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Betulin and its derivatives – precursors of new drugs

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ABSTRACT

White birch bark extract, used in traditional medicine, has many medical properties. Its main components are betulin and betulinic acid - lupane type pentacyclic triterpenes. The discovery of the anti-tumor and anti-viral properties of betulinic acid has become an impulse to modify the structures of these compounds in order to obtain better activity. Over twenty years of research, several derivatives have been selected that have significantly better anti-tumor or antiviral properties than their precursors. This paper presents all new derivatives of betulin and betulinic acid, which have been tested in phase I, II or III of clinical trials. Clinical experiments on animals with natural cancers have also been described. The results obtained by various research teams give hope for the potential use of these compounds in therapy. At the same time, the presented analysis shows that the implementation of the new drug on the market is a process that lasts 10-15 years, which, despite the involvement of huge financial and human resources, does not guarantee success.

Keywords: betulin, betulinic acid, bevirimat, oleogel-S10, episalvan

1. INTRODUCTION

The search for new medicinal substances is an extremely complex, expensive and time-consuming process. Implementation of the new drug on the market takes about 10-15 years,

and the total cost of such a venture is several billion dollars. Despite such large financial expenditures and the involvement of many specialists, there is no guarantee of success. Many promising substances are eliminated after many years of laboratory testing at the stage of clinical trials, because they appear to cause serious side effects [1].

Modern methods of searching for new drugs are based on *in silico* technologies. The first step in designing a drug is to determine the biological target, for example a protein whose function is to be modulated by the designed molecule. Then structure of the so-called lead structure is determined. The next steps are the optimisation of pharmacokinetic and pharmacodynamic properties as well as confirmation of the activity and mechanism of action of the new substance in *in vitro* studies on cellular and *in vivo* models in experimental animals. Pre-clinical and clinical trials are the last stage in the study of new drugs. Currently, the pharmaceutical industry uses advanced *in silico* technologies including molecular modeling, bioinformatic techniques - genomics, proteomics and chemoinformatics - combinatorial libraries, QSAR (*Quantitative Structure-Activity Relationship*), QSPR (*Quantitative Structure-Property Relationship*) and prediction properties of ADMET (*Absorption, Distribution, Metabolism, Excretion, Toxicity*) [1, 2].

In spite of the development of *in silico* technology, the search for new natural substances with biological activity, of which the plant kingdom is a virtually unlimited source, is still valid. For a long time scientists have been interested in the so-called secondary metabolites, especially those found in plants used in traditional medicine. For many years, the function of secondary metabolites was unknown, and therefore they were classified as metabolic waste. Recently, however, it has been shown that they play an important role in the adaptation of the plant to the environment through interaction with other organisms - pathogens or competitors. One of the most numerous secondary metabolite groups are terpenes and terpenoids [3, 4].

The terpene biosynthesis takes place by combining five-carbon isoprene units (C_5H_8) into chain or ring forms to form a compound of general formula $(C_5H_8)_n$. Derivatives of terpenes, which contain, in addition to the hydrocarbon skeleton, any heteroatoms are called terpenoids. Depending on the size of the molecule, the following are distinguished: hemiterpenes ($n = 1$), monoterpenes ($n = 2$), sesquiterpenes ($n = 3$), diterpenes ($n = 4$), triterpenes ($n = 6$), tetraterpenes ($n = 8$) and polyterpenes. Commonly known terpenes are isovalerate acid (hemiterpen), geraniol, menthol, camphor (monoterpenes), lycopene and carotenes (tetraterpenes) and natural gum (polyterpene). An example of a linear triterpene is squalene, an oil component derived from shark liver.

Pentacyclic triterpenes are betulin and betulinic acid. [5]

2. PENTACYCLIC TRITERPENES

The pentacyclic triterpene skeleton is composed of six isoprene units and is formed by cyclisation of the squalene molecule. The compounds are characterised by the presence of five six-carbon or four six-carbon rings and one five-carbon ring [5]. Depending on the structure, terpenes are divided into 5 types, which include various derivatives:

- 1) lupane type: lupeol, betulin, and betulinic acid;
- 2) oleanane type: oleanolic acid, β -amyrin, erythrodiol;
- 3) ursane type: ursolic acid, α -amyrin, uvaol;
- 4) taraxastane type: taraxasterol;

5) taraxerane type: taraxerol. [5, 6].

The general scheme for the structure of lupane type pentacyclic triterpenes is shown in Fig. 1.

