ISSN 1068-1620, Russian Journal of Bioorganic Chemistry, 2010, Vol. 36, No. 6, pp. 771–778. © Pleiades Publishing, Ltd., 2010.
Original Russian Text © O.B. Kazakova, N.I. Medvedeva, I.P. Baikova, G.A. Tolstikov, T.V. Lopatina, M.S. Yunusov, L. Zaprutko, 2010, published in Bioorganicheskaya Khimiya, 2010, Vol. 36, No. 6, pp. 841–848.

Synthesis of Triterpenoid Acylates: Effective Reproduction Inhibitors of Influenza A (H1N1) and Papilloma Viruses

O. B. Kazakova[a,1], N. I. Medvedeva[a], I. P. Baikova[a], G. A. Tolstikov[a], T. V. Lopatina[a], M. S. Yunusov[a], and L. Zaprutko[b]

[a] *Institute of Organic Chemistry, Ufa Scientific Center, Russian Academy of Sciences, pr. Oktyabrya 71, Ufa, 450054 Russia*
[b] *Organic Chemistry Department, Poznan University of Medical Sciences, Poznan, Poland*

Received March 18, 2010; in final form, May 5, 2010

Abstract—The synthesis of a new group of triterpenoid acylates has been conducted on the basis of oleanolic, glycyrrhetic, and ursolic acids and betulin. 28-*ortho*-Methoxycynnamoylbetulin has been demonstrated to possess high activity against the influenza type A (H1N1) virus with the selectivity index SI > 100 while studying the activity of the synthesized compounds in relation to the reproduction of viral pathogens of respiratory infections. The high activity of 3,28-dinicotinoylbetulin against the papilloma virus (strain HPV-11) was detected with the selectivity index SI 35.

Keywords: triterpenoids, antiviral activity

DOI: 10.1134/S1068162010060142

INTRODUCTION

The etherification reaction is widely used in the synthesis of medically important compounds based on pentacyclic triterpenoids. The most prominent examples are the antiulcer preparation carbenoxolon (hemisuccinyl-glycyrrhetic acid disodium salt) [1], anti-HIV agent Bevirimat with the new mechanism of action [(3',3'-dimethylsuccinyl)betulin acid] [2, 3], and glycyrrhizin (niglizin) nicotinoates possessing a wide spectrum of activity [4–8]. The present study describes the synthesis of triterpenoid acylates with nicotinic (3-pyridincarbon) acid and demonstrates the results of the screening of the antiviral activity of some new and several previously described compounds of this class.

RESULTS AND DISCUSSION

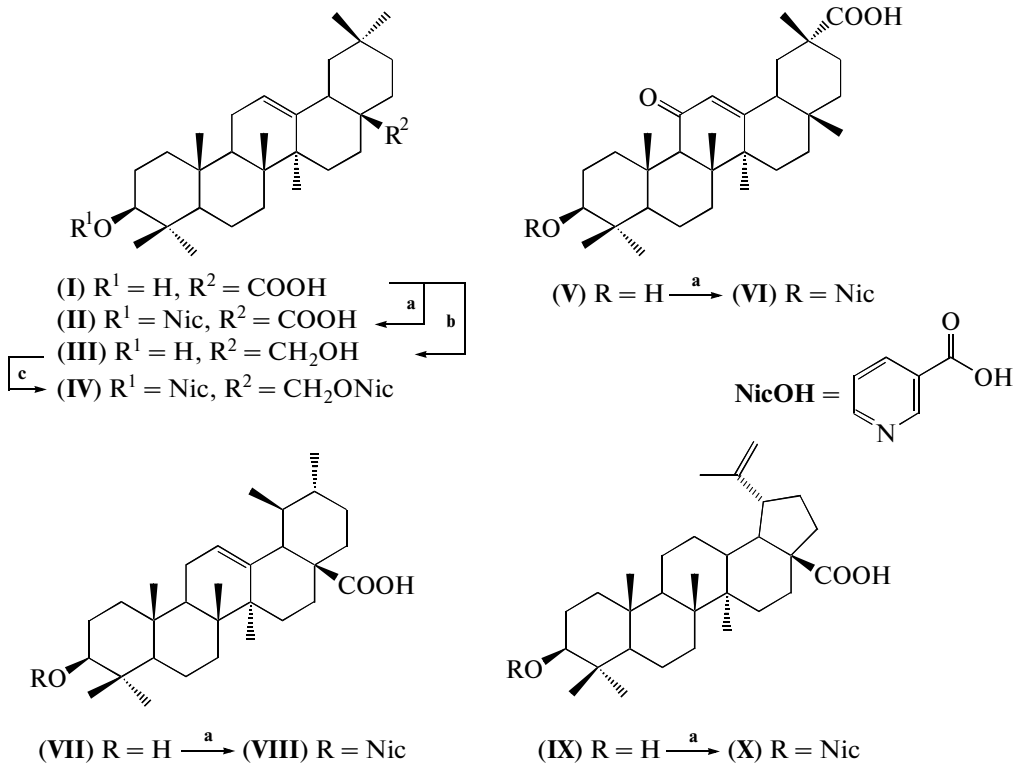
Oleanolic (**I**), glycyrrhetic (**V**), ursolic (**VII**), and betulinic (**IX**) acids and betulin (**XI**) have been used as the primary compounds for the synthesis.

