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Triterpenes and Triterpenoids Clinically Useful with Multiple Targets in Cancer, Malaria and More Treatment: Focus on Potential Therapeutic Value

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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Review Article

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ABSTRACT

Triterpenoids are the most promising plant secondary metabolites. Several triterpenoids, including ursolic acid, oleanolic acid, betulinic acid, celastrol, pristimerin, lupeol and avicins are representative group of phytochemicals possesses biological properties. Anti-tumor, anti-cancer, anti-biotic, cytotoxic, anti-inflammatory, anti-HIV, acetyl cholinesterase, anti-wrinkle and anti-feedant activities of these compounds is measured. Role for triterpenes in the cancer setting is also gradually emerging. Triterpenoids are highly multifunctional and structurally diverse organic compounds, characterized by a basic backbone modified in multiple ways, allowing the formation of more than 20,000 naturally occurring triterpenoid varieties.

Fusidic acid is a representative member of terpenes, which has found clinical applications. It is non-allergic, has relatively low toxicity and has little cross-resistance with other clinically used anti-biotics and it remains a unique and promising agent due to the significant potencies against *Staphylococci*. Synthesis of triterpene derivatives is a strategy to obtain compounds with enhanced

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bioactivity by the introduction of electron-withdrawing/donating groups.To improve anti-tumor activity, some synthetic triterpenoid derivatives cyano-3,12-dioxooleana-1,9 (11)-dien-28-oic (CDDO), its methyl ester (CDDO-Me) and imidazolide (CDDO-Im) derivatives have been synthesized. Of these, CDDO, CDDO-Me and betulinic acid have shown promising anti-tumor activities and are presently under evaluation in phase I studies. The present review provides updates on wide range of most important biological activities of triterpenoids and their role in the resolution of diseases to examine the mechanism by which they are useful as ethnopharmacological medicines. In this review efforts have been taken to review the potential use of triterpenoids to be used in the pharmaceutical industry as potential drug leads. In addition previous and current information regarding, its natural and semisynthetic analogs, focussing on its biological activities will be discussed. Thus, the present review investigates the potential use of these triterpenes against human pathogens, including their mechanisms of action, via *in vivo* studies, and the future perspectives about the use of compounds for human or even animal health are also discussed.

Keywords: Triterpenoid; bacteria; biological activities; anti-cancer; biotransformation.

1. INTRODUCTION

According to the World Health Organization for health care, about three-quarters of the world population relies upon traditional remedies [1]. Herbs/plants served the humanity to cure different ailments. The chief natural source of triterpenoids are plants but terpenoids includes diterpenes, sesquiterpenes etc. are also reported from other sources, such as microorganisms, sex hormones of insects, marine organisms, animals as well as fungi and exhibit a multiplicity of biological properties. Triterpenoids are a large naturally occurring and structurally diverse group of natural products whose structures comprise six isoprene units [2], that display nearly 200 distinct skeletons. Most triterpenoids are 6-6-6-5 tetracycles, 6-6-6-6-5 pentacycles, or 6-6-6-6-6 pentacycles, but acyclic, monocyclic, bicyclic, tricyclic, and hexacyclic triterpenoids have also been isolated from natural sources. A recent review covering mechanisms of their formation from squalene, oxidosqualene or bisoxidosqualene has been published [3]. Triterpenes are a wide-spread group of natural compounds with considerable practical significance which are produced by arrangement of squalene epoxide in a chair-chair- chair-boat arrangement followed by condensation.

During the last decade, there has been an unprecedented escalation of interest in triterpenes. It is estimated that well over 2400 subjects have taken part in clinical studies with different types of triterpenes with dosage up to 25 g or more per day with no adverse effect reported. Most of this interest has focused on the cholesterol-lowering properties of triterpenes and evidence of this phenomenon include at least 25 clinical studies, 20 patents and at least 10 major commercially triterpene-based products currently being sold all around the world.3a 4

For the last few years interest in drugs of plant origin has been reviving and the scientists are exploring the potential of natural products for the cures of diseases like AIDS and cancer [4,5]. About 80% of population in developing countries like China, India and Pakistan, relies on traditional medicines which make this region different from the West, that has lost this tradition in the process of modernization and rapid development in the last two centuries. According to an estimate only 25% of all prescriptions in the United States are from natural products. A knowledgeable review of triterpenes biotransformation has been recently published. This review covers the published literature in the field of the therapies of triterpenoids [5].

Parts of plants, for example rosemary leaves, birch bark, apple peel and mistletoe shoots are rich in triterpenes and provide different triterpene compositions. In the last decade more studies have shown further effects that justify the expectation that triterpenes are useful to treat cancer by several modes of action, thus, triterpene acids are known mainly for their differentiation inducing effects as well as their anti-angiogenic effects [6]. A large number of triterpenoids are known to exhibit, anti-cancer efficacy in preclinical animal models as well as cytotoxicity against a variety of tumor cells. Extensive phytochemical and pharmacological studies on genus *Anemone* have proved the

triterpenoid saponins to be the main bioactive substances, with potent biological properties including anti-bacterial, anti-tumor, insect deterrence, anti-peroxidation, etc [7-14].

2. OCCURANCE OF TRITERPENES

The term triterpenoid is more general refers to derivatives of triterpenes (i-e. glycosides) or modified terpenes (i-e. withanoides quassinoids, limonoids etc). Triterpenes are ubiquitously present in variety of ethnomedicinal plants. Triterpenes are also secondary metabolites. It has been estimated that 80 distinct types of both the structure and the chemical characteristics of triterpenes have been identified till today. Betulinic acid, a pentacyclic lupane-type triterpene can be isolated from the outer bark of many plants, that are valuable for timber purposes [15-36]. Isolation of ursolic acid, lupeol and oleanolic acid from various plants by different workers has been reported [37-44]. The leaves of little *periwinkle* and *Vinca minor*, revealed the presence of ursolic acid [45]. Isolation of ursolic acid from *Ilex aquifolium L.I*. has been reported [46]. Chemical investigation of bark, peel and leaf of *Punica granatum* L. also revealed the presence of ursolic acid. Ursolic acid has been reported from other plants [47-54]**.**

3. BIOLOGICAL PROPERTIES OF TRITERPENES

Triterpenes comprise one of the most interesting groups of natural products due to their diverse pharmacological activities. The majority of anti-cancer and anti-infectious agents are of natural origin. The anti-tumor activity of natural products has been linked to their ability to trigger cell death pathways including apoptosis in cancer cells. Programmed cell death or apoptosis is the cell's intrinsic death program that plays a pivotal role in maintaining tissue homeostasis and that is highly conserved among different species. Since apoptosis is involved in the regulation of many physiological processes, defective apoptosis signaling may lead to various pathological conditions [55].

Many triterpenes can either be used directly as active compounds or modified to increase their selectivity and potency. Several structural groups of triterpenes have demonstrated specificity against transcriptional factors which can be promising candidates for treating inflammation, cancer, and immune diseases [56].

3.1 Anti-cancer Activity

Triterpenes belonging to the lupane, oleanane or ursane group treat cancer by different modes of action. Today cancer treatment is not only a question of eliminating cancer cells by induction of cell death. New therapeutic strategies also include targeting the tumour micro environment, avoiding angiogenesis, modulating the chronic inflammation or the immune response that is often associated with cancer. Furthermore, the induction of redifferentiation of dedifferentiated cancer cells is an interesting aspect in developing new therapy strategies [57]. The pharmacological potential of triterpenes of the oleanane, lupane or ursane type seems high for cancer treatment. They provide a multi-target potential for coping with new cancer strategies. Whether this is an effective approach for cancer treatment has to be proven. Because many triterpenes are an increasingly promising group of plant metabolites, the utilisation of medicinal plants as their sources is of interest.

3.1.1 Cytotoxic activity of isomalabaricane-type triterpenoids stellettins

The isomalabaricane-type triterpenoids stellettins A-K (**1**–**13**) (Fig. 1) have been reported from the species of the genus *Jaspis* of marine sponge [56].

3.1.2 Multi-drug resistance reversal activity of triterpenes in cancer therapy

The alisol B analogue (**25**) was suggested to have effects towards standard chemotherapy on reversing the multidrug resistance of certain cancer cell lines. Compound **25** was found to restore the sensitivity of two MDR cell lines, K562-DR and HepG2-DR, towards anti-tumor agents which are substrates of P-glycoprotein but have different modes of action. For example, **25** restored the activity of vinblastine in causing G2/M arrest in MDR cells.

Compound **25** increased doxorubicin accumulation in a dose dependent manner and slowed down the efflux of rhodamin-123 from MDR cells. It inhibited the photoaffinity labeling of *p*-glycoprotein by iodoaryl azidoprazosin and stimulated the ATPase activity of P-glycoprotein. This suggested that it could be a transporter substrate for *p*-glycoprotein. **25** was also found to be a partial non-competitive inhibitor of *p*-glycoprotein when verapamil was used as a substrate [64].

The alisol B derivative **24** exhibited cytotoxic activity against several cancer cell lines SK-OV3, B16-F10 and HT1080, showing ED50 value of 7.5, 7.5, and 4.9 *μ*g/ml, respectively. The alisol B analogues **25** and **33**, alisol A analogue **2**, showed only weak activities in the same cell lines with ED_{50} values of 10–20 *μ*g/ml [65] (Fig. **2**).

Twelve triterpenes analogues were synthetically prepared from **25** and assessed for cytotoxicity against a panel of human and murine tumor cell lines. Among them, 23*S*-acetoxy-24*R*(25)-epoxy-11*β*,23*S*-dihydroxy-protost-13(17)-en-3-hydroxy-imine exhibited significant cytotoxic activities against A549, SK-OV3, B16-F10 and HT1080 tumor cells with ED_{50} values of 10.0, 8.7, 5.2, and 3.1 *μ*g/ml, respectively. Furthermore, 23*S*-acetoxy-13(17), 24*R*(25)-diepoxy-11*β*-hydroxy-protost-3-one,13(17), 24*R*(25)-diepoxy-11*β*,23*S*-dihydroxy-protostan-3-one, 24*R*,25-epoxy-11*β*,23*S*-dihydroxy-protost-13(17)-en-3-one, and 11*β*,23*S*,24*R*,25-tetrahydroxyprotost-13(17)-en-3-one displayed moderate cytotoxic activities against two of these cell lines, HT1080 and B16-F10. The findings seemed to suggest that a hydroxy-imino group at the C-3 position would enhance the cytotoxic activity of this class of compounds [66].

Compound **25** was found to induce apoptotic cell death in human hormone-resistant prostate cancer PC-3 cells. The mechanism was described to be mitochondria-mediated, causing the activation of caspases 3,8 and 9. Compound **25** was found not only to induce *Bax* expression, but also to cause the translocation of *Bax* from the cytosol to the nucleus [67].

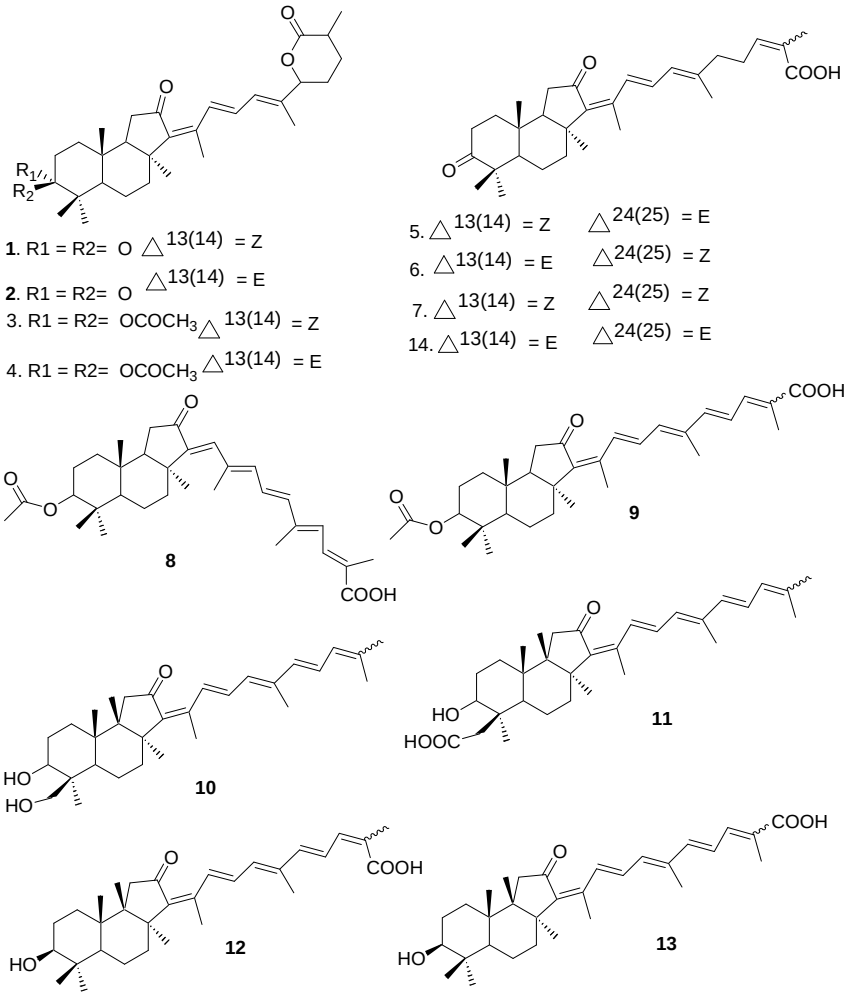


Fig.**1**. Isomalabaricane type triterpenoids stellettins from marine sponge.