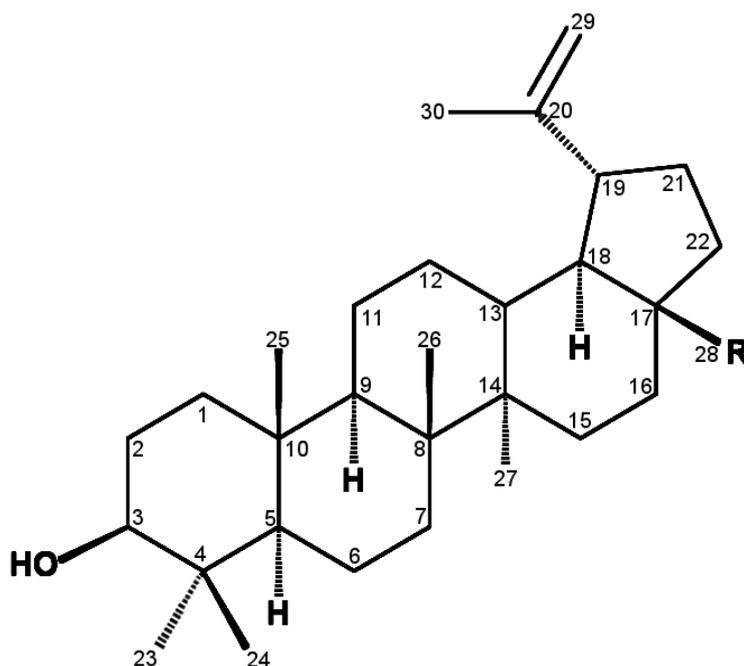


Figure 1. The general chemical structure of the lupane type pentacyclic triterpenes.
 R = OH – betulin; B); R = COOH – betulinic acid; C) R = CH₃ – lupeol.

Triterpene compounds are present in the bark, resin, fruit peel, leaves, flowers, essential oils and seeds, performing a protective function against microorganisms and insects. Their occurrence in the plant kingdom is common, and the same derivatives of lupane have been found in more than 300 species of plants, among others *Betula alba*, *Olea europaea*, *Rosmarinus officinalis*, *Sambucus nigra*, *Platanus acerifolia*, *Vitis vinifera*, *Calendula officinalis*, *Salvia officinalis* [6].

Plants with a high content of triterpenes are used in phytotherapy due to their healing properties. Usually, these are well-known plants used in folk medicine with anti-inflammatory, anti-bacterial, anti-fungal, anti-cancer and anti-allergic properties [6, 7].

Triterpene compounds are the subject of numerous phytochemical and pharmacological studies, and the increase in interest in betulinic acid and betulin dates from the publication of Pisha *et al.* in Nature Medicine in 1995 [8].

Pisha *et al.* were the first to report that betulinic acid is cytotoxic to human melanoma line MEL-1, -2, -3, -4 with no toxic effects on normal cells. The experiments were performed *in vitro* on cell lines and *in vivo* on mice. This publication was cited almost 600 times over 24 years.

3. BETULIN AND BETULINIC ACID

Betulin (BE) is one of the most commonly found in nature triterpenes. The richest source is the bark of white birch species (*Betula* sp.): *B. verrucosa*, *B. pendula*, *B. pubescens*, *B. alba*, in which it occurs in the amount of 20-35% [9]. BE occurs in the form of crystal clusters in large, thin-walled cells, arising in the birch bark in the spring. It is betulin that gives the bark of these trees a white colour [9]. This compound was isolated for the first time from birch bark and described by Lowitz in 1788. The isolation of BE from birch bark can be carried out by sublimation or by extraction with organic solvents such as chloroform, acetone, ethanol, tetrahydrofuran, n-heptane. The content of BE in the bark extracts of various birch species is 70-80%, the rest is made up of other terpenes: betulinic acid (4-12%), lupeol, betulinaldehyde, oleanolic acid. The exact structure of betulin was established in 1952 [10, 11]. This compound is made of a thirty-carbon skeleton, made up of four six-membered rings and one five-membered, trans-connected in relation to each other. This structure reminds steroids in structure. The chemical activity of betulin depends on the presence of hydroxyl groups at C3 and C28 and the isopropenyl group at C19 [10].

The natural derivative of betulin is betulinic acid (BA), which differs from BE by the presence of a carboxylic group instead of hydroxymethyl at C17 (Fig.1). BA occurs in the bark of birches and other plant species, but in an amount that does not allow for efficient extraction, that is why BE is a substrate for the synthesis of this natural derivative. It should be emphasised that both compounds are characterised by very low solubility in water. The chemical synthesis of BA relies on the oxidation of BE with Jones reagent ($\text{Na}_2\text{Cr}_2\text{O}_7$, H_2SO_4 in acetone, at a temperature close to 0 °C), and then reduction of the obtained betulonic acid with NaBH_4 in tetrahydrofuran. The specific betulinic acid epimer is isolated by crystallisation. This method is unfortunately not very efficient (about 60%). A more efficient reaction (86%) is chemoselective oxidation of the primary hydroxyl group of betulin directly to the carboxylic ones [12].