The acylation of triterpenoids (**I**), (**III**), (**V**), (**VII**), (**IX**), (**XI**), and (**XVI**) by nicotinic chloranhydride has been conducted via boiling in pyridine with a catalyzing amount of dimethylaminopyridine (Schemes 1, 2). The reactions with triterpenoids (**I**), (**V**), (**VII**), and (**IX**) involved a 1.5-fold excess of chloranhydride; reaction products (**II**), (**VI**), (**VIII**), and (**X**) have been purified via recrystallization. Regioselective betulin acylation (**XI**) has been conducted in an analogous way except for at room temperature with the formation of 28-*ortho*-nicotinoylbetulin (**XII**), also known as 3β-*ortho*-hemisuccinate (**XIII**). Interestingly, derivative (**XII**) is oxidized by chromium oxide (VI) via heating in acetic acid involving C3-OH groups and the isopropenyl fragment simultaneously with the formation of 3,20-dioxoderivative (**XIV**). Nicotinoylbetulin (**XII**) oxidation by the Jones reagent in acetone resulted in ketone synthesis (**XV**) (Scheme 2).

As a result of the reaction between diol (olean-12-en-3,28-diol) (**III**) and 20-oxobetulin (messagenin) (**XVI**) with a 3-fold excess of nicotinic chloranhydride, 3β,28-di-*ortho*-nicotinates (**IV**), (**XVII**) have been synthesized. 28-*ortho*-Nicotinoylbetulin (**XII**) has been transformed into the 2(3)-dehydroderivative (**XVIII**) characterized as a ketone (**XIX**) by pyridine treatment by thionyl chloride.

Natural and synthetic triterpenoid derivatives are considered to be a new group of viral reproduction inhibitors. The possibility of many betulin derivatives to suppress the reproduction of the influenza virus and enterovirus ECHO6 [11–13] have been detected in the present study together with their antiviral activity against HIV-1, HIV-2, and the herpes simplex virus [9, 10]. The present study included the investigation of the antiviral activity of several acylates (Schemes 1–3) relative to respiratory infections, B and C hepatitis viruses, and the papilloma virus. The activity has been studied in the departments of the National Institute of Allergy and Infectious Diseases (NIAID, United States) according to the methods described at www.niaid-aacf.org.

[1] Corresponding author; phone/fax: (347)2356066; e-mail: obf@anrb.ru.



Reaction conditions: (a) 1.5-fold excess of NicCl/pyridine, DMAP, 115°C; (b) $LiAlH_4$/THF; (c) 3-fold excess of NicCl/pyridine, DMAP, 115°C.

Scheme 1.

Compounds (**X**), (**XX**) have been established as possessing low toxicity (IC_{50} = 185 and >100 μM, respectively) and to have no impact on the replication of the DNA of hepatitis B viruses (HBV) under a 10-μM concentration. The degree of suppression of nucleic acid replication of the hepatitis C viruses (HCV) for compound (**X**) in a concentration of 20 μM was 88.3% and the cytotoxicity (doze of vial cells) was 35% (SI < 10). These parameters were 65.6 and 75.7%, respectively, SI > 1 for compound (**XX**). This points to the toxicity of compound (**X**) in the investigated concentration, while derivative (**XX**) was shown to be slightly toxic relative to the replication of the HCV nucleic acid.

