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C-28 Esters of Triterpenoid Acids Bearing Tris(hydroxymethyl)aminomethane: Synthesis and Anticancer/Antimicrobial Activity †

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† Presented at the 23rd International Electronic Conference on Synthetic Organic Chemistry, 15 November–15 December 2019; Available online: https://ecsoc-23.sciforum.net/.

Published: 14 November 2019

Abstract: Widespread secondary plant metabolites (betulinic, ursolic, and oleanolic acids) are promising scaffolds for the discovery of new drugs. Among the many semi-synthetic derivatives of lupane triterpenoids known today, the anticancer agent C-28 ester of betulinic acid with tris(hydroxymethyl)aminomethane (NVX-207) has been actively studied. This betulinic acid derivative, which has shown significant antitumor activity in vitro and in vivo against various malignant tumors, is a candidate drug. It is known that modification of the structure of the triterpenoid skeleton can lead to significant changes in biological properties. In this regard, we have synthesized C-28 esters of ursolic and oleanolic acids and C20-29 hydrogenated betulinic acid bearing tris(hydroxymethyl)aminomethane (TRIS), and transformed them into guanidinium salts by guanylation of the primary amino group in a branched ester fragment under the action of 1H-pyrazole-1-carboxamidine hydrochloride. The obtained compounds were tested in in vitro experiments on three human cancer cell lines. The presence of the TRIS-fragment in the triterpenoid conjugates markedly enhanced the cytotoxic action as compared to the parent compounds, dihydrobetulinic, ursolic, and oleanolic acids (IC_{50} values 2.8–7.6 μM for Jurkat cells and 1.1–6.8 μM for U937 cells), while the correlation between the cytotoxic activity and the chemical structure of the triterpenoid skeleton was not observed. Extended biological testing of these triterpenoids by using flow cytometry analysis showed that antitumor activity of compounds is caused by apoptotic processes and induction of cell cycle arrest in the S-phase. New triterpenoids were also tested for their antimicrobial activity against the growth of four bacterial strains (*Escherichia coli*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (MRSA)) and two fungal strains (*Candida albicans* and *Cryptococcus neoformans).* The TRIS-dihydrobetulinic acid conjugate and its guanidinium derivative did not exhibit antimicrobial properties. The corresponding ursane and oleane-skeleton pentacyclic tritrerpenoids showed good bacteriostatic activity against methicillin-resistant *S. aureus* (MICs 4 and 32 μg/mL) and excellent antifungal effect against *Cryptococcus neoformans* and *Candida albicans* (MICs 4 and 0.25 μg/mL).

Keywords: pentacyclic triterpene; betulinic acid; ursolic acid; oleanolic acid; amine and guanidine derivatives; NVX-207; cytotoxicity; antimicrobial activity

1. Introduction

Among natural compounds of plant origin, which are considered as the richest sources of leading structures for drug development, a significant place is occupied by pentacyclic triterpenoid acids of the lupane, ursane, and oleanane group (Figure 1) [1,2]. These compounds are produced as a

result of squalene cyclization and are present everywhere in various parts of plants—in the bark, in the wax coating of the leaves, or in the peel of the fruit.

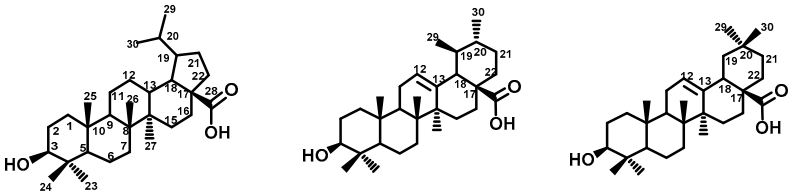


Figure 1. Dihydro betulinic, ursolic, and oleanolic acids.

Triterpenoid acids exhibit a wide variety of biological activities, which successfully combine with low systemic toxicity. The greatest interest in these compounds is due to their antitumor (anticancer), antiviral, antibacterial, and antiparasitic properties [3–6]. However, the relatively low potential biological activity of native triterpenoid acids, their poor solubility in aqueous medium, and insufficient bioavailability cause serious problems for promoting these compounds to clinical practice.

Natural pentacyclic triterpenoid acids have high synthetic potential due to the easily transformed functional groups (3-OH, 28-COOH) present in their molecules. During the last decade numerous derivatives of pentacyclic triterpenoids have been produced by modifying functional groups at the C-3 and C-28 atoms of triterpene core, which in some cases were more efficient in their biology action than their prototypes [3]. One of the most well-known compounds of this type is the C-28 ester of betulinic acid bearing the tris(hydroxymethyl)aminomethane fragment (NVX-207). (Figure 2).

