Synthesis of conjugates of lupane triterpenoids with chromane antioxidants and *in vitro* study of their influence on the production of nitrogen monoxide and on the arginase activity in activated macrophages

A. Yu. Spivak,[a] R. R. Khalitova,[a] Yu. P. Bel´skii,[b] A. N. Ivanova,[b] E. R. Shakurova,[a] N. V. Bel´skaya,[b] V. N. Odinokov,[a] M. G. Danilets,[b] and A. A. Ligacheva[b]*

[a] *Institute of Petrochemistry and Catalysis, Russian Academy of Sciences, 141 prosp. Oktyabrya, 450075 Ufa, Russian Federation. Fax: +7 (347) 284 2750. E-mail: ink@anrb.ru*

[b] *Research Institute for Pharmacology, Siberian Branch of the Russian Academy of Medical Sciences, 3 prosp. Lenina, 634028 Tomsk, Russian Federation. Fax: +7 (382) 241 8379*

Conjugates of lupane triterpenoids (betulin and betulonic and betulinic acids) with synthetic analogs of α-tocopherol were obtained *via* ester bond formation and tested *in vitro*. Showing low cytotoxicity, some of them suppress nitrogen monoxide production without affecting the activity of arginase, which suggests their antiinflammatory and immunomodulating properties.

Key words: lupane triterpenoids, antioxidants, chromanes, tocopherols, bioconjugates, nitrogen monoxide, activated macrophages, antiinflammatory activity.

Nitrogen monoxide (NO) is generated enzymatically in mammalian cells from L-arginine under the action of constitutive or inducible NO synthases (cNOS and iNOS, respectively). This diatomic molecule fulfills very important physiological functions and contributes greatly to the immune protection of living organisms from pathogenic microorganisms and transformed cells.[1] Under normal physiological conditions, the concentration of NO in cells and tissues is low. However, oxidative and toxic stresses caused by macrophage activators such as microbial agents and cytokines of T helpers (Th) increase the expression of the inducible NO synthase in macrophages. This type of macrophage activation is termed "classical activation" and the macrophage phenotype is denoted M1. Cells M1 exhibit pro-inflammatory properties, support Th1-dependent immunologic reactions, produce pro-inflammatory cytokines (such as interleukins-1, -6, -12 and tumor necrosis factor α), and very actively devour microorganisms, destroy the extracellular matrix, and promote the apoptosis of infected and transformed cells.[2—5]

L-Arginine is also a substrate for another enzyme (arginase 1) expressing on activated macrophages. The type of macrophage activation that imparts functionally opposite (with respect to M1) properties to macrophages is termed "alternative activation"; its inductors are glucocorticoids and cytokines Th2 (see Ref. 2). Macrophages M2 exhibit antiinflammatory activity, suppress Th1- and enhance Th2-immune response, secrete antiinflammatory substances (interleukin-10, an interleukin-1 receptor antagonist, which transforming the growth factor β), favor angiogenesis and remodeling, and produce chemoattractants for Th2 cells.[2—5]

It has been shown that the uptake of L-arginine follows only one pathway promoted by either NO synthase (giving nitrogen monoxide and citrulline) or arginase (giving polyamines and L-ornithine). This allowed one to consider the arginine metabolism to be an index of the macrophage activation type.[6,7] Regulation of the activity balance for these enzymes is of great importance for an organism since pathological excesses of nitrogen monoxide (the concentration of NO increases *ca.* 10^3 times),[8,9] a superoxide radical anion, and their reaction product (the potent oxidant peroxynitrite) initiate oxidation of the lipids of biological membranes, breaks of DNA chains, and other mutagenic phenomena, which are believed to cause serious diseases such as arthritis, diabetes, stroke, aseptic shock, autoimmune diseases, and chronic inflammation.

In the last decade, active research has been undertaken to find medicinal agents based on native substances that can inhibit the production of nitrogen monoxide.[10—15] In this respect, lupane triterpenoids and some of their synthetic derivatives are very promising.[16,17]

We assumed that synthetic combinations of betulin, betulinic acid, or betulonic acid with antioxidant molecules containing the pharmacophore chromane fragment of α-tocopherol will help to discover novel broad-spec-

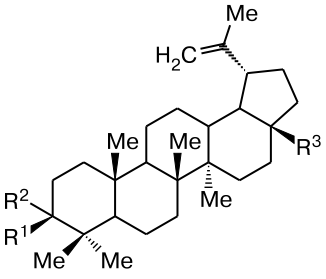
Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 12, pp. 2164—2174, December, 2010.

trum compounds with NO-modulating and antiradical activities. Hybrid compounds with a trimethylated chromane fragment that showed themselves as efficient polyfunctional agents have been reported earlier.[18—20]

In the present work, we obtained a number of acylates of betulin and betulinic and betulonic acids with pharmacologically significant analogs of α-tocopherol and studied *in vitro* the influence of some of the hybrid compounds on the NO production stimulated by lipopolysaccharides (LPS) and on the activity of arginase in mouse peritoneal macrophages. We also estimated their cytotoxic effects on macrophages. In addition, we tested as NO production inhibitors the earlier obtained conjugates of triterpene acids with Trolox acid, in which a hydrazine residue acts as a spacer.

Results and Discussion

For esterification of the functional groups at the C(3) and C(28) atoms of lupane triterpenoids **1a—f**, we used the following chromane-containing acids: 3-(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)propionic acid (**2**) (α-CEHC), a water-soluble metabolite of α-tocopherol; the accessible hydrophilic antioxidant 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (**3**) (Trolox acid); and a nonhydrolyzable ether analog of α-tocopherol with a redox-passive chromane fragment, referred to as α-tocopheryloxyacetic (2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yloxyethanoic) acid (**4**). The acid function in betulonic acid was esterified with chromanylmethanol **5** prepared in three steps from acid **3** as described earlier.[21]



R^1 = OH, R^2 = H:
R^3 = CH_2OH (**a**),
COOH (**b**), COCl (**c**);
$R^1 + R^2$ = O, R^3 = COCl (**d**);
R^1 = OH, R^2 = H:
R^3 = CH_2OTBS (**e**),
COOBn (**f**)

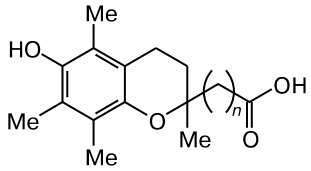
1a—f

Acylation of the OH group at the C(28) atom in betulin **1a** and at the C(3) atom in betulinic acid **1b** with acid **4** in the presence of dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine (DMAP) as activators regioselectively gave compounds **6** and **7**, respectively, in high yields (Scheme 1). Acylate **8** was obtained by prolonged reflux (48 h) of betulonyl chloride **1d** with chromanol **5** in CH_2Cl_2 (see Scheme 1). In this case, *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide (EDC) used in a double molar excess with respect to the alcohol is preferred as a condensation agent.

However, the carbodiimide method failed in condensation reactions of chromane acids **2** and **3** with betulin or betulinic acid. These reactions in the presence of various activators (DCC, EDC, and diisopropylcarbodiimide (DIC)) were substantially complicated by side processes leading to complex mixtures of products.

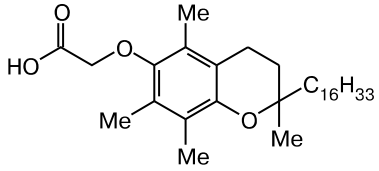
Our attempted syntheses of acid chlorides from acids **2** and **3** were also unsuccessful: the action of $COCl_2$ or $SOCl_2$ on compounds **2** and **3** in various solvents (benzene, diethyl ether, and pyridine) resulted in decomposition of the substrate molecules. The target acylates **9—11** were obtained using 6-*O*-protected acids **2** and **3** and 28-*O*-ether derivatives of betulin or betulinic acid. The protecting groups were selected so that they could be removed from acylates **9—11** in one step. Silyl ethers **12** and **13a** were obtained in three steps by transforming acids **2** and **3** into the corresponding methyl esters, which reacted with *tert*-butyl(dimethyl)silyl chloride (TBS-Cl) and imidazole to give compounds **14** and **15a** in good yields (Scheme 2). The acid function in methyl carboxylates **14** and **15a** was deblocked by halogenolysis with LiBr in DMF as described earlier.[13] Benzyl ether **13b** was obtained from acid **3** in a similar way (methanolysis of the acid function, benzylation of the phenolic hydroxyl, and halogenolysis of the ester group). Condensation of TBS ethers **12** and **13a** with TBS ether of betulin (**1e**) and condensation of 6-*O*-benzyl derivative **13b** with benzyl betulinate **1f** (in the presence of DCC and DMAP as activators) afforded acylates **9—11**, respectively, in 30—62% yields (see Scheme 2).

Compounds **8** and **11** were smoothly debenzylated by their hydrogenation over Pd/C in diethyl ether for 2 and 3 h, respectively, yielding products **16** and **17** (Scheme 3). It was found that hydrogenation of acylate **8** for 8 h results in the reduction of the C(20)=C(29) double bond (*cf.* Ref. 22). The conditions for removal of the TBS protection from

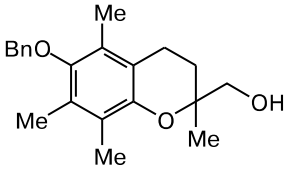


2, 3

n = 2 (**2**), 0 (**3**)

