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Analogues Of Betulin And Betulinic Acid With Biological Activity.

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ABSTRACT

Betulin **1** is a chemical that occurs naturally in birch bark. From a chemical point of view, it belongs to the group of pentacyclic triterpenes. A characteristic feature of betulin is its lack of toxicity both *in vitro* and *in vivo*. Betulin **1**, betulinic acid **2** and lupeol **3** are all characterized by anti-inflammatory and antiallergic properties. Betulinic acid **2** leads to the death of cancer cells in the human body, e.g. melanoma, as well as neuroblastoma and glioma cells. Moreover, it is used as a drug that is non-toxic to healthy human cells in the treatment of melanoma. It is worth emphasizing that botulin **1** and derivatives isolated from cap leaves inhibit the development of HIV in an infected cell in the initial stage. This article aims to present up-to-date information about betulin **1** derivatives and biological properties.

Keywords: betulin derivatives, cancer, inflammatory, antivirus, biological activity

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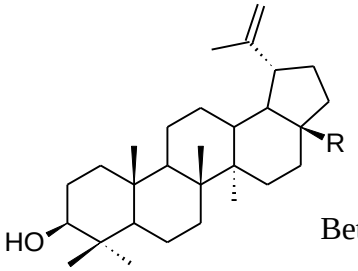
INTRODUCTION

Betulin ((3*β*)-lup20(29)-ene-3,28-diol) **1** was first isolated from the bark of the birch wart (*Betula verrucosa*) in 1788 by Lowitz, followed by detailed studies involving elemental analysis of betulin, which were described by Hausmann [1-3]. This compound due to its construction belongs to the pentacyclic triterpenes of the lupane type [3,4].



Betulin **1** R = CH_2OH

Betulinic acid **2** R = COOH

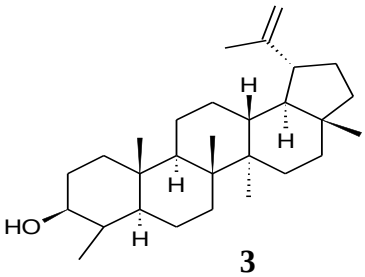


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Figure 1. Betulin and its derivatives.

It is composed of four five-carbon rings and one four-membered ring (**Figure 1**). All rings are arranged relative to each other in a trans configuration [5,6]. Thanks to the hydroxyl moieties at the 6 (secondary) and 28 (primary) carbon atom and the isopropenyl group at C-20, botulin **1** is a chemically active hydrocarbon. It is also known as betulin alcohol, betulinol or betulol [5].Betulin **1** derivatives differ in the substituents on the C-20 carbon atom: betulinic acid **2** has a carboxyl group, 3-methyl lupeol[4,5]. A feature that distinguishes them as potential therapeutic preparations is their lack of toxicity *in vitro* and *in vivo* [1]. In small quantities, betulin **1** can be isolated from many plant species, e.g. from hazel bark (*Corylus avellana*), from several alder species (*Alnus*) and from hornbeam (*Carpinus betulus*). Betulinic acid **2** and betulin **1** can be isolated from the rhizomes of the water plant *Nelumbo nucifera*. Betulin **1** is obtained on an industrial scale from two species of birch: warty (*Betula verrucosa*) and mossy (*Betula pubescens*). Betulin **1** content in birch bark ranges from 9 to 30% [6-8].