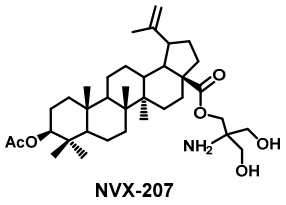


Figure 2. Structure of compound NVX-207.

This promising anticancer agent has shown higher cytotoxicity as compared to betulinic acid against diverse tumor cell lines in humans and dogs. In addition, the use of NVX-207 in the phase I/II clinical trials in large animals led to the complete remission of resistant tumors [7–10]. Small structural alterations of this lupane triterpenoid significantly influenced its anticancer activity.

In this connection, we have synthesized new structural analogues of NVX-207, namely, the C-28 esters of ursolic and oleanolic acids, as well as the ester of C20-29 hydrogenated betulinic acid having tris(hydroxymethyl)aminomethane moiety (TRIS). The resulting compounds were converted into guanidinium salts by guanilation of primary amine groups in the branched-chain ester fragment.

The guanidine group is a common key unit in various natural and synthetic compounds demonstrating antimicrobial, antiviral, and antitumor activities [11]. Furthermore, because of high basicity (pKa 13.5), the guanidinium group is important for selective delivery of cytotoxic molecules to tumor cells [12].

In this paper, we discuss the effect of these structural changes on the antitumor and antimicrobial activity.

2. Experimental

2.1. Chemistry

All reagents and solvents were of the purest grade available, and were generally used without further treatment. The starting compounds (ursolic, oleanolic acids) and reagents: oxalyl chloride,

dimethylaminopyridine (DMAP), tris(hydroxymethyl)aminomethane, triethylamine (Et_3N), *N*,*N*-diisopropylethylamine (DIPEA), and 1H-pyrazole-1-carboxamidine hydrochloride were purchased from Acros Organics (Geel, Belgium). Dihydrobetulinic acid was obtained from betulin according to the known procedure [13]. Acetates of oleanolic, ursolic, and dihydrobetulinic acids were synthesized according to the typical procedures. Synthesis and (^1H, ^{13}C) NMR spectra of compounds NVX-207, **2**, **5**, **8**, **5a**, and **8a** are in the works reported previously [7,14].

2.1.1. General Procedure for the Synthesis of Amines **2**, **5**, **8** and Amides **5a**, **8a**

Oxalyl chloride (0.13 mL, 1.5 mmol) was added with stirring to a solution of compounds **1**, **4**, or **7** (0.5 mmol) in dry CH_2Cl_2 (5 mL) precooled to 0 °C, and stirring of the reaction mixture was continued at room temperature for 2 h. Then the solvents with excess oxalyl chloride were removed under vacuum and the acid chlorides of **1**, **4**, and **7** (1 mmol), dissolved in a mixture of pyridine (4 mL), CH_2Cl_2 (1 mL), and DMAP (0.09 g, 0.7 mmol), were added. After complete dissolution of DMAP, a solution containing TRIS (tris(hydroxymethyl)aminomethane) (0.24 g, 2 mmol) in pyridine (0,5 mL) was added. The mixture was stirred for 10 h at room temperature and the solvent was removed rapidly under vacuum. The residue was chromatographed on silica gel, using CH_2Cl_2/MeOH 30:1→1:1, to obtain pure compounds **2**, **5**, **8**, **5a**, and **8a**.

2.1.2. General Procedure for the Guanilation of Amines **2**, **5**, and **8**

The amine (0.5 mmol) was dissolved in dry DMF (1 mL) and under vigorous stirring DIPEA (0.2 mL, 1.5 mmol) and 1H-pyrazole-1-carboxamidine hydrochloride (0.09 g, 0.6 mmol) were added. The mixture was stirred for 24 h (monitoring by TLC). The mixture is diluted with cold H_2O and the precipitate which formed was filtered off and washed with water to obtain pure compounds **3**, **6**, and **9**.