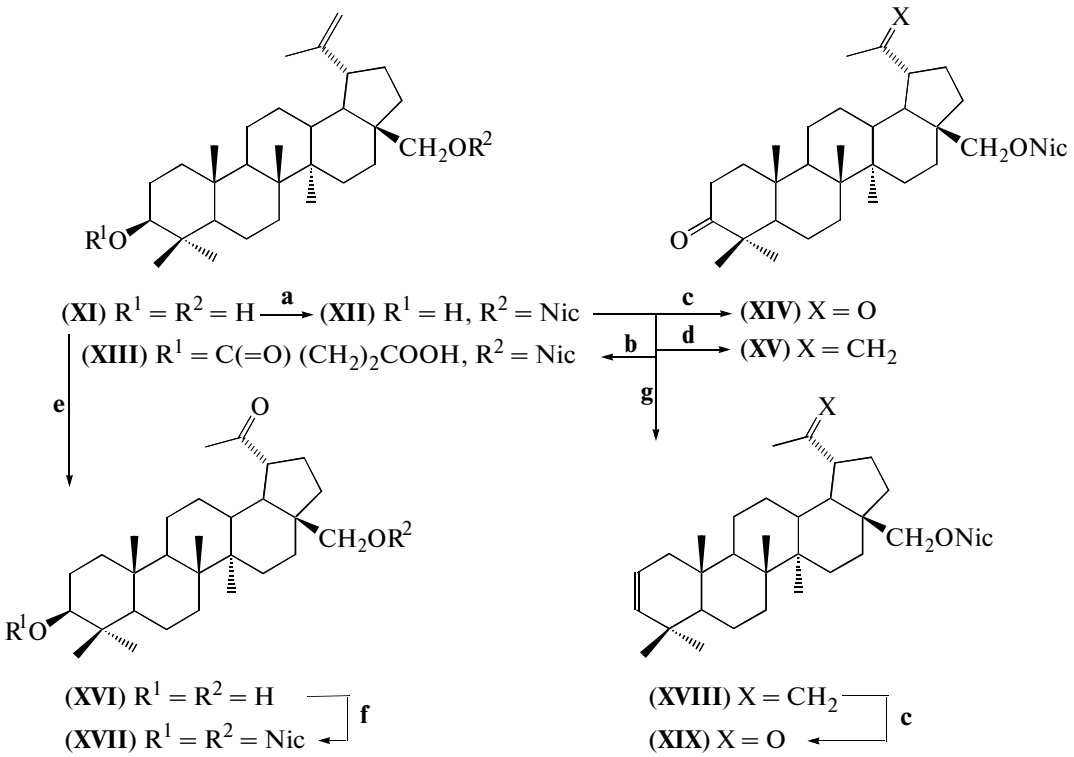
The high activity of compound (**XX**) relative to the papilloma virus (strain HPV-11) with the selectivity index SI 35 was detected. The SI was 10 for compound (**X**) with the demonstrated light cytotoxicity. Accordingly, this is the first time the triterpenoids activity was demonstrated against the papilloma virus.

While studying the activity of the synthesized compounds relative to the reproduction of viral respiratory infection agents (table), 28-*ortho*-methoxycynnamoylbetulin (**XXII**) was established to possess high activity against the influenza A virus (H1N1) with SI > 100. At the same time, compounds (**II**), (**IV**) demonstrated no suppressing activity against the influenza A virus (H7N1).

Accordingly, effective inhibitors of the influenza and papilloma viruses have been selected from triterpenoid acylates.

EXPERIMENTAL

^1H- and ^{13}C NMR spectrums have been registered via a Bruker AM-300 spectrometer (Germany), 300 and 75.5 MHz, respectively, (δ, ppm, KSSV, Hz) in $CDCl_3$, with tetramethylsilane as the internal standard. The melting temperatures have been detected via a Boetius microtable. The optical absorbance has been measured via a Perkin-Elmer 241 MC polarimeter (Germany) in a tube 1 dm in length. TLC analysis was conducted on Sorbfil plates (ZAO Sorbpolimer, Russia) using the system of solvents of chloroform–ethyl acetate 40 : 1. The substances were detected by 10% sulfuric acid with subsequent heating at 100–120°C over the course of 2–3 min. An Ozon-2K ozonizer (Russia) was used for ozonation. Column chromatography was carried out on neutral Al_2O_3 (Reachim). Oleanolic (**I**), glycyrrhetic (**V**), ursolic (**VII**), and betulinic (**IX**) acids have been obtained as previously described [14–17]. Betulin (**XI**) was extracted according to [4]. Nicotinic chloranhydride was synthesized



Reaction conditions: (a) 1.5-fold excess of NicCl/pyridine, DMAP, 122°C; (b) succinic anhydride, pyridine, 115°C; pyridine, (c) CrO_3, AcOH, 60°C; (d) Jones reagent/acetone; (e) O_3, CH_2Cl_2; (f) 3-fold excess of NicCl/pyridine, DMAP, 115°C; (g) $SOCl_2$, pyridine, 115°C.

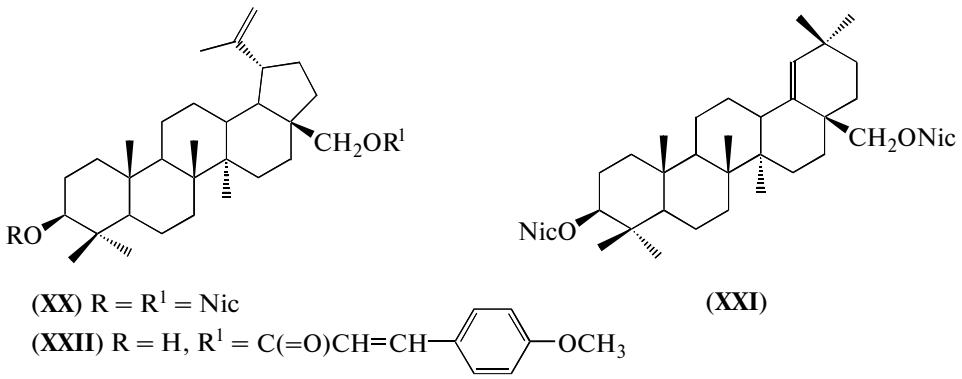
Scheme 2.

according to study [18]. Compounds (**XX**)–(**XXII**) were synthesized according to [4, 19, 20].