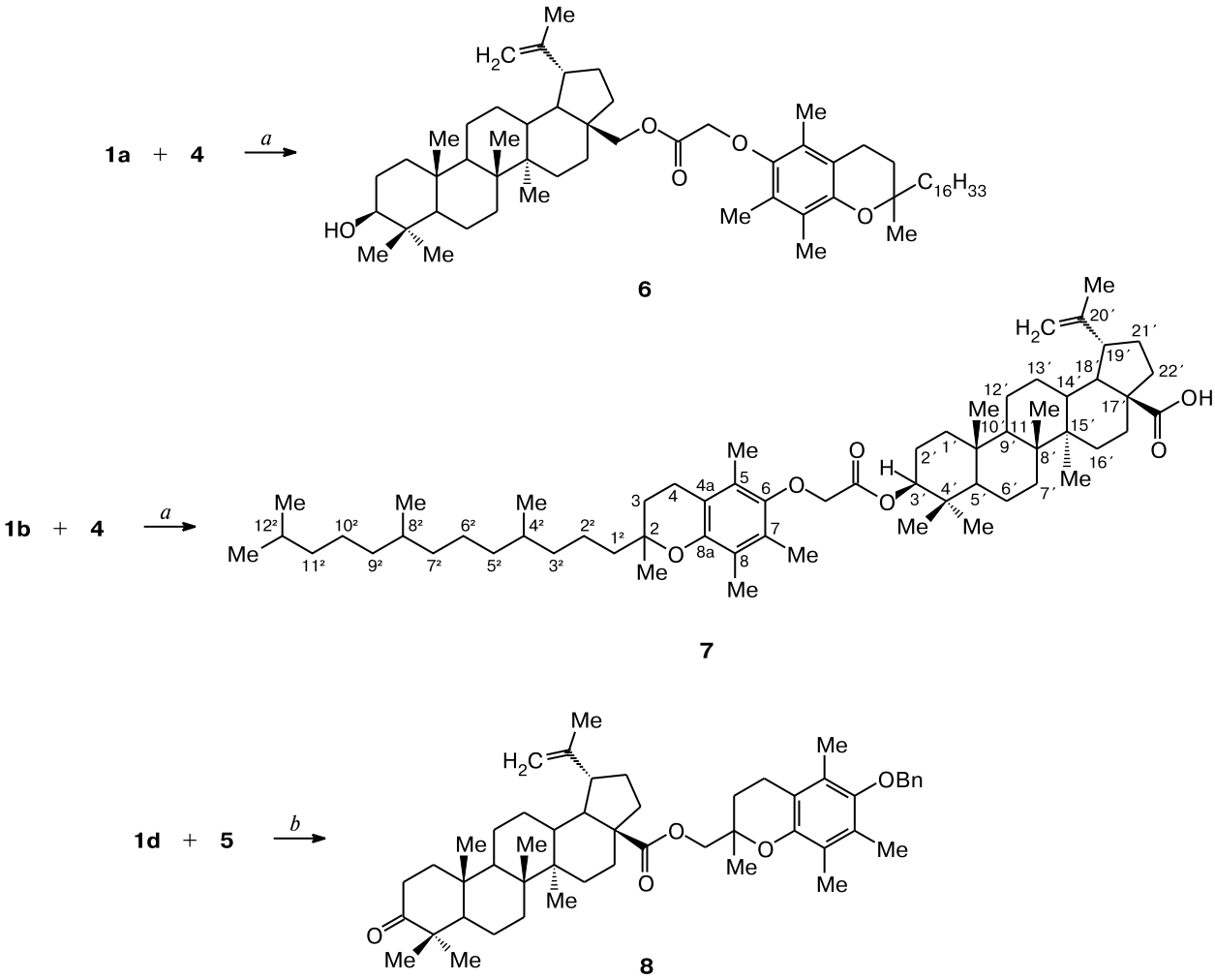


4



5

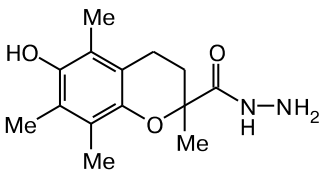
Scheme 1



Reagents and conditions: *a*. DCC, DMAP (10 mol.%), CH_2Cl_2, 20 °C; *b*. EDC, CH_2Cl_2, reflux, 48 h.

acylates **9** and **10** were studied with compound **10** as an example. Deblocking of the phenolic OH group in acylate **10** under the action of tetrabutylammonium fluoride (TBAF) in THF at 0 °C gave silyl monoether **18a** in 0.5 h. The next TBS group at the C(28) atom in compound **18a** was removed at ~20 °C for 24 h, yielding compound **18b** (see Scheme 3).

The syntheses of conjugates **19** and **20** by reactions of Trolox hydrazide **21** with betulinyl (**1c**) and betulonyl chlorides (**1d**), respectively (Scheme 4), have been described earlier.[23]

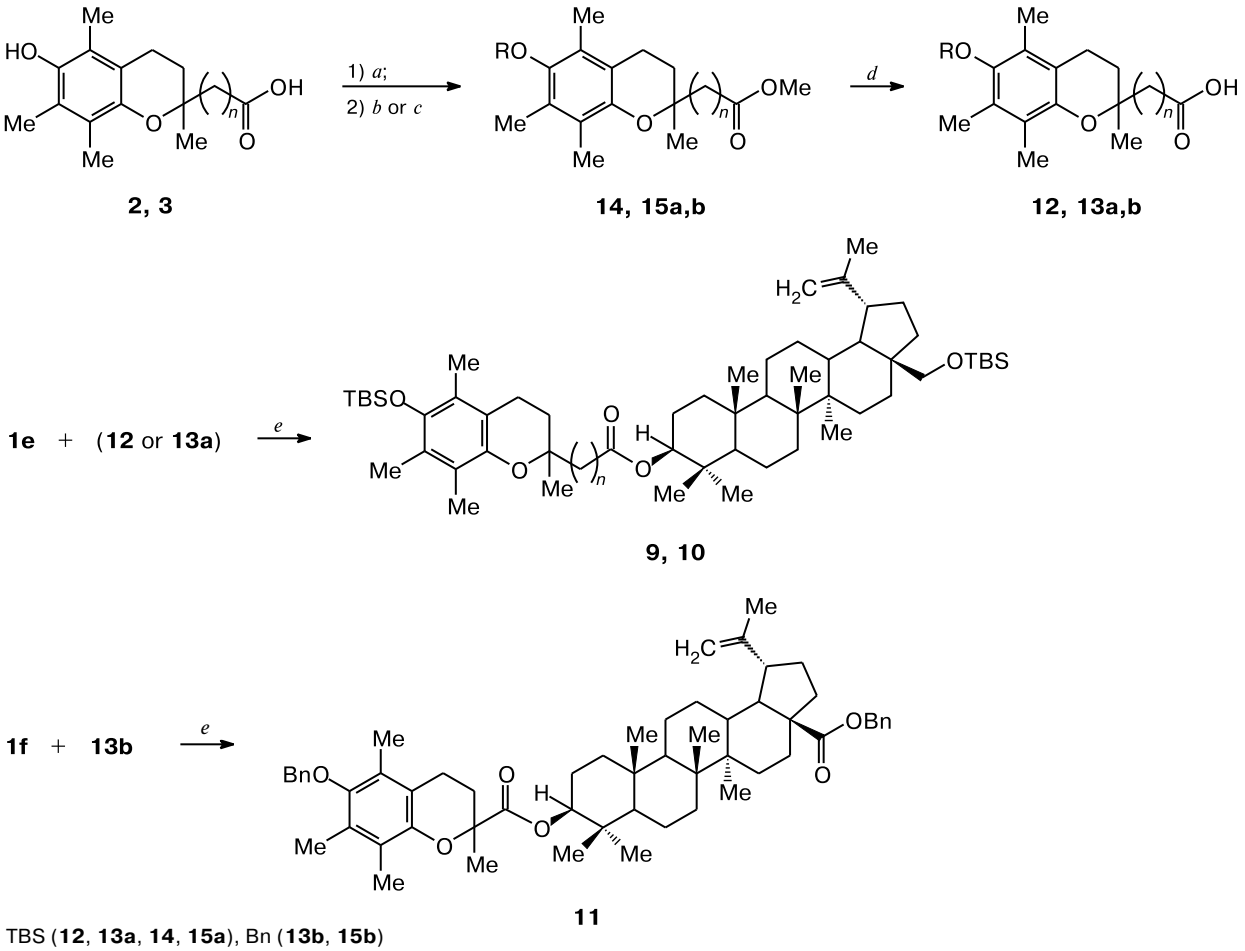


21

The structures of all the compounds obtained were identified from spectroscopic data. Their ^{13}C NMR spectra show signals for all the C atoms belonging to the triterpenoid and chromanol residues. Since acid **3** and chromanylmethanol **5** were used as racemic mixtures, their adducts with terpenoids were mixtures of two diastereomers. In some cases, this was evident from the presence of a double set of signals in the ^1H and ^{13}C NMR spectra (see, *e.g.*, the ^1H NMR spectra of compound **16b** and the ^{13}C NMR spectra of compound **18a**).

Pharmacological studies were carried out *in vitro* for compounds **6**, **7**, **19**, and **20** versus betulinic (**1b**) and α-tocopheryloxyacetic acids (**4**). The MTT assay data on the cytotoxic effects of betulinic acid and hybrid compounds **19** and **20** are summarized in Table 1. Betulinic acid was toxic in concentrations of 25 and 50 μg mL^{-1}, while hybrid compounds **19** and **20** in the same concentrations showed no cytotoxicity.

Scheme 2



R = TBS (**12**, **13a**, **14**, **15a**), Bn (**13b**, **15b**)
n = 2 (**2**, **9**, **12**, **14**); 0 (**3**, **10**, **13a,b**, **15a,b**)

Reagents and conditions: *a*. TsOH—MeOH, CH_2Cl_2, reflux; *b*. TBS-Cl—imidazole, DMF, 85 °C; *c*. BnCl—K_2CO_3, DMF, 20 °C; *d*. LiBr, DMF, reflux; *e*. DCC, DMAP (10 mol.%), CH_2Cl_2, 20 °C.