2.2. Biology

2.2.1. Anticancer Activity

Cell Culturing

Cells (Jurkat, K562, U937) were purchased from Russian Cell Culture Collection (Institute of Cytology of the Russian Academy of Sciences, Saint Petersburg, Russia) and cultured according to standard mammalian tissue culture protocols and sterile technique. All cell lines used in the study were tested and shown to be free of mycoplasma and viral contamination. Cells were maintained in RPMI 1640 Media (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 4 mM glutamine, 10% FBS (Sigma, Burlington, MA, USA), and 100 units/mL penicillin–streptomycin (Sigma Burlington, MA, USA). All types of cells were grown in an atmosphere of 5% CO_2 at 37 °C. The cells were sub-cultured at 2-day intervals with a seeding density of 1 × 105 cells per 24-well plate in RPMI with 10% FBS.

Cytotoxicity Assay

Viability (Live/dead) assessment was performed by staining cells with 7-aminoactinomycin D (7-AAD) (Biolegend, San Diego, CA, USA). Cells were treated of test compounds with six different concentrations (1, 5, 10, 15, 30, and 60 μM). After treatment, cells were harvested, washed 1–2 times with phosphate-buffered saline (PBS), and centrifuged at 400× *g* for 5 min. Cell pellets were resuspended in 200 μL of flow cytometry staining buffer (PBS without Ca^{2+} and Mg^{2+}, 2.5% FBS) and stained with 5 μL of 7-AAD staining solution for 15 min at room temperature in the dark. Samples were acquired on a NovoCyteTM 2000 FlowCytometry System (ACEA, San Diego, CA, USA) equipped with 488 nm argon laser. Detection of 7-AAD emission was collected through a 675/30 nm filter in FL4 channel.

Viability and Apoptosis

Apoptosis was determined by flow cytometric analysis of Annexin V and 7-aminoactinomycin D (7-AAD) staining. Briefly, 200 μL of Guava Nexin reagent (Millipore, Bedford, MA, USA) were added to 5 × 105 cells in 200 μL, and the cells were incubated with the reagent for 20 min at room temperature in the dark. The plates were treated with compound **2** and dihydrobetulinic acid at IC_{50} concentration (4 and 59 μM) for 24 h and 48 h. At the end of incubation, the cells were analyzed on NovoCyteTM 2000 FlowCytometry System (ACEA). Different states of cell death were defined as follows: normal cells are localized in the lower-left quadrant; early apoptotic cells are in the lower-right quadrant; late apoptotic cells and necrotic cells are in the upper-right quadrant; and necrotic cells are in the upper-left quadrant.

Cell Cycle Analysis

Cell cycle was analyzed using the method of propidium iodide staining. Briefly, cells were plated in 24-well round bottom plates at density 10 × 105 cells per well, centrifuged at 450× *g* for 5 min, and fixed with ice-cold 70% ethanol for 24 h at 0 °C. Cells were then washed with PBS and incubated with 250 μL of Guava Cell Cycle Reagent (Millipore, Burlington, MA, USA) for 30 min at room temperature in the dark. Samples were analyzed on NovoCyte™ 2000 FlowCytometry System (ACEA, San Diego, CA, USA).

2.2.2. Antimicrobial Activity

The minimum inhibitory concentrations (MICs) of compounds NVX-207, **2**, **3**, **5**, **6**, **8**, and **9** were determined against Gram-negative bacterial strains *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and Gram-positive bacteria *Staphylococcus aureus* methicillin-resistant (MRSA) strain types. Antifungal activity was determined against *Candida albicans* and *Cryptococcus neoformans*. The antimicrobial screening performed by CO-ADD (The Community for Antimicrobial Drug Discovery) was funded by the Wellcome Trust (UK) and The University of Queensland (Australia) [15].

Antibacterial Assays.

All bacteria were cultured in cation-adjusted Mueller–Hinton broth (CAMHB) at 37 °C overnight. A sample of each culture was then diluted 40-fold in fresh broth and incubated at 37 °C for 1.5–3 h. The resultant mid-log phase cultures were diluted (CFU/mL measured by OD_{600}), then added to each well of the compound-containing plates, giving a cell density of 5×10^5 CFU/mL and a total volume of 50 μL. All the plates were covered and incubated at 37 °C for 18 h without shaking. Inhibition of bacterial growth was determined measuring absorbance at 600 nm (OD_{600}), using a Tecan M1000 Pro monochromator plate reader.

The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (bacteria without inhibitors) on the same plate as references. The MIC was determined as the lowest concentration, at which the growth was fully inhibited, defined by an inhibition ≥ 80%. In addition, the maximal percentage of growth inhibition is reported as DMax, indicating any compounds with partial activity.

Hits were classified by MIC ≤ 16 μg/mL or MIC ≤ 10 μM in either replicate (n = 2 on different plates).

