Medical Prospects for Using Triterpenoids of Lupane Series

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Absract

Prospects are discussed concerning the application of triterpenoids belonging to lupane series in medical practice. Among naturally occurring compounds betulinic acid is attached with a particular significance as a highly efficient anticancer agent and the inhibitor of enteric virus ECHO6. Amides, ureides, dipeptides and acylates of betulinic, betulonic and 3-oximinobetulonic acids those exhibit a high anti-HIV activity, pronounced antineoplastic and organ-protective action belong to the most promising semisynthetic derivatives of the lupane series. It is demonstrated that the manufacture of biologically active additives with the extract of betulin is under active development.

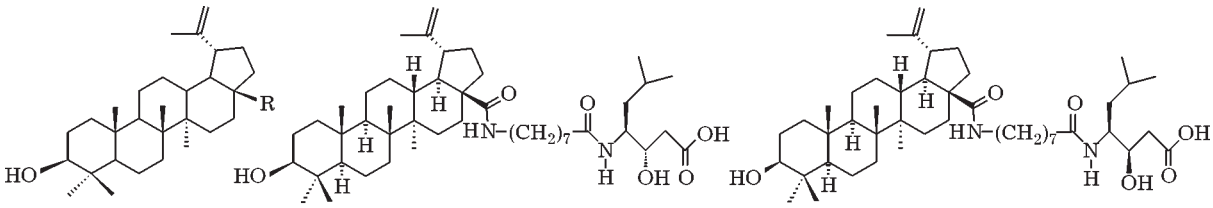
Key words: triterpenoids, betulin, betulinic acid, anticancer activity, antiviral activity

The class of triterpenoids widely occurring in nature attracts increasingly more attention of the experts in the field of medical chemistry and pharmacology. Biological activity of triterpenoids belonging to the lupine series was noted even in the 19th century. So, betulin **1** was offered as a component of antiseptic plasters [1]. The tar obtained via the thermal decomposition of birch bark, which tar contains lupane derivatives, is known as a component of the Vishnevsky ointment. A true burst of interest with respect to pharmacological properties of lupane derivatives was noted during the recent decade after revealing extremely prospective antiviral (anti-HIV) and antineoplastic agents in this group of compounds.

Lupeol **2** is considered to be among active citostatic agents; in particular, it exhibits the properties of a powerful inhibitor of skin cancer [2]. It is revealed that lupeol is capable of reducing the risk of neprolith formation in kidneys and acts as an urolytic agent since it prevents tissues from injuries and influences the process of the dissolution of concrements related to urolithiasis [3]. Unique properties of betulinic acid **3** as an anti-melanocarcinomatic agent have been found out as the result of the studies on the activity of more than 2500 natural extracts [4].

Powerful anti-HIV agents have been revealed among the synthetic derivatives of betulinic acid. So, the mechanism of anti-HIV action of betulinic acid peptides such as RPR 103611 and IC 9564 consists in inhibition of the stage of joining the envelope of a viral particle together with a cellular membrane [5].An anti-HIV preparation Bevirimat (Panacos Pharmaceuticals, the USA) based on 3-O-3′,3′-dimethylsuccinylbetulinic acid **4** is at the finishing stage of development [6].

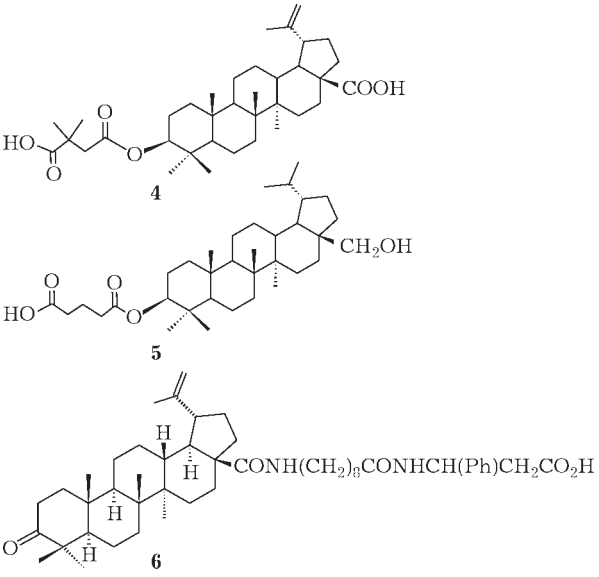
The activity parameters (EC_{50} < 0.00035 μmol, IC_{50} = 7 μmol, TI > 20 000) surpass those for azidothymidine (EC_{50} = 0.15 μmol, TI = 12 500), whereas the mechanism of anti-HIV action is connected with blocking the stage of viral and cellular membrane fusion. These properties cause the compound under consideration to differ favourably from the substances of nucleoside nature those influence directly the replication processes of the viral nucleic acid.