Table 1. Cytotoxic effects of betulinic acid **1b** and hybrid compounds **19** and **20** on peritoneal macrophages ($X \pm m$)[a]

Concentration /μg mL^{-1}	Optical density[b]		
	1b	**19**	**20**
—[c]	—	0.403±0.031	—
0.01	0.447±0.029	0.448±0.034	0.411±0.017
0.10	0.445±0.019	0.448±0.021	0.357±0.014
1.00	0.452±0.030	0.459±0.047	0.455±0.047
10.00	0.332±0.049	0.458±0.045	0.467±0.058
25.00	0.237±0.020[c]	0.478±0.028	0.477±0.042
50.00	0.273±0.046[c]	0.484±0.044	0.503±0.012[d]

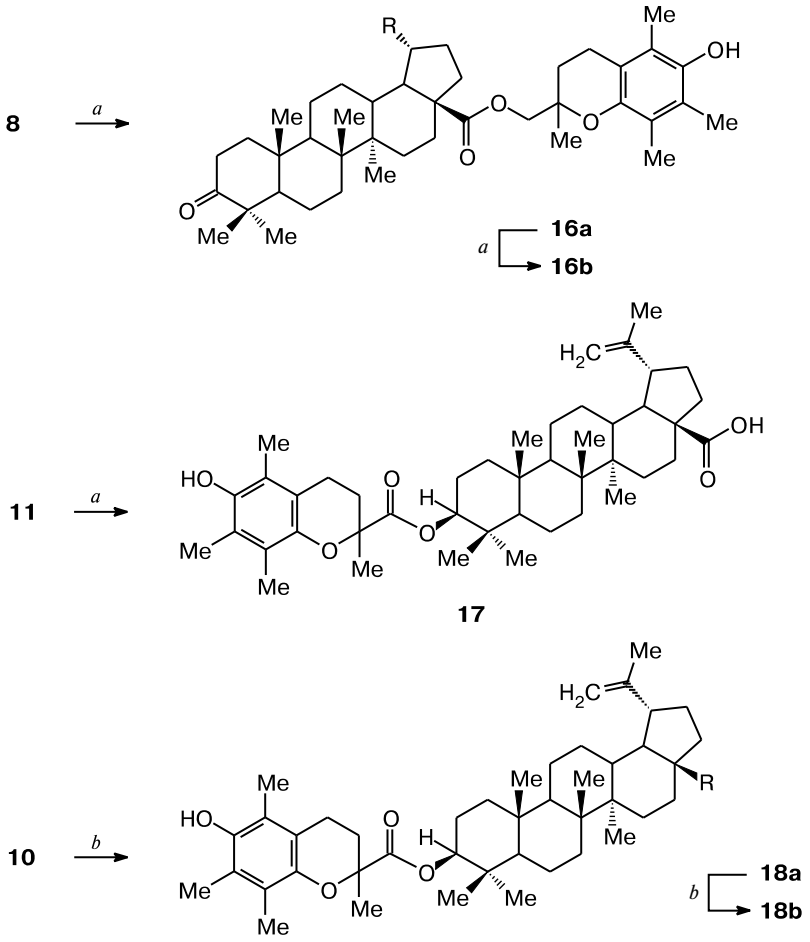
[a] Here and in Tables 2 and 3, X is the mean of experimental data and m is the standard mean error.
[b] The optical density in the wells containing only macrophages (without LPS) was 0.425±0.049.
[c] Blank entry.
[d] The differences from the blank entry are reliable for $p < 0.05$.

The influence of conjugates **6**, **7**, **19**, and **20** and acids **1b** and **4** on the functional state of macrophages was estimated from the NO production by LPS-activated macrophages and from the activity of macrophagal arginase.

To do this, we incubated the peritoneal macrophages of mice of the line C57Bl/6 with LPS and the above compounds in the concentrations specified in Table 2 for 48 h. After the incubation was completed, we estimated the NO production in the supernatants of cell cultures (from the nitrite content) and the activity of arginase in homogenate cells.

These studies showed that all the compounds tested have an effect on the NO production by LPS-stimulated macrophages (see Table 2). Betulinic (**1b**) and α-tocopheryloxyacetic acids (**4**) decreased the NO production in a concentration range from 0.1 to 50 μg mL^{-1}, depending on the dose used. Hybrid compounds **6**, **7**, **19**, and **20** had an inhibitive effect in higher concentrations (25 and 50 μg mL^{-1}).

Scheme 3



R = C(Me)=CH_2 (**16a**), $CHMe_2$ (**16b**), CH_2OTBS (**18a**), CH_2OH (**18b**)

Reagents and conditions: *a*. H_2, Pd/C, Et_2O; *b*. TBAF, THF.

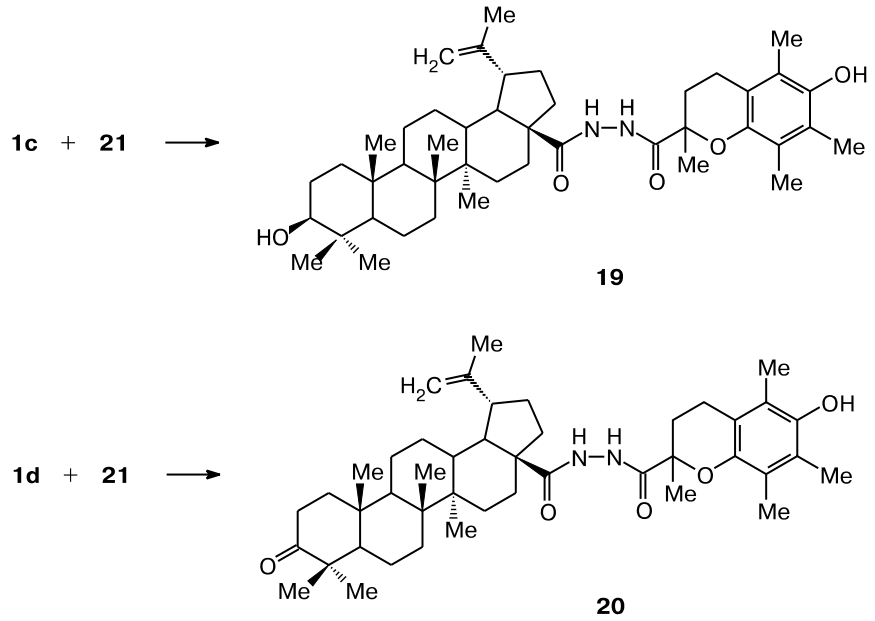
The study of the influence of these compounds on the arginase activity (Table 3) revealed that betulinic (**1b**) and α-tocopheryloxyacetic acids (**4**) decrease the arginase activity in concentrations of 10, 25, and 50 μg mL^{-1}. Conju-

Table 2. Influence of betulinic acid **1b**, α-tocopheryloxyacetic acid (**4**), and hybrid compounds **6**, **7**, **19**, and **20** on the NO production by peritoneal macrophages ($X \pm m$)[a]

Concentration /μg mL^{-1}	Concentration of nitrites (μmol L^{-1}) in the presence of compound					
	1b	**4**	**6**	**7**	**19**	**20**
—[b]			47.8±1.0[c]			
0.1	44.8±0.3[d]	43.50±1.2	54.20±2.5	46.39±1.0	47.7±0.7	45.0±1.4
1.0	41.2±0.9[d]	41.35±1.9[d]	48.46±1.7	44.22±0.6	47.8±0.4	47.7±0.8
10.0	29.2±1.2[d]	14.81±0.6[d]	38.59±2.1[d]	43.71±1.8	42.8±1.4[d]	44.1±1.7
25.0	5.9±0.5[d]	4.44±0.6[d]	28.66±0.3[d]	42.36±0.7[d]	34.4±1.1[d]	40.6±2.1[d]
50.0	1.4±0.8[d]	0.38±0.3[d]	26.41±3.1[d]	38.35±0.6[d]	13.1±1.3[d]	19.8±1.2[d]

[a] The concentration of nitrites in the wells containing only macrophages (without LPS) was 7.5±1.8 μmol L^{-1}.
[b] Blank entry.
[c] The differences from the entry with LPS-free culture for $p < 0.05$.
[d] The differences from the blank entry are reliable for $p < 0.05$.

Scheme 4



Reagents and conditions: EDC, CH_2Cl_2, reflux, 48 h.

gates **6**, **7**, **19**, and **20** did not affect the arginase activity in these concentrations.

Thus, in contrast to betulinic acid, the hybrid compounds showed no cytotoxicity in the MTT assay toward mouce peritoneal macrophages. Betulinic and α-tocopheryloxyacetic acids suppressed both the NO production (feature M1) and the arginase activity (feature M2), which can also be attributed to their direct cytotoxic effects. Unlike the starting acids **1b** and **4**, the hybrid compounds were not cytotoxic; they selectively influenced macrophages, suppressing the NO production without changing the arginase activity (in concentrations below 50 μg mL^{-1}). Based on the results obtained, one can state that compounds **6**, **7**, **19**, and **20** exhibit antiinflammatory effect. Therefore, these compounds can weaken the Th1-type of immune response by suppressing M1. Because the compounds obtained have lower cytotoxicity (compared to the starting acids) and selectivity toward activated macrophages, these compounds can probably serve as a basis in the design of antiinflammatory and immunocorrecting drugs for efficient control of various autoimmune diseases (rheumatoid arthritis and type I diabetes).

Experimental

IR spectra were recorded on a Specord IR-75 spectrometer (thin films or solutions in $CHCl_3$). UV spectra were recorded on a Specord M-40 spectrometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker AVANCE-400 instrument (400.13 (^1H) and 100.62 MHz (^{13}C)) in $CDCl_3$ with Me_4Si as the internal

Table 3. Influence of betulinic acid **1b**, α-tocopheryloxyacetic acid (**4**), and hybrid compounds **6**, **7**, **19**, and **20** on the arginase activity in peritoneal macrophages ($X \pm m$)[a]

Concentration /μg mL^{-1}	Arginase activity (in conventional units) in the presence of compound					
	1b	**4**	**6**	**7**	**19**	**20**
—[b]	31.2±1.6					
1	32.0±1.0	31.1±1.0	33.7±1.0	31.6±1.7	31.8±2.7	32.1±1.6
10	21.7±1.2[c]	19.0±0.3[c]	32.7±1.2	29.2±1.0	31.3±0.7	30.2±1.0
25	2.4±0.8[c]	4.3±0.9[c]	27.1±1.7	29.5±0.4	31.3±1.1	27.9±1.2
50	0.3±0.3[c]	1.1±0.6[c]	20.5±1.6[c]	25.5±1.2[c]	29.5±0.7	13.5±0.7[c]

[a] The arginase activity in the wells containing only macrophages (without LPS) was 29.7±1.28.
[b] Blank entry.
[c] The differences from the blank entry are reliable for $p < 0.05$.

standard. Mass spectra were measured on a Bruker-Autoflex III spectrometer (MALDI TOF, positive ion mode, 2,5-dihydroxybenzoic and α-cyano-4-hydroxycinnamic acids as the matrices). Optical rotation was measured on a Perkin—Elmer-141 polarimeter. Specific rotation was expressed in (deg mL) $(g\ dm)^{-1}$; the concentration of the solution was expressed in g $(100\ mL)^{-1}$. Elemental analysis was carried out on a Carlo Erba 1106 analyzer. TLC was carried out on Sorbfil plates (Sorbpolimer, Krasnodar, Russia) in hexane—ethyl acetate (3 : 1) (*A*) and chloroform (*B*); spots were visualized in a solution of anisaldehyde in ethanol. For column chromatography, silica gel L (KSKG grade, 50—160 μm) was employed. Racemic α-tocopherol, DCC, EDC, DMAP, TBAF, TBS-Cl, imidazole, Trolox, 10% Pd/C, and oxalyl chloride (Fluka) were used. Betulin was isolated in a known way.[24] Betulonic acid, betulinic acid, and betulonyl chloride were prepared as described earlier.[25—27] Chromanylpropionic acid **2** was prepared according to a known procedure.[28] Chromanylmethanol **5** was prepared from Trolox acid in three steps;[21] TBS ethers **1e** (see Ref. 29), **14**, and **15a** (see Ref. 30), α-tocopheryloxyacetic acid (**4**),[31] and benzyl betulinate **1f** (see Ref. 22) were prepared according to the corresponding procedures.