Antifungal Assays.

Fungi strains were cultured for 3 days on Yeast Extract-Peptone Dextrose (YPD) agar at 30 °C. A yeast suspension of 1×10^6 to 5×10^6 CFU/mL (as determined by OD_{530}) was prepared from five colonies. The suspension was subsequently diluted and added to each well of the compound-containing plates giving a final cell density of fungi suspension of 2.5×10^3 CFU/mL and a total volume of 50 μL. All plates were covered and incubated at 35 °C for 36 h without shaking.

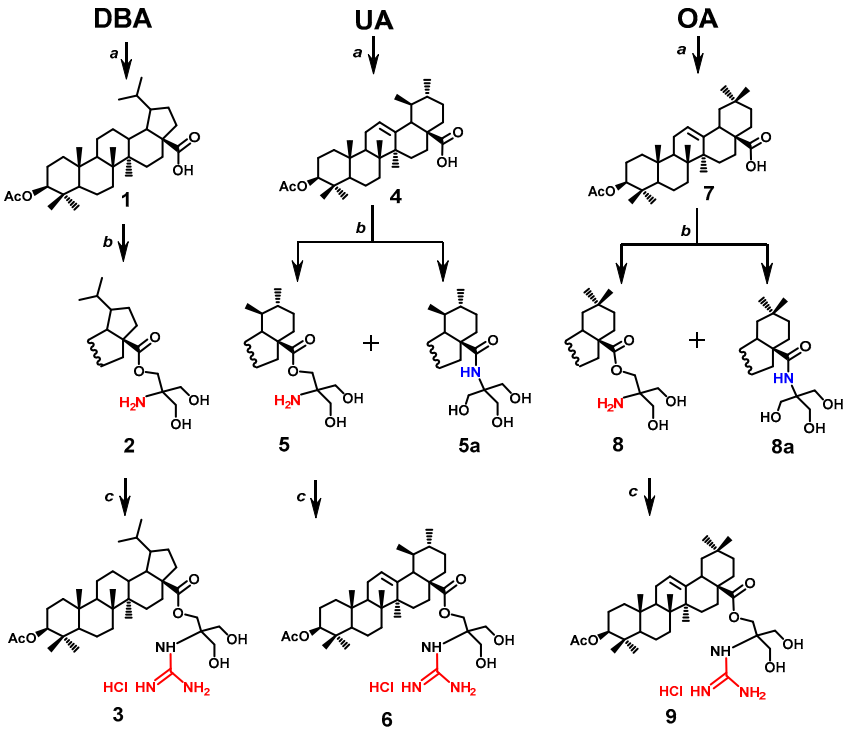
Growth inhibition of *C. albicans* was determined measuring absorbance at 630 nm (OD_{630}), while the growth inhibition of *C. neoformans* was determined measuring the difference in absorbance between 600 and 570 nm ($OD_{600–570}$), after the addition of resazurin (0.001% final concentration) and incubation at 35 °C for additional 2 h. The absorbance was measured using a Biotek Synergy HTX plate reader.

The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (fungi without inhibitors) on the same plate. The MIC was determined as the lowest concentration, at which the growth was fully inhibited, defined by an inhibition ≥ 80% for *C. albicans* and an inhibition ≥ 70% for *C. neoformans*. Due to a higher variance in growth and inhibition, a lower threshold was applied to the data for *C. neoformans*. In addition, the maximal percentage of growth inhibition was reported as DMax, indicating any compounds with marginal activity. Hits were classified by MIC ≤ 16 μg/mL or MIC ≤ 10 μM in either replicate (n = 2 on different plates).

3. Results and Discussion

Chemistry

Esters of dihydrobetulinic, ursolic, and oleanolic acids **2**, **5**, and **8** were prepared via the acetate protection of the 3-OH group in native triterpenoid acids and subsequent transformation of the resulting acetates **1**, **4**, and **7** to unstable acyl chlorides. Then the acyl chlorides were involved without further purification in the reaction with tris(hydroxymethyl)aminomethane under reported conditions [7]. The reactions afforded the desired esters **2**, **5**, and **8** in 15%–23% yields and amides **5a** and **8a** in 24%–32% yields. The resulting compounds were separated by column chromatography (Scheme 1). Guanylation of the amine group was carried out by a standard procedure [16] on treatment with commercially available guanylation reagent 1H-pyrazole-1-carboxamidine hydrochloride.