So far, total synthesis of BE and BA has not been carried out in laboratory conditions. The largest, constantly renewable source of betulin is the birch bark. German company Birken GmbH. has developed a new highly efficient way of obtaining betulin from a birch cork cambium (white part of the birch bark) which is a waste material from pulp mills. In the patent-protected method (EP 1 758 555 B1) betulin of 80-90% purity is obtained [13].

BE and BA are excellent substrates, or structures leading to further chemical transformations and the creation of new derivatives with stronger anti-cancer, anti-viral or anti-microbial properties. The latest report by Sousa *et al.* from 2019 discusses in detail the modifications of BE and BA, which were published in 2013-2018 [14]. The study presents over 200 new derivatives, including the synthesis of betulin esters with *L*-amino acids, which was carried out in the years 2013-2015 by our research team [15]. The possibilities of modifying BE and BA by simple transformations are described: amination (amide and amine derivatives), esterification, sulfonation, alkylation.

The creation of new compounds by click chemistry and modifications by hydroxylation or aldol condensation are discussed. The published report shows that modifications of the betulin and betulinic acid molecules mainly concern active groups at C3 and/or C28. New derivatives are designed to increase anti-tumor, anti-HIV and anti-malarial properties and are usually characterised by much better solubility in water than BE and BA [14, 15].

3. 1. Anti-tumor activity of betulinic acid and its derivatives

The first report on the anti-tumor activity of betulinic acid was Pisha's publication in 1995, in which selective cytotoxicity of this compound was found relative to human melanoma cells MEL-1, -2, -3, -4. *In vivo* studies - no BA toxicity for experimental animals - indicated the potential of BA in human melanoma therapy [8]. In 2001, Salti *et al.* reported the protective effect of BA against DNA damage induced by UV-C (254 nm) in cultured congenital melanocyte cells (primary lines were derived from a biopsy of a congenital melanocytic nevi from a healthy patient) [16].

Currently, the mechanism of anti-tumor activity of BA is well known and comes down to three main points:

- 1) anti-proliferative activity by inhibiting topoisomerase I and II in tumor cells;
- 2) activation of the mitochondrial apoptosis pathway in cancer cells;
- 3) the ability to inhibit angiogenesis within the tumor.

BA is an inhibitor of eukaryotic topoisomerase I, it causes its inactivation and prevents the formation of DNA-replicating enzyme complexes. This prevents replication and inhibits the proliferation of cancer cells. In turn, the inhibition of topoisomerase II leads to the inhibition of DNA repair processes [17].

The most important anti-tumor mechanism of action of BA is its ability to induce the mitochondrial pathway of apoptosis. Anti-cancer therapy is based mainly on the initiation of the mitochondrial pathway of apoptosis [18-21]. Many chemotherapeutic agents and radiotherapy have such an effect. BA induces the formation of free radicals (ROS) in various tumor lines, what increases the permeability of the outer mitochondrial membrane.

The regulation of the mitochondrial membrane permeability depends on the expression of proapoptotic proteins, e.g. Bax, Bak, Bad and on the expression of antiapoptotic proteins, e.g. Bcl-2, Bcl-XL, and Mcl-1.

BA has the ability to modulate the level of expression of these proteins, what may increase the level of mitochondrial membrane permeability and induce the process of apoptosis in cancer cells. Increasing the permeability of mitochondrial membranes results in the release into the cytosol of apoptogenic proteins from the intermembrane space of mitochondria: cytochrome c and AIF (*Apoptosis Induction Factor*).

Cytochrome c activates caspase-8, and it activates caspase-3, what in turn leads to the activation of endonucleases. The next step is the fragmentation of nuclear DNA, cell shrinkage and its phagocytosis by cells of the immune system.

Inhibition of angiogenesis is the result of inhibiting by BA the aminopeptidase N activity - an enzyme of key importance for the angiogenesis process [19]. BA also affects the selective, proteasome-dependent degradation of transcription factors: Sp1, Sp3 and Sp4, which regulate the expression of vascular endothelial growth factor (VEGF) [20, 22].

The study by Ali-Sayed *et al.* summarises many years of research on the anti-cancer activity of BA. BA exhibits anticancer activity against a variety of cell lines, e.g. melanoma, neuroblastoma, medulloblastoma, glioblastoma multiforme, ovarian, colon, prostate, lung, breast, cervix cancer. It should be emphasised that BA is characterised by selective cytotoxicity at low concentrations in relation to tumor cells, with the lack of toxicity of the same doses in relation to normal cells. There was no BA toxicity in relation to human normal fibroblasts, lymphocytes, keratinocytes [15, 18, 23].