Scheme 1.

The parameters describing the anti-HIV-1 activity of 3-O-glutaryldihydrobetuline **5** (EC_{50} = $2.0 \cdot 10^{-5}$ μmol, IC_{50} = 23.59 μmol, TI = 1 120 000, H9 lymphocytes) are two order of magnitude higher as compared to corresponding values for dimethylsuccinyl acylates [7] (Scheme 1).

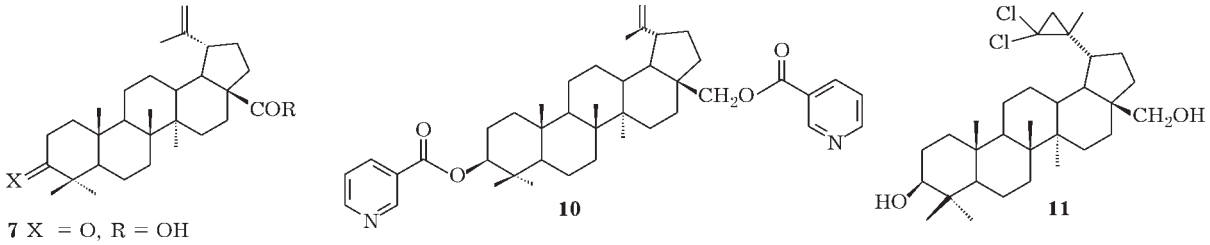
Betulinic acid dipeptide (N′{N-[3-oxolup-20(29)-ene-28-oyl]-9-aminononanoyl}-3-amino-3-phenylpropionic acid) **6** has been patented as an agent exhibiting antiviral (anti-HIV, anti-herpes) and immunostimulatingy activity [8]. The authors of [9] demonstrated that the mentioned compound inhibits the growth of tumorous cells MT-4, MOLT-4, CEM and Hep G2. The dipeptide represents an active inductor of apoptosis in leucaemic cells as well as in the cells of hepatocarcinoma *in vitro*. Betulonic acid and its derivative [3-oxo-20(29)-lupene-28-oyl]-3-aminopropionic acid have been revealed to exhibit the ability to reduce necrobiotic lesions of the hepatic parenchyma caused by cytostatic anticancer chemotherapy [10] (Scheme 2).



Scheme 2.

For the Russian Federation a triad of the most urgent viral infections is presented by flu, herpes and hepatitis. We have for the first time established virus-inhibiting properties of triterpenoids with respect to the influenza A virus [11, 12]. As a rule, the antiviral properties in this case are determined by the presence of oxime groups (C-3 or C-28) or an amide bond. The most pronounced activity is exhibited by betulonic acid oxime **7**, betulonic acid amide as well as conjugates of betulonic acid and betulonic acid oxime with *L*-methionine **8**, **9** [13]. Furthermore, pronounced antiviral properties are exhibited by betuline dioxime, betuline 28-oxime, hemiphthalate 3-acetylbetuline hemiphthalate. The results obtained indicate the fact that the studies on anti-influenza properties of triterpenoids as well as the development of novel preparations on their basis represent a new prospective scientific field (Scheme 3).

A considerable role in exhibiting antiviral activity with respect to type 1 herpes virus is played by the combination of substituents at C-3 and C-28 positions in the structure of lupane molecule. In the series of 3-hemisuccinates, 3-hydroxy and 3-oxo derivatives one can notice a positive influence of the carboxylic group and of relatively long-chain substituents whose significant part is presented by nitrogen-containing fragments (amides, conjugates with amino acids, ureides) [14]. Among active compounds one can note betuline, betulonic acid and methyl ether of dihydrobetulinic acid, betulinic acid hemisuccinate, betuline dimethoxycinnamate, betulonic acid amide with octadecylamine, conjugate of betulonic acid with *L*-methionine **8**, betulonic acid isocyanate and



10 **11**

7 X = O, R = OH
8 X = O, R = $NHCH(CO_2CH_3)(CH_2)_2SCH_3$
9 X = NOH, R = $NHCH(CO_2CH_3)(CH_2)_2SCH_3$

Scheme 3.

betulonic acid ureide with *L*-methionine [15], betulinic acid 4-chlorobenzalhydrazide and betulonic acid oxime benzalhydrazide. As far as the HIV-1 is concerned, betuline dinicotinate **10** [16], conjugate of betulonic acid with *L*-methionine **8** and betulinic acid hydrazide one could considered to be promising compounds. The combination of antiviral activity with dinicotinate hepatoprotective action offers considerable scope for using betuline in the field of viral hepatitis prophylaxis and treatment.