Scheme 1. Synthesis of compounds **2**, **3**, **5**, **5a**, **6**, **8**, **8a** and **9**. *Reagents and conditions:* (**a**) AcCl, THF, Py, dimethylaminopyridine (DMAP), r.t.; (**b**) 1. $(COCl)_2$, CH_2Cl_2; 2. tris(hydroxymethyl)aminomethane, DMAP, Py, CH_2Cl_2, r.t.; (**c**) 1H-Pyrazole-1-carboxamidine hydrochloride, *N*,*N*-diisopropylethylamine (DIPEA), DMF, r.t., 24 h.

All compounds prepared in this study, including NVX-207, were tested in vitro for their cytotoxic activity against human tumor cell lines: Jurkat (T-lymphoblastic leukemia), K562 (chronic myeloid leukemia), and U937 (histiocytic lymphoma). These compounds were tested in triplicates by standard MTS assay.

The introduction of the tris(hydroxymethyl)aminomethane moiety into the molecules of triterpene acids **1**, **4**, and **7** markedly enhanced the cytotoxic activity of the resulting conjugates **2**, **5**, **8** as well as their guanidinium derivatives **3**, **6**, and **9** against all tested cell lines irrespective of the triterpene skeleton type (Table 1).

Table 1. Cytotoxicity of dihydrobetulinic-, ursolic-, oleanolic acids and compounds NVX-207, **2**, **3**, **5**, **6**, **8**, and **9** against Jurkat, K562, and U937 cancer cells.

Compound	IC_{50} (μM) [a]		
	Jurkat	**K562**	**U937**
NVX-207	4.15 ± 0.21	4.42 ± 0.26	1.87 ± 0.11
2	2.78 ± 0.16	3.19 ± 0.17	1.14 ± 0.09
3	3.1 ± 0.41	2.3 ± 0.34	15 ± 0.23
5	6.19 ± 0.27	6.59 ± 0.29	2.91 ± 0.18
6	3.8 ± 0.23	11 ± 0.18	5.3 ± 0.29
8	2.96 ± 0.18	3.21 ± 0.22	2.81 ± 0.15
9	7.6 ± 0.33	13 ± 0.45	6.8 ± 0.11
DBA	**59 ± 0.31**	**44 ± 0.24**	**39 ± 0.38**
UA	**23 ± 0.34**	**68 ± 0.11**	**17 ± 0.12**
OA	**271 ± 0.19**	**235 ± 0.24**	**186 ± 0.18**

[a] IC_{50} (μM) is the half maximal inhibitory concentration for viable cells. Each IC_{50} (mean ± SE) has been derived from at least three experiments in duplicate.

For example, the IC_{50} values of compounds **2** and **3** were 2.8 and 3.1 μM for T-lymphoblastic leukemia cells and 3.2 and 2.3 μM for chronic myeloid leukemia cells, while IC_{50} values of dihydrobetulinic acid were 59 and 44 μM, respectively. The most pronounced differences in the antitumor activity were found for oleanolic acid and its conjugates **8** and **9**. Indeed, the IC_{50} values of oleanolic acid, its conjugates **8** and **9** for Jurkat cells were 271, 2.9, and 7.6 μM, respectively.

The identified lead molecule **2** with the highest antitumor characteristics was evaluated for the possible apoptosis induction in Jurkat cells using Annexin V/7-AAD staining [14]. The highest percentage of late apoptosis (91.8%) was detected upon the addition of compound **2** (4 μM) to cells followed by 48 h incubation (Figure 3E).

Dihydrobetulinic acid triggers apoptosis in Jurkat cells at higher doses as compared to ester **2**. The number of apoptotic cells on treatment with dihydrobetulinic acid (59 μM) for 48 h constituted around 24% (4.0% of early-stage and 20.5% of secondary necrotic/late-stage apoptotic), while the number of vital cells was 73.1% (Figure 3C).

DNA flow cytometry was also used to analyze the cell cycle kinetics in Jurkat cells pre-incubated with dihydrobetulinic acid and ester **2** at their IC_{50} concentration for 24 and 48 h (Figure 4).

The results of experiments have shown in significant S phase arrest in cells after treatment with dihydrobetulinic acid or compound **2**. The ratio of cells in the S phase increased from 38.3% (control) to 72.7% in cells treated with ester **2** (4 μM for 48 h) and increased to 47.2% in cells treated with dihidrobetulinic acid. The appropriate number of cells in the G2/M phase decreased. For example, the treatment of Jurkat cells with ester **2** resulted in a decrease of these cells in the G2/M phase from 14.4% (control) to 7% (Figure 4D). Considering these results, we assume that dihydrobetulinic acid and compound **2** are able to trigger the programmed cell death, including apoptotic mechanisms and cell cycle arrest in the S phase.