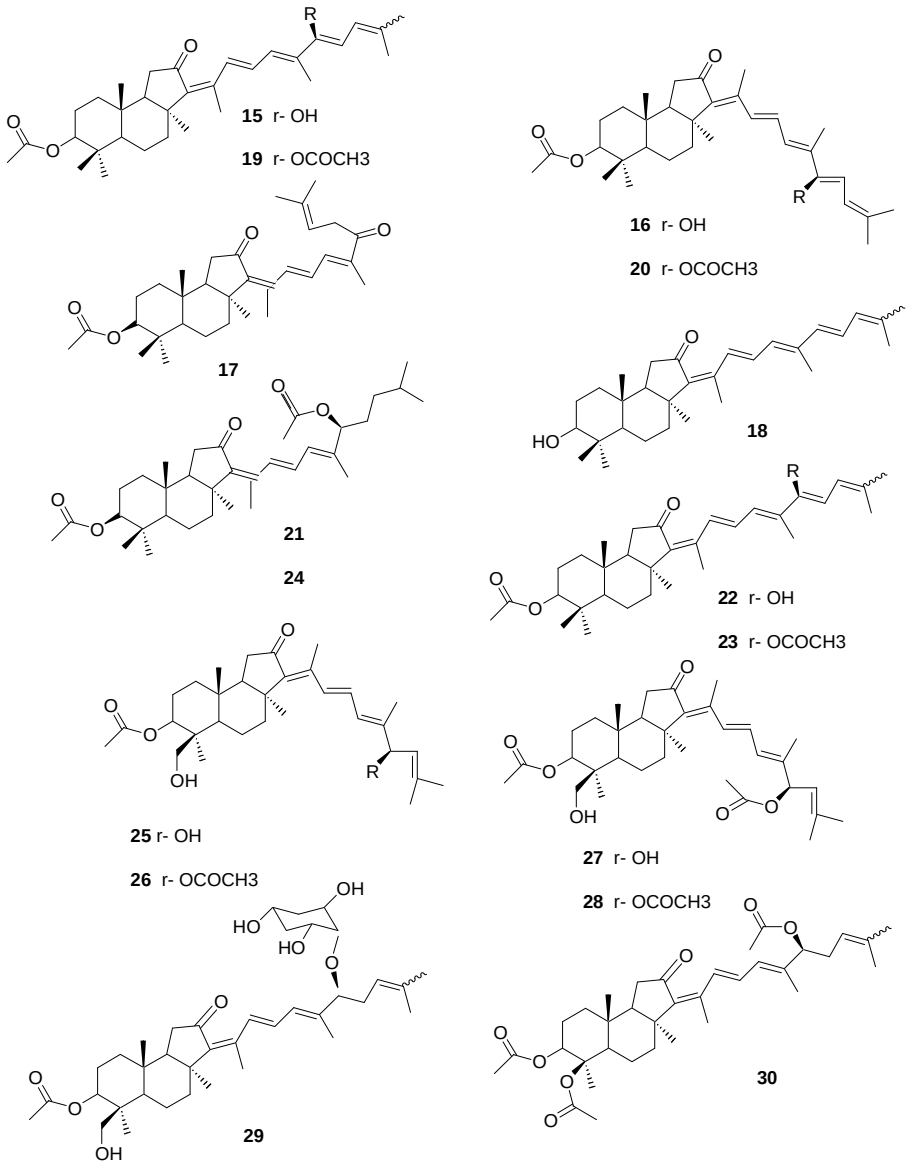


Fig. **2** Triterpenoid stelliferins from marine sponges

Anti-neoplastic isomalabaricane triterpenes, stelliferins A–F (**15–20**), were isolated from the *Okinawan* marine sponge *Jaspis stellifera* [58,59]. The isomalabaricane triterpenes, stelliferin G (**21**), 29-hydroxy-stelliferin A (**22**), 29-hydroxy stelliferin E (**23**), 3-epi-29-hydroxy-stelliferin E (**24**), 13(*E*)-29-hydroxy-stelliferin E (**25**), 29-hydroxy-stelliferin B (**26**), 13(*E*)-stelliferin G (**27**), and 13(*E*)-3-*epi*-29-hydroxy-stelliferin E (**28**), were isolated from the organic extract of the sponge *Jaspis sp*. All compounds were tested against *leukemia* (MOLT-4) cells and *melanoma* (MALME-3M). The mixtures of 29-hydroxy-stelliferin B (**26**) and 13E-stelliferin G (**27**) have shown highest growth-inhibitory [(IC_{50}) 0.11, 0.23 *μ*g/ml, respectively)] activities against MALME-3M [60] (Fig. 2 above).

Triterpenes, geoditin A (**31**), geoditin B (**32**), isogeoditin A (**33**) and isogeoditin B (**34**) were isolated from *Rhabdastrella aff. distincta* marine sponge. All compounds were tested against a small panel of human tumor cell lines [61]. Geoditin A (**31**) and geoditin B (**32**) have been isolated from *Geodia japonica* marine sponge. Geoditin A was the most cytotoxic to HL60 cells [IC_{50} Z3 mg/ml (<6.6 *m*M)] and geoditin B exhibited relatively weak cytotoxicity [62].

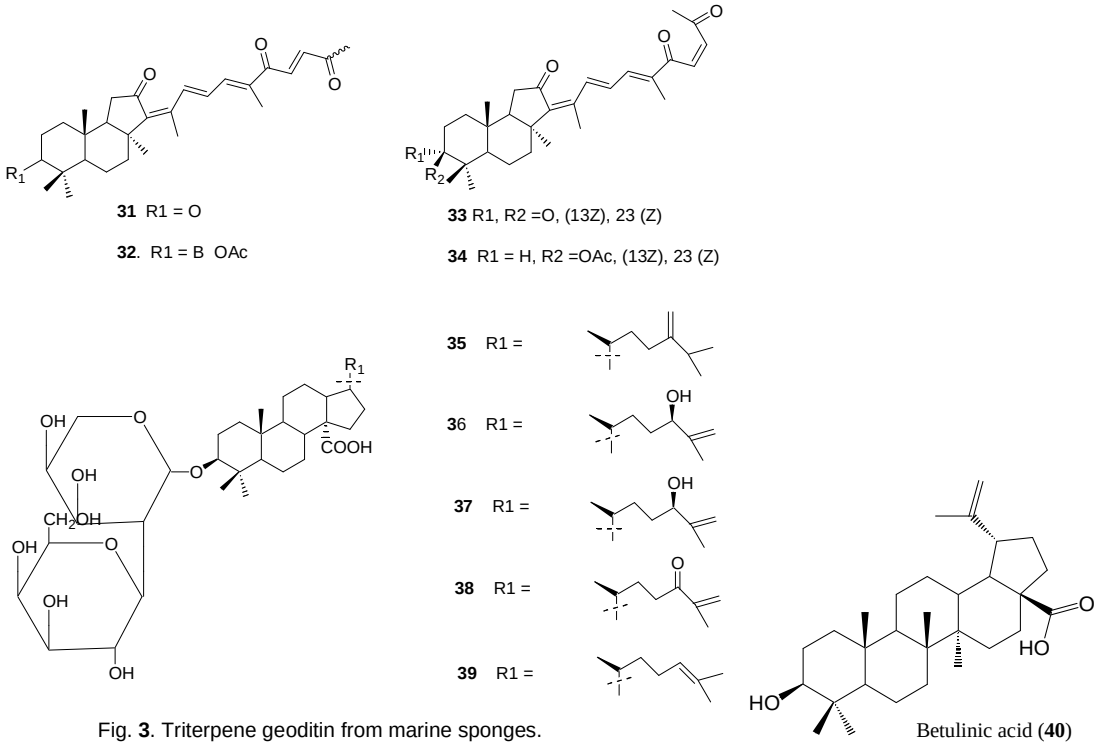


Fig. **3**. Triterpene geoditin from marine sponges. Betulinic acid (**40**)

Five cytotoxic triterpene glycosides, erylosides F1-F4 (**35**–**38**), and erylosides F (**39**) (Fig. **3**) were isolated from the sponge *Erylus formosus* collected from Mexico. Four compounds induced the early apoptosis of Ehrlich carcinoma cells, where erylosides F3 have shown excellent activity at a concentration of 100 *µ*g/ml [63].

3.1.3 Anti-cancer activity of betulinic acid/ betulinic acid derivatives

Betulinic acid is a natural product that exhibits potent anti-tumor activities by triggering the mitochondrial path to apoptosis. Betulinic acid (**40**) is currently under evaluation as a topical agent in a phase I/II clinical trial for the treatment of dysplastic nevi with the potential to transform into melanoma.

Betulinic acid is a highly promising anti-cancer drug after inducing apoptosis in melanoma cell lines *in vivo* and *in vitro*, experimental work focused on the apoptosis inducing mechanisms of betulinic acid and other triterpenes. The anti-tumour effects were subsequently confirmed in a series of cancer cell lines from other origins, for example lung, breast, colon and neuroblastoma.

The anti-tumor cytotoxicity of betulinic acid has been extensively studied in a panel of cancer cell lines, primary tumor samples and xenograft mouse models (Table 1). It is selectively cytotoxic against melanoma cell lines [68]. Anti-cancer activity was subsequently also reported against other types of human cancers including neuroblastoma, glioblastoma, medulloblastoma, ewing tumor, leukemia as well as several carcinoma, colon, breast, hepatocellular, lung, prostate, renal cell, ovarian or cervix carcinoma [69-81]. Moreover, betulinic acid cooperated with different cytotoxic stimuli to suppress tumor growth, chemotherapeutic drug [82,83] or the death receptor ligand trail [84]. This suggests that betulinic acid may be used as sensitizer in combination regimens to enhance the efficacy of anti-cancer therapy.

Table 1. *In vitro* cytotoxic effect of betulinic acid on human cancer cell lines

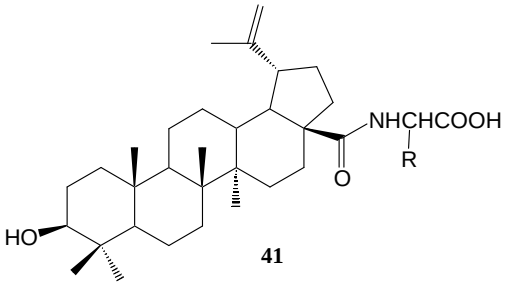
Cancer type	ED_{50} (*µ*g/ml)
Neuroblastoma	2-10 [180]
Melanoma	1.1-4.8 [174]
Glioblastoma	5-16 [176]
Medulloblastoma	3-15 [176]
Head & neck cancer	8 [181]
Ovarian carcinoma	1.8-4.5 [182]
Lung carcinoma	1.5-4.2 [182]
Cervix carcinoma	1.8 [182]
Leukemia	2-15 [183]

Betulinic acid suppressed tumor growth in a melanoma xenograft model [72]. Also *in vivo*, betulinic acid cooperated with chemotherapeutic agents [83]. No toxicities or weights loss were observed in betulinic acid-treated mice [72]. Pharmacokinetic studies in mice bearing melanoma xenografts demonstrated that betulinic acid was well absorbed and distributed within the tumor [85,86]. Phase I/II studies of 3-*O*-(3',3'-dimethylsuccinyl) betulinic acid in patients with human immunodeficiency virus infection demonstrated that plasma concentrations ranged from 8 to 58 *μ*g/ml [87,88].

Betulinic acid was reported to harbor anti-carcinogenic properties that could be exploited in cancer prevention settings [89]. Many betulinic acid derivatives were developed with the aim to improve the pharmacokinetic properties. For example, replacing the cyano group with a methoxy carbonyl was reported to markedly enhance the apoptosis inducing activities of betulinic acid [90]. These new betulinic acid analogues showed higher plasma and tissue levels compared to betulinic acid. Further, C-3 modified betulinic acid derivatives proved to have better *in vivo* anti-tumor efficacy as compared to betulinic acid *in vivo* against human colon cancer [91]. 17-Carboxylic acid modified 23-hydroxy betulinic acid ester derivatives demonstrated for cytotoxic activity *in vitro* on five cancer cell lines. All compounds showed higher cytotoxic activity as compared to 23-hydroxy betulinic acid and betulinic acid *in vivo* and also *in vitro* [92]. Betulinic acid killed melanoma cells in mice [90]. Betulinic acid showed anti-cancer activities [93-94].

