gp120 contributed to the **42** sensitivity, but on the contrary, a gp41 mutation did not appear to affect **42** sensitivity.

The last approach was represented by an estimation of **1** and its derivatives as enzymatic inhibitors, but none of them inhibited HIV-1 reverse transcriptase (RT) [66], [70], [71]. Moreover, **40** and its analogues did not inhibit HIV-1 integrase [70], and **1**, **20** and their derivatives [65] did not inhibit proteine kinase C. But while ω-aminoalkanoic acid derivatives did not show inhibition of the HIV-1 protease [70], [71], dicarboxylic acid hemiesters in C-3 position of **1** were found to be potent inhibitory principles against HIV-1 protease as glutaryl hemiester (**44**) with an IC_{50} value at 4.0 μM [76].

B. Ursolic Acid and Derivatives

According to the close structural similarity between **1** and **2**, and the interesting results obtained during the investigation of the anti-HIV activity of **1** and its derivatives, **2** was tested [77] and showed activity but was



slightly toxic (EC_{50} = 2.0 μg/ml, IC_{50} = 6.5 μg/ml (H9 cells), TI = 3.3). Derivatives were synthetized in reference to the corresponding derivative **43**, as 3-*O*-(3',3'-dimethylsuccinyl)-ursolic acid (**49**), which displayed only weak anti-HIV activity but was less toxic than **2** (TI = 23.6) [78]. As **2** and **1** differ only by the E-ring, the latter might play an important role in the anti-HIV potency.

Other bioassays were developed to test **2** and its derivatives as enzymatic inhibitors. While **2** showed moderate inhibitory effects on HIV RT (28 % at 100 μM) [49], isolated using a bioassay-linked extraction from the whole plant of *Geum japonicum* (Rosaceae) [79] and the leaves of *Crataegus pinatifida* (Rosaceae) [80], it appeared as a potent inhibitor of HIV-1 protease (85 % at 17.9 μg/ml [79] and IC_{50} = 8.0 μM [80]). From the stems of *Cynomorium songaricum* (Cynomoriaceae), **2**, acetyl ursolic acid (**46**) and malonyl ursolic acid hemiester (**47**) were isolated and showed potent inhibitory activity against HIV-1

protease (IC_{50} = 8.0, 13.0 and 6.0 μM, respectively) and several dicarboxylic acid hemiesters of **2** were synthesized [76]. As described for **1**, the most potent activity was observed for the glutaryl hemiesters (**48**) with IC_{50} of 4.0 μM.