Betulin **1** is also the main ingredient in the outer layer of the bark of birches growing in North America such as *Betula papyrifer*a, *B. populifolia* Marsh. (gray birch), *B. cordifolia* Regel. (mountain white birch) and *B. caerulea* Blanch. (blue birch). In these species, the bark contains 9-25% triterpenoids. Betulin **1** constitutes 5–22% of the mass of the outer layer of the bark and 75-80% of the triterpene fraction, while lupeol **3** 9-10% - 0.2-2% of the birch bark [9]. The triterpene fraction of black birch (*B. lenta*) contains mainly lupeol **3** (approx. 60%), betulin **1** (35%) and small amounts of lup-20(29)-en-3-on-28-ol (betulone) and acid betulinic 2,3-caffeoyl betulin (ester with caffeic acid). *Betula papyrifera* bark triterpenes apart from betulin **1** and lupeol **2** are: lupenone, erythrodiol, β-amyrin, sitosterol and methyl betulinate [10]. *Betula maximowicziana* Regal, very rare in Poland, in the outer layer of the bark contains: betulin **1**, 3-*O*-caffeoyl betulin, lupeol **3** and oleanolic acid acetate. The inner layer of the bark contains acerogenin E, 16-hydroxy-17-*O*-methylacerogenin E, β-*D*-glucopyranoside of alnusdiol, 7-*O*-β-*D*-xylopyranoside (+)-catechins, monogynol A, and others. There are no lupane **3** derivatives in the root bark, but dammarane derivatives (esters with caffeic acid) and 3-*O*-caroyl-oleanolic acid [11].

The outer layer of birch bark is very rich in betulin **1**; it undergoes only minor changes after a long time [11]. Betulin **1** occurs in the form of crystalline aggregates and is located in the cortex in large thin-walled cells. The characteristic white color of birches comes from betulin **1**, which fills the inside of periderm cells [11]. Betulin **1** can be obtained from birch bark through sublimation by extraction with organic solvents such as

dichloromethane, chloroform, acetone and crystallization with suitable solvents. In birch bark together with betulin **1** in trace amounts are: betulinic acid **2**, lupeol **3**, betulinic aldehyde and allobetulin [6,7].

BIOLOGICAL PROPERTIES OF BETULIN AND ITS DERIVATIVES

Betulin **1** and its derivatives are characterized by numerous biological properties. It is important that betulin **1** and betulinic acid **2** do not show toxicity up to a concentration of 500 mg/kg body weight, while lupeol 3 does not cause side effects after administration (per os) of a dose of 2000 mg/kg [7]. The latest *in vivo* studies have proven that betulin **1** has a lipotropic effect (reduces the level of lipids in the blood, liver, adipose tissue) and supports the body's metabolism. Betulinic acid **2** effectively inhibits the secretion of gastric acids, and therefore causes a decrease in inflammatory changes in the digestive tract [6]. There are no reports in human literature regarding the metabolism of betulinic acid **2** and betulin **1** *in vitro* in humans. Studies conducted in humans regarding the metabolism of betulin **1** and its derivatives would give a complete picture of their properties and lack of toxicity to the human body [7-11]. Betulin **1**, betulinic acid **2** and lupeol **3** have antiallergic and anti-inflammatory effects. Betulinic acid **2**, which is part of the extract of the common herb *Prunella vulgaris*, has antiallergic properties. The specificity of this acid consists in inhibiting mast cell degranulation and releasing β-hexosaminidase, which is found in the same histamine granules [7]. Betulinic acid **2** induces apoptosis of human tumor cells, such as melanoma cells [8]. In addition, this acid induces apoptosis of neuroblastoma, spinal cord and glioma cells, which are not susceptible to most proapoptotic factors [6]. However, with a lack of toxicity to healthy cells and high biological activity, it can be used as a new drug in the treatment of human melanoma [7-9].

Studies have shown that betulin **1** and its derivatives, isolated from the extract of the cap leaf (*Syzygium claviflorum*), stop the life cycle of the HIV virus in the infected cell at an early stage and protect the surrounding cells from spreading. The mechanism of action of these compounds probably consists in blocking the viral protein coat, and consequently blocking the binding of the virus to the outer membrane of host cells. Disabling this connection results in lack of reproduction. In order to explain their antiviral activity more precisely, these compounds require further laboratory and clinical tests and selection of the most active compounds from this group [12-13]

Betulin **1** among many biological properties has a hepatoprotective effect, consisting in reducing the cytotoxicity of cadmium (II) chloride in HepG2 cells. In 80% of cases, cadmium (II) compounds cause death of HepG2 cells that have not been given betulin **1**. The best protective effect is obtained by administering betulin 24 hours before intoxication with cadmium (II) chloride. Hepato-protective effects are probably caused by an unidentified protein whose synthesis is caused by betulin **1**. Preliminary sequence analysis showed that the expression of betulin-induced genes is the same as the human mitochondrial cytochrome b gene [12].