3.1.3.1 Additional anticancer effects of betulinic acid

Betulinic acid (**40**) has also been reported to inhibit aminopeptidase N [95-97]. Betulinic acid was reported to exert anti-angiogenic effects by inhibiting growth factor induced *in vitro* angiogenesis in endothelial cells [95]. Further, the anti-angiogenic activity of betulinic acid was attributed to activation of selective proteasome dependent degradation of the transcription factors specificity protein Sp1, Sp3, and Sp4. Compared to betulinic acid, 20,29-dihydro-betulinic acid derivatives were claimed to posses better antiangiogenic properties as betulinic acid [98,99]. Betulinic acid was shown to inhibit the catalytic activity of topoisomerase. Furthermore, betulinic acid exerts context-dependant effects on the cell cycle [100,101]. Alterations in cell cycle progression in response to betulinic acid were also highly dependant on individual cell lines [101].

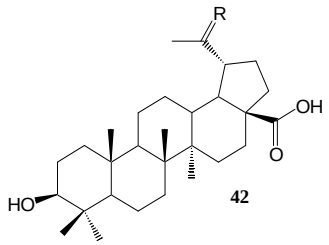


41

	R		R
41a	H	41e	$CH(CH_3)CH_2CH_3$
41b	CH_3	41f	$CH_2CH_2SCH_3$
41c	$CH(CH_3)_2$	41g	$CH_2C_6H_5$
41d	$CH_2CH(CH_3)_2$	41h	CH_2(3-indole)

Fig. **4.** C- 28 Peptide derivatives of betulinic acid

Betulinic acid is a highly selective growth inhibitor of malignant tumor cells and human melanoma and in these cells induce apoptosis.



42

	R
42a	O
42b	NOH
42c	$NOCH_3$

Fig. **5.** C-20 modified betulinic acid derivatives

The cytotoxicity of betulinic acid was studied *in vitro* in non-melanoma and melanoma tumor cell lines. It was also tested on cell lines expressing a different p53 status. It exerted its anti-proliferative activity. Betulinic acid was found active *in vitro* against neoplastic cell lines. Betulinic acid is a cytotoxic agent against neuroectodermal tumor cells, which represent the most common solid tumors of childhood [102]. The betulinic acid was coupled with a series of amino acids at the C-28 carboxylic acid side and evaluated the cytotoxicity of the modified compounds against epidermal carcinoma (KB) and cultured human melanoma (MEL-2) of the mouth cell lines [103].

The free acid of the alanine (**41b**) and valine (**41c**) analogues showed toxicity against KB. Methyl ester of alanine and valine conjugates and the free acid of the glycine (**41a**) conjugate showed toxicity against MEL-2. The free acid of the alanine conjugate showed the best toxicity profile against MEL-2; however it also showed toxicity against KB. Meanwhile the methyl ester of **41a**, and methionine (**41f**), alanine (**41b**) and tryptophan (**41h**), analogues showed improved cytotoxicity against MEL-2. The methyl ester of the phenyl alanine (**41g**), leucine (**41d**), glutamic acid (**41e**) and valine analogues showed the loss of cytotoxicity against MEL-2 when converted to the corresponding free acid conjugates. The C-20 alkene functional group of betulinic acid was modified [104]. When the double bond was oxidized to a ketone (**42a**), loss of cytotoxicity was observed. Converting to oximes **42b** and **42c** result in the loss of cytotoxicity. The cytotoxicity profile of betulinic acid derivatives may be sensitive to both the size of substituent at the C-20 position and its electrostatic properties (Fig. 4, 5).

3.1.3.2 Anti-cancer and pharmacological activity of betulinic acid isolated from plants

Betulinic acid from *Dillenia indica* L. fruits showed significant anti-leukemic activity in human leukemic cell lines U937, HL60 and K562 with IC_{50} values at 13.73, 12.84, 15.27 *mg*/ml respectively [105].

Betulinic acid isolated from *Orthosiphon stamineus* extract was tested for its cytotoxicity towards liver metastatic murine colon 26-L5 carcinoma cells [106]. It was found that betulinic acid showed the cytotoxicity with an ED_{50} value of 75.4 *µ*g/ml. Betulinic acid obtained from *Engelhardtia serrata* Bl. was tested for its cytotoxic and apoptosis inducing activities against the K562 cell line [107] and showed an inhibitory activity on the growth of K562 tumor cell line with IC_{50} value of 6.25 *µ*g/ml and also induced 35% apoptosis at 25 *µ*g/ml. Betulinic acid isolated from the *Bischofia javanica* was evaluated for its inhibitory effects on DNA topoisomerases II activity [108] and was found catalytic inhibitor of Topo II activity with IC_{50} value of 56.12 *µ*M which was comparable to that of 52.38 *µ*M for a classic Topo II inhibitor, etoposide. The ED_{50} values of betulinic acid and etoposide were found 7.19 and 2.59 *µ*M against A549 cancer cell line. Betulinic acid isolated from *Nerium oleander* was tested for anti-cancer activity toward three kinds of human cell lines, *i.e*. VA-13 malignant tumor cells, WI-38 fibroblast cells, and HepG2 human liver tumor cells [109]. Betulinic acid showed significant cell growth inhibitory to WI-38 cells, moderate cell growth inhibitory activity to VA-13 cells and HepG2 with IC_{50} values of 1.3, 11.6 and 21 *µ*M, respectively.

3.1.4 Anti-cancer activity of ursolic acid and oleanolic acid

Ursolic acid is a pentacyclic triterpenoid derived from berries, fruits, leaves and flowers of medicinal plants, such as *Rosemarinus officinalis*. Ursolic acid induced bax up-regulation and Bcl-2 down-regulation and release of cytochrome C to the cytosol from mitochondria. Moreover, ursolic acid cleaved caspase-9 and decreased mitochondrial membrane potential as shown with JC-1 staining. So ursolic acid induce apoptosis through both mitochondrial death pathway and extrinsic death receptor dependent pathway in MDA-MB-231 cells. So ursolic acid could be used as a potential anti-cancer drug for breast cancer [110].

Ursolic acid has been shown to inhibit tumorigenesis, tumor promotion and suppress angiogenesis. Scientists found that ursolic acid decreased cell proliferation rate and induce apoptosis in human breast cancer cell line, MDA-MB-231. When scientists checked the expression levels of proteins associated with apoptosis signal, they found that ursolic acid induces various apoptotic molecules related to either intrinsic or extrinsic apoptosis signal pathway in MDA-MB-231 cells [110].

3.1.4.1 Effects of ursolic acid and oleanolic acid on human colon carcinoma cell line HCT15

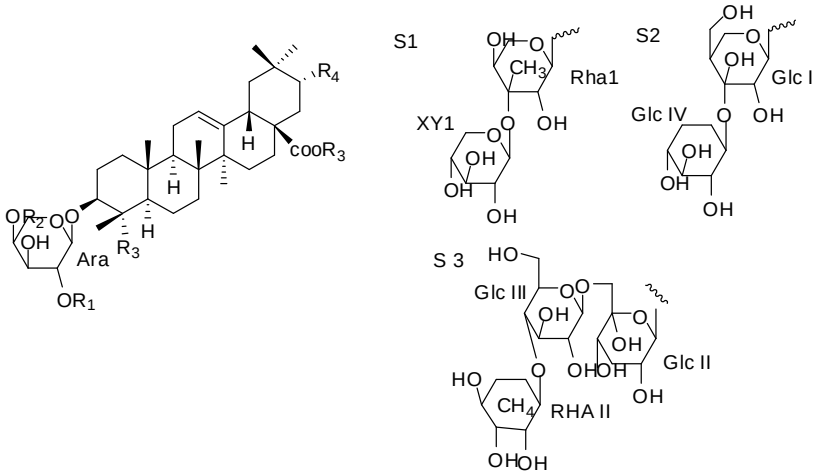
Ursolic acid inhibitory effects on human colon carcinoma cell line HCT15 were investigated. HCT15 cells were cultured with different drugs. A number of cells were degenerated, but cell fragments were rarely seen. The IC_{50} values for ursolic acid and oleanolic acid were 30 and 60 *µ*M/L, respectively. Proliferation assay showed that proliferation of ursolic acid and oleanolic acid treated cells was slightly increased at 24 h and significantly decreased at 48 h fand 60 h, whereas untreated control cells maintained an exponential growth curve. Ursolic acid and oleanolic acid have significant anti-tumor activity. The effect of ursolic acid is stronger than that of oleanolic acid. The possible

mechanism of action is that both drugs have an inhibitory effect through cell-cycle arrest on tumor cell proliferation [111].

3.1.4.2 Anti-tumor activity of oleanolic acid and its derivatives

Oleanolic acid had excellent anti-tumor effects and exhibited cytotoxic activity towards many cancer cell lines in culture. Ursolic acid and oleanolic acid were examined for their ability to inhibit the tumor growth and modify hematopoiesis after irradiation in three experimental systems: (a) *in vivo* anti-tumor activity of implanted tumor by asciatic cells was found to be augmented by addition of ursolic acid and oleanolic acid at a high concentration and inhibited in a dose-dependant manner; (b) in the sublethal whole body irradiated mice treated with the drugs in the 30 min preirradiation period, enhanced effects of ursolic acid and oleanolic acid on peripheral leukocytes were observed by a different significance, (c) when these chemicals were administered i.p. to mice 30 min before 4 Gy irradiation, both ursolic acid and oleanolic acid enhanced the postirradiation responses of splenic blastogenesis by PHA. Ursolic acid was a more potent tumorigenic inhibitor than oleanolic acid. Combining with the gamma-irradiation, however, there was no significant synergetic effect on their anti-tumor activity. The beneficial effects of ursolic acid and oleanolic acid on hematopoiesis and immunocompetence, suggested they might partially play a role in anti-cancer and furthermore with the ability to decrease undesirable radiation damage to the hematopoietic tissue after radiotherapy [112,113].

Phytochemical investigation of the rhizomes of *Anemone rivularis* var. *flore-minore* led to the isolation of five triterpenoid saponins **43-47**, along with five saponins **48-52**. The aglycone of **46** and **47** was determined as 21*α*-hydroxy-oleanolic acid. The cytotoxicity of these compounds was evaluated against four human cancer cell line, including HL-60, HepG2, A549 and HeLa. The monodesmosidic saponins **48-50** exhibited cytotoxic activity toward all tested cancer cell lines, with IC50 values in the 7.25–22.38 *μ*M range [114] (Fig. 6).



	43	44	45	46	47	48	49	50	51	52
R1	S1	S1	S1	RHa1	RHa1	S1	RHa1	S1	S1	H
R2	GLC1	DLC1	S2	DLC1	S2	H	GLC1	GLC1	H	H
R3	CHO	CHO	CH2	CH3	CH3	CH2OH	CH2OH	CH3	CH3	CHO
R4	H	H	H	OH	OH	H	H	H	H	H
R5	GLC 11	S3	GLC 11	S3	S3	H	H	H	S3	S3

Fig.**6** Structures of compounds **43-52.**

The anti-tumor and differentiation-inducing effects of a derivative of oleanolic acid modified at C-3, 3-oxo oleanolic acid (3-oxo-olea-12-en-28-oic acid, 3-oxo-OA) was studied. *In vitro*, 3-oxo-olea-12-en-28-oic acid were found to inhibit significantly the growth of cancer cells derived from different tissues. 3-Oxo-olea-12-en-28-oic acid had inhibitory effect on melanoma *in vivo*. This selection may relate to the differentiation induced by 3-oxo-olea-12-en-28-oic acid. The inhibition of 3-oxo-olea-12-en-28-oic acid on B16-BL6 suggests that 3-oxo-olea-12-en-28-oic acid may be a useful anti-cancer agent for melanoma [115,116].

3.1.4.3 Pharmacological activity of ursolic acid isolated from plant extracts

Plant extracts containing ursolic acid have been tested for various microbial and biological activities. It has been reported to possess a wide range of diverse pharmacological properties, including strong anti-inflammatory activity, anti-cancer, anti-oxidant and is one of the most promising chemopreventive agents for cancer and showed that ursolic acid can inhibit proliferation and induce apoptosis of many tumor cell lines.

Vauquelinia corymbosa correa plant has shown activity against P-388 lymphocytic leukemia test. The constituents responsible for this activity were identified as uvaol, ursolic acid and betulinic acid [117]. *Ledum groenlandicum retzius* has shown anti-oxidant, anti-inflammatory and anti-cancer activities [118,119].

Anti-proliferative activities of thirteen triterpenoids from apple peels were evaluated against human MCF-7 breast cancer cells, HepG2 liver cancer cells, and Caco-2 colon cancer cells. Most of the triterpenoids showed high potential anti-cancer activities against the three human cancer cell lines. The isolated compounds, 2α-hydroxy-3β-[(2E)-3-phenyl-1-oxo-2-propenyl]oxy-olean-12-en-28-oic acid, 2*a*-hydroxy ursolic acid and 3β-trans-p-coumaroyloxy-2α hydroxyolean-12-en-28-oic acid showed higher anti-proliferative activity toward HepG2 cancer cells. Ursolic acid, 3β-trans-*p*-coumaroyloxy-2α-hydroxy-olean-12-en-28-oic acid and 2α-hydroxy ursolic acid exhibited higher anti-proliferative activity against MCF-7 cancer cells. All triterpenoids showed anti-proliferative activity against caco-2 cancer cells, especially maslinic acid, 2α-hydroxy ursolic acid, 3β-trans-p-coumaroyloxy-2α-hydroxyolean-12-en-28-oic acid and 2α-hydroxy-3β–{[(2E)-3-phenyl-1-oxo-2-propenyl]oxy}olean-12-en-28-oic acid, showed much higher anti-proliferative activities [120].