Halogenolysis of methyl carboxylates 14 and 15a,b with LiBr (general procedure). Compound **14** or **15a,b** (0.3 mmol) and LiBr (4.4 mmol) were dissolved in dry DMF (2.5 mL) and refluxed with stirring for 6 h (monitoring by TLC in system *A*). The reaction mixture was cooled, diluted with water (2.5 mL), and neutralized with 5% HCl; the product was extracted with AcOEt. The extract was concentrated and the residue was chromatographed on SiO_2 with hexane—AcOEt (3 : 1) as an eluent to give compounds **12** and **13a,b**, respectively.

3-[6-*tert*-Butyl(dimethyl)silyloxy-2,5,7,8-tetramethyl-3,4-dihydro-2*H*-1-benzopyran-2-yl]propionic acid (12). Yield 82%, amorphous powder. Found (%): C, 67.13; H, 9.31; Si, 7.22. $C_{22}H_{36}O_4Si$. Calculated (%): C, 67.30; H, 9.24; Si, 7.15. IR, ν/cm^{-1}: 1710 (COOH). UV ($CHCl_3$), λ_{max}/nm (ε): 294 (2588). ^{1}H NMR, δ: 0.12 (s, 6 H, Me—Si); 1.06 (s, 9 H, Me in Bu^t); 1.24 (s, 3 H, Me—C(2)); 1.77—1.92 (m, 2 H, H(1′) + 2 H, H(3)); 2.04, 2.06, 2.10 (all s, 3 H each, Me—Ar); 2.52—2.61 (m, 2 H, H(2′) + 2 H, H(4)); 8.04 (s, 1 H, COOH). ^{13}C NMR, δ: −3.36 (Me—Si); 11.92, 13.39, 14.31 (Me—Ar); 18.59 (Bu^t—Si); 20.71 (C(4)); 23.32 (Me—C(2)); 26.09 (Me in Bu^t); 28.58 (C(3)); 31.66 (C(2′)); 34.34 (C(1′)); 73.34 (C(2)); 117.16 (C(5)); 122.72 (C(7)); 123.60 (C(8)); 126.01 (C(4a)); 144.32 (C(8a)); 145.44 (C(6)); 179.07 (COOH).

6-*tert*-Butyl(dimethyl)silyloxy-2,5,7,8-tetramethyl-3,4-dihydro-2*H*-1-benzopyran-2-carboxylic acid (13a). Yield 72%, amorphous powder. Found (%): C, 65.74; H, 8.92; Si, 7.78. $C_{20}H_{32}O_4Si$. Calculated (%): C, 65.89; H, 8.85; Si, 7.70. IR, ν/cm^{-1}: 1720 (COOH). UV ($CHCl_3$), λ_{max}/nm (ε): 292 (2700). ^{1}H NMR, δ: 0.13 (s, 6 H, Me—Si); 1.06 (s, 9 H, Me in Bu^t); 1.62 (s, 3 H, Me—C(2)); 1.85—1.93 (m, 1 H, H(3)); 2.04, 2.12, 2.15 (all s, 3 H each, Me—Ar); 2.36—2.42 (m, 1 H, H(3)); 2.60 (m, 2 H, H(4)); 8.05 (m, 1 H, COOH). ^{13}C NMR, δ: −3.45, −3.30 (Me—Si); 12.01, 13.39, 14.31 (Me—Ar); 18.59 (Bu^t—Si); 20.96 (C(4)); 25.10 (Me—C(2)); 26.09 (Me in Bu^t); 30.28 (C(3)); 76.74 (C(2)); 117.18 (C(5)); 122.61 (C(7)); 123.53 (C(8)); 126.12 (C(4a)); 144.87 (C(8a)); 145.70 (C(6)); 177.65 (COOH).

6-Benzyloxy-2,5,7,8-tetramethyl-3,4-dihydro-2*H*-1-benzopyran-2-carboxylic acid (13b). Yield 73%, amorphous powder. Found (%): C, 74.18; H, 7.06. $C_{21}H_{24}O_4$. Calculated (%): C, 74.09; H, 7.11. IR, ν/cm^{-1}: 1718 (COOH). UV ($CHCl_3$), λ_{max}/nm (ε): 286 (2092). ^{1}H NMR, δ: 1.69 (m, 3 H, Me—C(2)); 1.94—2.00 (m, 1 H, H(3)); 2.23, 2.26, 2.32 (all s, 3 H each, Me—Ar); 2.47—2.50 (m, 1 H, H(3)); 2.80—2.95 (m, 2 H, H(4)); 4.81 (s, 2 H, OCH_2Ph); 7.28—7.64 (m, 5 H, Ph); 8.13 (s, 1 H, COOH). ^{13}C NMR, δ: 11.96, 12.08, 12.97 (Me—Ar); 20.75 (C(4)); 25.21 (Me—C(2)); 30.16 (C(3)); 74.74 (OCH_2Ph); 76.94 (C(2)); 117.28 (C(5)); 123.02 (C(7)); 126.20 (C(8)); 127.12, 127.81, 128.55, 137.92 (Ph); 127.71 (C(4a)); 147.52 (C(8a)); 149.13 (C(6)); 179.62 (COOH).

(6-Benzyloxy-2,5,7,8-tetramethyl-3,4-dihydro-2*H*-1-benzopyran-2-yl)methyl 3-oxolup-20(29)-en-28-oate (8). *N*-Ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide (0.23 mL, 1.28 mmol) and freshly prepared betulonyl chloride **1d** (0.48 g, 1.04 mmol) in dry CH_2Cl_2 (5 mL) were added dropwise to a stirred solution of benzylated chromanylmethanol (**5**) (0.21 g, 0.64 mmol) in dry CH_2Cl_2 (5 mL). The reaction mixture was refluxed with stirring for 48 h, diluted with CH_2Cl_2 (10 mL), washed with water, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on SiO_2 with $CHCl_3$ as an eluent. The yield was 57%, white crystals, m.p. 96—98 °C, $[\alpha]_D^{20}$ +18.5° (*c* 0.27, $CHCl_3$). Found (%): C, 80.19; H, 9.31. $C_{51}H_{70}O_5$. Calculated (%): C, 80.27; H, 9.25. IR, ν/cm^{-1}: 1725 (C=O). UV ($CHCl_3$), λ_{max}/nm (ε): 288 (2000). ^{1}H NMR, δ: 0.92, 1.00, 1.02, 1.06, 1.11 (all s, 3 H each, H(23′), H(24′), H(25′), H(26′), H(27′)); 1.25—2.60 (m, 24 H, CH_2, CH in the betulonic acid residue + 5 H, Me—C(2), H(3) in the chromanol residue); 1.73 (s, 3 H, H(30′)); 2.14, 2.21, 2.26 (all s, 3 H each, Me—Ar); 2.68 (t, 2 H, H(4), *J* = 6.4 Hz); 3.08 (m, 1 H, H(19′)); 4.08, 4.20 (both d, 1 H each, CH_2—O, *J* = 12.2 Hz); 4.62, 4.75 (both s, 1 H each, H(29′)); 4.70 (s, 2 H, OCH_2Ph); 7.28—7.54 (m, 5 H, Ph). ^{13}C NMR, δ: 11.90, 12.04, 12.90 (Me—Ar); 14.68 (C(27′)); 15.84 (C(25′)); 15.99 (C(26′)); 19.41 (C(6′)); 19.68 (C(30′)); 20.26 (C(4)); 21.07 (C(24′)); 21.47 (C(11′)); 22.40 (Me—C(2)); 25.59 (C(12′)); 26.65 (C(23′)); 28.85, 28.95 (C(3)); 29.73 (C(15′)); 30.66 (C(21′)); 32.21 (C(16′)); 33.67 (C(7′)); 34.15 (C(2′)); 36.92 (C(10′)); 37.08 (C(22′)); 38.49 (C(13′)); 39.66 (C(1′)); 40.68 (C(8′)); 42.52 (C(14′)); 47.08 (C(19′)); 47.34 (C(4′)); 49.39 (C(18′)); 49.93 (C(9′)); 55.00 (C(5′)); 56.77 (C(17′)); 68.44, 68.63 (CH_2—O); 74.76 (CH_2—Ph); 76.81 (C(2)); 109.79 (C(29′)); 117.19 (C(5)); 123.04, 126.04, 128.28 (C(4a), C(7), C(8)); 127.71, 127.84, 128.50, 137.94 (Ph); 147.31 (C(8a)); 148.63 (C(6)); 150.38 (C(20′)); 175.96 (COO); 217.98 (C(3′)). MS, *m/z*: 786.151 $[M + Na]^+$.

Synthesis of acylates 6, 7, and 9—11 (general procedure). Dimethylaminopyridine (0.03 mmol), an appropriate compound **4**, **12**, or **13a,b** (0.27 mmol) (see Schemes 1 and 2), and DCC (0.27 mmol) were added with stirring to compound **1a,b,e,f** (0.24 mmol) in dry CH_2Cl_2 (5 mL). The reaction mixture was stirred at ~20 °C for 12—20 h, filtered, and concentrated *in vacuo*. The residue was chromatographed on SiO_2 with $CHCl_3$ as an eluent to give compounds **6**, **7**, and **9—11**, respectively.