In the years 2006-2013, a phase I/II clinical trial (NCT00346502, University of Illinois, Chicago, USA, www.clinicaltrials.gov) was carried out to evaluate the safety and effectiveness of experimental ointment with 20% BA for the treatment of dysplastic melanocytic nevus. It was planned to include 28 patients from 200 after the initial screening in the study. The study consisted of using ointment with 20% BA for dysplastic nevus for four weeks. After this period, the dysplastic nevus treated was removed surgically. For control, a similar nevus not treated with an ointment was removed.

The removed nevi were examined histopathologically. Unfortunately, the results of the experiments were not published. The study was suspended in 2013 due to lack of financing. Simone Fulda published the information on this program for the first time in 2008 without providing any references [19]. Probably the researcher collaborated with the American centre in the field of research on the mechanism of anti-tumor activity of BA. Although the clinical trial was completed in 2013, there are still publications which emphasise that BA is currently in the phase I/II of clinical trials assessing its effectiveness in the treatment of dysplastic nevus [18, 24].

The next phase I pilot clinical trial (NCT00701987, www.clinicaltrials.gov) to evaluate the safety, tolerability and effectiveness of ALS-357 (BA) in patients with cutaneous metastatic melanoma was conducted in 2008-2009. 12 participants took part in the study. The drug was applied topically in the form of an ointment for 4 weeks in four arms: twice a week, every other day, once a day and twice a day. Unfortunately, the BA concentration in the preparation was not included in the study information. In biopsies, apoptosis was evaluated by the TUNEL method and the expression of genes and proteins of the apoptotic pathway (day: 15, 29, 43). At each visit, the ALS-357 concentration in serum (day: 1, 8, 15, 22, 29, 43) was evaluated. Unfortunately, no research results have been published.

Clinical studies evaluating the effectiveness of BA in melanoma treatment may have been discontinued due to the progress in the treatment of this cancer, which took place in 2011, when FDA (*Food and Drug Administration*, USA) registered a new drug, an inhibitor BRAF, vemurafenib for the treatment of melanoma [25]. This initiated a new era in the treatment of melanoma. Betulinic acid derivatives that show significantly greater cytotoxicity to tumor cells than BA alone are NVX-207 and B10. The molecular structures are shown in Fig. 2.

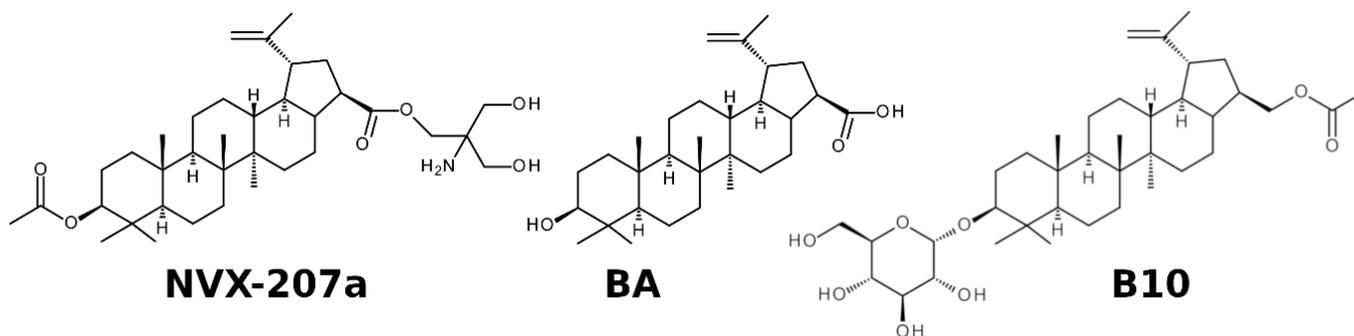


Figure 2. Chemical structure of betulinic acid (BA), NVX-207 and B10.

The first report on the significant anti-cancer activity of NVX-2017 in *in vitro* and *in vivo* studies dates from 2009 [26]. The activity of the compound (mean $IC_{50} = 3.5 \mu M$) *in vitro* was

determined using normal and tumor cell lines (human and canine). The studies confirmed that NVX-207 activates the mitochondrial pathway of apoptosis. Phase I/II trials in dogs with natural tumors showed excellent clinical response after the topical treatment with NVX-207 (application once per week). Complete tumor remission was obtained in all five treated animals [27]. In 2016, Liebscher *et al.* presented the results of research conducted on melanoma tumor lines (MelDuWi and MellJess) and human melanoma lines (A375) using BA and its derivatives: NVX-207 and B10 [28].