Regulation of the phosphatidylinositol 3-kinase-Akt and the mitogen-activated protein kinase pathways were observed by ursolic acid in human endometrial cancer cells [121]. Ursolic acid demonstrates anti-cancer activity on human prostate cancer cells [122]. The cytotoxic activity of α-amyrine and ursolic acid has been assessed against three human cancer cell lines, TK-10, UACC-62 and MCF-7. Ursolic acid was found to possess the highest cytotoxic activity [123]. Proliferative inhibition, cell-cycle dysregulation, and induction of apoptosis by ursolic acid in human non-small cell lung cancer A 549 cells was studied [124]. Ursolic acid induces Bax-dependent apoptosis through the caspase-3 pathway in endometrial cancer SNG-II cells. Proteomic analysis of ursolic acid induced apoptosis in cervical carcinoma cells. Molecular mechanism of ursolic acid induced apoptosis in poorly differentiated endometrial cancer HEC108 cells [125-127].

The effect of ursolic acid on Bcl-2, COX-2 and Bax expression in human gastric cancer cell line SGC7901 was investigated, and explore its potential mechanisms of inhibiting proliferation and inducing apoptosis. Agents that can suppress STAT3 activation have potential for prevention and treatment of cancer. Ursolic acid tested, for its ability to suppress STAT3 activation. Ursolic acid, inhibited both constitutive and interleukin-6-inducible STAT3 activation in a time and dose dependant manner in multiple myeloma cells [128- 130].

3.2 Anti-Hiv Activity

3.2.1 Oleanolic acid /oleanolic acid derivatives: *In vitro* Anti-Hiv activity of oleanolic acid and its derivatives on infected human mononuclear cells

Oleanolic acid inhibited HIV-1 replication in acutely infected H9 cells and inhibited H9 cell growth with an IC_{50} value of 21.8 μg/ml. Pomolic acid (**63**) from *R. woodsii* and *H. capitata*, was also identified as an anti-HIV agent. Although ursolic acid did show anti-HIV activity, it was slightly toxic (IC_{50} 6.5 μg/ml, T. I. 3.3). The derivatives of betulinic acid, isolated from *S. claviflorum* as an anti-HIV principle, exhibited

extremely potent anti-HIV activity. Accordingly, derivatives of oleanolic acid (**53-62**) showed their anti-HIV activity [131] (Fig. 7).

Oleanolic acid derivatives with different lengths of 3-*O*-acidic acyl chains were synthesized and evaluated for their inhibitory activity against HIV-1 protease. The lengths of the acidic chains were optimized to 6 and 8 carbons. Changing a 3-ester bond to an amide bond or dimerization of the triterpenes retained their inhibitory activity against HIV-1 protease. Introduction of an additional acidic chain to C-28 of oleanolic acid increased the inhibitory activity, though a derivative with only one acidic chain linked at C-28 also showed potent activity against HIV-1 protease. The ester bonds of the triterpene derivatives were found to be stable to lipase. The inhibitory mechanism was proved directly by size exclusion chromatography to be inhibition of dimerization of the enzyme polypeptides [132].

The effect of oleanolic acid on the growth of human immune deficiency virus-1 in cultures of human peripheral mononuclear cells and of monocyte/macrophages was studied. Its inhibitory activity was also evaluated on peripheral mononuclear cells. Results showed that oleanolic acid inhibits the HIV-1 replication in all the cellular systems used (EC_{50} values: 22.7μM, 24.6μM and 57.4μM for *in vitro* infected peripheral mononuclear cells (naturally infected peripheral mononuclear cells and monocyte/macrophages, respectively). Oleanolic acid inhibits *in vitro* the HIV-1 protease activity [131]. Anti-HIV activity of oleanolic acid and structurally related triterpenoids was studied [132].

3.2.2 Anti-Hiv activity of ursolic acid/ ursolic acid derivatives

Ursolic acid have shown to inhibit protease [132-134,135,136]. Treatment with ursolic acid increases phospho-JNK in a time and dose dependent manner but does not alter phospho-Erk1/2 and phospho-P38. This shows that JNK may participate in ursolic acid induced apoptosis in K562 cells [135]. Reduction of DNA-damaging effects of anti-HIV drug 3'-azido-3'-dideoxythymidine on human cells by ursolic acid was studied [137]. Ursolic acid did show anti-HIV activity (EC_{50} 2.0 μg/ml) and was found slightly toxic (IC_{50} 6.5 μg/ml). Ursolic acid, isolated from *P. glandulosa, S. claviflorum* and *H. capitata*, was found to show similar anti-HIV activity, with an EC_{50} value of 2.0 μg/ml and IC_{50} values of 6.5 μg/ml [138,139,140].

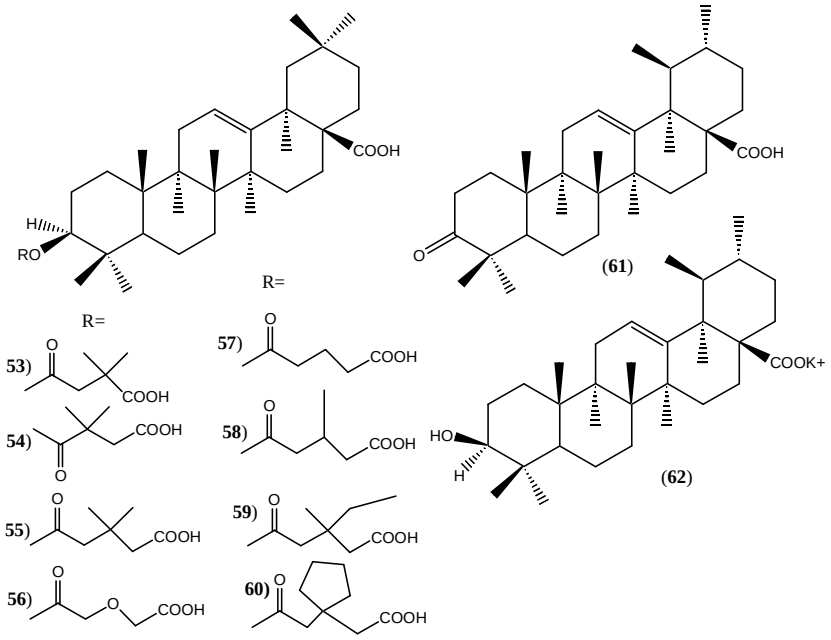


Fig.**7.** Derivatives of oleanolic acid (**53-62**) with different lengths of 3-O-acidic acyl chains.

Several 3-*O*-acyl ursolic acids were evaluated for anti-HIV activity. Ursolic acid was found equipotent (EC_{50} 4.4 μM) with oleanolic acid (EC_{50} 3.7 μM), although it was slightly toxic (IC_{50} 14.3 μM). 3-*O*-diglycoryl ursolic acid (**64**) demonstrated relatively potent anti-HIV activity with an EC_{50} of 0.31 μM and a therapeutic index of 155.5. In contrast, 3-*O*-(3', 3'-dimethylsuccinyl) ursolic acid (**65**), which is analogous to the extremely potent anti-HIV betulinic acid derivatives, displayed only weak anti-HIV activity (EC_{50} 2.1 μM) (Fig. **8**) [141].

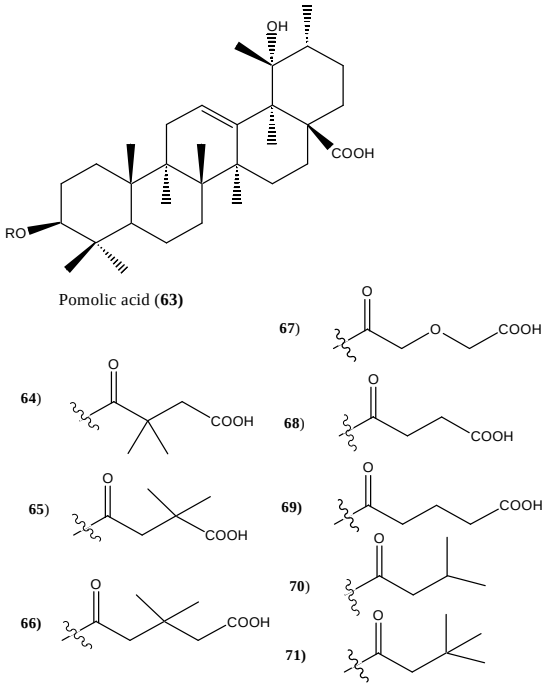


Fig.**8.** Several 3-*O*-acyl ursolic acid derivatives.

Ursolic acid and its hydrogen malonate isolated from *Cynomorium songaricum RUPR* as inhibitors of human immunodeficiency virus type 1 protease, with 50% inhibitory concentrations IC_{50} of 8μM and 6μM. Amongst various dicarboxylic acid hemiesters of related triterpenes, inhibitory activity increase in the order of oxalyl, malonyl, succinyl and glutaryl hemiesters, for ursolic acid. The most potent inhibition was observed for the glutaryl hemiesters, with an IC_{50} of 4 μM [142].

3.2.3 Anti-Hiv activity of betulinic acid/ betulinic acid derivatives

Betulinic acid from *Syzrgium claviflorum* was tested on HIV-1 replication in H9 lymphocyte cells. It showed an inhibitory activity against HIV-1 replication with an EC_{50} value of 1.4 μM and inhibited uninfected H9 cell growth with an IC_{50} value of 13μM.

Betulinic acid isolated from the stem bark of *Peltophorum africanum* evaluated for the inhibitory activities against HIV-1NL4-3 (X4-HIV-1) and HIV-1JRCSF (R5-HIV-1). It inhibited HIV-1NL4-3 and HIV-1JRCSF with IC_{50} values of 0.04 and 0.002 μg/ml, respectively. It showed that betulinic acid could be used as potential therapeutics for HIV-1 [143]. Betulinic acid from *Cratoxylum arborescens* tested in the HIV-1 RT assay and syncytium assay and it showed anti-HIV-1 activity in the syncytium assay with IC_{50} value of 9.8 μg/ml and in the RT assay with IC_{50} of 10.8 μg/ml [144].

Betulinic acid derivatives have been reported as inhibitors of HIV-1[145], HIV-protease [146] or of reverse transcriptase [147]. Since a number of betulinic acid derivatives have been shown to inhibit HIV-1 at a very early stage of the viral life cycle, these compounds have the potential to become useful additions to current anti-HIV therapy, which relies primarily on combination of protease inhibitors and reverse transcriptase.

3.2.4 Structure activity relationship studies of betulinic acid with reference to anti-Hiv activity

Betulinic acid from *Syzrgium claviflorum* exhibited inhibitory activity against HIV-1 replication in H9 lymphocyte cells with an EC_{50} value of 1.4 μM.

Hydrogenation of betulinic acid yielded dihydro-betulinic acid (**74**) which showed slightly more potent anti-HIV activity with an EC_{50} of 0.9 □M and a selectivity index of 14. Mayaux *et al*. synthesized certain amide derivatives of betulinic acid (**75a-c**). Among them, the compound N'-{N-[36-hydroxyl-20(29)ene-28-oyl]-8-amino octanoyl}-1-statin (**75c**) was found to be the most potent anti-HIV compound against HIV-1 strain IIIB/LAI with a selectivity index of 200. Compound **75c** was also examined for a possible inhibition of the *in vitro* activity of several purified HIV-1 enzymes (Fig.9).

Syntheses and anti-HIV activities of some derivatives by modifying the C-3 hydroxyl group in betulinic acid and dihydro betulinic acid was studied. Compounds **40** and **74** were treated with 3,3-imethylglutaric anhydride and diglycolic anhydride to furnish the corresponding 3-*O*-acyl derivatives (**76c**, **76d**, **76c** and **76d**) [147].

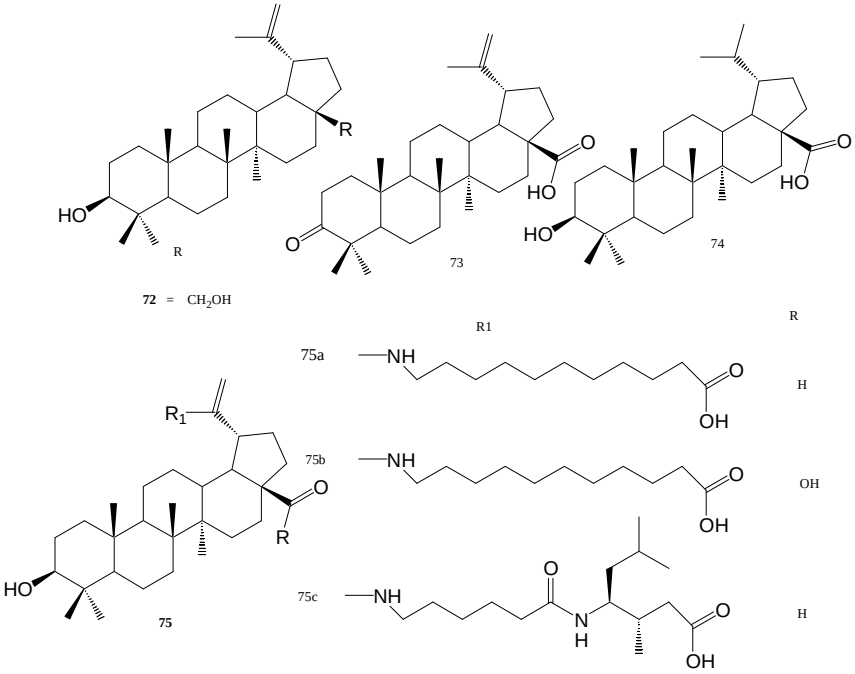


Fig.**9** Structures of betulinic acid , betulin, dihydrobetulinic acid, and amide derivatives of betulinic acid.