28-{[2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)-3,4-dihydro-2*H*-1-benzopyran-6-yloxy]acetoxy}lup-20(29)-en-3β-ol (6). Yield 63%, amorphous powder, $[\alpha]_D^{20}$ +6.9° (*c* 4.97, $CHCl_3$). Found (%): C, 80.15; H, 11.09. $C_{61}H_{100}O_5$. Calculated (%): C, 80.21; H, 11.03. IR, ν/cm^{-1}: 1759 (O—C=O). UV ($CHCl_3$), λ_{max}/nm (ε): 289 (2438). ^{1}H NMR, δ: 0.84—0.98 (m, 12 H, Me—C(4″), Me—C(8″), Me—C(12″)); 0.98, 1.00, 1.12 (all s, 3 H each, H(23′), H(24′), H(25′), H(26′), H(27′)); 1.20—2.17 (m, 24 H, CH_2, CH in the betulin residue + 26 H, Me—C(2), CH_2, CH in the tocopherol residue); 1.70 (s, 3 H, H(30′)); 2.09,

2.16, 2.18 (all s, 3 H each, Me—Ar); 2.50 (m, 1 H, H(19′)); 2.52 (t, 2 H, H(4), J = 6.5 Hz); 3.20 (dd, 1 H, H(3′)); 4.12—4.45 (both d, 1 H each, C(28′), J = 11.2 Hz); 4.32 (d, 2 H, CH_2—O, J = 3.2 Hz); 4.45 (s, 1 H, OH); 4.62, 4.75 (both s, 1 H each, H(29′)). ^{13}C NMR, δ: 11.78, 11.92, 12.79 (Me—Ar); 14.80 (C(27′)); 15.40 (C(24′)); 16.11 (C(25′)); 16.12 (C(26′)); 18.30 (C(6′)); 19.63 (C(30′)); 19.70, 19.77 (Me—C(4″), Me—C(8″)); 20.63 (C(11′)); 20.80, 21.03 (C(4)); 22.65, 22.74 (Me—C(12′)); 23.87 (C(2″)); 24.44 (C(6″), C(10″)); 24.82 (C(12′)); 25.21 (Me—C(2)); 27.10 (C(15′)); 27.42 (C(2′)); 27.98 (C(12″)); 28.00 (C(23′)); 29.59 (C(16′)); 29.78 (C(21′)); 31.21 (C(3)); 32.69, 32.77 (C(4″), C(8″)); 34.21 (C(22′)); 34.55 (C(7′)); 37.15 (C(3″)); 37.29 (C(10′), C(5″)); 37.41 (C(7″)); 37.46 (C(9″), C(13′)); 38.73 (C(1′)); 38.86 (C(4′)); 40.02 (C(11″)); 40.90 (C(8′), C(1″)); 42.72 (C(14′)); 46.52 (C(17′)); 47.70 (C(18′)); 48.84 (C(19′)); 50.39 (C(9′)); 55.32 (C(5′)); 63.37 (C(28)); 70.07 (CH_2—O); 74.87 (C(2)); 78.93 (C(3′)); 109.95 (C(29′)); 117.62 (C(5)); 123.02 (C(7)); 125.63 (C(8)); 127.56 (C(4a)); 147.93 (C(8a)); 148.19 (C(6)); 150.01 (C(20′)); 169.85 (COO). MS, m/z: 913.591 $[M]^+$, 936.599 $[M + Na]^+$, 952.549 $[M + K]^+$.

3β-{[2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)-3,4-dihydro-2*H*-1-benzopyran-6-yloxy]acetoxy}lup-20(29)-en-28-oic acid (7). Yield 81%, amorphous powder, $[\alpha]_D^{20}$ +9.35° (*c* 1.07, $CHCl_3$). Found (%): C, 78.83; H, 10.69. $C_{61}H_{98}O_6$. Calculated (%): C, 79.00; H, 10.65. IR, ν/cm^{-1}: 1732 (CO—OH). UV ($CHCl_3$), λ_{max}/nm (ε): 289 (2649). ^{1}H NMR, δ: 0.84—0.98 (m, 12 H, Me—C(4″), Me—C(8″), Me—C(12″)); 0.88, 0.90, 0.95, 1.03, 1.10 (all s, 3 H each, H(23′), H(24′), H(25′), H(26′), H(27′)); 1.10—2.05 (m, 24 H, CH_2, CH in the betulinic acid residue + 26 H, Me—C(2), CH_2, CH in the tocopherol residue); 1.70 (s, 3 H, H(30′)); 2.09, 2.16, 2.20 (all s, 3 H each, Me—Ar); 2.57 (t, 2 H, H(4), J = 6.4 Hz); 3.04 (m, 1 H, H(19′)); 4.29 (br.s, 2 H, CH_2—O); 4.63, 4.76 (both s, 1 H each, H(29′)); 4.67 (dd, 1 H, H(3′)); 7.20 (s, 1 H, COOH). ^{13}C NMR, δ: 11.79, 11.91, 12.78 (Me—Ar); 14.68 (C(27′)); 16.03 (C(24′)); 16.19 (C(25′)); 16.49 (C(26′)); 18.16 (C(6′)); 19.36 (C(30′)); 19.70, 19.77 (Me—C(4″), Me—C(8″)); 20.87 (C(4)); 21.03 (C(11′)); 22.65, 22.74 (Me—C(12′)); 23.86 (Me—C(2), C(2″)); 24.45 (C(10″)); 25.45 (C(12′)); 27.60 (C(2′)); 27.99 (C(12″), C(23′)); 29.71 (C(15′)); 30.58 (C(21′)); 31.26 (C(3)); 32.18 (C(16′)); 32.70, 32.79 (C(4″), C(8″)); 34.24 (C(7′)); 37.13 (C(10′), C(22′), C(3″)); 37.29 (C(5″)); 32.79 (C(6″)); 37.41 (C(7″)); 37.46 (C(9″)); 38.42 (C(1′), C(13′)); 39.38 (C(4′)); 40.01 (C(1″), C(11″)); 40.69 (C(8′)); 42.43 (C(14′)); 47.80 (C(18′)); 48.90 (C(19′)); 50.39 (C(9′)); 55.50 (C(5′)); 56.42 (C(17′)); 70.02 (CH_2—O); 74.85 (C(2)); 81.77 (C(3′)); 109.78 (C(29′)); 117.59 (C(5)); 122.99 (C(7)); 125.66 (C(8)); 127.60 (C(4a)); 148.03 (C(8a)); 148.14 (C(6)); 150.35 (C(20′)); 169.30 (COO); 182.54 (C(28′)). MS, m/z: 927.565 $[M]^+$, 950.575 $[M + Na]^+$, 966.499 $[M + K]^+$.

28-[*tert*-Butyl(dimethyl)silyloxy]-3β-(3-{6-[*tert*-butyl(dimethyl)silyloxy]-2,5,7,8-tetramethyl-3,4-dihydro-2*H*-1-benzopyran-2-yl}propionyloxy)lup-20(29)-ene (9). Yield 62%, white crystals, m.p. 122—124 °C, $[\alpha]_D^{20}$ +9.3° (*c* 0.55, $CHCl_3$). Found (%): C, 74.63; H, 10.64; Si, 6.09. $C_{58}H_{98}O_5Si_2$. Calculated (%): C, 74.78; H, 10.60; Si, 6.03. IR, ν/cm^{-1}: 1721 (O—C=O). UV ($CHCl_3$), λ_{max}/nm (ε): 294 (2440). ^{1}H NMR, δ: 0.07, 0.14 (both s, 12 H, Me—Si); 0.93, 1.07 (both s, 18 H, Me in Bu^t); 0.85, 0.86, 0.87, 0.99, 1.05 (all s, 3 H each, H(23′), H(24′), H(25′), H(26′), H(27′)); 1.20—2.05 (m, 24 H, CH_2, CH in the betulin residue + 7 H, Me—C(2), H(3), H(1″) in the chromanylpropionic acid residue); 1.70 (s, 3 H, H(30′)); 2.08, 2.10, 2.13 (all s, 3 H each, Me—Ar); 2.40 (m, 2 H, H(2″)); 2.50 (m, 1 H, H(19′)); 2.62 (t, 2 H, H(4), J = 6.5 Hz); 3.26, 3.70 (both d, 1 H each, CH_2—O, J = 9.2 Hz); 4.50 (m, 1 H, H(3′)); 4.60, 4.71 (both s, 1 H each, H(29′)). ^{13}C NMR, δ: −5.43, −3.34 (Me—Si); 11.99, 13.40, 14.33 (Me—Ar); 14.74 (C(27′)); 15.90 (C(24′)); 16.16 (C(25′)); 16.57 (C(26′)); 18.17, 18.33 (Bu^t—Si); 18.60 (C(6′)); 19.11 (C(30′)); 20.76, 20.88 (C(4)); 23.69 (C(11′)); 23.70 (Me—C(2)); 25.22 (C(12′)); 25.99, 26.12 (Me in Bu^t); 27.03 (C(2′)); 27.44 (C(15′)); 28.01 (C(23′), (C(3)); 29.20 (C(16′)); 29.93 (C(21′)); 31.66 (C(2″)); 34.13 (C(22′)); 34.33 (C(7′)); 34.67 (C(1″)); 37.07 (C(10′)); 37.37 (C(13′)); 37.87 (C(1′)); 38.37 (C(4′)); 40.94 (C(8′)); 42.68 (C(14′)); 48.06 (C(18′), C(17′)); 48.39 (C(19′)); 50.32 (C(9′)); 55.38 (C(5′)); 60.45 (C(28′)); 73.48 (C(2)); 80.85 (C(3′)); 109.43 (C(29′)); 117.23 (C(5)); 122.71 (C(7)); 123.59 (C(8)); 125.99 (C(4a)); 144.29 (C(8a)); 145.52 (C(6)); 150.89 (C(20′)); 173.77 (COO). MS, m/z: 931.632 $[M]^+$, 954.659 $[M + Na]^+$.