It has been shown that all three compounds, in particular NVX-207, are characterised by high cytotoxicity towards equine melanoma cells, activating the mitochondrial apoptosis pathway. The studies were also carried out *in vivo* on 2 horses (13- and 18-year-old) suffering from melanoma. The experiment consisted of intratumoral injections of NVX-207 once a week for the next 19 weeks. No irritation was observed, the animals tolerated the therapy well. In laboratory tests, no abnormalities were observed, except for transient increases in hepatic transaminases [28]. One horse was euthanised in the 19th week of the experiment due to colon torsion. Unfortunately, it has not been described whether changes in melanoma lesions in the form of tumor regression or cure were observed.

Compound B10, a glycosylated derivative of BA, has a different mechanism of cytotoxic activity than BA and NVX-2017. It turned out that B10 induces autophagy, destabilises lysosomes and releases cathepsins Z and B to the cytoplasm [29]. This report and studies from 2014 indicate the possibility of using B10 in the treatment of tumors under hypoxic conditions due to the increased cytotoxicity of this compound in hypoxia, e.g. in the treatment of malignant glioma [30].

Despite the large potential anti-tumor activity and the well-known mechanism of cytotoxicity against cancer cells, BA and its derivatives have not been used in therapy so far. In addition to *in vitro* experimental studies, there are reports of the use of two potential drug precursors in cancer therapy in dogs and equine melanoma.

3. 2. Anti-viral activity of betulinic acid and its derivatives

The search for herbal medicinal agents capable to fight HIV infection resulted in the discovery in 1994 of the anti-viral properties of the extract from the leaves of *Syzygium claviflorum*. The active compounds of this extract are betulinic acid and its derivative of platanic acid [31]. In the course of further research, a semi-synthetic derivative of BA with stronger anti-viral properties was discovered.

This derivative, being 3-O-(3',3'-dimethylsuccinyl) betulinic acid, is now known as bevirimat. The structure of the molecule is shown in Fig. 3.

This preparation has very strong inhibitory properties of HIV-1 replication, because as a protease inhibitor it leads to inhibition of virus maturation in infected lymphocytes. This compound has been characterised as a maturation inhibitor - a representative of a new group of anti-HIV drugs [32]. In 2007, two reports were published describing the results of the first and the second phase of bevirimat clinical trials [33, 34].

The compound was evaluated for safety, pharmacokinetics and pharmacodynamics in a double-blind study in healthy volunteers and in HIV-infected adults (phase IIa and IIb, 57 people infected with HIV). The drug has been found to be safe and well tolerated, with no dose-limiting toxicity or serious side effects. The most frequently reported adverse effects of bevirimat were headache and discomfort in the throat, but none of the study participants discontinued using the preparation [33, 34].

In the years 2008-2009 another phase II of clinical trial was conducted (NCT01097070, www.clinicaltrials.gov), which included 35 HIV-positive volunteers. Then, clinical trials of the drug were suspended due to the take over of rights to bevirimat by the company Myriad Genetics, which stopped work on HIV maturation inhibitors in 2010.