Triterpenes can now be used to obtain potential drugs. The chemical structures of many currently used drugs of plant origin, such as vincristine, vinblastine, colchicine, paclitaxel or quinine, are complicated, and thus the profitability of synthesis of these compounds is very low. Betulin **1** is one of the few exceptions as a secondary metabolite, constituting over 20% of the outer layer of the bark of various birch species. Thanks to this, it can be a valuable raw material for the synthesis of new therapeutic agents: anti-inflammatory, antiviral, antimalarial and anticancer [12,13].

ANTIALLERGIC AND ANTI-INFLAMMATORY PROPERTIES

Betulin **1**, betulinic acid **2** and lupeol **3** isolated from numerous plants have antiallergic and anti-inflammatory effects. Betulinic acid **2** contained in the extract of the common herb *Prunella vulgaris* has antiallergic properties. It inhibits mast cell degranulation and release of β-hexosaminidase and histamine found in the same granules [13].

Betulin **1** and betulinic acid **2** isolated from the rhizomes of the water plant *Nelumbo nucifera* show high anti-inflammatory activity. These substances inhibit carrageenan and serotonin-induced edema of the rat's paw, comparable to standard anti-inflammatory substances. Also, lupeol **3** isolated from the bark (*Crataeva nurvala*) has an anti-inflammatory effect in acute and chronic inflammation in rats. It reduces swelling of paws in rats with artificially induced arthritis by CFA (from *Mycobacterium tuberculosis*) up to 39%. Anti-inflammatory effects of lupeol **3** have also been observed in rats with formaldehyde-induced arthritis. Lupeol **3** was effective

in reducing the volume of exudate and inhibiting the overall leukocyte count in rats. Probably the anti-inflammatory activity of this compound results from immunosuppressive activity and is associated with inhibition of cell migration to inflammatory sites as well as reduction of pro-inflammatory chemotactic factors [4,14,15].

ANTICANCER PROPERTIES

Betulinic acid **2** induces cell apoptosis in many human tumor lines. The efficacy of betulinic acid **2** against tumor cells was tested in mice infected with human MEL-1, -2, -3 and -4 melanoma cells (**Table 1**)[7,11,33]. Tumor growth inhibition was demonstrated at very low compound concentrations without causing side effects such as weight loss. The selective action of betulinic acid **2** against human melanoma cells does not affect the vital functions of healthy cells. Betulinic acid **2** is devoid of cytotoxicity to healthy cells (pH > or = 7.0), whereas it shows cytotoxicity under conditions prevailing inside the tumor cells (pH < or = 6.8). Given the lack of toxicity to healthy cells and the high activity of this compound, it can be presumed that it will be introduced into therapy as a new drug in the treatment of human [9].

Table 1. Cytotoxicity of betulinic acid towards cancer cell lines

Compound	Cell line		EC50	
			[µM]	[µg/ml]
BETULINIC ACID	human gliomas	LN-229	20	-
		U-87MG	25	
		T98G	25	
		LN-18	70	
	human medulla	Daoy	-	3
		D283		3
		D341		7.5
		MHH1		9
		MHH3		10
		MHH4		4
		MEB1		15
	human melanoma	MEL-1	-	1.1
		MEL-2		2.0
		MEL-3		4.8
		MEL-4		3.3

Betulinic acid **2** induces apoptosis not only of melanoma cells, but also of neuroblastoma, spinal cord and glioblastoma cells, which are poorly affected by most proapoptotic factors [4, 22].

The likely mechanism of action of betulinic acid **2** is induction of apoptosis, i.e. programmed cancer cell death. This process occurs bypassing the relay cascades that trigger programmed cell death and without the involvement of the p53 protein, which is responsible for triggering apoptosis in many cell lines. In contrast, some cells treated with betulinic acid **2** show increased expression of p21 protein, whose increased content is associated with cell cycle arrest [24].