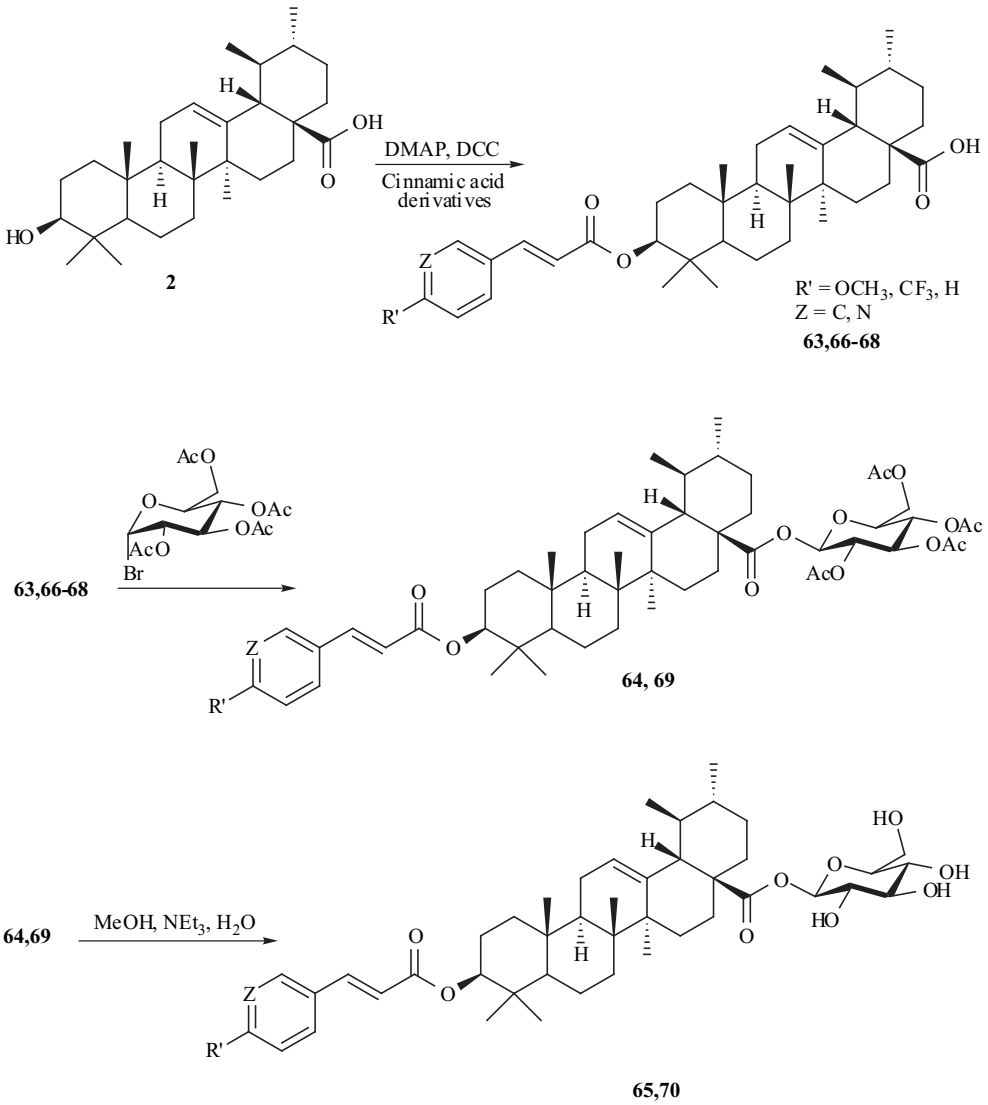
To understand how **2** derivatives inhibited HIV-1 protease, the mechanism of action was analyzed and **2** was shown to inhibit dimerization of HIV-1 protease [81].

C. Echinocystic Acid and Derivatives

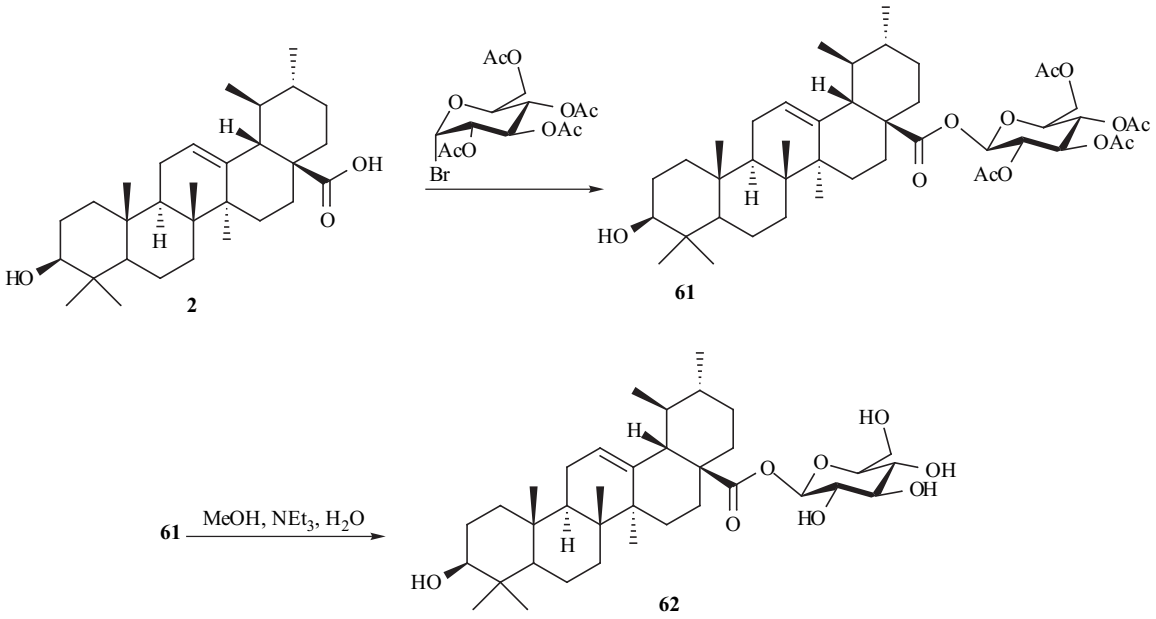
During a bioassay-directed fractionation of an extract of fruits from *Gleditsia japonica* and *Gymnocladus chinensis* (Leguminosae), gleditsia saponin (**39**) which possesses **3** as aglycon, was isolated as anti-HIV principle [82]. The prosapogenins and the aglycon of **39**, and **3** derivatives were prepared and evaluated for inhibitory activity against HIV replication. The entire saponin **39** and 3,16-di-*O*-acetylechinocystic acid (**50**) were shown to be the most potent inhibitors (EC_{50} = 1.1, IC_{50} = 9.8 μM, and EC_{50} = 2.3, IC_{50} = 13 μM, H9 cells, respectively) with bad TI (8.9 and 5.4, respectively). Regarding gleditsia saponin, monoterpenyl moities appeared to be essential for its anti-HIV activity.

