UDC 615.2

Computer Analysis of Biological Activity Spectrum for Novel Betulonic Acid Derivatives

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Abstract

Analysis of the structure-to-properties relations for the new derivatives of betulonic acid was carried out with the help of PASS program. Agents with potentially high antitumor, hepatoprotective and anti-inflammatory activity were revealed among these compounds. Analysis of the possible mechanisms of the action of novel betulonic acid derivatives was carried out.

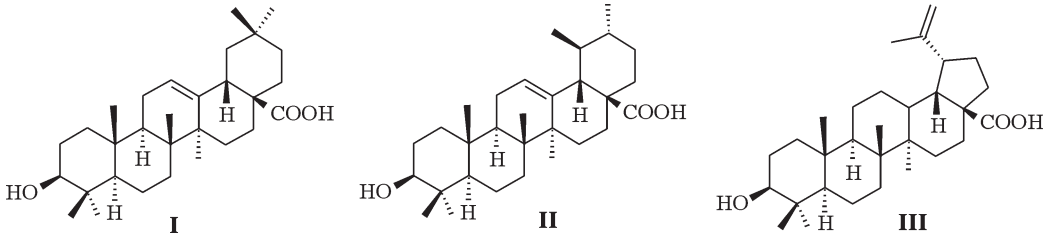
Key words: derivatives of betulonic acid, structure-to-properties relations, PASS program

INTRODUCTION

In connection with extensively developing modern combinatorial chemistry, there is a certain difficulty in choosing candidates for the screening of medicinal agents among an enormously great amount of potentially highly active molecules. The methods widely applied nowadays *in vitro* for estimating main types of biological activity (macromolecular targets) do not allow covering all the amount of synthesized compounds. Besides, they not always provide the reliability of data concerning the mechanism of action, toxicity, pharmacokinetics, pharmacodynamics and the metabolism of novel agents. As a consequence, with such an approach, there is an increase in the probability of incorrectly choosing basic structures (lead compounds), used for further synthesizing the "libraries" of derivatives. In addition, out of the framework of scientific interest there are compounds with a potentially high activity, those however did not fall into the focus of screening.

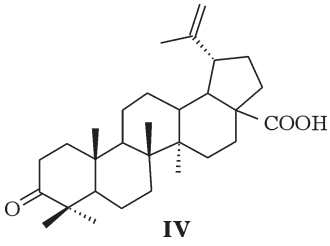
Fast, efficient and low-cost screening new agents could be performed by means of computer-based forecasting the spectrum of biological activity (structure-activity relationship analysis, SAR). With the help of this approach one could obtain much data concerning potential biological activity, toxicity, the mechanism of action and the metabolism of chemical compounds, as well as perform the selection of potential highly active agents at an early stage of research, which promotes the optimization of synthesizing new derivatives on the basis of the structure-activity relationship revealed [1–3].

Last time, of great interest are research works devoted to studying compounds with a wide spectrum of biological action synthesized on the basis of naturally occurring triterpene platforms such as oleanolic acid **I**, ursolic acid **II** and betulinic acid **III** [4] (Scheme 1).



Scheme 1.

For these triterpenoids, researchers revealed antioxidative, antineoplastic, anti-inflammatory, antiviral and cytoprotective activity, the mechanisms of action and biological targets: proteins the regulators of separate signal pathways, transcription factors [5–9]. Owing to a variety of biological effects and the presence of many molecular targets these compounds could be considered a basis for creation multitarget preparations. Nowadays, betulinic acid and a number of its derivatives are under clinical testing as antineoplastic preparations. According to our data, another triterpenoid such as betulonic acid **IV** could be also considered to be a base compound for obtaining potentially multitarget agents.



IV

As the result of experimental investigation of biological activity for the derivatives of betulonic acid synthesized at the Novosibirsk Institute of Organic Chemistry of the Siberian Branch of the RAS, it was established that its amides exhibit anti-inflammatory, antioxidative, hepatoprotective and antitumor activity with a correcting action under the conditions of highly toxic cytostatic polychemotherapy [10–16].