28-[*tert*-Butyl(dimethyl)silyloxy]-3-({6-[*tert*-butyl(dimethyl)silyloxy]-2,5,7,8-tetramethyl-3,4-dihydro-2*H*-1-benzopyran-2-yl}carbonyloxy)lup-20(29)-ene (10). Yield 62%, white crystals, m.p. 118—120 °C, $[\alpha]_D^{20}$ +11.2° (*c* 0.75, $CHCl_3$). Found (%): C, 74.36; H, 10.53; Si, 6.28. $C_{56}H_{94}O_5Si_2$. Calculated (%): C, 74.44; H, 10.49; Si, 6.22. IR, ν/cm^{-1}: 1723 (O—C=O). UV ($CHCl_3$), λ_{max}/nm (ε): 293 (2743). ^{1}H NMR, δ: 0.06 (s, 6 H, Me—Si); 0.09, 0.10 (both s, 3 H each, Me—Si); 0.91 (s, 9 H, Me in Bu^t); 0.70, 0.76, 0.84, 0.96, 1.01 (all s, 3 H each, H(23′), H(24′), H(25′), H(26′), H(27′)); 1.05, 1.06 (both s, 9 H, Me in Bu^t); 1.10—1.90 (m, 24 H, CH_2, CH in the betulin residue + 5 H, C(2)Me, H(3) in the Trolox acid residue); 1.69 (s, 3 H, H(30′)); 2.01, 2.09, 2.15 (all s, 3 H each, Me—Ar); 2.39—2.60 (m, 3 H, H(4), H(19′)); 3.25, 3.68 (both d, 1 H each, CH_2—O, J = 9.6 Hz); 4.39 (m, 1 H, H(3′)); 4.58, 4.68 (both s, 1 H each, H(29′)). ^{13}C NMR, δ: −5.45, −3.52, −3.44 (Me—Si); 11.98, 13.31, 14.33 (Me—Ar); 14.73 (C(27′)); 15.87 (C(24′)); 16.07 (C(25′)); 16.37 (C(26′)); 18.07, 18.31 (Bu^t—Si); 18.58 (C(6′)); 19.08 (C(30′)); 20.84 (C(4)); 21.22 (C(11′)); 25.54 (Me—C(2)); 25.19 (C(12′)); 25.97, 26.08 (Me in Bu^t); 27.02 (C(2′)); 27.21 (C(15′)); 27.95 (C(23′)); 29.44 (C(16′)); 29.71 (C(21′)); 29.91 (C(3)); 34.07 (C(22′)); 34.32 (C(7′)); 37.02 (C(10′)); 37.36 (C(13′)); 37.73 (C(1′)); 38.28 (C(4′)); 40.91 (C(8′)); 42.66 (C(14′)); 48.06 (C(17′), C(18′)); 48.37 (C(19′)); 50.24 (C(9′)); 55.25 (C(5′)); 60.47 (C(28′)); 73.50 (C(2)); 81.60, 81.69 (C(3′)); 109.39 (C(29′)); 117.05, 117.23 (C(5)); 122.47, 122.56 (C(7)); 123.41, 123.58 (C(8)); 125.86, 126.04 (C(4a)); 144.62 (C(8a)); 146.49 (C(6)); 150.90 (C(20′)); 173.96, 174.15 (COO). MS, m/z: 903.598 $[M]^+$.

Benzyl 3β-[(6-benzyloxy-2,5,7,8-tetramethyl-3,4-dihydro-2*H*-1-benzopyran-2-yl)carbonyloxy]lup-20(29)-en-28-oate (11). Yield 30%, amorphous powder, $[\alpha]_D^{20}$ +12.0° (*c* 0.22, CH_2Cl_2). Found (%): C, 80.07; H, 8.86. $C_{58}H_{76}O_6$. Calculated (%): C, 80.14; H, 8.81. IR, ν/cm^{-1}: 1725 (O—C=O). UV ($CHCl_3$), λ_{max}/nm (ε): 287 (2052). ^{1}H NMR, δ: 0.74, 0.79, 0.90, 0.97, 0.98 (all s, 3 H each, H(23′), H(24′), H(25′), H(26′), H(27′)); 1.15—2.70 (m, 24 H, CH_2, CH in the betulinic acid residue + 7 H, Me—C(2), (3), H(4) in the Trolox acid residue); 1.71 (s, 3 H, H(30′)); 2.15, 2.22, 2.28 (all s, 3 H each, Me—Ar); 3.05 (m, 1 H, H(19′)); 4.40 (dd, 1 H, H(3′), J = 11.2 Hz, J = 4.0 Hz); 4.62, 4.75 (both s, 1 H each, H(29′)); 4.70 (s, 2 H, OCH_2Ph in the Trolox acid residue); 5.10—5.16 (m, 2 H, OCH_2Ph in the betulinic acid residue); 7.28—7.51 (m, 10 H, Ph). ^{13}C NMR, δ: 11.92, 11.96, 12.88 (Me—Ar); 14.70 (C(27′)); 15.85 (C(24′));

16.14 (C(25′)); 16.36 (C(26′)); 18.14 (C(6′)); 19.37 (C(30′)); 20.90 (C(11′)); 21.10 (C(4)); 25.50 (C(12′)); 25.86 (Me—C(2)); 27.17 (C(2′), C(15′)); 28.02 (C(23′)); 29.74 (C(21′)); 30.77 (C(3)); 32.13 (C(16′)); 34.21 (C(7′)); 37.00 (C(22′)); 37.08 (C(10′)); 38.19 (C(13′)); 38.34 (C(1′), C(4′)); 40.68 (C(8′)); 42.41 (C(14′)); 46.98 (C(18′)); 49.47 (C(19′)); 50.43 (C(9′)); 55.38 (C(5′)); 56.38 (C(17′)); 65.74, 75.00 (O$\underline{C}H_2$Ph); 78.90 (C(2)); 82.00 (C(3′)); 109.60 (C(29′)); 117.36 (C(5)); 122.92 (C(7)); 125.86 (C(8)); 127.79 (C(4a)); 127.89, 128.07, 128.27, 128.51, 136.53, 137.94 (Ph); 147.32 (C(8a)); 148.77 (C(6)); 150.52 (C(20′)); 173.68 (COO); 175.80 (C(28′)). MS, *m/z*: 892.052 $[M + Na]^+$, 908.014 $[M + K]^+$.

Debenzylation of compounds 8 and 11 (general procedure). A catalyst (10% Pd/C; 20 wt.% of compound **8** or **11**) was added to a solution of compound **8** or **11** (0.1 mmol) in anhydrous Et_2O (7 mL). The mixture was stirred in a hydrogen atmosphere for 2—8 h. After the reaction was completed (monitoring by TLC in system *B*), the catalyst was filtered off and washed with Et_2O. The filtrate was concentrated and the residue was chromatographed on SiO_2 with $CHCl_3$ as an eluent to give compound **16a,b** or **17**, respectively.

(6-Hydroxy-2,5,7,8-tetramethyl-3,4-dihydro-2*H*-1-benzopyran-2-yl)methyl 3-oxolup-20(29)-en-28-oate (16a). Yield 59%, white crystals, m.p. 98—100 °C, $[\alpha]_D^{20}$ +11.3° (*c* 0.15, CH_2Cl_2). Found (%): C, 78.41; H, 9.62. $C_{44}H_{64}O_5$. Calculated (%): C, 78.53; H, 9.59. IR, ν/cm^{-1}: 1724 (C=O). UV ($CHCl_3$), λ_{max}/nm (ε): 295 (3123). ^{1}H NMR, δ: 0.89, 0.93, 0.99, 1.04, 1.09 (all s, 3 H each, H(23′), H(24′), H(25′), H(26′), H(27′)); 1.20—2.60 (m, 24 H, CH_2, CH in the betulonic acid residue + 5 H, Me—C(2), H(3) in the chromanol residue); 1.71 (s, 3 H, H(30′)); 2.12, 2.14, 2.20 (all s, 3 H each, Me—Ar); 2.68 (t, 2 H, H(4), *J* = 6.4 Hz); 3.08 (m, 1 H, H(19′)); 4.15 (m, 2 H, CH_2—O); 4.40, 4.61 (both s, 1 H each, H(29′)); 4.75 (s, 1 H, OH). ^{13}C NMR, δ: 11.30, 11.82, 12.23 (Me—Ar); 14.65 (C(27′)); 15.85 (C(25′)); 15.94 (C(26′)); 19.37 (C(6′)); 19.64 (C(30′)); 20.29 (C(4)); 21.04 (C(24′)); 21.43 (C (11′)); 22.06 (Me—C(2)); 25.57 (C(12′)); 26.62 (C(23′)); 28.96, 29.06 (C(3)); 29.67 (C(15′)); 30.63 (C(21′)); 32.19 (C(16′)); 33.63 (C(7′)); 34.15 (C(2′)); 36.90 (C(10′)); 37.09 (C(22′)); 38.46 (C(13′)); 39.64 (C(1′)); 40.64 (C(8′)); 42.50 (C(14′)); 47.04 (C(19′)); 47.35 (C(4′)); 49.34 (C(18′)); 49.90 (C(9′)); 54.99 (C(5′)); 56.76 (C(17′)); 68.00 (CH_2—O); 73.36 (C(2)); 109.72 (C(29′)); 116.90 (C(5)); 118.55, 121.35, 122.69 (C(4a), C(7), C(8)); 144.89 (C(8a)); 145.04 (C(6)); 150.42 (C(20′)); 175.98 (COO); 218.18 (C(3′)). MS, *m/z*: 673.189 $[M]^+$.

(6-Hydroxy-2,5,7,8-tetramethyl-3,4-dihydro-2*H*-1-benzopyran-2-yl)methyl 3-oxolupan-28-oate (16b). Yield 75%, white crystals, m.p. 110—112 °C, $[\alpha]_D^{20}$ +5.6° (*c* 0.55, CH_2Cl_2). Found (%): C, 78.21; H, 9.90. $C_{44}H_{66}O_5$. Calculated (%): C, 78.29; H, 9.86. IR, ν/cm^{-1}: 1723 (C=O). UV ($CHCl_3$), λ_{max}/nm (ε): 295 (3244). ^{1}H NMR, δ: 0.78, 0.88 (both d, 3 H each, Me in Pr^i, *J* = 7 Hz); 0.93, 0.95, 0.99, 1.04, 1.09 (all s, 3 H each, H(23′), H(24′), H(25′), H(26′), H(27′)); 1.25—2.55 (m, 31 H, CH_2, CH in the betulonic acid residue + Me—C(2), H(3) in the chromanol residue); 2.12, 2.14, 2.20 (all s, 3 H each, Me—Ar); 2.68 (t, 2 H, H(4), *J* = 6.4 Hz); 4.02, 4.04, 4.13, 4.15 (all d, 0.5 H each, CH_2—O, *J* = 11.2 Hz); 4.35 (s, 1 H, OH). ^{13}C NMR, δ: 11.30, 11.82, 12.22 (Me—Ar); 14.58 (C(27′)); 14.69 (C(25′), Me in Pr^i); 15.85, 15.91 (C(26′)); 19.65 (C(6′)); 20.29 (C(4)); 21.06 (C(24′)); 21.48 (C(11′)); 22.15 (Me—C(2)); 22.70 (Me in Pr^i); 26.63 (C(12′)); 26.96 (C(23′)); 28.94, 29.07 (C(3)); 29.70 (C(15′)); 32.02 (C(21′)); 33.71 (C(16′)); 34.15 (C(2′), C(7′)); 36.88 (C(10′), C(22′)); 37.36 (CH in Pr^i); 38.40 (C(13′)); 39.62 (C(1′)); 40.66 (C(8′)); 42.65 (C(14′)); 44.28 (C(19′)); 47.35 (C(4′)); 49.84 (C(18′)); 49.67 (C(9′)); 54.97 (C(5′)); 57.21 (C(17′)); 73.32 (CH_2—O); 73.36 (C(2)); 116.92 (C(5)); 118.53, 121.32, 122.71 (C(4a), C(7), C(8)); 144.91 (C(8a)); 145.02 (C(6)); 176.26 (COO); 218.16 (C(3′)). MS, *m/z*: 675.077 $[M]^+$, 698.064 $[M + Na]^+$.