III. OUR INVESTIGATIONS ON THE CHEMISTRY OF BIOLOGICALLY ACTIVE TRITERPENOIDS

As described at the beginning of this review, betulinic and ursolic acids (**1** and **2**) are well known natural cytotoxic and anti-HIV agents. The literature reports only a few synthetic analogs of these structures for their cytotoxicity [27-29, 55]. In our search for novel antitumor compounds, we choose to introduce new substituents at C-3 and C-28 positions because some esters or amides were previously described in the literature to possess either cytotoxic [28] or antiviral [68, 78] (as HIV inhibitors) activities. Indeed, we synthesized almost exclusively esters at C-3 and/or C-28 positions. For these products the nature was our guide, whereas many osidic moieties were described in plants either at C-3 or at C-28 position, so we synthesized glucoside esters at C-28 position. For C-3 position, we introduced



Scheme 1. Acylation of ursolic acid by cinnamic acid derivatives at the C-3 position as an example.



Scheme 2. Glycosylation of ursolic acid at C-28 position as an example.

cinnamic acid derivatives to obtain the corresponding cinnamoyl esters.

In a continuation of our search and to enlarge the field of our investigations, we also studied chemical reactivity and biological activity of echinocystic acid (**3**), because of its structural relationship with the two other compounds.

A. Synthesis of Triterpenoid Analogs (Scheme 1 and 2)

1. Acylation of Betulinic and Ursolic Acids by Cinnamic Acid Derivatives at the C-3 Position

The hydroxy group at C-3 position could be acylated by various acylating reagents, by using a coupling agent, the dicyclohexylcarbodiimide (DCC) and a catalyst, the 4-dimethylaminopyridine (DMAP) [84].

In a typical reaction, a solution of **1** or **2** in THF was reacted with 2eq. of cinnamic acid, or of its derivatives, 2eq. of DCC and 2eq. of DMAP, under nitrogen at room temperature. The reaction was followed by TLC, and after 18h one more equivalent of each compound, coupling agent, catalyst and cinnamic acid, was added. This addition was repeated three times at 24, 34 and 45h. After completion the DCC urea formed was filtreted off and the filtrate was evaporated under vacuum. The residue was dissolved in dichloromethane washed with water, 1N HCl, three times with water and then dried ($MgSO_4$) and evaporated in vacuum. The crude product was purified by flash chromatography, eluted with appropriate mixture of hexane/ethyl acetate, to afford the attempted products with good yields (66-94%).

2. Particularity of Echinocystic Acid Acylation

In this case, the acylation was not obtained selectively at C-3 position because this compound possesses two hydroxyl groups at C-3 and C-16 positions. The size of the acylating group will determine the substitution. For example, the acetylation of **3** with acetic anhydride in pyridine afforded the diacetyl compound in thirty minutes. The acylation with more hindered/less reactive acids, as cinnamic acid and its derivatives in the presence of a coupling agent (DCC or EDCI, [1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride]) and a catalyst as described for other triterpenes, could afford the monosubstituted compound in small amounts, but generally the disubstituted derivative was obtained as the major product. These compounds could be separated by flash chromatography on silica gel using a mixture of hexane/ ethyl acetate as an eluant.