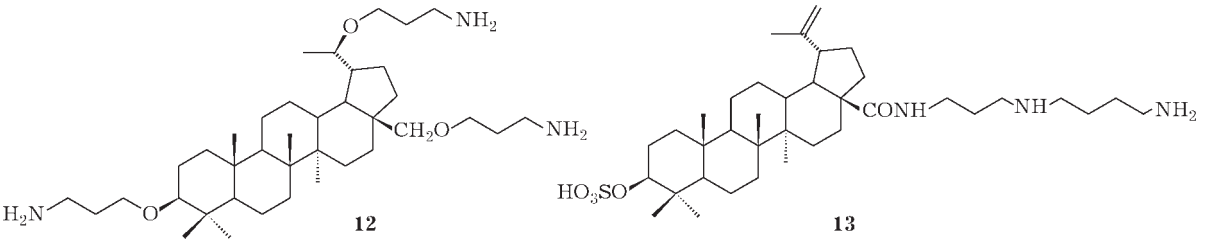
We have for the first time revealed that betulinic acid **3** and its derivatives should be considered to be promising inhibitors of envelope-free RNA-containing virus ECHO6 belonging to the family of poliomyelitis activators, rhinoviral infection, feverish and respiratory diseases caused by enteroviruses [17]. Betulinic acid surpasses a novel preparation Plekonaril in the efficiency parameters (EC_{50} = 0.007 μmol, $MPC/EC_{50} > 4000$).

Methods are developed for the standardization of the betuline substance and its pharmaceutical dosage forms [18]. Such a compound as 20,29-dichloromethanolupane-3β,28-diol **11** (Cyclobet) was shown to exhibit a pronounced antituberculosis activity [19].

The manufacture of betuline in Russia could be established commercially at a reasonable price. Russia takes a leading position in the manufacture of biologically active supplements containing betuline. Chemical-and-biological Association "Firma Vita" attached to the Russian Academy of Sciences offers such bioactive supplements as "Betulavitin" with a strongly pronounced immunomodulating, antiviral, antioxidative, regenerating action (http://www.preobrajenie.com/). Such a company as "Berezovy Mir" ("Birch world") produces biologically active supplements containing birch-bark extract whose properties could be judged by the names of preparations: Betula-Hepat, Diabetuline, Tubelon, Betusil, Betula-Hit, *etc*. (http://www.birchworld.ru/).

The studies of recent years are aimed at the modifying of betuline and obtaining methylene polyamine and 1,2,4-trioxalane derivatives basing on this substance. Triterpenes such as **12**, **13**, containing various polyamine chains are of interest not only as the analogues of the steroid antibiotic Squalamine, but also as the subject of inquiry for medical and supramolecular chemistry [20] (Scheme 4).

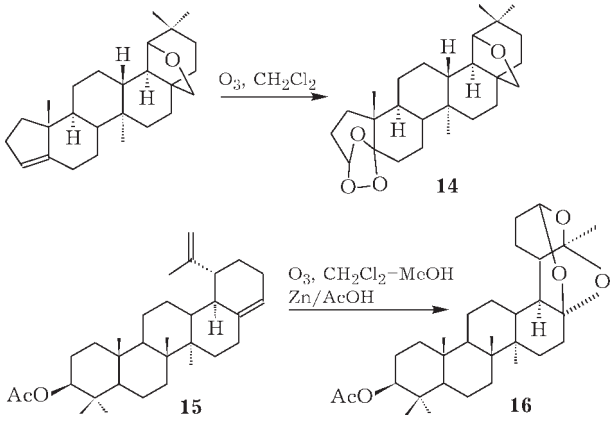
As the result of the ozonolysis of unsaturated triterpenoids we have synthesized stable secondary ozonides such as **14** representing the analogues of antimalarial preparation Artemisinin [21]. Triterpenoide **16** with the trioxane fragment in the cycle E have been obtained *via* the reductive



12 **13**

Scheme 4.

conversion of peroxide products of 22(17→28)-*abeo*-17(28),20(29)-lupdiene **15** ozonolysis [22]:



The consolidation of the efforts of Russian scientific groups working in the field of isolation, synthetic transformations and pharmacological studies of betuline and its derivatives aimed at prompt obtaining of Russian domestic pharmaceutical products of high social significance as well as the products for agricultural purposes is considered to be of crucial importance.

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