Studies have shown that betulinic acid **2** affects mitochondria. It causes a change in the permeability of the mitochondrial membrane protein complex, which is responsible for maintaining the appropriate potential difference. The mitochondrial potential disorder is caused by a modulating effect on BCL/BAX family proteins. These proteins are endogenous inhibitors of changes in mitochondrial membrane permeability and control caspase activation. Under the influence of betulinic acid **2**, the level of BCL/BAX is elevated, but the balance between them is maintained. The increased amount of BAX proapoptotic protein is compensated by the increased content of anti-apoptotic BCL-2. The BCL-2 protein forms a heterodimer with the BAX protein, inactivates it and thus prevents the death program from starting. The level of other proteins controlling the release of cytochrome c is unchanged. Cells overexpressing BCL-2 or BCL-XL genes are not subject to botulinic acid **2** induced apoptosis, which confirms the hypothesis that this compound affects mitochondrial potential

controlling proteins. As a consequence, apoptogenic proteins are released to the cytosol from the mitochondrial mesothelial space: cytochrome c and apoptosis induction factor (AIF). These proteins activate caspases. Cytochrome c activates caspase-3 and AIF both caspase-3 and caspase-8. Endonucleases are excited, causing fragmentation of nuclear DNA, contraction of the cell and its phagocytosis by cells of the immune system. Isolated mitochondria treated with betulinic acid **2** cause apoptosis of whole tumor cells in the cytoplasmic extract. In contrast, mitochondrial free cytoplasmic extract treated with betulinic acid **2** has no apoptotic properties [14-19].

Betulin **1**, betulinic acid **2** and lupeol **3** were also converted into glycosidic derivatives to increase their solubility in water. These compounds were combined with glucose, rhamnose and arabinose to obtain β-*D*-glucosides, α-*L*-rhamnosides and α-*D*-arabinosides, respectively. Studies were conducted on the cytotoxic activity of the obtained glycosides on three tumor lines, i.e. lung A-549, colon DLD-1, and melanoma B16-F1, and one healthy line, WS1 skin fibroblastoids. It was found that the introduction of the sugar residue at the C-3 or C-28 position in betulin **1** resulted in a loss of cytotoxicity relative to the tested lines. In contrast, targeting monosaccharides to the C-3 position proved beneficial. The greatest activity was shown by 3-*O*-α-rhamnopyranoside betulinic acid **5** (**Figure 2**), which obtained IC_{50} = 2.6, 3.9 and 3.9 µM, respectively, relative to the A-549 lung tumor line, DLD-1 colon and B16-F1 melanoma [28,29].

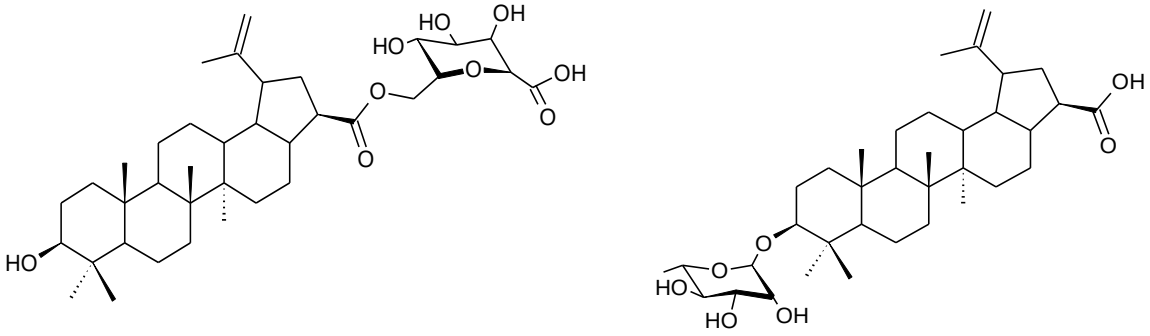

Figure 2. Betulinic acid 28-*O*-β-*D*-glucuronide 4 and betulinic acid 3-*O*-α-rhamnopyranoside 5

By contrast, for the healthy WS1 dermal fibroblastoid line, the value was only IC_{50} = 31 µM. Synthesis of glucuronide with betulinic acid **2** was also carried out. As a result of this reaction, 28-*O*-β-*D*-glucuronide betulinic acid **4** was obtained, which dissolved better in water than the starting betulinic acid **2**. This compound, in cytotoxicity studies, showed no inhibition of the growth of A-549 lung cancer cells and DLD-1 colon and healthy cells, skin fibroblastoids WS1 [5,26,27].