3. Glycosylation of Triterpenoids at C-28 Position

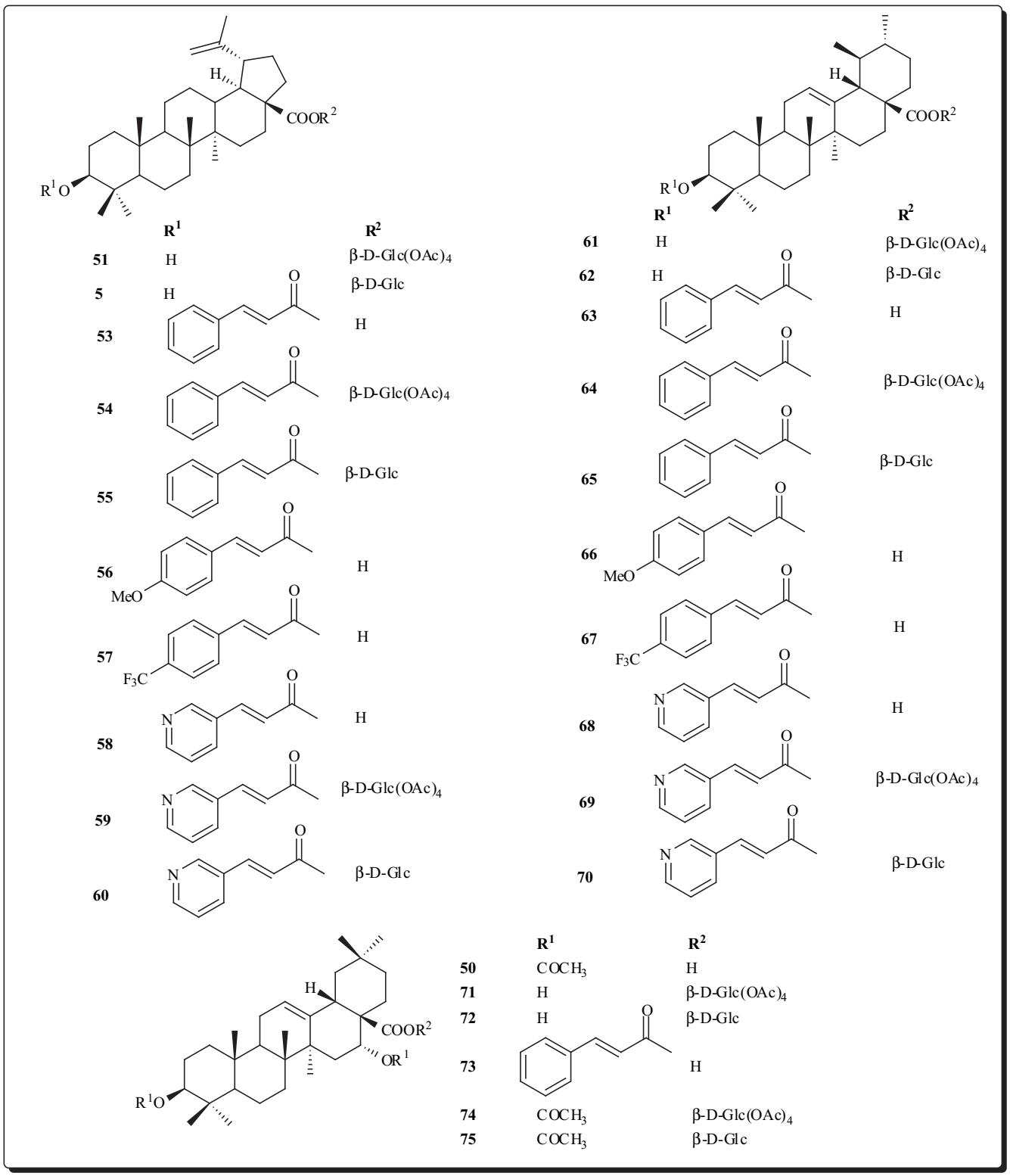
This reaction was performed under the same conditions no matter which acid was used. The condensation of triterpenoids (**1**, **2**, **3**) or 3-acetyltriterpenoids with 2, 3, 4, 6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide, was carried out in acetone in the presence of potassium carbonate at room temperature overnight. The crude 28-*O*-β-D-tetra-*O*-acetylglucopyranosides [85, 86] were purified by flash chromatography to afford the desired pure compounds in 90% yield. The latter were then quantitatively deacetylated according to the method described by Lubineau *et al* [87], by alkaline hydrolysis (16h, room temperature) with a mixture of $MeOH/N(Et)_3/H_2O$ (8:1:1) to give the attempted products.