Methods of the synthesis of compounds (II), (IV), (VI), (VIII), (X), (XII), and (XVII). Nicotinic chloranhydride in an amount of 1.5 mmol (for substances (**I**), (**III**), (**V**), (**VII**), (**IX**), (**XI**), (**XVI**)) or 3 mmol (for substances (**II**), (**VI**), (**VIII**), (**X**), (**XII**))—the catalytic amount of dimethylaminopyridine—was added to a solution containing 1 mmol of compounds (**I**), (**III**), (**V**), (**VII**), (**IX**), (**XI**), and (**XVI**) in dry pyridine. To obtain compound (**XII**), the reaction mixture was mixed at the room temperature, while to obtain compounds (**II**), (**IV**), (**VI**), (**VIII**), (**X**), (**XVII**), it was boiled for 4 h with a backflow condenser. The reaction mixture was poured into 200 ml 5% HCl; the pellet was filtered, washed with water, and dried; and the residue was recrystallized from the ethanol and purified on a chromatographic column.

3β-*ortho*-Nicotinoylolean-12-ene-28-oic acid (II). Yield was 0.47 g (85%) after recrystallization. R_f 0.15. T_m 220–222°C. $[\alpha]_D^{20}$ +98° (*c* 0.5, $CHCl_3$). Found, %: C 77.08; H 9.05; N 2.28. $C_{36}H_{51}NO_4$ (M_r 561.80). Calculated, %: C 76.97; H 9.15; N 2.49. ^{1}H NMR spectrum: 0.79, 0.81, 0.87, 0.91, 0.99, 1.01, 1.11 (21 H, 7 s, 7 CH_3), 1.13–2.03 (23 H, m, CH, CH_2), 2.85 (1 H, d, *J* 10.4, H18), 4.75 (1 H, t, *J* 8.4, H3), 5.30 (1 H, s, H12), 7.41 (1 H, dd, *J* 4.9, 4.7, H5'), 8.24 (1 H, ddd, *J* 5.1, 2.1, 1.8, H4'), 8.75 (1 H, t, *J* 4.6, H6'), 9.21 (1 H, dd, *J* 1.9, 7.0, H2'). ^{13}C NMR spectrum: 14.2, 15.3, 16.9, 17.1, 18.2, 22.9, 23.4, 23.6, 25.6, 27.4, 28.2, 30.6, 31.4, 32.6, 32.9, 33.6, 36.9, 38.0, 38.1, 39.4, 41.2, 41.7, 45.7, 47.5, 48.3, 55.3, 82.2 (C3), 122.9 (C12), 123.2 (C5'), 126.7 (C3'), 136.9 (C4'), 143.2 (C13), 150.8 (C2'), 153.2 (C6'), 164.8 (OC=O), 172.8 (COOH).

3β,28-Di-*ortho*-nicotinoylolean-12-ene (IV). $LiAlH_4$ in an amount of 155.8 mg (4.1 mmol) was added to a solution containing 1 mmol oleanolic acid (**I**) in dry THF and boiled for 3 h. H_2O in an amount of 3 ml was added to the reaction mixture, the $Al(OH)_3$ pellet was filtered, the water layer was extracted by chloroform (3 × 60 ml) and dried by $CaCl_2$, and the solvent was evaporated in a vacuum. The residue containing erythrodiol (**III**) was acylated by nicotinic chloranhydride according to the method mentioned above. Yield was 0.44 g (68%). R_f 0.27. T_m 99–101°C. $[\alpha]_D^{20}$ +76° (*c* 0.5, $CHCl_3$). Found, %: C 77.05; H 8.70; N 4.19.



Scheme 3.