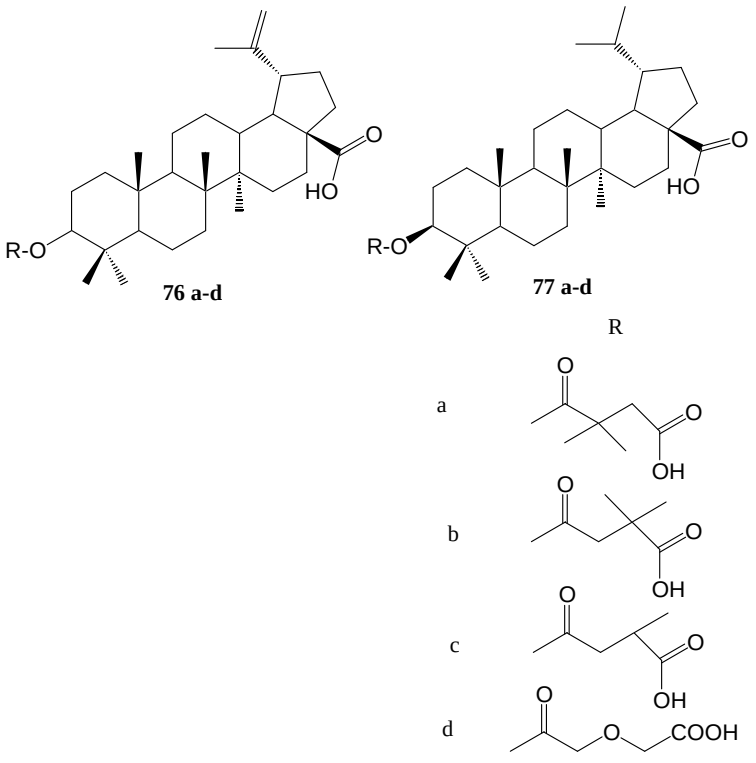
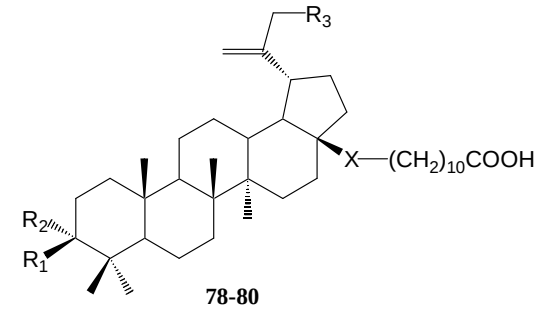
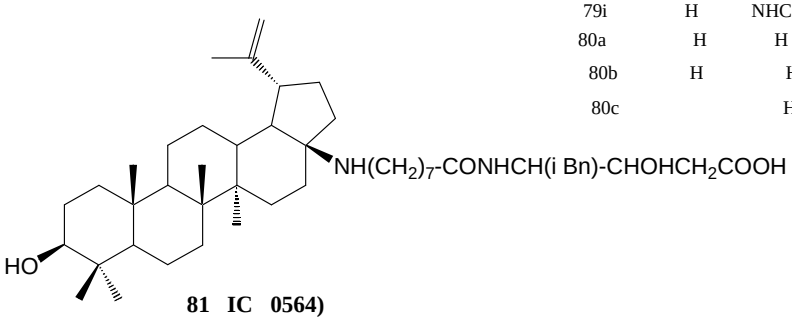


Fig. **10.** C-3 modified derivatives of betulinic acid and dihydrobetulinic acid



78-80

	R2	R3	X
78a	H	H	CONH
78b	OH	H	CONH
78C	=O	H	CONH
78d	H	H	CONH
78e	H	H	COO
78f	H	H	$CONCH_3$
78g	H	H	CONH
78h	H	H	CONH
79a	H	SCH_2CH_2OH	CONH
79b	H	$SCH_2CH_2N(Et)_2$	CONH
79c	H	SCH_2COOH	CONH
79d	H	$NHCH_2CH_2OH$	CONH
79e	H	1-pyrrolidinyl	CONH
79f	H	$NHCH_2COOH$	CONH
79g	H	OH	CONH
79h	H	NH2	CONH
79i	H	$NHCOCH_3$	CONH
80a	H	H	NHCO
80b	H	H	NHCONH
80c		H	CH2NH



81 IC 0564)

Fig. **11.** C_{17} modiffied betulinic acid derivatives.

In contrast, similar treatment of 7**2** and **74** with dimethylsuccinic anhydride afforded a mixture of 3-*O*-(2',2'-dimethylsuccinyl) and 3-*O*-(3',3'-dimethylsuccinyl) betulinic acid derivatives (**7**6**a** and **7**6**b**) and dihydro betulinic acid derivatives (**7**7**a** and **7**7**b**), respectively. The compounds **7**6**b** and **7**7**b** were extremely potent in acutely infected H9 lymphocytes with EC_{50} values less than 3.5 x 10^{-4} μM and selectivity index values > 20,000 and > 14,000, respectively.

In contrast, compounds **7**6**a** and **7**6**c** showed anti-HIV activities with SI values of 5.9 and 13.8□M, and EC_{50} values of 2.7 and 0.56 μM, respectively (Fig. **10**). Compounds **76c**, **76d**, **77c** and **77d** exhibited anti-HIV activities with EC_{50} values ranging from 0.01 to 2.3 x 10^{-3} μM. None of these compounds inhibited the HIV-RT in the concentration range of 167-219 μM. In the HIV-induced membrane fusion inhibition assay, compounds **76a-d** and **77a-d** inhibited syncytia formation in the concentration range of 33-70 μM. By modifying the C30-methyl and C17-COOH groups of betulinic acid a series of undecanoic acid and amides of lup-20(29)-en-28-oic acid derivatives synthesized and evaluated for activity in MT-4 and CEM4 cell cultures against HIV-1 strain IIIB/LAI [150] (Fig. **10**). Epimerization of the hydroxyl group at C-3 from 3β (**78a**) to 3α(**78a**) led to a 10-fold drop in activity. The 3-keto derivative **78c** showed intermediate activity, whereas the 3-deoxy derivative **78g** were found inactive. These point to a critical hydrogen bond interaction involving the oxygen at the 3 position, preferentially occurs in the 3β-position. The 3β-methoxy **78d** and 3-amino **78h** derivatives were found inactive. The inactivity of **78d** might be due to the methyl group steric hindrance. The introduction of a second hydroxyl group led to a complete loss of activity. The anti-viral properties of 30-(hydroxy-ethyl) thio **79a**, 30-[2-(diethylamino) ethyl]thio (**79b**), 30-(1-pyrrolidinyl)-(**79e**) and 3,30-dihydroxy (**79g**) derivatives remained high, but were not better than that of the unsubstituted derivative **78a**. This illustrated a lack of steric requirements by the HIV-1 molecular target for groups at C_{30}

position. However, an acidic substitution such as 30-(carboxymethyl) thio (**79c**) was clearly detrimental to potency. A similar decrease in activity was observed when a secondary nitrogen was directly attached at C_{30} position (**79d** and **79i**).

The combination of a free carboxyl and a secondary amine as well as an unsubstituted amino moiety (**79f** and **79h**) led to decrease in activity. Variations at the C-28 carboxyl position such as N-methylation of the amide moiety in **78f**, replacement of the amide by an ester in **78e,** and replacement of the carbonyl by a methylene as in **80c** led to a complete loss of activity. The importance of the hydrogen donating NH group was highlighted by the fact that the corresponding ester **78e** was found inactive. This was further corroborated by the lack of biological activity of the reversed amide **80a** and the urea derivative **80b** in which the NH group occupied a different special position. The dramatic loss of activity for most of the modification on the triterpene skeleton suggests a specificity for the compounds. All these compounds were found to interfere with HIV-1 entry in the cells at a post-binding step (Fig.11). All the compounds were inactive against HIV-2 ROD.

Soler *et al*. synthesized a series of ω-aminoalkanoic acid derivatives of betulinic acid and evaluated for their HIV-1 activity. The anti-HIV-1 activities of several members of this new series were found to be in the nanomolar range in CEM-4 and MT-4 cell cultures. Among them, compound **81** was found to display the best overall activity with an EC_{50} of 50 ±26 *n*M (CEM4) and 40±19 *n*M (MT-4) with a SI of >100. Nevirapine displayed an IC_{50} value of 84 ±21 *n*M (CEM4). Among various dihydro betulin and *O*-acyl betulinic derivatives, the most potent compound **82a** with two 3',3'-dimethylglutaryl groups displayed anti-HIV activity with an EC_{50} value of 0.66 *n*M and SI of 21,515 [148]. The dihydro betulin derivative **82a** showed a SI of 2253. Monoacylbetulin (**82b**), containing a substituted glutaryl group at C-28 position, had an EC_{50} value of 3.6 μM and a SI of 7.8. Conversion of the 3β–hydroxy group of **82b** to the monoketo derivative led to **82c**, which showed slightly lesser activity (EC_{50} = 29.2 μM; SI = 2.9) than the corresponding nonketone derivative **82b**. The triacylated compound **82d** displayed potent anti-HIV activity with an EC_{50} value of 0.045 μM.

The 3-epi derivative of **82a**, displayed lower inhibition of HIV. Bioisosteric replacement of **82a** to an amide derivative (**82f**) resulted in reduction of anti-HIV activity (EC_{50} = 0.5 μM; SI = 36.6). So the acylation only at the C-28 position did not result in significant increase/decrease of activity. However, compounds with acyl side chains at both C-28 and C-3 positions reached optimal activity. Among four isomeric 3,28-di-*O* (dimethylsuccinyl) betulin derivatives, **83c** demonstrated the highest anti-HIV activity in acutely infected H9 cells with an EC_{50} value of 0.87 *n*M [149]. Compound **83a** was also extremely potent with an EC_{50} value of 0.02 μM. Compound **83b** displayed fair activity (EC_{50} = 0.4 βM; SI = 96.5), while **83d** was toxic. Synthesized compound **81** analogues and compounds **83a** and **83b** were the most promising compounds against HIV infection with EC_{50} values of 0.33 and 0.46 μM respectively [151]. Both compounds inhibited syncytium formation with EC_{50} value of 0.40 and 0.33 μM, respectively. Hence the double bond in **81** can be eliminated and the statin moiety can be replaced with L-leucine while retaining anti-HIV activity [Fig. 12].

Two triterpene derivatives **84** and **85**, from *Alisma* genus, were reported to display inhibitory activity against HIV-I reverse transcriptase with an IC50 of 15.5 and 12.2 μg/ml [152].

3.3 Lipotropic and Liver-Protective Activity

Compounds belonging to the alisol A group **86** and **87** and the alisol B derivatives alisol B 23-acetate **88** and alisol C-23 acetate derivative **89** displayed good anti-cholesterolemic effects. Inclusion of 0.1% of either of these compounds in the diet for hypercholesterolemic rats would reduce the cholesterol levels by more than 50%, compound **87** being most potent, resulting in 61% reduction [153] (Fig. **13**). Alisol A 24-acetate **87**, **88** and **89** were able to protect mice against CCl4-induced liver damage with **89** being the most effective protectant [154]. Moreover, alisol A derivatives **90** and **91** were found to inhibit 100% and 60% of D-galactosamine induced liver damage *in vitro*, respectively [155,156]. The alisol A derivatives **87, 92, 93, 90, 94** and the alisol B type **88** were reported to exhibit protective activity against hepatitis B viral infections [157] (Fig. 14).

Compound **94** showed the most promising effects by inhibiting HBsAg and HBeAg (HBV e

antigen) with IC50 of 7.7μM and 5.1μM, respectively, while cytotoxic effects were only observed at much higher concentrations [50% cytotoxicity concentration (CC50) = 142.7 μM].