B. Cytotoxic Activities

The cytotoxic effect of triterpenes was evaluated on human colon adenocarcinoma cell lines HT 29 (Table 1).

These preliminary results allowed us to make some hypothesis on the required groups to obtain a good cytotoxic activity.

Our investigations demonstrated (Table 1), as expected, that **1, 2** and **3** acids are effective cytotoxic agents against HT 29 cells. Surprisingly, the potassium salts of **1** and **2**, which were supposed to be more soluble, didn't give more



potent cytotoxic potency. However, we could observe that the betulinic acid potassium salt was more potent than the corresponding ursolic acid derivative. This observation was confirmed with the 3-*O*-cinnamoyl potassium salt derivative of **53**, which demonstrated a good cytotoxic activity, whereas ursolic derivative didn't have any.

First, we investigated the cytotoxicity of both acetylglycopyranosyl esters and free glycopyranosyl esters at C-28 position of the 3 series, but, except for **62**, no cytotoxic activity was discovered. So these results were in accordance with a loss of cytotoxicity for glycopyranosyl derivatives either acetylated or not. The loss of cytotoxic effect by glycosylation at C-28 position suggested the importance of the hydrogen bonding capability and/or acidity in the expression of the cytotoxic effect. These observations are consistent with the reported *in vitro* cytotoxic activities described by Kim *et al.* [27] who

Table 1. Cytotoxic Activity of Hemisynthetic Compounds on Human HT 29 Colon Cancer Cells

Betulinic derivatives		Ursolic derivatives	
Compounds	**Cytotoxic activity IC_{50} µM**	**Compounds**	**Cytotoxic activity IC_{50} µM**
1	26	**2**	30
1 K salt	10	**2 K salt**	60
51	nc	**61**	nc
5	nc	**62**	8
53	nc	**63**	nc
53 K salt	8	**63 K salt**	nc
54	nc	**64**	nc
55	nc	**65**	nc
56	nc	**66**	350
57	nc	**67**	nc
58	nc	**68**	45
59	nc	**69**	nc
60	nc	**70**	nc

Echinocystic derivatives	
Compounds	**Total cytotoxic activity µM**
3	25
50	45
71	nc
72	nc
73	nc
74	nc

nc: no cytotoxicity

especially worked on betulinic analogs. Chatterjee *et al.* [22] did the same observation with **1** and its glucopyranosyl ester at C-28 position. Martin-Cordero's *et al.* [83] conclusion is also in accordance with these observations; indeed they postulate that the acid position at C-28 in **2** is essential for cytotoxic activity. The lack of references on the cytotoxicity of echinocystic derivatives did not allow us to state that the glycosyl moiety is responsible of the loss of cytotixicity. However Bader *et al.* [63] had demonstrated a loss of cytotoxic activity with a simple glycosyl moiety (arabinosyl) at C-28 position. But they also found an increase of cytotoxic activity with higher glycoside substituents at this same position. So the decrease of cytotoxicity of compounds **71** and **72** could be attributed to the glycosyl moiety as it was postulated with both other acids.

We also investigated the influence of the substitution at C-3 position. In each series whatever the cinnamoyl moiety substituted, we were able to see the loss of cytotoxicity against HT 29 cell lines. So, in accordance with observations of Kim *et al.* [27], the loss of toxic effect suggested a size limitation at C-3 position. However the use of pyridylpropenoyl moiety for the C-3 substitution gave with ursolic serie a compound **68** with a significant cytotoxic activity. This result suggested more than a size limitation at C-3 position, the great significance of the electronic density of the moiety use, for the toxic effect. This toxic effect wasn't observed with betulinic derivative **58**, so these first results suggested that the relatively parent structure of **2** and **1** is not so close and that these differences play a significant role in the potency of the derivatives. Surprisingly, the di-*O*-acetylechinocystic acid derivative **50** had a significant cytotoxic activity, whereas ursolic and betulinic acetyl compounds didn't have any.