EXPERIMENTAL

Last time, at the Institute of Organic Chemistry of the UrB RAS (Yekaterinburg) there were synthesized a number of novel betulonic acid derivatives with different substituents at the positions 2, 3, 20 and 28 in the triterpene structure (Table 1). The research work was aimed at searching for efficient correctors of chemotherapeutic preparations among these compounds. The investigation task included revealing prospective agents with antineoplastic, anti-inflammatory and hepatoprotective activity by means of computer forecasting, studying the properties of those using experiments in animals, as well as comparison the properties revealed with the properties of betulonic acid, its methyl ester and amides revealed earlier.

Forecasting the spectrum of biological activity for betulonic acid derivatives was carried out by means of PASS (Prediction of Activity Spectra for Substances, 2007 V. Poroikov, D. Filimonov *et al.*) program. The spectrum of biological activity is determined as a set of pharmacological effects, biochemical mechanisms of action and types of specific toxicity those could be exhibited by a chemical agent under interaction with biological objects [13, 14].

For forecasting, according to the PASS program we used the structural formulas of compounds in the standard of MDL molecular file, created within the framework of ISIS Draw 2.4 and ChemDraw Ultra 9.0 editors. For every derivative we estimated the parameter of drug-likeness within the range of 0–1 as the probability of complete correspondence of the activity profile to a known medicinal preparation. The term of drug-likeness reflects the general biological activity of a molecule determined on the basis of the analysis concerning various properties of its molecular structure (hydrophobicity, electron density distribution, the features of hydrogen bonds, the size of a molecule and its flexibility, the presence of various pharmacophore groups in the structure of a molecule). These properties affect the behaviour of a molecule in an organism determining its bioavailability, transport properties, affinity with respect to proteins, reactivity, toxicity, metabolic stability and many other parameters [1].

In order to estimate potential corrective properties of novel betulonic acid derivatives we took into account under SAR analysis, first of all, the whether antineoplastic, anti-inflammatory and hepatoprotective activity is exhibited by compounds under investigation. The probability of the presence of each kind of activity (P_a) was estimated within the range of 0.4–1 to compare with drug-likeness probability in order to estimate the contribution of separate activity kinds to the total potential pharmacological activity of the agent under investigation. Besides, for each of agents we performed the screening of possible molecular targets.