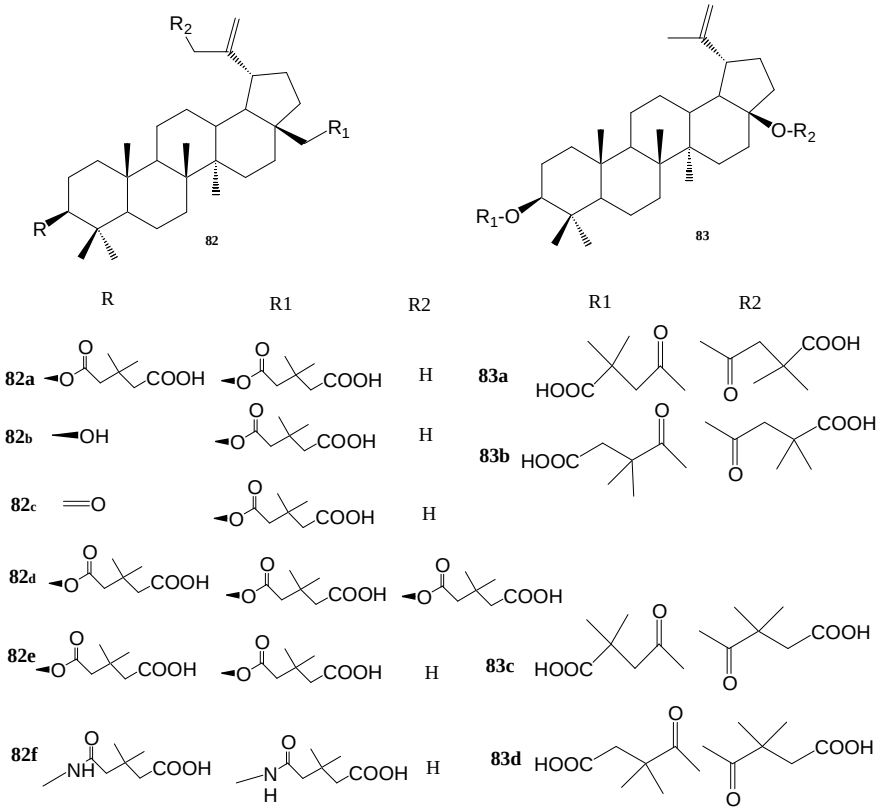


Fig.**12.** *O*-Acyl derivatives of betulinic acid and dihydrobetulinic acid.

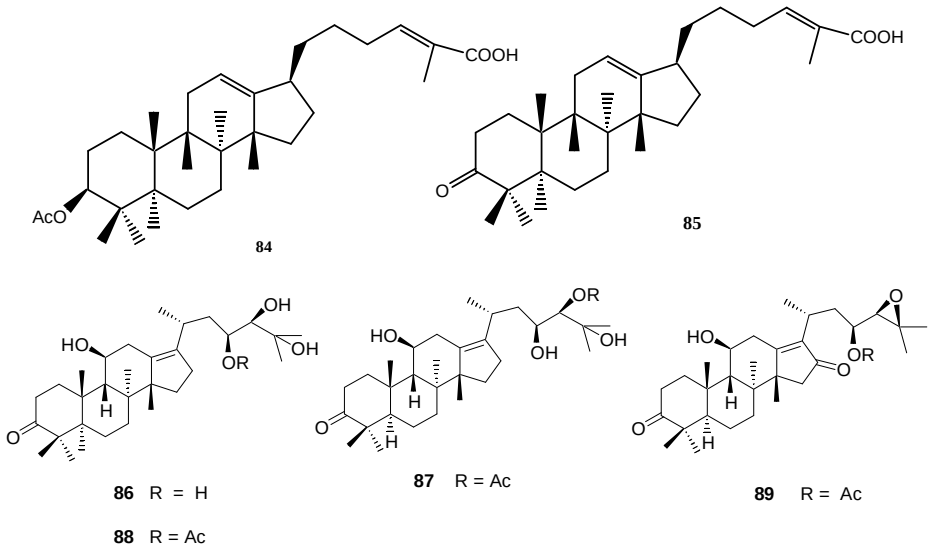


Fig. **13.** Alisole A derevatives **(86, 87)** and Alisole B derivatives **(88, 89)**

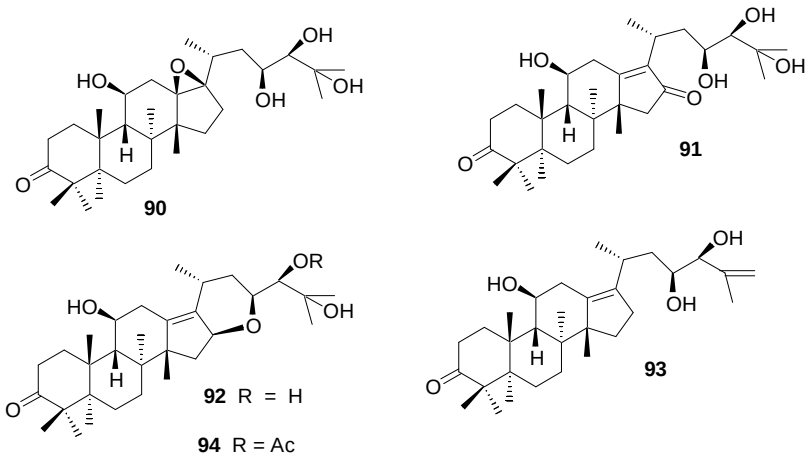


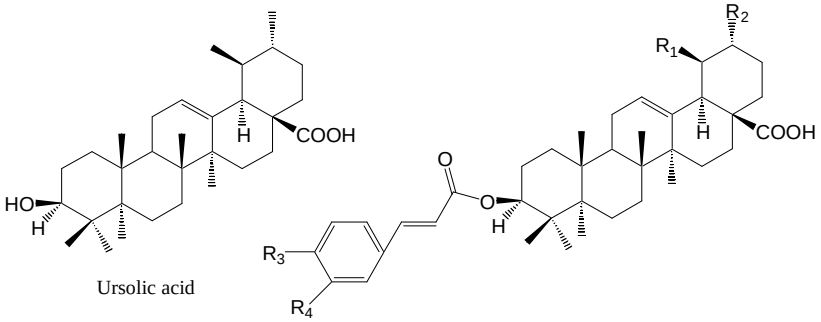
Fig. **14.** Alisole A derivatives.

A number of alisol A derivatives evaluated for their cytotoxicity and *in vitro* anti-HBV activity. Acylation of the hydroxy groups at positions 23, 24, and 11 decreased the cytotoxicity [158-161].

3.4 Anti-Bacterial activity

Oleanolic acid and ursolic acid have been modified at the C-3 position to cinnamate-based esters (**95-103**) and *in vitro* anti-mycobacterial activity against *Mycobacterium tuberculosis* H (37) has been determined.

Modification of oleanolic acid and ursolic acid to the *p*-coumarate and, the ferulate ester analogues resulted in high anti-mycobacterial activity (Fig. **15**). Ursolic acid exhibited anti-micobacterial activity, whereas its cinnamoyl and *p*-chlorocinnamoyl analogues **95** and **97** were found inactive. The *p*-coumarate ester **97** was found highly active (MIC = 6.25 *µ*g/ml). The corresponding acetate and methyl ether derivatives, **96** and **100**, showed lower activity with MIC's of 25 and 100 *µg/*ml respectively. Both the ferulate ester **109** and its acetate **100** were highly active. However, the corresponding caffeate ester **102** and its acetate **101** were much less active, with respective MICs of 200 and 100 *µ*g/ml [162].



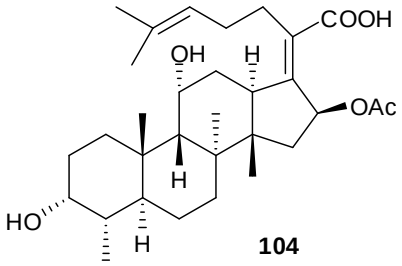
95) R1= Me R2= R3= R4= H
96) R1= Me R2= R4= H R3= OAC
97) R1= Me R2= R4= H R3= OH
98) R1= Me R2= R4= H R3= OMe
99) R1= Me R2= H R3= OAC R4= OMe
100) R1= Me R2= H R3= OH R4= OMe
101) R1= Me R2= H R3= R4= OAc
102) R1= Me R2= H R3= R4= OH
103) R1= Me R2= R4= H R3= Cl

Fig.15. Synthesis of Cinnamate -based esters of ursolic acid

Betulinic acid isolated from *Argentinean* legume and *Caesalpinia paraguariensis* bark and tested it against *Bacillus subtilis*, *methicillin* sensitive and resistant *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* [160]. Betulinic acid was found to be inactive against the tested organisms with MICs greater than 128 *μ*g/ml. Betulinic acid was isolated from *Syncarpia glomulifera* [161]. The crude extract of *S. glomulifera* showed anti-bacterial and cytotoxic activity. Betulinic acid was isolated from *Zizyphus joazeiro* showed a considerable activity against Gram-positive bacteria. The anti-bacterial activity of betulinic acid from *Vitex neg*undo L. was tested against *Bacillus subtilis* and *Escherichia coli* [164]. Betulinic acid did not show any inhibition zone against *Escherichia coli* at concentrations of 1000 *μ*g/disc, 500 *μ*g/disc, 250 *μ*g/disc and 125 *μ*g/disc. However, betulinic acid showed 18.8 mm of inhibition zone against *Bacillus subtilis* at concentration of 1000 *μ*g/disc while at a concentration of 500 *μ*g/disc and below, the betulinic acid did not show any inhibition zone. Standard compound Kanamycin showed an inhibition zone of 207.2 *mm* at a concentration of 0.05 *μ*g/ml.

Two active compounds, betulinic acid and oleanolic acid, were found in the extract of *Forsythia suspense*. Betulinic acid inhibited urease activity of *H. pylori* stronger than oleanolic acid [162].

Betulinic acid from *Vitex negundo* demonstrated anti-bacterial activity against *Bacillus subtilis* at a concentration of 1000 *μ*g/disc with a zone of inhibition of 18.8 *mm* [163]. Similarly betulinic acid and its three new derivatives namely 7*a*-(4-hydroxy-benzoyloxy) betulinic acid, 7□-(4-hydroxy-3'-methoxy-benzoyloxy), betulinic acid and 27-(4-hydroxy-3'-methyl-benzoyloxy) betulinic acid, were isolated from *Zizyphus jaazerio* [164] showed considerable activity against Gram-positive bacteria. Fusidic acid (**104**) was first isolated from *Fusidium coccineum* [167-170].



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It has been clinically used as an antibiotic topical therapies for *Staphylococcal* infections [171]. It exhibits potent effects against *Staphylococci*, including the methicillin-resistant *Staphylococcus aureus* and the coagulase-negative *Staphylococcal* species. Fusidic acid distributes well in various tissue, exhibits low toxicity and allergic reactions; and it has little cross-resistance with other clinically used antibiotics. Though never approved for use in the United States, fusidic acid is marketed with 21 million annual prescriptions in more than twenty countries [172]. The global problem of microbial resistance has now led to a renewed interest in its use. Since 2006, this "old" antibiotic has received attention in the US mainly because no recommended oral antibiotics have shown useful activity against methicillin-resistant *Staphylococcus aureus*. To date, phase 2 clinical trials has finished and the results supported the proceeding to phase 3 studies [173].

3.5 Antimalarial Activity of Triterpenes

Triterpenes and their derivative might be considered as potential lead compounds for the development of new antimalarial drugs, since the structural changes in the molecule significantly alter the anti-*Plasmodium* activity.

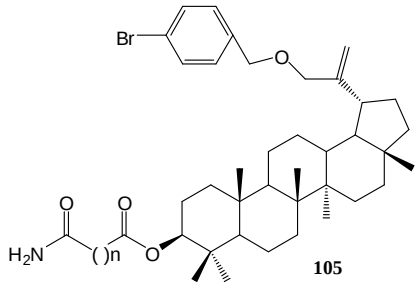
The ursolic acid from *Uapaca nitida* Müll-Arg. exhibited anti-malarial activity with IC_{50} values of 36.5 *μ*gs/ml and 28 *μ*gs/Ml [176]. Ursolic acid, purified from the hydromethanol extract of *M. inermis* induced a significant decrease of parasite proliferation. The *in vitro* and *in vivo* anti-malarial activity of betulinic acid was investigated [174, 175]. The *in vitro* anti-plasmodial activity of betulinic acid isolated from *Tanzanian* tree against Chloroquine resistant and sensitive (T9-96) *Plasmodium falciparum* were found to be 19.6 *μg*/ml and 25.9 □g/ml, respectively. When betulinic acid was tested for *in vivo* activity in a murine malaria model (*P.berghi*), the top dosage employed (250 *mg*/kg/day) was ineffective at reducing parasitemia and exhibited some toxicity. Betulinic acid from *Triphyophyllum peltatum* and *Ancistrocladus heyneanus* exhibited moderate to good *in vitro* anti-malarial activity against asexual erythrocytic stages of the human malaria parasite *P. falciparum* [177, 165-166].

The hexane extract from *Vernonia brasiliana* (L.) Druce was active *in vitro* against *Plasmodium falciparum* and *in vivo* in mice infected with *Plasmodium berghei*. Lupeol from hexane extract was identified as a compound responsible for the activity, inhibiting the *P.falciparum* growth by 45% when tested at 25 *μg*/ml. However, this triterpene was inactive *in vivo* to mice infected with *P.berghei*. *β*-Amyrin and germanicol, from the same fraction that yielded lupeol, were inactive in the *in vitro* assay [178]. A novel friedelane triterpenoid endodesmiadiol, assayed against the W2 chloroquine-resistant strain of *P. falciparum*, was found to be active with IC50 values ranging from 7.2 to 23.6 *μ*M.