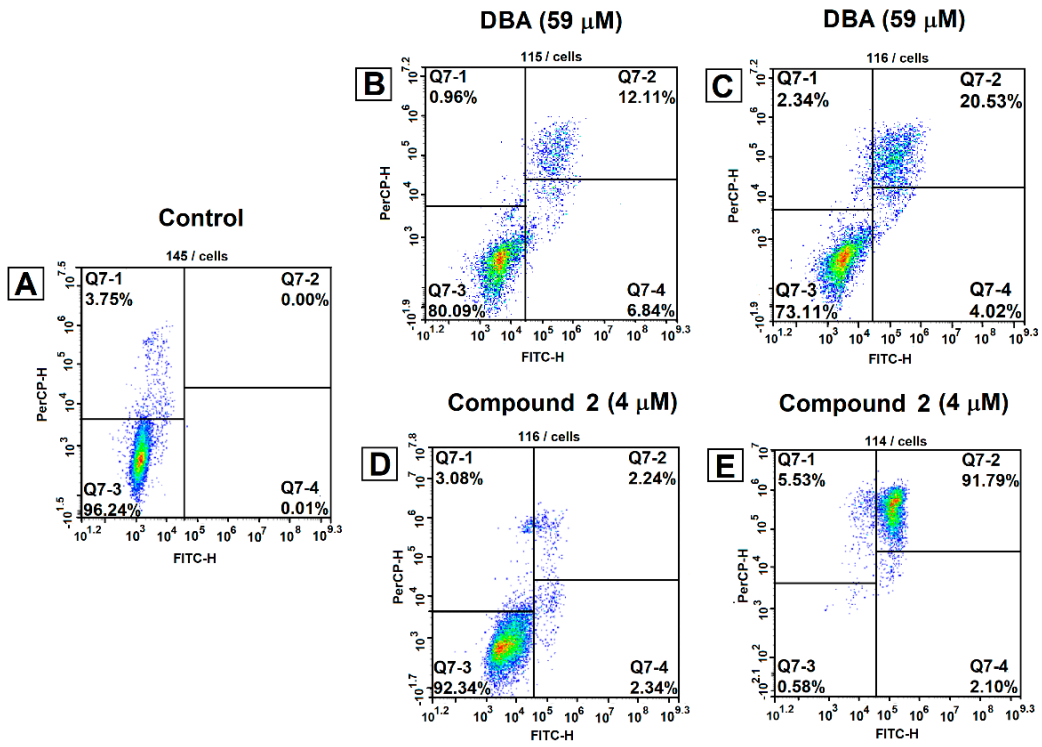


Figure 3. AnnexinV/7-AAD staining upon induction of apoptosis in Jurkat cells. Cells were treated with compound **2** and dihydrobetulinic acid at their IC_{50} concentration for 24 and 48 h. Then, the cells were harvested, stained with Annexin V/7-AAD, and analyzed by flow cytometry. The experiments were performed three times, and the results of the representative experiments are shown. The first cytogram represents an untreated cell sample (**A**); after incubation with dihydrobetulinic acid for 24 h (**B**) and for 48 h (**C**); and after incubation with compound **2** for 24 h (**D**) and for 48 h (**E**). Q7-1, necrotic cells; Q7-2, late apoptotic cells; Q7-3, living cells; Q7-4, early apoptotic cells.

Previously, it was shown that triterpene acids, either in a pure state or as parts of plant extracts, exhibit moderate bacteriostatic activity, mainly against Gram-positive bacteria [17–19]. The minimum inhibitory concentrations (MICs) of ursolic and oleanolic acids against various *Staphylococcus aureus* strains, including the methicillin-resistant (MRSA) strain, were in the range from 8 to 64 μg/mL, while betulinic acid was less active against these strains [20]. Some semisynthetic analogues of betulinic, ursolic, and oleanolic acids were synthesized and studied *in vitro* as potential antimicrobial agents [21–23]. We have studied the antibacterial activities of dihydrobetulinic, ursolic, and oleanolic acids and their conjugates **2**, **3**, **5**, **6**, **8**, and **9** using four bacterial strains, including Gram-negative *Escherichia coli, Acinetobacter baumannii,* and *Pseudomonas aeruginosa* and Gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA). The antifungal activity was determined against *Candida albicans* and *Cryptococcus neoformans.* The antimicrobial screening of compounds was performed by the Community for Open Antimicrobial Drug Discovery (CO-ADD, Institute for Molecular Bioscience, University of Queensland, Brisbane, Australia). The primary antimicrobial screening of compounds was performed at a single point concentration of 32 μg/mL. MICs were determined for the "hit" compounds from the primary screening (samples with bacterial growth inhibition value of above 80%).