TABLE 1

Keys, names and formulas of betulonic acid derivatives

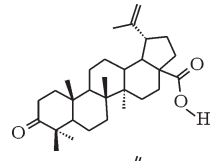
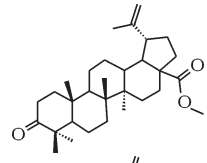
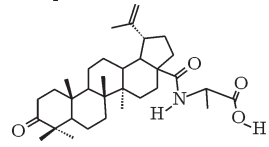
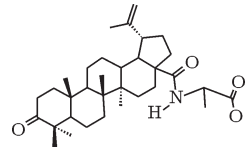
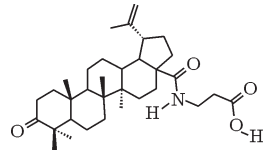
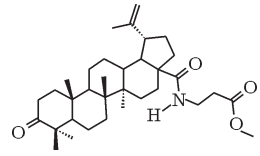
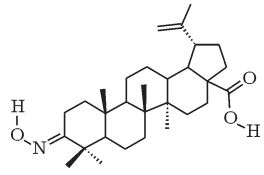
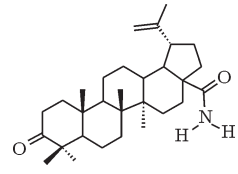
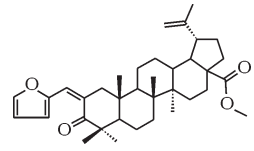
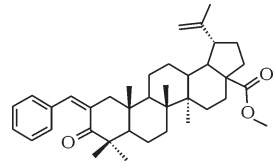
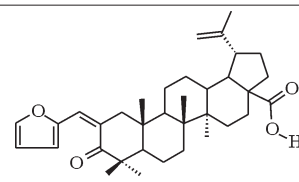
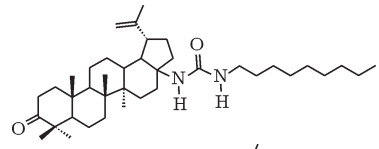
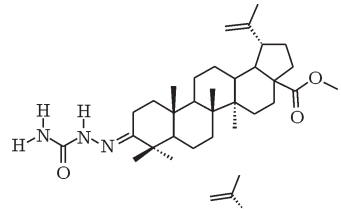
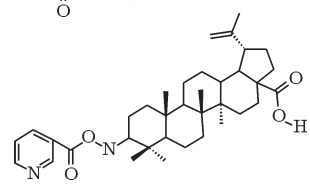
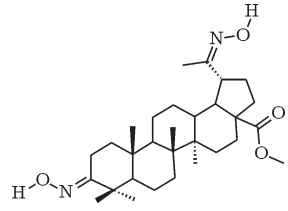
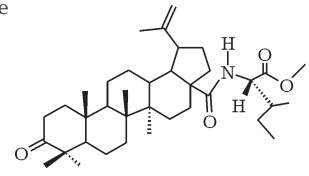
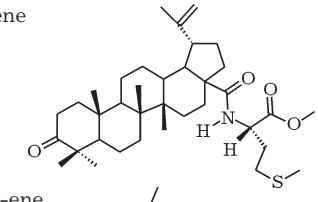
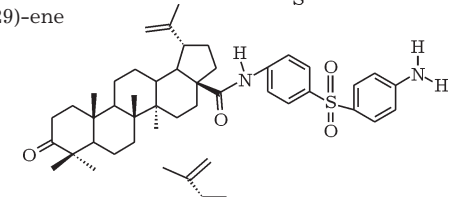
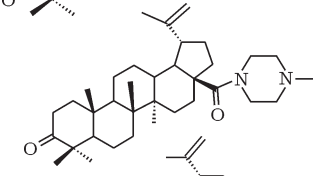
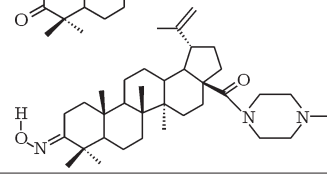
Compounds	Chemical name	Formulas
1	Betulonic acid	
2	Methyl ester of betulonic acid	
3	2-[3-Oxo-20(29)-lupen-28-oylamino]-propionic acid	
4	Methyl ester of 2-[3-oxo-20(29)-lupen-28-oylamino]-propionic acid	
5	3-[3-Oxo-20(29)-lupen-28-oylamino]-propionic acid	
6	Methyl ester of 3-[3-oxo-20(29)-lupen-28-oylamino]-propionic acid	
7	Betulonic acid 3-oxime	
8	3-Oxo-17b-carbamoyl-(28)-norlup-20(29)-ene	
9	Methyl ester of 2-furfurylidene-betulonic acid	
10	Methyl ester of 2-benzylidene-betulonic acid	

TABLE 1 (the End)

Compounds	Chemical name	Formulas
11	2-Furfurylidene-betulonic acid	
12	3-Oxo-17b-(N-decylureido)-(28)-norlup-20(29)-ene	
13	Methyl ester of 3-semicarbazone-betulonic acid	
14	Betulonic acid 3-oxime nicotinate	
15	Methyl ester of 3,20-dioximino-29-norlup-28-oic acid	
16	3-Oxo-28-(O'-methyl-L-isoleucino)-carbonyl-28-lup-20(29)-ene	
17	3-Oxo-28-(N-carbonyl-O'-methyl-L-methionine)-lup-20(29)-ene	
18	3-Oxo-28-(4-4'-diamimophenylsulphone)-carbonyl-lup-20(29)-ene	
19	3-Oxo-28-(N-methylpiperazine)-carbonyl-lup-20(29)-ene	
20	Betulonic acid 3-oxime N-piperazide	

RESULTS AND DISCUSSION

Fig. 1 demonstrates drug-likeness probability values for betulic acid derivatives.

The forecast executed with the use of the PASS program, confirms a high potential pharmacological activity of compounds **1–6** those we studied earlier. The novel derivatives of betulonic acid are also potentially active agents: the values of drug-likeness probability are ranging within 0.79–0.99. Maximum probability values were registered for 17b-carbamoyl derivative **8**, the nicotinate of betulonic acid 3-oxime **14**, betulonic acid derivative **16** with the residue of *L*-isoleucine at 28 position and 28-*N*-methylpiperazine derivative **19**. Thus, the value of drug-likeness probability for derivative **19** is almost coinciding with that for betulonic acid **1** (0.988 and 0.99, respectively).

The potential antitumor effect (antineoplastic) was estimated comparing to drug-likeness probability and possible mechanisms of antitumor action such as proapoptotic activity (apoptosis agonist), immunosuppressing action (immunosuppressant) and the ability of reducing the probability of tumor development (chemopreventive), for the estimation of every mechanism contribution to the total antineoplastic activity of novel derivatives. Figure 2 demonstrates the results of forecasting the antineoplastic activity of betulonic acid derivatives.