Betulinic acid and its derivative compounds betulonic acid, betulinic acid acetate, betulinic acid methyl ester and betulinic acid methyl ester acetate have been evaluated for their anti-malarial properties. These substances showed anti-plasmodial activity against W2 chloroquine-resistant *P. falciparum* parasites *in vitro*, with IC50 values of 9.89, 10.01, 5.99, 51.58 and 45.79 *μ*M, respectively. Moreover, since betulinic acid acetate displayed the best selectivity index among all substances tested. Betulinic acid and its derivative compounds might be considered as potential lead compounds for the development of new anti-malarial drugs, since the structural changes in the molecule significantly alter the anti-*Plasmodium* activity [179].

Emodin and lupeol were found to be the active principles responsible for the anti-plasmodial property of *C. siamea* [180]. Alisol A analogues **86** and **93** and the alisol B analogue **88** showed anti-plasmodial activities against *Plasmodium falciparum* K1 strain [181-183].

Diospyros rubra extracts were screened for anti-microbial, anti-malarial and cytotoxic activities. The extracts with good anti-malarial activity were isolated and extensively purified to give lupeol, lupenone, betulin, lupeol acetate, 28-*O*-acetyl betulin, *β*-sitosteryl-3-*O*-*β*-*D*-glucopyranoside and a mixture of *β*-sitosterol and stigmasterol. Compounds bearing an oxygen substituent in the B-ring had better activity. Kundu et al. used lupeol a pentacyclic triterpene, as a scaffold for the synthesis of lupeol based libraries. Compounds like **105** containing suberic acid and 4-bromobenzyl alcohol moieties exhibited 7–9 fold increase in the biological activity in comparison to lupeol (MIC = 13.07 lM) [192].



105

Isolated betulinic acid was screened *in vitro* for inhibitory activity against *Trypanosoma brucei* glycolytic enzyme GAPDH. Betulinic acid inhibited *T. brucei* GAPDH with an IC_{50} value of 240 *μ*M and was a competitive reversible inhibitor of this enzyme with respect to its cofactor NAD. Betulinic acid was obtained from *Clusia nemorosa* L. was tested for its anti-obese activity [226]. It was found that mice treated with betulinic acid and fed a HFD showed significantly decreased body weights, abdominal fat accumulation, blood glucose, plasma triglycerides, and total cholesterol relative to their respective controls fed no betulinic acid during 15 weeks. Betulinic acid has an antiobese potential through modulation of fat and carbohydrate metabolism.

3.6 Analgesic and Anti-inflammatory Activity

Lupane-type triterpenes, such as betulin, betulinic acid and lupeol, display anti-inflammatory activities. Pharmacological properties of the ubiquitous triterpene betulin was studied [184-187]. Anti-inflammatory activity of extract and fractions from *Nepeta sibthorpii* bentham was also studied [188 -194].

Anti allergic and anti-inflammatory ursolic acid derivative studied from the herb of *Prunella vulgaris* [195]. *In vitro* anti-inflammatory activity of 23-hydroxy-ursolic acid studied from *Cussonia bancoensis* in murine macrophage RAW 264.7 cells [196]. Ursolic acid, corosolic acid, 3-epicorosolic acid, pomolic acid, tormentic acid and hyptadienic acid, studied from *Perilla frutescens*. Except 3-epicorosolic acid all compounds, were evaluated for its inhibitory effects on 12-*O*-tetradecanoylphorbol-13-acetate-induced inflammation in mice. It showed a marked anti-inflammatory effect, with a 50% inhibitory dose of 0.09-0.3 mg per ear. In addition, an evaluation against the Epstein-Barr virus early antigen (Epstein-Barr virus-EA) activation induced by 12-*O*-

tetradecanoylphorbol-13-acetate-showed five compounds, ursolic acid, corosolic acid, 3-epicorosolic acid, tormentic acid and 3-epimaslinic acid, with a potent inhibitory effect on Epstein-Barr virus-EA induction (91-93% inhibition at 1x10(3) mol ratio/TPA) [197].

Ursolic acid and 23-hydroxy ursolic acid significantly inhibited 1%-carrageenan-induced edema in the rat. Ursolic acid and 23-hydroxy ursolic acid, are responsible for the anti-nociceptive and anti-inflammatory effect of *Cussonia bancoesnsis***.** Ursolic acid and 2α–hydroxy ursolic exhibited strong anti-allergic and anti-inflammatory inhibitory activities (IC_{50} values 17 and 27 μM, respectively) [196,197].

In vitro anti-inflammatory activity of triterpenoid compounds from *Phillyrea latifolia L.* was observed [198,199]. Substantial inhibition by amyrin, β-boswellic acid and ursolic acid, but not by 18β-glycyrrhetinic acid was observed. The data show that the dual inhibition of 5-lipoxygenase and human leukocyte elastase is unique to boswellic acids. Other triterpenes with human leukocyte elastase inhibitory activities do not inhibit 5-lipoxygenase, and leukotriene biosynthesis inhibitors from different chemical classes do not impair human leukocyte elastase activity. Because leukotriene formation and human leukocyte elastase release are increased simultaneously by neutrophil stimulation in a variety of inflammation and hypersensitivity based human diseases. The reported blockade of two proinflammatory enzymes by boswellic acids might be the rationale for the putative anti-phlogistic activity of acetyl-11-keto-β-boswellic acid and derivatives [200,201].

Ursolic acid not only inhibits human leukocyte elastase, but also 5-lipoxygenase and cyclooxygenase activity. It inhibited TPA-induced mouse ear edema by 72.4%. Hirota and coworkers determined that 200g and 50g applications of ursolic acid inhibited 12-*O*-hexadecanoyl-16-hydroxyphorbol-13-acetate-induced inflammatio. It inhibited concanavalin A induced histamine release, which can cause severe inflammation, at a concentration of 0.001 M [202].

Betulinic acid from *Diospyros leucomelas* showed anti-inflammatory activity in the Carrageenan and serotonin paw edema tests and TPA [203]. Betulinic acid form *Ipomoea pescaprae* showed pronounced anti-nociceptive properties in the writhing test in mice [204]. Anti-nociceptive effect of triterpenes from cacti was observed [205, 207]. Betulinic acid was isolated from *Ipomoea pescaprae* (L.) R. Br, shows pronounced anti-nociceptive properties in the writhing test in mice.

(The alisol A analogues **92**, **110**, and **111** the aliol B analogues **112**, **113**, and **114,** the alisol E analogue **115** and the *seco*-PT analogues **116**, and **117** were found to inhibit nitric oxide production in lipopolysaccharide-induced macrophages (IC50 = 8.4–68 μM). This action is suggestive of anti-inflammatory activity. In addition, the alisol A analogues **86**, **87** and **93** and the alisol B derivatives **118**, **119** and **86** showed potent inhibitory activity in the same bio-assay, but exhibited cytotoxic effects at concentrations above 30 μM. Compound **92** was found to suppress inducible nitric oxide synthase induction [213, 212] (Fig. 16).

Betulinic acid was isolated from rose hip was tested *in vitro* for inhibition of cyclooxygenase (COX-1, COX-2) and 5-LOX-mediated leukotriene B4 (LTB4) formation [228]. Betulinic acid was found to act as moderate inhibitors of COX-1, COX-2 and LT formation *in vitro* with IC_{50} values of >125, >125 and 102.2 μM, respectively.

3.7 Anthelmintic Activity

Anthelmintic activity of the extracts of *Berlina grandiflora* and one of its active principle, betulinic acid was studied. The anthelmintic activity of methanol, hexane and ethyl acetate extracts of *Berlina grandiflora* examined, which contain betulinic acid as the major component. *Caenorhabditis elegans*, a free living soil nematode, was used as an *in vitro* model in the study. A suspension of worms was treated with the extracts. Activity was assessed in terms of number of worms exhibiting motility. The results showed that the crude extracts showed anti-helmintic activity in the order ethyl acetate > methanol > hexane. Betulinic acid isolated from the ethyl acetate fraction showed strong anthelmintic activity at 100 ppm comparable to piperazine. The anthelmintic activity of the methanol, hexane and ethyl acetate extracts of the stem bark of *Berlina grandiflora* examined. The isolated betulinic acid from the ethyl acetate fraction at 100 and 500 ppm showed stronger anthelmintic activities than piperazine [206].

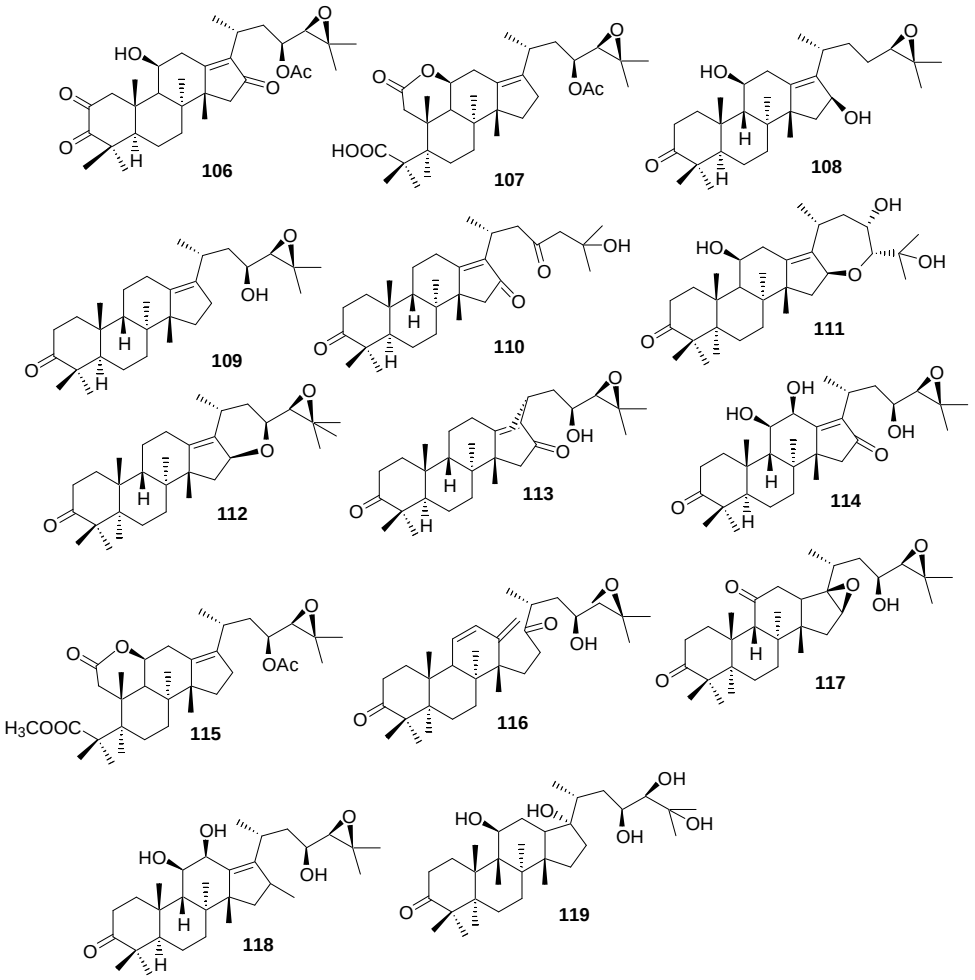


Fig. **16.** Alisol A, alisol B, alisol E, seco-PT analogues

3.8 Other Biological Activities

The isomalabaricane triterpenoids stelliferin riboside and 3-*epi*-29-acetoxy stelliferin E were isolated from an extract of the sponge *Rhabdastrella globostellata,* which was active in an assay measuring stabilization of the binding of DNA with DNA polymerase-*β*. Both compounds have shown to induce 29% and 23% binding, respectively [61].

Betulinic acid was isolated from the methyl ethyl ketone extract of *Tetracera boiviniana* and was monitored for DNA polymerase *β* inhibition [208]. Betulinic acid showed an inhibition of DNA polymerase *β* with IC_{50} value of 14 *μ*M in the presence of bovine serum albumin and 6.5 *μ*M in the absence of bovine serum albumin.

The alisol B derivatives **73** and **88** were reported to exhibit muscle relaxant effects on isolated rat ileum against contractions induced by 5-isoleucine-angiotensin I, bradykinin, and acetylcholine [209]. Alisol B analogue **106** and the *seco*-PT analogue **107** showed concentration-dependent (10^{-5}–10^{-4} M) inhibitory activities on the contractions induced by K+ in isolated aortic strips of rats [210]. The alisol B derivatives **73**, **88**, **108**, **109**, and **89** were found to be effective in restoring choline acetyltransferase activity, and were suggested to have potential for the treatment of Alzheimer's disease, myasthenia gravis, and gastrointestinal disorders [211]. Compounds **87** and **73** produced a significant increase in Na+ excretion in saline loaded rats when administered orally at a dose of 30 mg/kg [213, 212].