Betulinic acid **2** is poorly soluble in aqueous solutions, due to which its biological activity can often not be revealed. This limits the penetration of the compound through cell membranes and reaching target organelles. Research is being conducted on the synthesis of betulinic acid **2** derivatives that show greater polarity and activity compared to the parent compound. Many amide betulinic acid **2** conjugates with α-amino acids were obtained in the form of methyl esters and with a free carboxyl group. The best results were obtained when combining betulinic acid **2** with glycine or alanine. Betulinic acid **2** amide with glycine dissolved in water 100 times better than the acid itself, while the derivative with alanine dissolved 50 times better [27,28].

A study was conducted on the cytotoxic activity of betulinic acid **2** derivatives with α-amino acids against the Mel-2 melanoma and fibrous sarcoma of the mouth KB. This study showed that conjugates with alanine, valine methyl esters and free glycine residues are the most active against the Mel-2 line (EC_{50} = 3.5, 2.1 and 4.2 µg x ml^{-1}, respectively). Compared to the KB line, derivatives with a free alanine and valine residue were found to be the most active (EC_{50} = 4.6 and 9.0 µg x ml^{-1}, respectively) [28,29].

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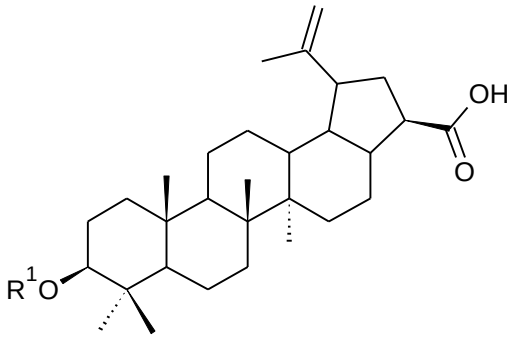
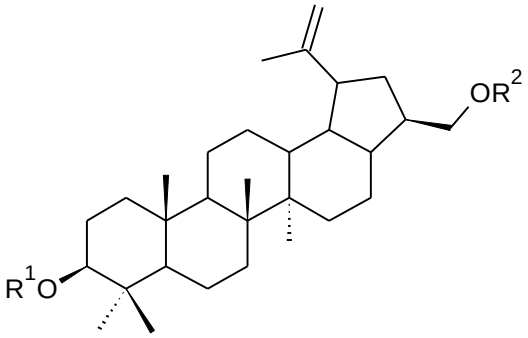


Derivative	6a	6b	6c	6d
R^1	Lys	Abu	Ala	Phe



Derivative	7a	7b	7c	7d	7e	7f	7g
R^1	His	Gly	His	Ala	Phe	Met	Lys
R^2	Gly	Gly	Ala	Ala	Phe	Met	Lys

Figure 3. Ester derivatives of botulin 6 and betulinic acid 7 with α-amino acid

The team of Drąg-Zalesińska synthesized a similar group of derivatives with α-amino acids. Ester derivatives of botulin **1** and betulinic acid **2** were obtained (**Figure 3**) [30]. The obtained derivatives were tested for cytotoxicity on lines of tumor cells: two lines of gastric cancer (EPG85-257P and EPG85-257RDB) and two pancreatic tumor lines (EPP85-181P and EPP85-181RDB). Betulin diesters with lysine **7g** and alanine were found to be the most active against the EPG85-257P line **7d** (IC_{50} = 4.6 and 3.8 µM, respectively), according to the rules for the line EPP85-181P cytotoxicity level of lysine esters of betulinic acid **6 a** and betulin **7g** (IC_{50} = 9.7 and 4.1 µM) (**Figure 3**). These derivatives also showed very good solubility in water. The effect of apoptosis in cancer cells treated with the above esters in comet tests was 100% [30].