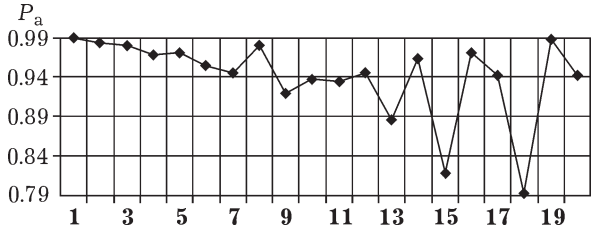


Fig. 1. Drug-likeness probability values for betulonic acid derivatives.

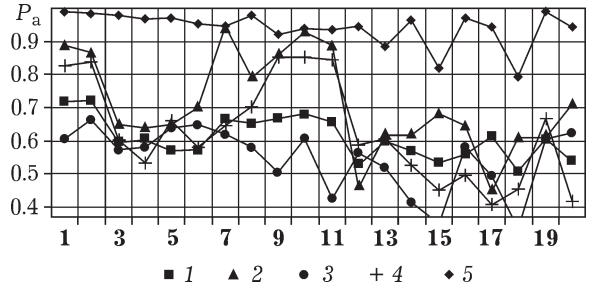


Fig. 2. P_a values for antineoplastic (*1*), proapoptotic (*2*), immunosuppressing (*3*) and oncoprophylactic (*4*) activity for the derivatives of betulonic acid comparing to drug-likeness probability (*5*).

Basing on the forecast data one could conclude that there is a high correlation level between all the parameters of the antineoplastic activity of betulonic acid amides. This fact indicates that there is complex influence of these compounds upon the processes of tumor initiation and growth, which, in general, corresponds to experimental data. Coincidence between the values of proapoptotic activity and drug-likeness probability for betulonic acid (position 3) oxime **7** and the methyl ester of 2-benzylidene-betulonic acid **10** indicates that there is a determining contribution of apoptosis stimulating properties to the potential action mechanism for these derivatives. For the methyl ester of 2-furfurylidene-betulonic acid **9** and the methyl ester of 3-semicarbazone-betulonic acid **13** the values of probabilities proapoptotic and oncoprotective activity correlate well. At the same time, 28-*N*-methylpiperazine derivative **19**, to all appearance, exhibits any complex mechanism of antitumor action.

Basing on the data of forecasting the spectrum of the biological activity of betulonic acid derivatives we predicted potential targets in the mechanisms of the antineoplastic action of compounds. Figure 3 demonstrates P_a values determined with the use PASS for some mechanisms of antitumor action, inherent in the derivatives of betulonic acid: the inhibition of phosphatase (phosphatase inhibitor), the inhibition of phospholipase C and the agonism with respect to interleukins (interleukin agonist).

The data obtained indicate that the main molecular mechanism of antineoplastic action

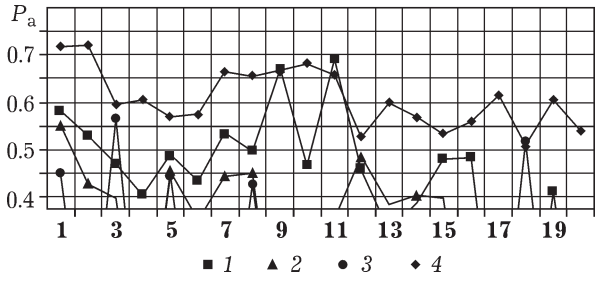


Fig. 3. P_a values for the inhibition of phosphatase (*1*), phospholipase C (*2*), for the agonism with respect to interleukin (*3*) and for the direct antineoplastic activity of betulonic acid derivatives (*4*).

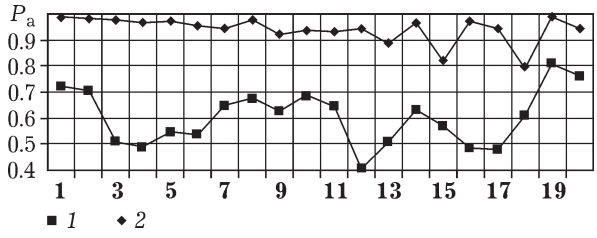


Fig. 4. P_a values for the anti-inflammatory activity of betulonic acid derivatives (*1*) comparing to drug-likeness probability (*2*).