3β-[(6-Hydroxy-2,5,7,8-tetramethyl-3,4-dihydro-2*H*-1-benzopyran-2-yl)carbonyloxy]lup-20(29)-en-28-oic acid (17). Yield 75%, amorphous powder, $[\alpha]_D^{20}$ +14.8° (*c* 0.58, $CHCl_3$). Found (%): C, 76.57; H, 9.40. $C_{44}H_{64}O_6$. Calculated (%): C, 76.70; H, 9.36. IR, ν/cm^{-1}: 1717 (O—C=O). UV (CH_2Cl_2), λ_{max}/nm (ε): 296 (2858). ^{1}H NMR, δ: 0.78, 0.81, 0.86, 0.89, 0.96 (all s, 3 H each, H(23′), H(24′), H(25′), H(26′), H(27′)); 1.29—2.31 (m, 24 H, CH_2, CH in the betulinic acid residue + 7 H, Me—C(2), H(3), H(4) in the Trolox acid residue); 1.71 (s, 3 H, H(30′)); 2.08, 2.16, 2.21 (all s, 3 H each, Me—Ar); 3.02 (m, 1 H, H(19′)); 4.29 (dd, 1 H, H(3′), *J* = 11.6 Hz, *J* = 4.4 Hz); 4.63, 4.76 (both s, 1 H each, H(29′)); 7.28 (s, 1 H, OH); 7.39 (s, 1 H, COOH). ^{13}C NMR, δ: 11.18, 11.84, 12.92 (Me—Ar); 14.71 (C(27′)); 15.90 (C(24′)); 16.10 (C(25′)); 16.39 (C(26′)); 18.10 (C(6′)); 19.34 (C(30′)); 20.84 (C(11′)); 21.18 (C(4)); 25.42 (C(12′)); 25.70 (Me—C(2)); 27.23 (C(2′)); 28.04 (C(23′)); 29.69 (C(21′)); 30.57 (C(15′)); 30.74 (C(3)); 32.16 (C(16′)); 34.19 (C(7′)); 37.09 (C(22′), C(10′)); 37.82 (C(13′)); 38.28 (C(1′)); 38.41 (C(4′)); 40.68 (C(8′)); 42.43 (C(14′)); 46.95 (C(18′)); 49.26 (C(19′)); 50.33 (C(9′)); 55.36 (C(5′)); 56.42 (C(17′)); 77.23 (C(2)); 81.81 (C(3′)); 109.75 (C(29′)); 116.90 (C(5)); 118.51 (C(7)); 121.25 (C(8)); 122.48 (C(4a)); 145.11 (C(8a)); 146.03 (C(6)); 150.35 (C(20′)); 173.73 (COO); 182.47 (C(28′)). MS, *m/z*: 689.145 $[M]^+$, 712.136 $[M + Na]^+$, 728.099 $[M + K]^+$.

28-[*tert*-Butyl(dimethyl)silyloxy]-3β-[(6-hydroxy-2,5,7,8-tetramethyl-3,4-dihydro-2*H*-1-benzopyran-2-yl)carbonyloxy]-lup-20(29)-ene (18a). A solution of TBAF (0.03 g, 0.11 mmol) in THF (0.11 mL) was added dropwise under argon at 0 °C to a solution of compound **10** (0.09 g, 0.10 mmol) in dry THF (2 mL). The reaction mixture was stirred at 0 °C for 0.5 h and diluted with water. The product was extracted with AcOEt and the extract was washed with brine, dried over $MgSO_4$, and concentrated. The residue was chromatographed on SiO_2 with $CHCl_3$ as an eluent. The yield of compound **18a** was 62%, amorphous powder, $[\alpha]_D^{20}$ +11.7° (*c* 1.52, $CHCl_3$). Found (%): C, 75.99; H, 10.25; Si, 3.59. $C_{50}H_{80}O_5Si$. Calculated (%): C, 76.09; H, 10.22; Si, 3.56. IR, ν/cm^{-1}: 1727 (O—C=O). UV ($CHCl_3$), λ_{max}/nm (ε): 295 (2814). ^{1}H NMR, δ: 0.07 (s, 6 H, Me—Si); 0.92 (s, 9 H, Me in Bu^t); 0.91, 0.95, 0.97, 1.00, 1.03 (all s, 3 H each, H(23′), H(24′), H(25′), H(26′), H(27′)); 1.10—1.92 (m, 24 H, CH_2, CH in the betulin residue + 5 H, Me—C(2), H(3) in the Trolox acid residue); 1.70 (s, 3 H, H(30′)); 2.08, 2.17, 2.19 (all s, 3 H each, Me—Ar); 2.37—2.68 (m, 3 H, H(4), H(19′)); 3.26, 3.70 (both d, 1 H each, CH_2—O, *J* = 9.6 Hz); 4.27 (s, 1 H, OH); 4.40 (m, 1 H, H(3′)); 4.58, 4.68 (both s, 1 H each, H(29′)). ^{13}C NMR, δ: −5.36 (Me—Si); 11.22, 11.84, 12.18 (Me—Ar); 14.76 (C(27′)); 15.88 (C(24′)); 16.09 (C(26′)); 16.41 (C(25′)); 18.10 (Bu^t—Si); 18.32 (C(6′)); 19.09 (C(30′)); 20.86 (C(11′)); 21.10 (C(4)); 25.41 (Me—C(2)); 25.20 (C(12′)); 25.98 (Me in Bu^t); 27.03 (C(2′)); 27.21 (C(15′)); 28.03 (C(23′)); 29.44 (C(16′)); 29.93 (C(21′)); 30.74 (C(3)); 34.08 (C(22′)); 34.32 (C(7′)); 37.04 (C(10′)); 37.37 (C(13′)); 37.81 (C(1′)); 38.16 (C(4′)); 40.92 (C(8′)); 42.68 (C(14′)); 48.06 (C(17′),

C(18′)); 48.39 (C(19′)); 50.26 (C(9′)); 55.31 (C(5′)); 60.47 (C(28′)); 73.50 (C(2)); 81.79 (C(3′)); 109.40 (C(29′)); 116.80, 116.90 (C(5)); 118.32, 118.46 (C(7)); 121.08, 121.20 (C(8)); 122.48 (C(4a)); 145.13, 145.18 (C(8a)); 145.85, 146.03 (C(6)); 150.91 (C(20′)); 173.69, 173.87 (COO). MS, *m/z*: 789.819 $[M]^+$, 812.638 $[M + Na]^+$.

3β-[(6-Hydroxy-2,5,7,8-tetramethyl-3,4-dihydro-2*H*-1-benzopyran-2-yl)carbonyloxy]lup-20(29)-en-28-ol (18b). A solution of TBAF (0.04 g, 0.15 mmol) in THF (0.15 mL) was added dropwise under argon at 0 °C to a solution of compound **18a** (0.06 g, 0.08 mmol) in dry THF (3 mL). The reaction mixture was stirred at ~20 °C for 24 h (monitoring by TLC in system *B*) and diluted with water. The product was extracted with AcOEt and the extract was washed with brine, dried over $MgSO_4$, and concentrated. The residue was chromatographed on SiO_2 with $CHCl_3$ as an eluent. The yield of compound **18b** was 57%, amorphous powder, $[\alpha]_D^{20}$ +17.4° (*c* 0.69, $CHCl_3$). Found (%): C, 78.21; H, 9.89. $C_{44}H_{66}O_5$. Calculated (%): C, 78.29; H, 9.86. IR, ν/cm^{-1}: 1726 (O—C=O). UV ($CHCl_3$), λ_{max}/nm (ε): 295 (2153). ^{1}H NMR, δ: 0.84, 0.88, 0.90, 0.96, 1.02 (all s, 3 H each, H(23′), H(24′), H(25′), H(26′), H(27′)); 1.20—1.70 (m, 24 H, CH_2, CH in the betulin residue + 5 H, Me—C(2), H(3) in the Trolox acid residue); 1.70 (s, 3 H, H(30′)); 2.06, 2.16, 2.19 (all s, 3 H each, Me—Ar); 2.37—2.68 (m, 3 H, H(4), H(19′)); 3.34, 3.82 (both d, 1 H each, CH_2—O, J = 10.5 Hz); 4.27, 8.13 (s, 1 H, OH); 4.39 (m, 1 H, H(3′)); 4.60, 4.70 (both s, 1 H each, H(29′)). ^{13}C NMR, δ: 11.21, 11.88, 12.18 (Me—Ar); 14.76 (C(27′)); 15.91 (C(24′)); 16.09 (C(26′)); 16.40 (C(25′)); 18.11 (C(6′)); 19.05 (C(30′)); 20.82 (C(11′)); 21.17 (C(4)); 25.39 (Me—C(2)); 25.14 (C(12′)); 27.03 (C(2′)); 27.21 (C(15′)); 28.02 (C(23′)); 29.73 (C(16′)); 30.73 (C(3), C(21′)); 33.96 (C(22′)); 34.10 (C(7′)); 37.03 (C(10′)); 37.28 (C(13′)); 37.81 (C(1′)); 38.17 (C(4′)); 40.91 (C(8′)); 42.71 (C(14′)); 47.77 (C(17′)); 47.80 (C(18′)); 48.73 (C(19′)); 50.23 (C(9′)); 55.30 (C(5′)); 60.56 (C(28′)); 77.34 (C(2)); 81.78 (C(3′)); 109.40 (C(29′)); 116.91 (C(5)); 118.44 (C(7)); 121.05 (C(8)); 122.50 (C(4a)); 145.17, 145.18 (C(8a)); 145.85 (C(6)); 150.90 (C(20′)); 173.70 (COO). MS, *m/z*: 674.548 $[M]^+$, 697.560 $[M + Na]^+$.