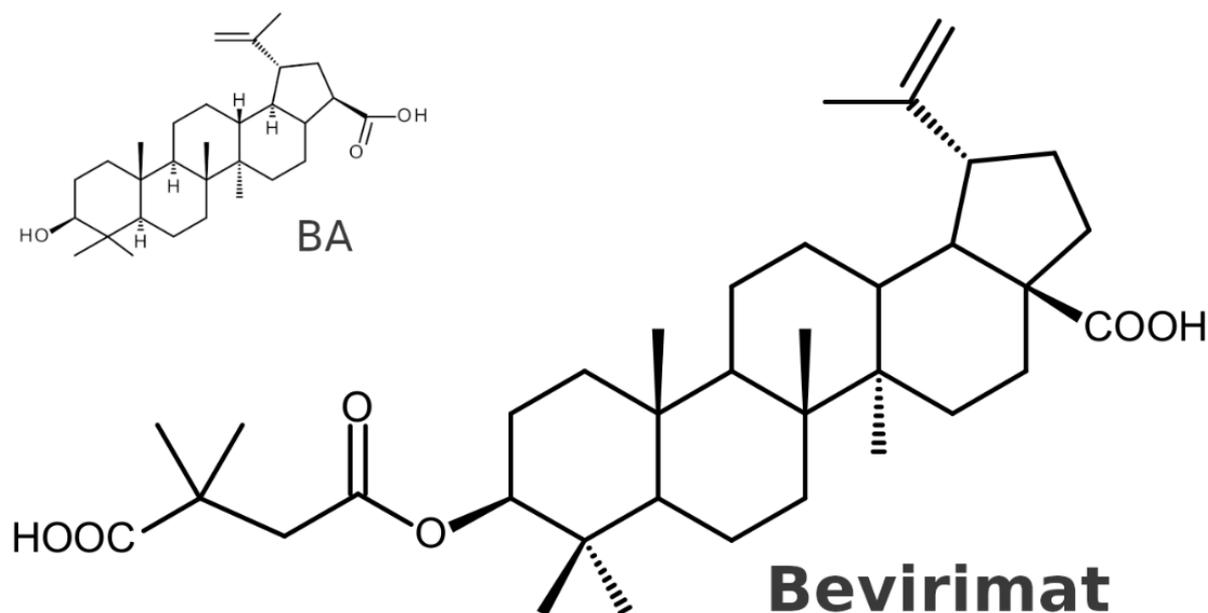


Figure 3. Chemical structure of bevirimat [3-O-(3', 3'- dimethylsuccinyl) betulinic acid];
BA – betulinic acid

Further *in vitro* studies of bevirimat showed a build-up of drug resistance, what was related to nucleotide polymorphisms at the CD capsid/SP1 protein cleavage site and mutations at the CD/SP1 site. These mutations were not found during clinical trials, what may indicate their much slower appearance in *in vivo* conditions [35, 36]. Therefore, clinical studies of bevirimat were definitively terminated. However, *in vitro* experimental studies were continued, what led to the discovery of second generation maturation inhibitors, the representative of which is BMS-9551766. The chemical structure of the molecule is shown in Figure 4.

The BMS-9551766 synthesis pathway (GSK3532795) was published in 2016 [37]. Phase II of clinical trials sponsored by ViiV Healthcare were conducted in cooperation with GlaxoSmithKline in 2013-2014 (NCT01803074, www.clinicaltrials.gov) and in 2015-2016 (NCT02415595, www.clinicaltrials.gov). Unfortunately, they were discontinued due to the strong gastrointestinal intolerance of the preparation and the increase in drug resistance in patients [38].

The representative of the second generation inhibitors is also a derivative GSK2838232. The chemical structure of the molecule is shown in Fig. 5. This compound in 2013-2016 was tested in phase I clinical trials in four different programs (NCT01802918, NCT02289482, NCT02289495, NCT02795754, www.clinicaltrials.gov) sponsored by GlaxoSmithKline. The results of these studies were published in 2018 [39]. A good safety profile as well as

pharmacokinetic and dynamic properties were found to allow the continuation of clinical trials in phase II in 2017-2018 (NCT03045861, www.clinicaltrials.gov). It has been given to HIV-infected patients in combination with other antiretroviral therapy: Cobicistat. The results of these studies have not yet been published.

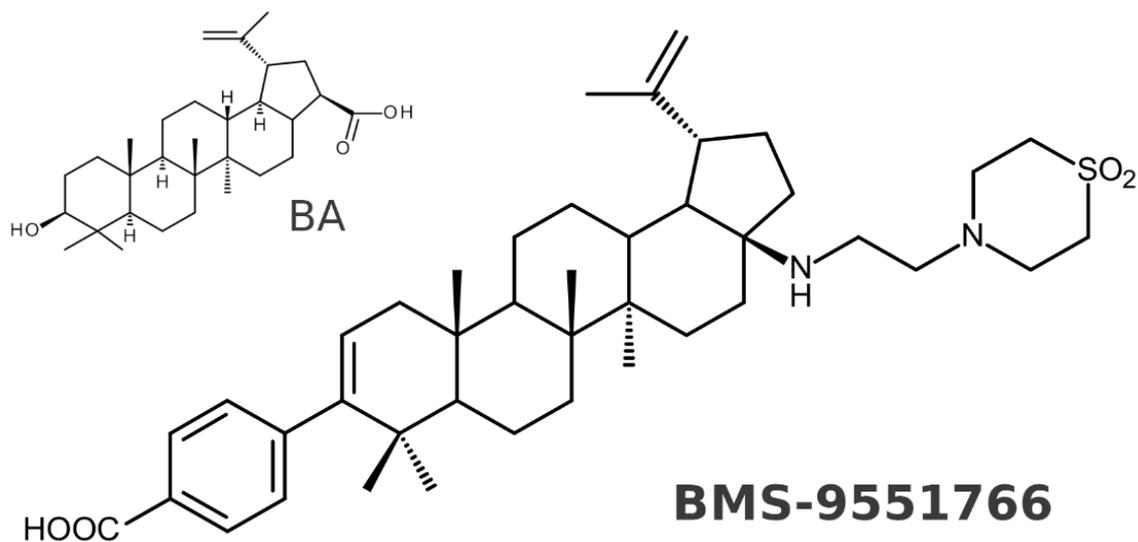


Figure 4. Chemical structure of BMS-9551766 and BA – betulinic acid.