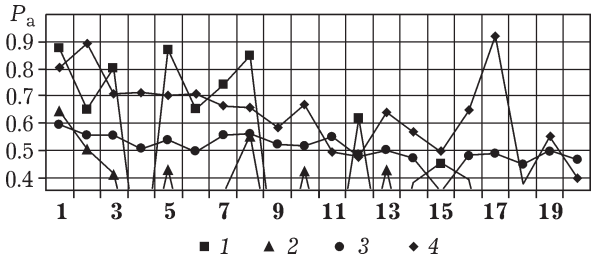


Fig. 6. P_a values for transferase stimulation (*1*), for the inhibition of oxidoreductase (*2*) and bilirubin oxidase (*3*), for the hepatoprotective activity of betulonic acid derivatives (*4*).

is inherent in 2-furfurylidene-betulonic acid **11** and its methyl ester **9** is the process phosphatase inhibition. The agonism with respect to interleukin could play a considerable role in the mechanism of potential antineoplastic action of the α-alaninamide derivative of betulonic acid **3** and the 4,4′-diaminodiphenyl sulphone derivative of betulonic acid **18** at position 28. The inhibition of phospholipase C could determine the antitumor action of a derivative **12** with *N*-decylureide fragment at 17b-position.

Data resulted from forecasting the anti-inflammatory activity (Fig. 4) indicate a high potential of anti-inflammatory activity of all the derivatives of betulonic acid. For betulonic acid and its amides, the presence of anti-inflammatory activity is confirmed experimentally.

The results of forecasting hepatoprotective activity (hepatoprotectant) for the derivatives of betulonic acid (Fig. 5) correspond to exerting hepatoprotective effect experimentally confirmed for betulonic acid **1**, its ester **2** and amides **4–6**. A high probability of hepatoprotective action was demonstrated for all the novel derivatives of betulonic acid, except for compounds **18** and **20**.

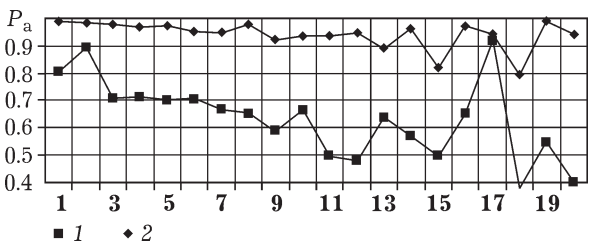


Fig. 5. P_a values for the hepatoprotective activity of betulonic acid derivatives (*1*) comparing to drug-likeness probability (*2*).

Potential mechanisms for the hepatoprotective effect of betulonic acid derivatives are demonstrated in Fig. 6 and include the stimulation of transferase (transferase stimulant), the inhibition of oxide reductase (oxidoreductase inhibitor) as well as of bilirubin oxidase (bilirubin ox. inhibitor).

The forecast data indicate a considerable contribution of inhibiting bilirubin oxidase in a potential hepatoprotective effect inherent in all the derivatives of betulonic acid. The stimulation of transferase correlates especially well with the hepatoprotective activity for betulonic acid **1**, its α-alaninamide derivative **3**, β-alaninamide of the methyl ester of betulonic acid **6**, for a novel derivative with *N*-decylureide fragment in 17b-position **12** and 3,20-dihydroxyimino derivative **15**. The inhibition of oxide reductase does not draw any considerable contribution to the mechanism of hepatoprotection for these compounds.

CONCLUSION

As the result of the investigation performed for the derivatives of betulonic acid it was demonstrated that there is a high correlation level for the data concerning pharmacological activity, obtained from the experiments with animals and predicted by means of computer forecasting according to program PASS. The results of the computer analysis confirmed the multitarget character of the compounds synthesized and allowed us to suggest possible molecular targets. An important role of positions 28 and 3 in the triterpene structure of betulonic acid for modifying aimed at obtaining any novel highly efficient agents is confirmed. The results

of the SAR-analysis performed for a wide spectrum of betulonic acid derivatives allow researchers to determine a group of highly prospective agents – correctors for pathological processes, which agents nowadays undergo profound pharmacological investigations at the Laboratory of Pharmacological Researches of the NIOCh, SB RAS (Novosibirsk).

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