Research conducted by Bębenek et all showed that betulin derivatives containing the 1,2,3-triazole ring have a broad spectrum of biological activities, including antiviral, anticancer and antibacterial activity. The cytotoxic activity of the obtained compounds **8** and **9** (**Figure 4**) was determined using five human cancer cell lines (T47D, MCF-7, SNB-19, Colo-829 and C-32) in the WST-1 assay. Bistriazol **8** showed a promising IC_{50} value (0.05 µM) against human T47D ductal carcinoma [16].

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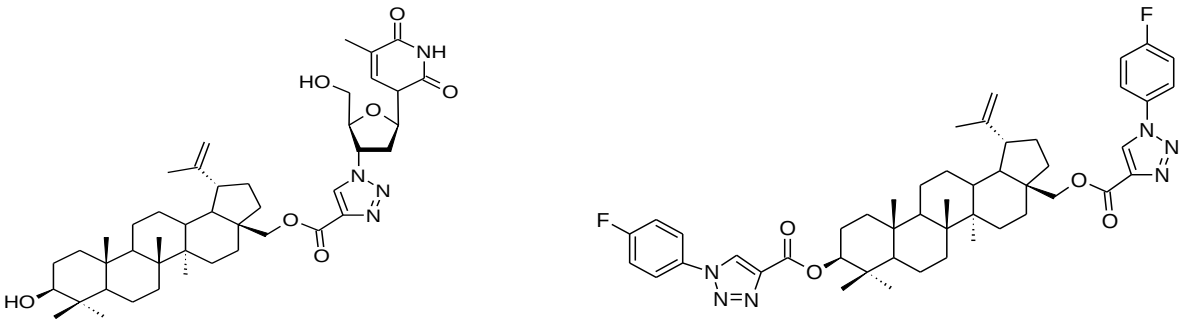

Figure 4. Compound 8 and 9

The triazole **8** and bistriazole **9** were tested for their cytotoxic activity against human cancer cell lines such as: T47D (human ductal carcinoma), MCF-7 (human adenocarcinoma), SNB-19(glioblastoma), Colo-829 (human malignant melanoma), and C-32 (human amelanotic melanoma).The WST-1 assay is used to evaluate the cytotoxic activity of triterpene derivatives. The cytotoxic activity data were expressed as the concentration of compounds (μM), which inhibits the proliferation of 50% of tumor cells as compared with the control untreated cells. Betulin **1**, was used as a positive control. The resulting IC50 values are summarized in **Table 2** [16].

Table 2. The cytotoxic activity of derivatives 8 and 9

Compound	Human Cell Line /IC50±SD [μM]				
	T47D	**MCF-7**	**SNB-19**	**Colo-829**	**C-32**
1	Neg	Neg	17.7±1.2	15.3±2.2	Neg
8	Neg	Neg	Neg	35.1±6.2	Neg
9	0.05±0.01	0.09±0.01	0.08±0.01	Neg	Neg

**Neg - negative in the concentration used.*

The tested compounds were the most active towards the human breast cancer cells T47D (caspase-3 positive) compared with MCF-7 cells. The bistriazole **9**, which contains two fluorine moieties, showed a good cytotoxic activity againstT47D, MCF-7, and SNB-19 (IC_{50} = 0.05μM, 0.09μM, and 0.08μM, respectively). Additionally, a lower cytotoxic activity of the tested compounds against the C-32 cell line was observed [16].

ANTIVIRUS PROPERTIES

Betulinic acid **2** and its derivatives, isolated from *Syzygium claviflorum* leaf extract, have been shown to inhibit HIV replication in H9 lymphocytes. Anti-HIV activity is determined compared to the drug azidothymidine (AZT). These compounds inhibit the life cycle of the virus in the infected cell at an early stage and protect the surrounding cells from the spread of HIV. The likely mechanism of action of betulinic acid **2** and its derivatives is by blocking the virus protein coat, which prevents it from binding to the outer membrane of host cells. Without this connection, the virus cannot reproduce. Betulinic acid **2** and its derivatives require further laboratory and clinical tests to clarify their antiviral activity and to select the most active compounds from this group [19,20].