$C_{42}H_{56}N_2O_4$ (M_r 652.92). Calculated, %: C 77.26; H 8.64; N 4.29. ^{1}H NMR spectrum: 0.89, 0.91, 0.99, 1.00, 1.11, 1.19, 1.21 (21 H, 7 s, 7 CH_3), 1.22–2.20 (28 H, m, CH, CH_2), 4.00 and 4.36 (2 H, both d, *J* 11.1, H28), 4.76 (1 H, t, *J* 11, H3), 5.22 (1 H, d, *J* 3.4, H12), 7.38 and 7.40 (2 H, both dd, *J* 8.0, 4.0, H5', H5''), 8.28 and 8.31 (2 H, both ddd, *J* 8.0, 1.9, 4.0, H4', H4''), 8.76 and 8.78 (1 H, both dd, *J* 4.0, 1.9, H6', H6''), 9.22 and 9.24 (2 H, both d, *J* 1.9, H2', H2''). ^{13}C NMR spectrum: 15.5, 16.7, 16.9, 17.1, 18.2, 22.4, 23.5, 25.6, 26.0, 28.2, 29.6, 30.9, 31.6, 32.4, 33.1, 33.9, 36.2, 36.8, 38.0, 38.2, 39.8, 41.6, 42.5, 46.1, 47.5, 55.3, 71.5 (C28), 82.2 (C3), 122.9 (C5'), 123.2 (C5''), 123.3 (C12), 126.4 (C3'), 126.7 (C3''), 136.9 (C4'), 136.9 (C4''), 143.4 (C13), 150.8 (C2'), 150.8 (C2''), 153.2 (C6'), 153.3 (C6''), 164.9 and 165.2 (OC=O).

3β-*ortho*-Nicotinoyl-11-oxoolean-12-ene-30-oic acid (VI). Yield was 0.50 g (87%) after recrystallization. R_f 0.17. T_m 194–196°C. $[\alpha]_D^{20}$ + 138° (*c* 0.5, $CHCl_3$). Found, %: C 75.24; H 8.48; N 2.33. $C_{36}H_{49}NO_5$ (M_r 575.79). Calculated, %: C 75.10; H 8.58; N 2.43. ^{1}H NMR spectrum: 0.81, 0.92, 1.00, 1.11, 1.14, 1.15, 1.41 (21 H, 7 s, 7 CH_3), 1.00–2.21 (20 H, m, CH, CH_2), 2.38 (1 H, s, H9), 2.90 (1 H, d, *J* 12.2, H18), 4.80 (1 H, t, *J* 5.6, H3), 5.72 (1 H, s, H12), 7.41 (1 H, dd, *J* 7.9, 4.0, H5'), 8.31 (1 H, d, *J* 7.9, H4'), 8.80 (1 H, dd, *J* 4.0, 7.9, H6'), 9.21 (1 H, d, *J* 1.6, H2'). ^{13}C NMR spectrum: 16.3, 16.9, 17.3, 18.6, 23.3, 23.5, 26.3, 26.41, 28.1, 28.4, 28.4, 30.9, 31.8, 32.6, 36.9, 37.7, 38.3, 38.6, 40.9, 43.1, 43.7, 45.4, 48.1, 54.9, 61.5 (C8), 82.1 (C3), 123.4 (C5'), 126.9 (C3'), 128.3 (C12), 137.4 (C4'), 150.2 (C2'), 152.6 (C6'), 164.6 (C13), 169.5 (OC=O), 180.9 (COOH), 200.0 (C11).

3β-*ortho*-Nicotinoylursan-12-ene-28-oic acid (VIII). Yield was 0.50 g (90%) after recrystallization. R_f 0.16. T_m 152–154°C. $[\alpha]_D^{20}$ + 66° (*c* 0.3, $CHCl_3$). Found, %: C 76.87; H 9.27; N 2.33. $C_{36}H_{51}NO_4$ (M_r 561.80). Calculated, %: C 76.97; H 9.15; N 2.49. ^{1}H NMR spectrum: 0.87, 0.91, 0.97, 0.99, 1.00, 1.02, 1.11 (21 H, 7 s, 7 CH_3), 1.16–2.00 (23 H, m, CH, CH_2), 2.2 (1 H, d, *J* 11.1, H18), 4.75 (1 H, t, *J* 8.0, H3), 5.21–5.31 (1 H, m, H12), 7.38 (1 H, dd, *J* 5.1, 4.8, H5'), 8.25 (1 H, ddd, *J* 4.1, 1.0, 1.6, H4'), 8.76 (1 H, t, *J* 5.2, H6'), 9.21 (1 H, dd, *J* 2.1, 5.9, H2'). ^{13}C NMR spectrum: 15.5, 17.1, 17.4 (C29), 18.2, 21.1 (C30), 23.4, 23.6, 24.2, 27.9, 28.3, 30.6, 33.0, 35.6, 36.9, 38.1, 38.13, 38.3, 39.0, 39.1, 39.7, 42.2, 47.5, 49.8, 52.6, 55.4, 82.3 (C3), 123.3 (C5'), 126.1 (C3'), 126.8 (C12), 137.0 (C13), 137.9 (C4'), 150.9 (C2'), 153.3 (C6'), 165.0 (OC=O), 172.6 (COOH).