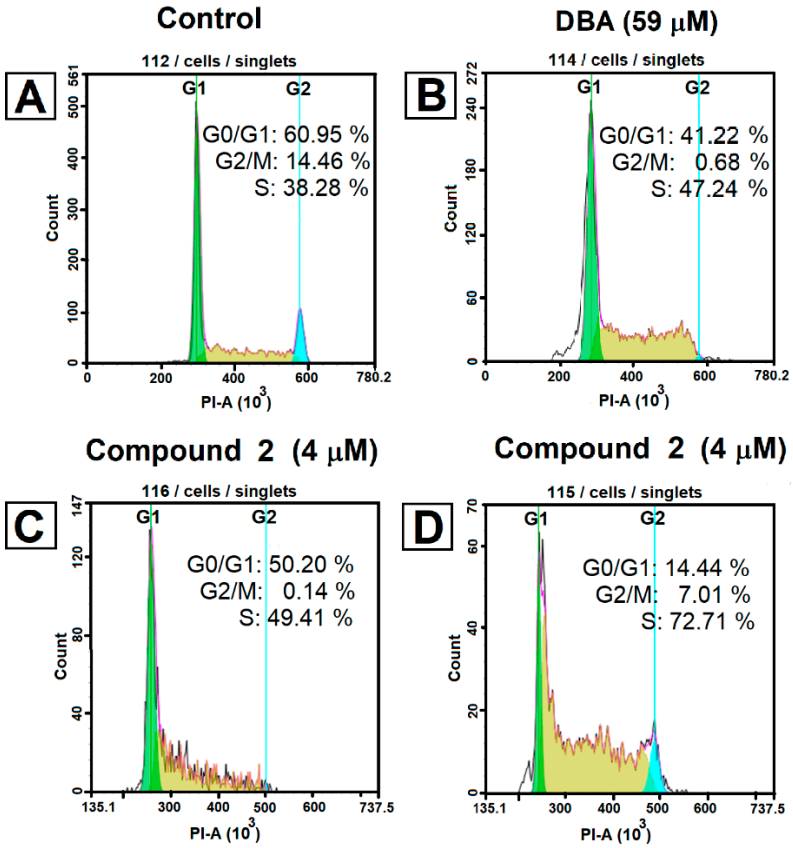


Figure 4. Cell cycle analysis in Jurkat cells. Jurkat cells were treated with tested compound **2** at the IC_{50} concentration for 24–48 h and dihydrobetulinic acid for 48 h. The cells were trypsinized, harvested, and washed three times with ice-PBS for PI-stained DNA content detected by flow cytometry. The experiments were performed three times, and the results of the representative experiments are shown. The first cytogram represents an untreated cell sample (**A**); after incubation with dihydrobetulinic acid for 48 h (**B**); and after incubation with compound **2** for 24 h (**C**) and for 48 h (**D**).

Our experimental results demonstrated the absence of significant antimicrobial effect of native triterpenoid acids and lupane triterpenoids **2** and **3** (Table 2).

Table 2. Antimicrobial activities of dihydrobetulinic-, ursolic-, oleanolic acids and compounds **2**, **3**, **5**, **6**, **8**, and **9** [a].

Compound	Gram-Positive Bacteria [b], Gram-Negative Bacteria [c] and Fungal Genetic [d] Strains						
	Sa [b]	*Ec* [c]	*Kp* [c]	*Pa* [c]	*Ab* [c]	*Ca* [d]	*Cn* [d]
2	29.41	37.71	4.97	−10.92	−28.05	72.24	−10.5
3	63.83	−19.19	−12.78	2.65	−42.67	72.1	69.21
5	49.64	4.43	9.19	8.96	9.71	98.8	67.64
6	79.47	0.39	−0.57	−5.98	8.11	23.07	118.6
8	14.95	5.63	13.25	13.37	3.01	40.42	1.16
9	72.3	3.74	12.35	−12.6	−10.66	76.58	106.1
DBA	15.59	8.16	4.77	1.38	8.37	9.31	−52.68
UA	4.69	8.78	−2.51	−3.62	4.19	2.10	−84.32
OA	7.17	6.40	2.82	−3.76	0.97	3.22	−92.51