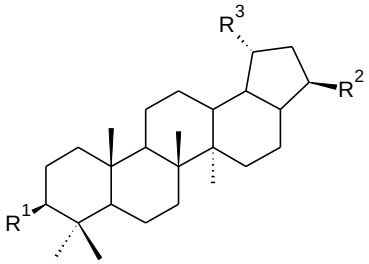

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Table 3. Anti-HIV activity of betulin 1 and betulinic acid 2 derivatives

Compound	R^1	R^2	R^3	EC_{50}(μM)	TI
10	OH	COOH	CH_3CHCH_3	0.9	-
11	OH	COOH	$CH_3C(O)$	6.5	-
12	$C_7H_{10}O_4$	COOH	CH_3CHCH_3	5.7×10^{-3}	1017
13	$C_7H_{10}O_4$	COOH	$CH_3C=CH_2$	2.3×10^{-3}	1974
14	$C_6H_8O_4$	CH_2OH	CH_3CHCH_3	1.7×10^{-3}	16160
15	$C_7H_{10}O_4$	CH_2OH	CH_3CHCH_3	1.3×10^{-3}	19530
16	$C_7H_{10}O_4$	$C_7H_{10}O_4$	$CH_3C=CH_2$	8.7×10^{-4}	42400
17	$C_6H_8O_4$	COOH	$CH_3C=CH_2$	3.5×10^{-4}	20000
18	$C_6H_8O_4$	COOH	CH_3CHCH_3	3.5×10^{-4}	14000
19	$C_5H_7O_4$	CH_2OH	CH_3CHCH_3	2.0×10^{-5}	1.3×10^{5}
AZT	-	-	-	0.02	25000

Research demonstrates that dihydrobetulinic acid **10** and platanic acid **11** are characterized by similar action (**Table 3**). The above reports resulted in the synthesis of derivatives of these acids, including acid **17**, also known as PA-457 or DSB and acid **18**, which showed extremely low EC_{50} values. Betulinic **1** and dihydrobetulinic acid **10** derivatives esterified with 3,3'-dimethylglutaric acid **12** and **13** in the C-3 position also obtained very interesting EC_{50} and TI results [21]. A number of betulin diesters and dihydrobetulin with succinic and glutaric acid derivatives have also been synthesized, among which the most active anti-HIV factors in the H9 lineage were compounds **12**, **13**, **14** and **17** (**Table 3**) [22, 23].

The 1,2,3-triazole ring is an attractive structural unit, which exhibits a high stability underacid/base hydrolysis conditions. Moreover, triazoles are capable of forming hydrogen bonds, which can be important for their bioavailability and solubility [16]. Additionally, the 1,2,3-triazoles are a class of heterocyclic compounds that possess a broad spectrum ofbiological activities, such as antibacterial, anticancer, antiviral activity [16].

In order to characterize the antiviral activity against VSV, HSV-1, ECBO and HAdV-5, various experimental approaches were applied to the prepared betulin **1** and betulinic acid **2** derivatives.

At first, the compounds were screened for their cytotoxic activity against the human lung adenocarcinoma epithelial (A549) and the human ovarian cancer (SKOV-3) cells using a two-fold dilution series of the tested substance ranging from 100 μg/mL to 0.5 μg/mL (**Table 4**) [16].

Table 4. Antiviral activity of betulin derivative.

Compound	CC50(μg/mL)	EC50(μg/mL)	SI
1	18.5	0.5	37.0
8	10.3	1.0	10.3

CC50—50% cytotoxic concentration; concentration required to reduce A549 cells viability by 50%. EC50—50%effective concentration; concentration required to inhibit ECBO cytopathic effect in A549 cells by 50%. SI—selectivityindex, or ratio of CC50 to EC50.

Chemical compound **8**, which contains the pharmacophoric moiety (3'-azido-3'-deoxythymidine),shows a good antiviral activity toward the screening viruses.