3β-*ortho*-Nicotinoyl-lupa-12-ene-28-oic acid (X). Yield was 0.45 g (80%) after recrystallization. R_f 0.19. T_m 155–157°C. $[\alpha]_D^{20}$ + 28° (*c* 0.5, $CHCl_3$). Found, %: C 77.08; H 9.03; N 2.29. $C_{36}H_{51}NO_4$ (M_r 561.80). Calculated, %: C 76.97; H 9.15; N 2.49. ^{1}H NMR spectrum: 0.82, 0.89, 0.91, 0.98, 0.99 (15 H, 5 s, 5 CH_3), 1.67 (3 H, s, H30), 1.10–2.00 (25 H, m, CH, CH_2), 2.28 (1 H, m, H19), 4.61–4.81 (3 H, m, H3, H 29), 7.42 (1 H, dd, *J* 7.8, 4.9, H5'), 8.32 (1 H, ddd, *J* 7.8, 1.1, 1.3, H4'), 8.80 (1 H, dd, *J* 4.1, 1.1, H6'), 9.21 (1 H, d, *J* 1.3, H2'). ^{13}C NMR spectrum: 14.6, 16.0, 16.1, 16.7, 18.2, 19.4, 20.9, 23.7, 25.4, 28.1, 29.7, 30.6, 32.2, 34.2, 37.09, 37.13, 38.15, 38.2, 38.4, 40.7, 42.4, 46.6, 49.3, 50.4, 55.5, 56.3, 82.4 (C3), 109.6 (C29), 123.4 (C5'), 126.9 (C3'), 137.3 (C4'), 150.4 (C2'), 150.5 (C20), 152.8 (C6'), 164.8 (OC=O), 181.3 (C28).

3β-Hydroxy-28-*ortho*-nicotinoyl-lupa-20(29)-ene (XII). Yield was 0.49 g (90%) after recrystallization. R_f 0.59. T_m 112–114°C. $[\alpha]_D^{20}$ + 16° (*c* 0.5, $CHCl_3$). Found, %: C 79.04; H 9.80; N 2.45. $C_{36}H_{53}NO_3$ (M_r 547.82). Calculated, %: C 78.93; H 9.75; N 2.56. ^{1}H NMR spectrum: 0.77, 0.85, 0.98, 1.01, 1.08 (15 H, 5 s, 5 CH_3), 1.01–2.00 (25 H, m, CH, CH_2), 1.70 (3 H, s, H30), 2.50 (1 H, dt, *J* 5.8, 10.6, H19), 3.20 (1 H, dd, *J* 10.6, 5.2, H3), 4.12 and 4.55 (2 H, both d, *J* 10.9, H28), 4.54 and 4.66 (2 H, both s, H29), 7.42 (1 H, dd, *J* 8.0, 4.0, H5'), 8.31 (1 H, ddd, *J* 8.0, 1.2, 1.3, H4'), 8.78 (1 H, dd, *J* 4.0, 1.3, H6'), 9.23 (1 H, d, *J* 1.2, H2').

Antiviral activity of triterpenoid acylates against respiratory infections*

Compound	Virus**	Strain	EC_{50}, μg/ml	IC_{50}, μg/ml	SI
(II)	H1N1	California/07/2009	30	>100	>3.3
	H3N2	Brisbane/10/2007	32	33	1
	H5N1	Vietnam/1203/2004H	>100	>100	0
	Virus type B	Florida/4/2006	>100	>100	0
(IV)	H1N1	California/07/2009	31	>100	>3.2
	H3N2	Brisbane/10/2007	>100	>100	0
(VIII)	H1N1	California/07/2009	>100	>100	0
	H3N2	Brisbane/10/2007	>100	>100	0
(X)	H1N1	California/07/2009	31	>100	>3.2
	H3N2	Brisbane/10/2007	>100	>100	0
	H5N1	Vietnam/1203/2004H	26	>100	>3.8
	Virus type B	Florida/4/2006	29	86	3
	SARS	Urbani	>5.6	5.6	0
	Adeno	65089/Chicago	>24	24	0
	Rhinovirus type 2	HGP	>17	17	0
(XII)	H5N1	Vietnam/1203/2004H	>12	12	0
	H3N2	Brisbane/10/2007	>100	>100	0
	H5N1	Vietnam/1203/2004H	>34	34	0
	Virus type B	Florida/4/2006	>19	19	0
(XVII)	H1N1	California/07/2009	16	>200	>13
	H3N2	Brisbane/10/2007	>100	>100	0
(XIX)	H1N1	California/07/2009	46	91	2
	H3N2	Brisbane/10/2007	>9.5	9.5	0
	H5N1	Vietnam/1203/2004H	>100	>100	0
	Virus type B	Florida/4/2006	57	>100	>1.8
(XX)	H1N1	California/07/2009	61	>100	>1.6
	H3N2	Brisbane/10/2007	>100	>100	0
	H5N1	Vietnam/1203/2004H	44	>100	>2.3
	Virus type B	Florida/4/2006	29	>100	>3.4
	SARS	Urbani	>42	42	0
	Adeno	65089/Chicago	>87	87	0
	Rhinovirus type 2	HGP	>60	60	0
(XXI)	H1N1	California/07/2009	23	>100	>4.3
	H3N2	Brisbane/10/2007	>100	>100	0
(XXII)	H1N1	California/07/2009	2	>200	>100
	H3N2	Brisbane/10/2007	>100	>100	0
	H5N1	Vietnam/1203/2004H	39	72	1.8
	Virus type B	Florida/4/2006	>48	48	0

*The study was conducted in departments of the National Institute of Allergy and Infectious Diseases, Maryland, United States (www.niaid-aacf.org).