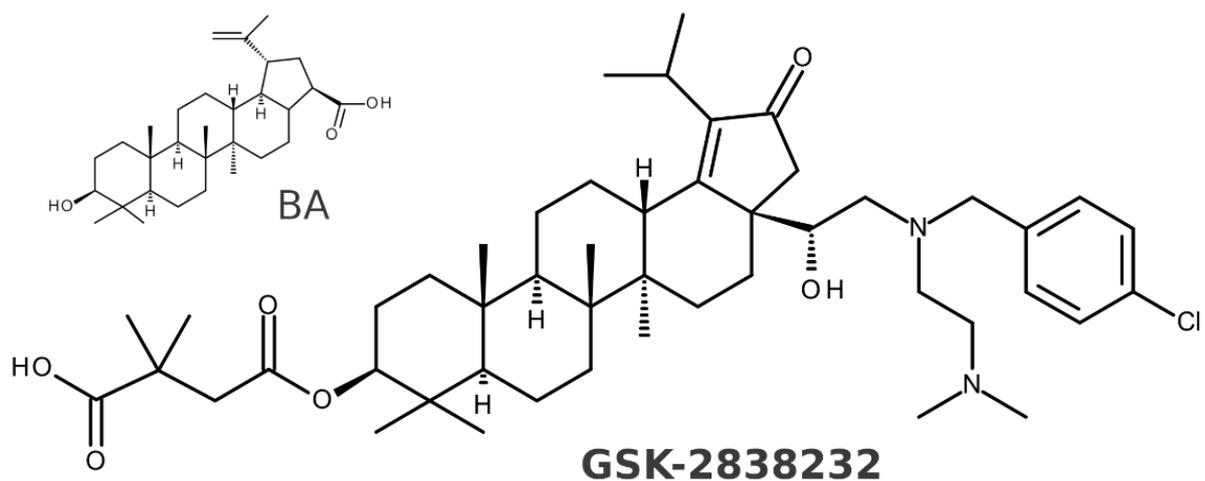


Figure 5. Chemical structure of GSK-2838232 and BA – betulinic acid.

Studies on the anti-viral activity of betulinic acid contributed to the discovery of a new group of anti-HIV drugs – first generation maturation inhibitors, which is represented by bevirimat and second generation maturation inhibitors - BMS-9551766 (GSK3532795) and GSK2838232. Although none of these molecules have been introduced for the treatment of

HIV, these studies have had a significant impact on the progress in HIV treatment that has occurred over the past 20 years.

3. 3. Anti-tumor activity of betulin

Betulin (BE) has much lower anti-tumor activity than BA. It inhibits weaker the proliferation of melanoma cells in comparison with BA and its derivatives. It has been shown that betaulin induces the mitochondrial pathway of apoptosis in lung cancer, breast cancer, colorectal cancer, and cervical cancer [11]. However, reports of BE anti-tumor activity are few and have not been a starting point for further studies of BE anti-tumor activity. During the last 20 years, the German company Birken GmbH conducted research on the biological activity of birch bark extract (TE - triterpen extract). A first description of an emulsion containing TE is provided in the international patent EE 200200552(A) (PL 202948) [13].

The TE used to prepare the emulsion contains at least 80% betulin, a maximum of 10% betulinic acid, a maximum of 3% lupeol and 4% oleanolic acid. The emulsion, in turn, contains from 2-10% of TE and avocado oil, almond oil, water, glycerol or urea. Due to its preservative (anti-bacterial, anti-fungal) and emulsifying properties of triterpenes, this composition does not have to contain additional preservatives. The emulsion was used for the preparation of medicaments in the form of ointment, cream, gel for application to the skin for the treatment of, among others, precancerous conditions and damaged skin. The patent also describes in detail the toxicological tests carried out on experimental animals - mice and rats. During intraperitoneal administration of TE, toxic symptoms were seen in mice and rats at doses above 500 mg/kg body weight. It was also found that one-off subcutaneous administration of the product at 2000 mg/kg body weight over 14 days does not cause mortality. Another study on TE kinetics was performed on rats and dogs by subcutaneous and intraperitoneal administration. The experiment showed no toxicity of TE for rats and dogs at a daily dose of 300 mg/kg body weight during a 28-day application [40].