Betulin esters with fatty acids have been known for a long time and are used in the production of cosmetics (e.g. hair care products) and as plasticizers in PVC production [5,26]. The series of betulin derivatives with fatty acids such as lauric, palmitic, stearic, oleic and linoleic acids have been obtained by esterification as mono-(C-28) and diesters [26]. The hydroxyl group was esterified to the C-3 position in the acid betulin fatty acids of various lengths (C-4 and C-22) [32]. As a factor the coupling used EDCI (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide) in the presence of DMAP in pyridine. The obtained derivatives were tested in *in vitro* tests for tumor inhibition in the Raji lymphoblast line (infected with *Epstein-Barr* virus) induced by TPA (12-*O*-myristoyl-

13-*O*-acetylphorbol). The ester derivatives betulinic acid **2** with crotonic, sorbic, 3,3-dimethylacrylic and linoleic acids and oleic inhibitors inhibited the activity of the *Epstein-Barr* virus in Raji cells [31,32].

HEPATOPROTECTIVE PROPERTIES

Betulin **1** is responsible for the hepatoprotective effect of HepG2 human hepatocytes. It reduces the cytotoxicity of cadmium (II) chloride in HepG2 cells. Cadmium (II) compounds in cells not treated with betulin **1** cause 80% mortality. The best hepatoprotective effect is obtained by administering betulin **1** 24 hours prior to intoxication with cadmium (II) chloride, which suggests that the protective effect is caused by an unidentified protein whose synthesis is caused by betulin **1**[25].

ANTI-STONE PROPERTIES

Studies in rats with hyperoxaluria due to pyridoxine deficiency have shown that betulin **1** and lupeol **3** reduce renal tubular damage and lower the crystal deposition rates in these animals' kidneys. These compounds increase the volume of urine excreted (urine supersaturation decreases) and reduce the amount of excreted oxalate. This indicates the inhibition of some enzymes that synthesize oxalate from glycolic acid given in the diet. The concentration of glycosaminoglycans and magnesium in the urine is normalized. Magnesium oxalate has a greater solubility product than calcium oxalate, and glycosaminoglycans hinder crystal formation. These factors have a protective effect against stone precipitation. At the same time, the concentration of protein in the urine decreases. These results show that betulin **1** and lupeol **3** reduce the risk of calcium oxalate deposition in the kidneys and thus the formation of kidney stones [4, 26].

The discovery of the selective effect of betulinic acid **2** only on cancer cells, without damaging healthy cells, contributed to the start of research on the modification of the structure of betulin **1** and betulin acid **2** by chemical and microbiological methods [26].

RECEPTOR BINDING.

Betulinic acid **2** is inactive at 16 receptor types, including α1-, α2- and β-adrenoreceptors, adenosine A_1, dopamine DA_1 and DA_2, serotonin 5-$HT_{1/1A}$ and 5-HT_2, histamine ç1, benzodiazepine and opiate receptors; GABAergic $GABA_A$ and $GABA_{Breceptors}$; as well as Na^+ / K^+ ATPase and DHP Ca^{2+} channels. This compound shows a weak affinity for muscarinic receptors [34]. The authors Shon et all. [34, 35] emphasize the advantages of betulinic acid **2** solubilization with liposomes, which may contribute to the use of this substance as a new drug. This approach is preferable to the use of synthetic polymers because the lipids contained in liposomes are natural components of the human body. Liposomal preparations may appear to be particularly effective when interacting with perforated capillaries usually supplying blood to tumors [35].

CONCLUSION

Betulin **1** and its derivatives exhibit a broad spectrum of biological activity at low concentrations. An additional feature of these substances, distinguishing them as new, potential therapeutic preparations, is the lack of toxicity, both *in vitro* and *in vivo*.

The most important pharmacological properties of betulin **1** and its derivatives are inhibitory effects on the development of some chemically resistant cancers, such as melanoma and gliomas. Betulinic acid **2** induces the process of apoptosis – cell suicide – only in cancerous cells. Thanks to this, some side effects associated with chemotherapy can be excluded. Other effects of betulin **1** and its derivatives include inhibition of bacterial and virus growth, anti-inflammatory, antiallergic, analgesic, and liver protective effects. There is also information that betulin **1** promotes the healing of severe burn wounds and relieves swelling after insect bites. Currently, betulin **1** is considered a potential precursor of many new medicinal preparations.

The availability of betulin **1** is high, up to 25% of the outer layer of the bark of white birch species, so it can be easily obtained and subjected to various chemical or biotechnological transformations to obtain new medicinal preparations.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article and the Supplementary Material.

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