**In other cases, virus type A strain.

^{13}C NMR spectrum: 14.7, 15.3, 16.0, 16.7, 18.3, 19.1, 20.8, 22.6, 23.6, 25.2, 27.1, 28.0, 29.2, 29.8, 34.0, 34.6, 36.9, 37.3, 38.2, 38.7, 40.9, 42.7, 46.7, 48.8, 50.3, 55.7, 63.0 (C28), 78.8 (C3), 109.9 (C29), 123.3 (C5'), 126.4 (C3'), 137.1 (C4'), 149.8 (C2'), 150.7 (C20), 153.3 (C6'), 165.5 (OC=O).

3β-Hemisuccinyl-28-*ortho*-nicotinoyl-lupa-20(29)-ene (XIII). Succinic anhydride in an amount of 4 mmol was added to a solution containing 0.55 g (1 mmol) of compound (**XII**) in 10 ml pyridine and boiled for 4 h with a backflow condenser and poured into 50 ml 5% HCl; the residue was filtered, washed by water, dried, and crystallized from the ethanol. Yield was 0.55 g (85%). R_f 0.20. T_m 130–132°C. $[\alpha]_D^{20}$ + 13° (*c* 0.3, $CHCl_3$). Found, %: C 74.25; H 8.77; N 2.05. $C_{40}H_{57}NO_6$ (M_r 647.89). Calculated, %: C 74.15; H 8.87; N 2.16. ^{1}H NMR spectrum: 0.83, 0.88, 0.92, 0.99, 1.01 (15 H, 5 s, 5 CH_3), 1.10–2.00 (25 H, m, CH, CH_2), 1.65 (3 H, s, H30), 2.50 (1 H, dt, *J* 5, 11, 11, H19), 2.62–2.69 (4 H, m, H1", H2"), 4.05 (1 H, d, *J* 11, H28), 4.48–4.73 (3 H, m, H28, H29), 4.11–4.20 (1 H, m, H3), 7.45 (1 H, dd, *J* 7.9, 4.8, H5'), 8.33 (1 H, ddd, *J* 7.9, 1.2, 1.8, H4'), 8.81 (1 H, dd, *J* 4.8, 1.8, H6'), 9.21 (1 H, d, *J* 1.2, H2'). ^{13}C NMR spectrum: 14.8, 16.0, 16.1, 16.5, 18.1, 19.1, 20.8, 23.6, 25.2, 27.1, 27.9, 29.1, 29.5 (C1"), 29.9 (C2"), 34.1, 34.6, 37.1, 37.7, 37.8, 38.4, 40.9, 42.7, 46.7, 47.7, 48.8, 50.3, 55.4, 60.4, 63.9 (C28), 81.4 (C3), 110.0 (C29), 123.5 (C5'), 126.0 (C3'), 137.5 (C4'), 149.9 (C2'), 150.2 (C20), 152.8 (C6'), 165.0, 172.0 (OC=O), 176.6 (COOH).

3,20-Dioxo-28-*ortho*-nicotinoyl-29-norlupan (XIV). CrO_3 in an amount of 0.6 g was added to a solution containing 0.55 g (1 mmol) of compound (**XII**) in 50 ml acetic acid and boiled for 4 h with a backflow condenser and poured into 50 ml of water; the residue was filtered, washed by water, dried, and purified by column chromatography; the eluent was chloroform, chloroform–methanol, 10 : 1. Yield was 0.35 g (65%). R_f 0.30. T_m 195–197°C. $[\alpha]_D^{20}$ + 28° (*c* 0.3, $CHCl_3$). Found, %: C 76.60; H 8.89; N 2.36. $C_{35}H_{49}NO_4$ (M_r 547.76). Calculated, %: C 76.74; H 9.02; N 2.56. ^{1}H NMR spectrum: 0.86, 0.98, 1.00, 1.02, 1.08 (15 H, 5 s, 5 CH_3), 1.10–2.10 (24 H, m, CH, CH_2), 2.18 (3 H, s, H30), 2.70 (1 H, dt, *J* 11.3, 5.2, H19), 4.06 and 4.50 (2 H, both d, *J* 11.0, H28), 7.31 (1 H, dd, *J* 8.0, 4.8, H5'), 8.21 (1 H, ddd, *J* 8.0, 1.7, 1.3, H4'), 8.70 (1 H, dd, *J* 4.8, 1.7, H6'), 9.18 (1 H, d, *J* 1.3, H2'). ^{13}C NMR spectrum: 14.7, 15.8, 15.9, 19.6, 21.0, 21.4, 26.7, 27.1, 27.3, 27.5, 29.5, 29.7, 33.5, 34.0, 34.5, 36.7, 36.9, 39.5, 40.8, 42.7, 46.8, 47.3, 49.3, 49.6, 51.9, 54.9, 63.4 (C28), 123.4 (C5'), 126.9 (C3'), 137.0 (C4'), 150.9 (C2'), 153.5 (C6'), 165.3 (OC=O), 211.2 (C20), 217.8 (C3).