Finally the combination of the substitution at C-3 and C-28 position resulted in the loss of cytotoxicity in all series examined. Indeed, on comparing the glycosides **69** and **70** with **68**, we confirmed this loss of activity. This result was also confirmed in the echinocystic series. Indeed

we could observe a loss of cytotoxicity with glycoside derivatives of di-*O*-acetylechinocystic acid **74** and **75**.

The above investigations suggest that simple modifications of the parent structure either in ursane or lupane series can produce new potentially interesting derivatives, which may modify the cytotoxic profile. However this study led us to think that no correlation could be done between ursane and lupane series even if they seem to have close structure. These observations confirmed suggestions of Kashiwada *et al.* [78], that the structure of the E ring, which differs from ursane and lupane series, might play an important role in the cytotoxic potency.

Finally, a more extensive investigation using a greater number of compounds is in progress in our laboratory. This work would be used to do a detailed structure/activity relationship (SAR) study for designing and synthesizing more potent betulinic, ursolic and echinocystic derivatives as cytotoxic agents.

C. Anti-HIV Activity

Anti-HIV activity of the compounds was evaluated on the T cell line, H9, infected with HIV-1 (Table 2).

Table 2. Anti-HIV Activity of Hemisynthetic Compounds

Betulinic derivatives		Ursolic derivatives	
Compounds	**EC_{50} µM**	**Compounds**	**EC_{50} µM**
1	25	**2**	10
1 K salt	50	**2 K salt**	60
51	50	**61**	low activity
5	25	**62**	4
53	-	**63**	-
53 K salt	50	**63 K salt**	400
54	na	**64**	na
55	-	**65**	-
56	-	**66**	30
57	na	**67**	na
58	-	**68**	7.5
59	-	**69**	-
60	-	**70**	-

n.a.: no activity
EC_{50}: the agent concentration that inhibited viral replication in H9 cell by 50%

Our first investigations in the field of terpenoids and anti-HIV led us to some interesting observations which could be correlated to those of the literature.

Initially, we observed in accordance with the literature [65, 78] that **1** and **2** exhibited an effective and similar level of anti-HIV activity with EC_{50} of 25 μM and 10 μM, respectively. Surprisingly our results on betulinic and ursolic potassium salts were in disagreement with those of Kashiwada *et al.*[77, 78], indeed our compounds both displayed weaker anti-HIV activity with an EC_{50} of 50 μM and 60 μM, respectively, than the corresponding acids. These results were confirmed with two other compounds, the potassium salts of **53** and **63** which exhibited weak activity with an EC_{50} of 50 µM and very low activity with an EC_{50} of 400µM, respectively.

In our continuing search for potent anti-HIV agents, we investigated the introduction of glycosyl moiety at C-28 position. We could observe that tetra-*O*-acetylglucopyranosyl esters from the betulinic **51** or ursolic **61** series exhibited a very low activity , whereas free glucopyranosyl esters 5 and **62** inhibited HIV replication in H9 lymphocytes with EC_{50} values of 25 µM and 4 µM, respectively. As it was already observed with cytotoxic properties, the ursolic derivative is much more efficient than its corresponding betulinic derivative. The literature doesn't report any active compounds with a simple glycosyl ester moiety at C-28, but only saponins with several hexosyl moieties at C-3 and C-28 positions. We reported only one example of active compound **39** (as an echinocystic saponin) in the first part of this review, which had been reported to be a potent anti-HIV agent, but its activity had been attributed to the presence of an unusual monoterpenyl moiety [82]. This example, in which all glycosyl moieties were shown to have free hydroxyl groups, correlated with our results allowing us to point out the importance of the free hydroxyl groups for the anti-HIV activity. This observation is interesting because other esters at this position didn't exhibit anti-HIV activity, or a very weak one [67, 68]. This suggested that the glycosyl group would be important for the activity either due to binding to a hydrophilic pocket or due to hydrogen bonds between the receptor and the product. The lack of activity of the other esters would suggest a size limitation at this position.

Anti-HIV activity of C-3 esters (cinnamoyl related esters) was also investigated. Our first results highlight the importance of the aromatic ring. Indeed the derivative **67** with a trifluoride moiety on the ring didn't exhibit any activity whereas a methoxy moiety on this ring in the derivative **66** was responsible of a weak anti-HIV activity, and finally, the replacement of the phenyl by a pyridyl ring in **68** gave a potent anti-HIV agent with an EC_{50} value of 7.5 µM. These investigations suggest, as for cytotoxic tests, the importance of electronic density of the C-3 substituent in the expression of the activity.