The first report on the effectiveness of TE in the treatment of the precancerous state of the skin - AK (*actinic keratosis*) comes from 2006 [41]. 28 patients with AK were involved in this pilot study, 14 patients were treated with TE only, and 14 patients underwent TE cryotherapy and ointment with TE. The results showed the effectiveness of ointment with TE in the treatment of AK with very good tolerability, without side effects. In the next phase II clinical trial, conducted on a group of 45 patients who received oleogel with TE, the possibility of using this preparation as an effective medicine for AK treatment was confirmed [42].

In this study, an improved formula of the original formulation was used, which was named Oleogel-S10. This formula has patent protection in Europe since 2004 and in the US since 2010 [43, 44].

In the years 2008-2010, a multi-centre, randomised, double-blind phase II trial was conducted to evaluate the effectiveness of Oleogel-S10 in the treatment of AK and to plan phase III trials (NCT00786994, www.clinicaltrials.gov). Unfortunately, the results published in 2015 did not confirm the effectiveness of Oleogel-S10 in the treatment of AK on a group of 165 participants. It was only shown that the drug was well tolerated during the 3-month period of administration [45].

Unfortunately, the search for an effective preparation in the treatment of the precancerous skin condition has not been successful. However, it turned out that Oleogel-S10 can be used in other skin diseases, what is associated with anti-inflammatory, anti-microbial and wound healing properties in the case of birch bark extract.

3. 4. Anti-inflammatory, anti-microbial and wound healing effects of pentacyclic triterpenes

Already in 1899, Wheeler stated that betulin had antiseptic properties and he used it to sterilise dressing materials and wound patches [13]. There are reports of anti-bacterial and anti-fungal effects of betulin and its derivatives on the bacterial strains of *Enterobacter aerogenes*, *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and on fungi strain *Candida albicans*. Anti-bacterial properties are also demonstrated by lupeol, oleanolic acid and betulinic acid, which are components of the birch bark extract [12, 43].

Triterpenes are known to have anti-inflammatory activity by inhibiting enzymes involved in the inflammatory reaction such as: phospholipase A2, cyclooxygenase, nitric oxide synthase. In addition, they reduce the production of prostaglandins and proinflammatory cytokines: tumor necrosis factor (TNF- α), interferon (INF- γ) and interleukins. These properties are characteristic in particular for betulin, betulinic acid, lupeol and oleanolic acid [7, 46, 47].

The Oleogel-S10 formula is slightly different from the original emulsion developed at Birken GmbH. Instead of avocado and almond oil, sunflower oil was used, with the same composition of triterpenes [45]. The product has successfully passed phase III of clinical trials (NCT01657305, NCT01807650, www.clinicaltrials.gov). Three phase III trials were conducted to evaluate the effectiveness and safety of this product. Two studies concerned wounds from the sites of skin fragment removal for split-thickness skin graft (STSG) (219 patients) [48, 49, 50]. One study was conducted in patients with second degree burns (61 people) [51]. In all studies, the acceleration of epithelization of wounds and the creation of a more elastic scar after the use of the tested drug was demonstrated.

The preparation affects all three phases of wound healing: inflammation, migration and differentiation. After activation of the inflammatory phase, faster epithelization is connected with the migration and differentiation of keratinocytes [52, 53]. In 2016, Oleogel-S10 was registered by EMA (*European Medicines Agency*, EU) as a preparation for treating second-degree burns with the name Episalvan. The drug is available on prescription. In 2018, Episalvan also obtained a positive FDA opinion and is currently in phase III of clinical trials (NCT03068780, www.clinicaltrials.gov) to evaluate the efficacy and safety of use in the treatment of difficult to heal wounds in *Epidermolysis bullosa* (EB). EB is a rare group of genetically conditioned skin diseases characterised by the formation of blisters on the skin after even minor injuries. There is currently no effective treatment for EB. The first reports from phase II trials show faster re-epithelization of EB wounds following the use of Episalvan [54].

A summary of the results of phase III clinical trials is planned for 2020 [55]. Oleogel-S10 (Episalvan) is the first registered medicine containing a herbal ingredient - an active triterpene extract, mainly composed of betulin, derived from birch bark. Undoubtedly, this is a new and significant achievement in phytotherapy.

4. CONCLUSIONS

Betulin and betulinic acid due to their chemical structure are excellent structures leading to the formation of new derivatives with potentially better anti-tumor, anti-viral or anti-inflammatory properties. Undoubtedly, the success of studies on the anti-HIV effect of betulinic acid was the discovery of a new group of antiretroviral drugs - maturation inhibitors. In turn,

many years of Birken Gmbh's experiments contributed to the marketing of the first medicine containing a triterpene extract intended for wound healing. There are still experimental and clinical trials in numerous centres, and there will certainly be new reports on the use of lupane type pentacyclic triterpenes in therapy.

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