Alisol B derivative **73** was reported to inhibit cell proliferation and induce apoptosis in both rat aortic smooth muscle A7r5 cells and human CEM lymphocytes. The effect was suggested to be partly due to the induction of c-Myc expression as well as the collapse of *Bax/Bcl*-2-mediated mitochondrial membrane potentials.

In addition to apoptotic effect, **73** showed anti-inflammatory, and hypolipidemic effects, and it was proposed to be useful for the development of drugs to prevent pathological changes associated with atherosclerosis and post-angioplasty restenosis. Compounds **73**, **88**, and **89** were observed to regulate the 5-HT3A receptor expression in *Xenopus* oocytes. All were reported to regulate the 5-HT-induced inward peak current mediated by the human 5-HT3A receptor in a concentration-dependent reversible, but non-competitive manner with relatively low IC50 values (1.7–3.5 *μ*M) [214].

Betulinic acid showed inhibitory activity to alpha-glucosidase in a dose-dependent manner (0.125–2.5 *m*M). Since the total aqueous ethanol extract of *Alismatis rhizoma* (25 *μ*g/ml) could inhibit alpha-glucosidase activity by 34.06%, comparing to acarbose (0.5 *m*M) by 47.08% [215], the triterpenes present in the plant are likely involved in the inhibitory process.

Anti-viral activities of triterpenes has been reported [218,219]. Scientists used it in tea and other beverages to treat stomach and intestinal problems such as dysentry and diarrhoea. In Russia, it has been reportedly used since 1834. In 1994, scientists at the University of North Carolina reported that chemicals found in white birch bark reduced the growth of human immunodeficiency virus [220].

3.9 Anti-Complement Activity

Alisol A analogues **86** and **87** as well as alisol B analogues **73** and **88** were reported to inhibit the complement-induced hemolysis through the classical pathway [216]. Alisol **87** and **73** (Fig. **2**) exhibited anti-complement activity with IC50 values of 130 and 150 *μ*M, respectively. 11*β*,23*S*,24*R*,25-tetrahydroxyprotost-13(17)-en-3-one, a synthetic derivatives of **73** showed moderate inhibitory activity with an IC50 value of 97.1 *μ*M. The presence of an aldehyde group at C-23 was found to produce the most potent inhibitory effect (IC50 47.7 *μ*M) on the complement system *in vitro* [216-217].

The anti-feedant activity of isolated betulinic acid from the leaves of *Vitex negundo* L. was studied against the third instar larvae of castor semilooper [220]. The percentage feeding reduction, at a dosage of 10 *μ*g/cm^2 for betulinic acid was 71.18, 84.75 and 73.34% after 24, 48 and 72 hr time period, respectively. Betulinic acid isolated from *Pentalinon andri-euxii* [221,222] and tested for its anti-protozoal activity against *Leishmania amazonensis*, *Leishmania braziliensis* (M2903), *Trypanosoma cruzi tulahuen* and *Plasmodium falciparum* (F32). Betulinic acid showed a moderate trypanocidal activity against *T. cruzi* with IC_{50} value of 50.0 *μ*M and a good anti-plasmodial activity with IC_{50} value of 22.5 *μ*M against *P. falciparum*. Leishmanicidal activity was not detected for betulinic acid against *L. amazonensis* and *L. braziliensis*. Betulinic acid isolated from *Saussurea lappa* C. B. Clarke and was evaluated *in vitro* for protein tyrosine phosphatase 1B inhibitory activity [222] with IC_{50} value of 0.7 *μ*g/ml, which was comparable to those of RK-682 and ursolic acid used as positive controls.

Substantial amounts of betulinic acid was obtained from five *Uapaca* species include *Uapaca acuminata*, *Uapaca guineensis*, *Uapaca heudolotti*, *Uapaca paludosa* and *Uapaca vandhoutei* [223,224]. Betulinic acid was isolated from *Clusia ellipticifolia* was studied for its anti-nociceptive activity [225]. The pharmacological study using the abdominal contortions model induced by acetic acid showed significant anti-nociceptive activities to the compounds and the highest effect was attributed to the betulinic acid.

4. BIOLOGICAL ACTIVITY ENHANCEMENT OF TRITERPENOIDS BY MICROBIAL TRANSFORMATIONS OF METABOLITES

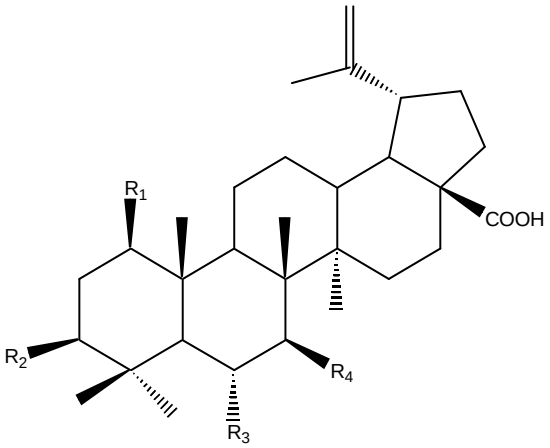
Enzymatic biotransformation is a very useful approach to expand the chemical diversity of natural products. Microbial transformation of triterpenoids has provided new derivatives that are potentially useful for pharmacological studies. These biotransformations have also been used as *in vitro* models to mimic and predict the mammalian metabolism of biologically active triterpenoids.

Recent enzymatic biotransformation studies on terpenoids have resulted in the isolation of novel compounds. 14-Hydroxy methyl caryophyllene oxide produced from caryophyllene oxide

showed a potent inhibitory activity against the butyryl cholinesterase enzyme, and was found to be more potent than parent caryophyllene oxide. The metabolites 3*β*,7*β*-dihydroxy-11-oxo-olean-12-en-30-oic acid, betulin, betulonic acid, argentatin A, incanilin, 18*β* glycyrrhetinic acid, 3,11-dioxo-olean-12-en-30-oic acid produced from 18*β* glycyrrhetinic acid were screened against the enzyme lipoxygenase. 3,11-dioxo-olean-12-en-30-oic acid, was found to be more active than the parent compound.

The metabolites 3*β*-hydroxy sclareol 18*α*-hydroxy sclareol, 6*α*,18*α*-dihydroxy sclareol, 11*S*,18*α*-dihydroxy sclareol, and 1*β*-hydroxy sclareol and 11*S*,18*α*-dihydroxy sclareol produced from sclareol were screened for anti-bacterial activity. 1*β*-Hydroxy sclareol was found to be more active than parent sclareol. There are several reports on natural product enzymatic biotransformation, but few have been conducted on terpenes. Examples of transformation of triterpenes include the microbial transformation of two lupine-type triterpenes, betulin and betulonic acid by the fungus *Chaetomium longirostre* and microbial transformation of a mixture of *argentatin* A and *incanilin* by *Gibberella suabinetti* and *Septomyxa affinis* [227]. 18□-Glycyrrhetinic acid was subjected to fermentation with *Cunninghamella elagans* and *Fusarium lini* afforded metabolite, 3,11-dioxo-olean-12-en-30-oic acid [228-229]. Biotransformation of three cycloartane-type triterpenes, cycloartenol, 24-methylene cycloartanol, and cycloartenone, by the fungus *Glomerella fusarioides* was studied [230]. The microbial transformation of *β*-amyrin acetate occurred in the culture of *Rhodobacter sphaeroides* [231].

Screening experiments showed a number of microorganisms capable of biotransforming betulinic acid **(40)**. Bioconversion of **40** with resting-cell suspensions of phenobarbital-induced *B. megaterium* ATCC 14581 resulted in the production of the known betulonic acid (**120**) and two new metabolites: 3â,7â-dihydroxy-lup-20(29)-en-28-oic acid (**121**) and 3â,6*R,*7â-trihydroxy-lup-20(29)-en-28-oic acid (**122**). Biotransformation of **40** with growing cultures of *C. elegans* ATCC 9244 produced one new metabolite characterized as 1â*,*3â,7â-trihydroxy-lup-20(29)-en-28-oic acid (**123**). Incubation of **40** with growing cultures of *M. mucedo* UI-4605 afforded metabolite **121**. Further, the anti-melanoma activity of metabolites **120-123** was evaluated against two human melanoma cell lines, Mel-1 (lymph node) and Mel-2. Fig. 17.



	R_1	R_2	R_3	R_4
120	H	=O	H	H
121	H	OH	H	OH
122	H	OH	OH	OH
123	OH	OH	H	OH

Fig. **17** Microbial transformation of betulinic acid.

Microbial transformation of the betulinic acid was studied to utilize microorganisms as *in vitro* models to prepare potential mammalian metabolites of this compound. Preparative-scale biotransformation with resting-cell suspensions of *Bacillus megaterium.* ATCC 13368 resulted in the production of four metabolites, which were identified as 3-oxo-lup-20(29)-en-28-oic acid (**124**), 3-oxo-11□-hydroxy-lup-20(29)-en-28-oic acid (**125**), 1□-hydroxy-3-oxo-lup-20(29)-en-28-oic acid (**126**), and 3□,7□,15□-trihydroxy-lup-20(29)-en-28-oic acid (**127**)[Fig. **18**). In addition, the anti-melanoma activities of these metabolites were evaluated with two human melanoma cell lines, Mel-2 and Mel-1[232].

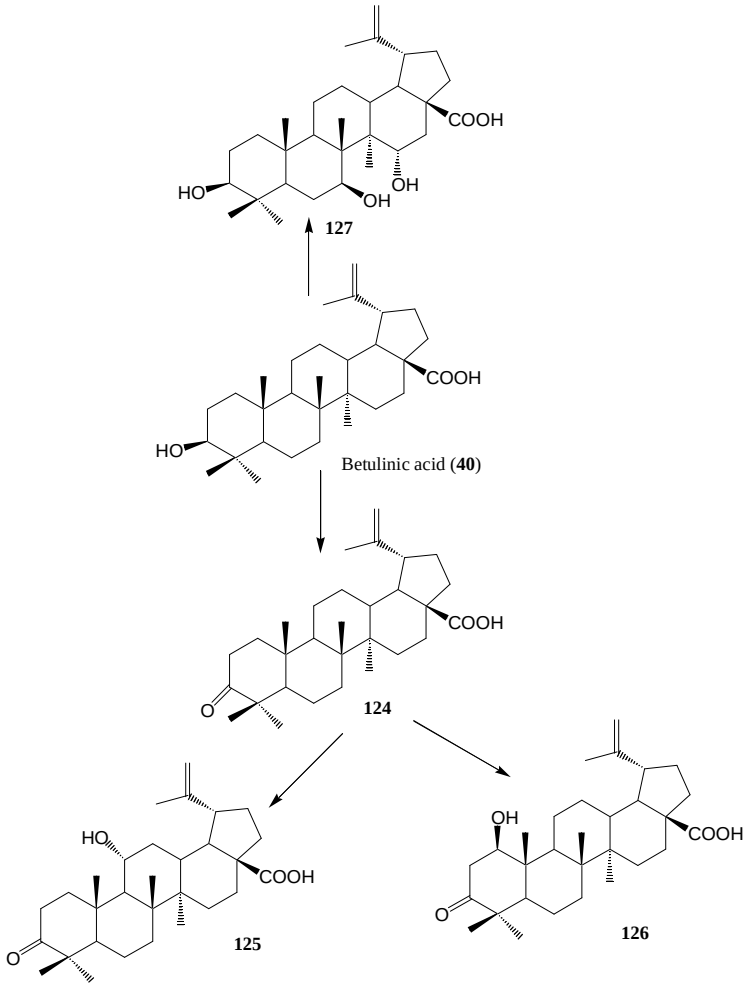


Fig.**18**. Proposed metabolic pathway for betulinic acid in *Bacillus megaterium ATCC 13368*

5. CONCLUSION

As part of a review dedicated to the triterpenes and triterpenoids clinically useful with multiple targets in cancer, malaria and more treatment: focus on potential therapeutic value. It is interesting to note that many of these triterpenes are known to possess significant biological properties. However, we have shown the inhibitory activity of pentacyclic triterpenes on various viruses. New unique triterpenes belonging to the lanostanes, dammarane, cucurbitanes, oleananes and ursane chemical series were isolated and were shown to possess significant activity against cancer, malaria, chikungunya etc. Betulinic acid is currently under evaluation as a topical agent in a phase I/II clinical trial for the treatment of dysplastic nevi with the potential to transform into melanoma. It has been clinically used as an anti-biotic topical therapies for *Staphylococcal* infections. A large number of triterpenoids are known to exhibit, anti-cancer efficacy in preclinical animal models as well as cytotoxicity against a variety of tumor cells.

Since the results obtained with triterpenes or derivatives of triterpene acids support the use of different medicinal plants thereby opening up a new frontier for future studies. Bringing together the efforts of both academy and industry, this review highlights the most significant research results achieved with triterpenic compounds, providing clues for lead compound selection and targets to be exploited. It will work as an extension of the continuous discussion concerning the introduction of these agents into the clinical settings for activity purposes. Much remains to be done to determine and understand the exact mechanism of action of these active

triterpenes, to design and synthesize structural analogues and select the best candidates for *in vivo* studies.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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