The influence of conjugates 6, 7, 19, and 20 and acids 1b and 4 on the functional state of macrophages was studied in both male and female mice (line C57BL/6) aged eight through twelve weeks (first-quality category), which had been provided by the Department of Experimental Biological Models of the Research Institute for Pharmacology, Siberian Branch of the Russian Academy of Medical Sciences. The animals were housed in an incomplete barrier system (light-dark cycle 12/12) with free access to water and granulated food.

Macrophages were isolated from the mouse peritoneal fluid. To do this, the abdominal cavities of mice were washed with cold physiological fluid (an isotonic 0.9% solution of NaCl, OOO "Zavod Medsintez"). Cells were precipitated, resuspended in a nutrient medium consisting of RPMI 1640 (GNTs VB "Vektor"), 10 vol.% fetal calf serum (FCS) ("HyClone"), 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES) (20 mmol L^{-1}, Sigma), 2-mercaptoethanol (0.05 mmol L^{-1}, Sigma), gentamicin (50 μg mL^{-1}, Sigma), and L-glutamine (2 mmol L^{-1}, Sigma), transferred to plastic Petri dishes ($c = (1.5—2.0) \cdot 10^6$ cells per 1 mL, and cultured for 2 h (37 °C, 5% CO_2). Only the cells adhered to the plastic were collected.

To study the influence of hybrid compounds on the functional state of macrophages and estimate their cytotoxicity, the collected cells were placed in flat-bottomed 96-well cell culture plates ($2.0 \cdot 10^5$ cells per well) and cultured with LPS (Sigma) and the test compounds for 48 h (37 °C, 5% CO_2). Then a part of wells were used to estimate the cytotoxicity in an MTT assay.[32] Another part of wells was used to determine the nitrite content in their supernatants with the Griess reagent on a Titertek spectrophotometer (λ = 540 nm); the arginase activity in cells was estimated according to a modified procedure.[33] To do this, 0.1% Triton X-100 (0.1 mL) was added to the wells with macrophages and cells were cultured with constant agitation at ~20 °C for 30 min. Then 0.025 *M* Tris-HCl (0.1 mL) and 0.01 *M* $MnCl_2$ (0.035 mL) were added to test tubes for activation of arginase and cells were incubated at 56 °C for 10 min, whereupon 0.5 *M* L-arginine (pH 9.7, 0.05 mL) (Sigma) was added to the lysate (0.05 mL) and cells were cultured at 37 °C for 60 min. The reaction was terminated by adding a mixture of conc. H_2SO_4 and H_3PO_4 with water (1 : 3 : 7 v/v, 0.8 mL). The urea concentration in the resulting solution was determined on a spectrophotometer (540 nm) using a Mochevina-450 test system ("Bio-LA-Test") according to the enclosed protocol. The amount of arginase catalyzing the formation of 1 mmol of urea per minute was taken to be a unit of enzyme activity. To estimate cytotoxicity, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Serva) was added to the wells four hours before the termination of the cultivation; the MTT concentration in the wells was 200 μg mL^{-1}. Then the supernatants were removed from the wells and the precipitate was dissolved in DMSO (dimexid, "Tatkhimfarmpreparaty"). Absorption of the resulting solutions was measured on a multichannel spectrophotometer at λ = 550 nm.

The data obtained were processed using a *t*-test procedure. The difference between experimental values were believed to be reliable at $p < 0.05$.

This work was financially supported by the Russian Foundation for Basic Research (Project No. 10-03-00105) and the Division of Chemistry and Materials Science of the Russian Academy of Sciences (Program "Medicinal and Biomolecular Chemistry").

References

1. S. V. Vasil′eva, in *Khimicheskaya i biologicheskaya kinetika. Novye gorizonty. Biologicheskaya kinetika: Sb. obzornykh statei* [*Chemical and Biological Kinetics. New Horizons. Biological Kinetics: A Collection of Reviews*], Khimiya, Moscow, 2005, **2**, p. 579 (in Russian).
2. S. Goerdt, C. E. Orfanos, *Immunity*, 1999, **10**, 137.
3. S. Ehrt, D. Schnappinger, S. Bekiranov, *J. Exp. Med.*, 2001, **194**, 1123.
4. D. M. Mosser, *J. Leukoc. Biol.*, 2003, **73**, 209.
5. A. Mantovani, *Blood*, 2006, **108**, 408.
6. M. Munder, K. Eichmann, M. Modolell, *J. Immunol.*, 1998, **160**, 5347.
7. T. Kreider, R. M. Anthony, J. F. Urban, Jr., W. C. Gause, *Curr. Opin. Immunol.*, 2007, **19**, 448.
8. H. Maeda, T. Akaike, *Biochemistry (Moscow)*, 1998, **63**, 854.
9. T. Akaike, M. Suga, H. Maeda, *Pros. Soc. Exp. Biol. Med.*, 1998, **217**, 64.
10. J. Wang, G. Mazza, *J. Agric. Food Chem.*, 2002, **50**, 850.
11. T. Motai, S. Kitanaka, *J. Nat. Prod.*, 2005, **68**, 1732.

12. Li-Yue Nie, J.-J. Qin, Y. Huang, L. Yan, Yu-Bo Liu, Yue-Xing Pan, Hui-Zi Jin, Wei-Dong Zhang, *J. Nat. Prod.*, 2010, **73**, 1117.
13. T. Honda, B.-A. V. Rounds, L. Bore, H. J. Finlay, F. G. Favaloro, N. Suh, Y. Wang, M. B. Sporn, G. W. Gribble, *J. Med. Chem.*, 2000, **43**, 4233.
14. T. Honda, G. W. Gribble, N. Suh, H. J. Finlay, B.-A. V. Rounds, L. Bore, F. G. Favaloro, Jr., Y. Wang, M. B. Sporn, *J. Med. Chem.*, 2000, **43**, 1866.
15. T. Honda, Y. Honda, F. G. Favaloro, G. W. Gribble, N. Suh, A. E. Place, M. H. Rendi, M. B. Sporn, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 1027.
16. T. Honda, K. T. Liby, X. Su, C. Sundararajan, Y. Honda, N. Suh, R. Risingsong, C. R. Williams, D. B. Royce, M. B. Sporn, G. W. Gribble, *Bioorg. Med. Chem. Lett.*, 2006, **15**, 6306.
17. K. Liby, T. Honda, C. R. Williams, R. Risingsong, D. B. Royce, N. Suh, A. T. Dinkova-Kostova, K. K. Stephenson, P. Talalay, C. Sundararajan, G. W. Gribble, M. B. Sporn, *Mol. Cancer Ther.*, 2007, **6**, 2123.
18. V. N. Odinokov, A. Yu. Spivak, O. V. Knyshenko, *Bioorg. Khim.*, 2007, **33**, 387 [*Russ. J. Bioorg. Chem.* (*Engl. Transl.*), 2007, **33**].
19. A. Detsi, D. Bouloumbasi, K. C. Prousis, M. Koufaki, G. Athanasellis, G. Melagraki, A. Afantitis, O. Igglessi-Markopoulou, Ch. Kontogiorgis, D. J. Hadjipavlou-Litina, *J. Med. Chem.*, 2007, **50**, 2450.
20. A. Di Stefano, P. Sozio, A. Cocco, A. Iannitelli, E. Santussi, M. Costa, L. Pessi, C. Nasuti, F. Cantalamessa, F. Pinnen, *J. Med. Chem.*, 2006, **49**, 1486.
21. M. Nozawa, K. Takhashi, K. Kato, H. Akita, *Chem. Pharm. Bull.*, 2000, **48**, 272.
22. S. Genet, A. Strehle, C. Schmidt, G. Boudjelal, A. Lobstein, K. Schoonjans, M. Souchet, J. Auwerx, R. Saladin, A. Wagner, *J. Med. Chem.*, 2010, **53**, 178.
23. A. Yu. Spivak, R. R. Mufazzalova, E. R. Shakurova, V. N. Odinokov, *Zh. Org. Khim.*, 2010, **46**, 1355 [*Russ. J. Org. Chem.* (*Engl. Transl.*), 2010, **46**].
24. G. A. Tolstikov, M. I. Goryaev, Kh. O. Kim, R. A. Khegai, *Zh. Prikl. Khim.*, 1967, **40**, 920 [*J. Appl. Chem. USSR* (*Engl. Transl.*), 1967, **40**].
25. O. B. Flekhter, E. I. Boreko, L. R. Nigmatullina, E. V. Tret´yakova, N. I. Pavlova, L. A. Baltina, S. N. Nikolaeva, O. V. Savinova, V. F. Eremin, F. Z. Galin, G. A. Tolstikov, *Bioorg. Khim.*, 2004, **30**, 89 [*Russ. J. Bioorg. Chem.* (*Engl. Transl.*), 2004, **30**].
26. R. M. Kondratenko, L. A. Baltina, E. V. Vasil´eva, L. A. Baltina, Jr., A. F. Ismagilova, Kh. M. Nasyrov, N. Zh. Baschenko, R. M. Kireeva, S. M. Fridman, G. A. Tolstikov, *Bioorg. Khim.*, 2004, **30**, 61 [*Russ. J. Bioorg. Chem.* (*Engl. Transl.*), 2004, **30**].
27. K. Hiroya, T. Takahashi, N. Miura, A. Naganuma, T. Sakamoto, *Bioorg. Med. Chem.*, 2002, **10**, 3229.
28. S. A. S. Pope, G. E. Burtin, P. T. Clayton, D. J. Madge, D. P. R. Muller, *Free Radical Biol. Med.*, 2002, **33**, 807.
29. H. Lei, J. Atkinson, *J. Org. Chem.*, 2000, **65**, 2560.
30. K. Hiroya, T. Takahashi, N. Miura, A. Naganuma, T. Sakamoto, *Bioorg. Med. Chem.*, 2002, **10**, 3229.
31. K. A. Lawson, K. Anderson, M. Menchaca, J. Atkinson, L. Sun, V. Knight, B. E. Gilbert, C. Conti, B. G. Sanders, K. Kline, *Mol. Cancer Therapeutics*, 2002, **2**, 437.
32. *ISO 10993-5. Biological Evaluation of Medical Devices*, *Pt 5: Tests for Cytotoxicity, in vitro Methods*, International Standardization Organisation, Geneva, 1992.
33. M. G. Danilets, Yu. P. Bel´skii, N. V. Bel´skaya, E. S. Trofimova, E. G. Uchasova, V. I. Agafonov, *Byull. Eksp. Biol. Med.* [*Bulletin of Experimental Biological Medicine*], 2007, App. 1, 97 (in Russian).

Received October 17, 2010