Comparing these results with the literature highlights the importance of the ester moiety at C-3 position, as described by all studies of Kashiwada *et al.* [66-69, 78, 88] and others, either in betulinic or ursolic series. For these authors the ester at C-3 position is essential to obtain a potent anti-HIV agent. They all pointed out the importance of the isovaleryl moiety in the antiviral activity of their products, more than the ester moiety the electronic density of this one seems to be very important for anti-HIV activity.

The differences of activity observed between betulinic and ursolic series suggested, as we explained with cytotoxic activity, that the E ring might play an important role in the anti-HIV activity.

In conclusion, our first results correspond with those of the literature allowing us to focus on some required moieties for an anti-HIV activity: an ester at the C-3 β position and a high electronic density at the end of the chain. At C-28

position, a free carboxylic acid, a high hydrophilic group or a hydrogen donating group might be required. Importance of the E ring: in our study the six-membered ring seems to give a better result than the five-membered one. The oxidation or the elimination of the hydroxyl group at C-3 position or the substitution of the A ring decrease the anti-HIV activity [77].

CONCLUSION

This review presented the updated reports of the literature on betulinic (**1**), ursolic (**2**), echinocystic (**3**) acids and their natural and semisynthetic analogs as potent anticancer and anti-HIV agents. Furthermore, the results of the authors' team on the chemical modifications of **1**-**3** and the biological evaluation of the resulting derivatives are shown.

1 was reported to be a melanoma specific agent in cell culture as well as an antitumor compound in several *in vivo* model systems. The apoptosis inducing ability in melanoma and other cell types, and the favorable therapeutic index suggests **1** to be a promising new lead compound supporting preclinical developments. **2** inducing cell death by apoptosis in HL-60 cells and others, was also shown to be an effective anticancer agent acting at various stages of tumor development. There are only few reports on the antitumor activity of **3** glycosides. The natural analogs and modified triterpenoids of **1**-**3** at C-3, C-20 and C-28 were assessed for cytotoxicity in several malignant cell lines, allowing interesting structure/activity relationships studies. Concerning the semisynthetic derivatives of **1**, the size limitation of the substituents at C-3, the importance of a free COOH group at C-28 in comparison with CHO, $COOCH_3$ and CH_3 substituents, and the importance of the substitution by different aminoacids at C-28 were highlighted for a good cytotoxicity toward melanoma cells. In the contrary, the modification at C-20 was not favorable to improve the cytotoxicity. For the derivatives of **2**, the most significant structural features were the 1-ene-one functionalities for a potent anticarcinogenic activity. With respect to the natural derivatives of **3**, the glycosidation at C-3 and the unusual acylation at C-28 were important for antitumor promoting activity.

Similar conclusions were made on the structural requirements for a good anti-HIV activity in M9 cells: esterification at C-3 for **1**, role of the E-ring, free COOH group at C-28 or chemical optimization yielding statine derivatives or undecanoic amides, role of the monoterpenyl moiety in **3** glycosides. Furthermore the di-*O*-acetyl-3,16-derivative of **3** was a potent HIV-inhibitor. Among the enzymes (reverse-transcriptase, integrase and proteine kinase C) inhibitors, the glutaryl hemiesters of **1** and **2** were potents inhibitors of the HIV-1 protease, whereas almost derivatives of **1** were inactive on the other enzymes.

The potentially interesting derivatives obtained in the authors' team by introducing new substituents at C-3 and C-28 positions might modify the cytotoxic profile on HT 29 human colon cancer cells. The first conclusions correlated with those of the literature, allowing us to focus on the importance of some structure requirements for the cytotoxic and anti-HIV activity. The next step of our investigations is to synthesize other derivatives allowing extensive structure/activity relationship studies and to bring molecular biological data in order to explain the mechanism of action. The most important targets on which we should focus on, are the cytotoxic activity against melanoma cells by induction of apoptosis, the anti- HIV effect by inhibiting the HIV-1 entry and the HIV-1 maturation and the inhibitory effect against HIV-1 protease. Hovewer a confirmation of all the *in vitro* findings concerning the compounds of this review by *in vivo* antitumor and anti-HIV assays might bring a rational basis to further potential therapeutic developments.

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