[a] Concentration was set at 32 μg/mL in DMSO. [b] Gram-positive bacterial strain: *Staphylococcus aureus* (Sa), ATCC 43300. [c] Gram-negative bacterial strains: *Escherichia coli* (Ec), ATCC 25922; *Acinetobacter baumannii* (Ab), ATCC 19606; *Pseudomonas aeruginosa* (Pa), ATCC 27853. [d] Fungal genetic strains: *Candida albicans* (Ca), ATCC 90028; *Cryptococcus neoformans* (Cn), ATCC 208821.

Ursane and oleane conjugates have shown the higher antimicrobial effect than their lupane analogues**.** Ursolic and oleanolic acid conjugates with guanidinium group **6** and **9** showed a moderate activity against *S. aureus* (MIC 4 μg/mL). Ursolic ester **5** was highly active against *C. albicans* and *C. neoformans* (MICs < 0.25 and 2.0 μg/mL) (Figure 5).

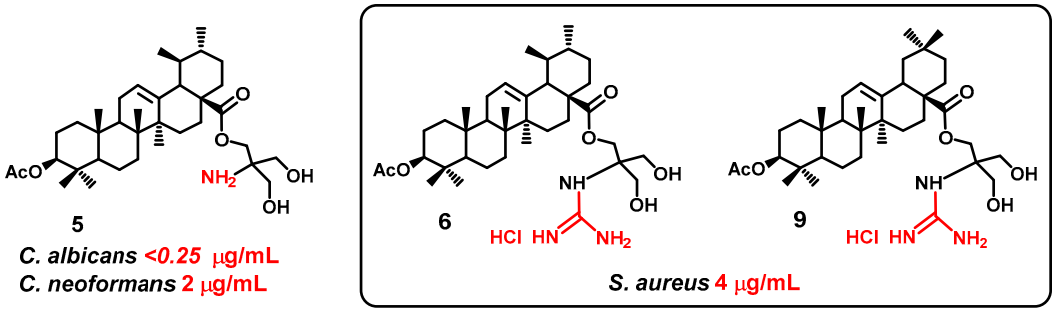


Figure 5. Structures of lead compounds **5**, **6,** and **9.**

4. Conclusions

In summary, we have synthesized new esters of dihydrobetulinic, ursolic, and oleanolic acids with tris(hydroxymethyl)aminomethane moiety at the C-28 position and tested them *in vitro* for anticancer and antimicrobial activity. The introduction of the TRIS-moiety into the molecules of native triterpene acids markedly enhanced the cytotoxic activity of the resulting conjugates **2**, **5**, and **8**, as well as their guanidinium derivatives **3**, **6**, and **9** against tumor cell lines Jurkat, K562, and U937, irrespective of the triterpene skeleton type. The ester of dihydrobetulinic acid with tris(hydroxymethyl)aminomethane (**2**) as a lead molecule with the highest antitumor characteristics was selected for extensive biological testing, which showed that this compound is capable of triggerring programmed cell death, including apoptotic mechanisms and cell cycle arrest in the S phase. Primary antimicrobial screening showed the absence of significant antimicrobial effect of native triterpenoid acids and lupane triterpenoids **2** and **3.** Conjugates with the ursane and oleane skeleton type demonstrated a higher antimicrobial effect than their lupane analogues. Ursolic and oleanolic acid conjugates with guanidinium group **6** and **9** displayed moderate activity against *S. aureus*, while the ursolic ester **5** was highly active against *C. albicans* and *C. neoformans.*

Author Contributions: Validation and writing—review and editing, A.S.; performing the chemistry experiments, R.K. and D.N.; performing the biology experiments, L.D. The manuscript was prepared through the contributions of A.S., L.D., and D.N. All authors have read and agreed to the published version of the manuscript.

Funding: This work was performed under financial support from the Russian Science Foundation (Grant 19-73-00155).

Acknowledgments: The antimicrobial screening performed by CO-ADD (The Community for Antimicrobial Drug Discovery) was funded by the Wellcome Trust (UK) and The University of Queensland (Australia).

Conflicts of Interest: The authors declare no conflict of interest.

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