3-Oxo-28-*ortho*-nicotinoyl-lupa-20(29)-ene (XV). The Jones reagent in an amount of 1.6 ml in 5 ml acetone was added by drops to a solution containing 0.55 g (1 mmol) of compound (**XII**) in 30 ml of acetone at 0°C within 30 min. The reaction mass was mixed at 0°C for 4 h, followed by the addition of 1 ml MeOH and was poured into ice (10 g). The residue was filtered, washed by water, and dried; the residue was dissolved in 20 ml $CHCl_3$ and passed through a layer (1 cm) of Al_2O_3; the solvent was evaporated in a vacuum; and the residue was recrystallized from the ethanol. Yield was 0.46 g (85%). R_f 0.30. T_m 128–130°C. Found, %: C 79.01; H 9.48; N 2.37. $C_{36}H_{51}NO_3$ (M_r 545.80). Calculated, %: C 79.22; H 9.42; N 2.57. ^{1}H NMR spectrum: 0.85, 0.93, 0.93, 0.98, 1.02 (15 H, 5 s, 5 CH_3), 1.63 (3 H, s, H30), 0.99–2.30 (24 H, m, CH, CH_2), 2.42 (1 H, m, H19), 4.06 and 4.50 (2 H, both d, *J* 11, H28), 4.53 and 4.63 (2 H, both s, H29), 7.31 (1 H, dd, *J* 8.0, 4.8, H5'), 8.21 (1 H, ddd, *J* 8.0, 1.7, 1.3, H4'), 8.71 (1 H, dd, *J* 4.8, 1.7, H6'), 9.18 (1 H, d, *J* 1.3, H2'). ^{13}C NMR spectrum: 14.7, 15.8, 15.9, 19.1, 19.5, 21.0, 21.2, 25.1, 26.5, 27.0, 29.5, 29.8, 33.4, 34.1, 34.6, 36.8, 37.7, 39.5, 40.8, 42.7, 46.6, 47.3, 47.7, 48.7, 49.6, 54.9, 63.6 (C28), 110.0 (C29), 123.2 (C5'), 126.2 (C3'), 136.9 (C4'), 149.8 (C2'), 150.8 (C20), 153.3 (C6'), 165.5 (OC=O), 217.8 (C3).

3β,28-Di-*ortho*-nicotinoyl-20-oxo-29-norlupan (XVII). Ozone was passed through 1 mmol betulin (**XI**) in 20 ml CH_2Cl_2 at –60°C until the disappearance of the primary substance (THF control). The temperature was brought to room temperature; the solvent was evaporated in a vacuum. The residue consisting of 20-oxobetulin (**XVI**) was acylated by nicotinic chloranhydride according to the abovementioned technique. The reaction product (**XVII**) was purified by the column chromatography method; the eluents were benzene and chloroform. Yield was 0.50 g (76%). R_f 0.28. T_m 138–140°C. $[\alpha]_D^{20}$ + 7° (*c* 0.5, $CHCl_3$). Found, %: C 75.10; H 8.22; N 4.08. $C_{41}H_{54}N_2O_5$ (M_r 654.89). Calculated, %: C 75.20; H 8.31; N 4.28. ^{1}H NMR spectrum: 0.72, 0.81, 0.89, 0.99, 1.09 (15 H, 5 s, 5 CH_3), 1.11–2.12 (24 H, m, CH, CH_2), 2.11 (3 H, s, H30), 2.65 (1 H, dt, *J* 11.1, 5.9, H19), 3.98 and 4.44 (2 H, both d, *J* 11, H28), 4.65 (1 H, dd, *J* 10.1, 5.7, H3), 7.29 and 7.35 (2 H, both dd, *J* 8.0, 3.9, H5', H5"), 8.23 and 8.26 (2 H, both ddd, *J* 8.0, 1.6, 1.3, H4', H4"), 8.72 and 8.74 (2 H, both dd, *J* 3.9, 1.6, H6', H6"), 9.14 and 9.17 (2 H, both d, *J* 1.3, H2', H2"). ^{13}C NMR spectrum: 14.6, 15.9, 16.6, 18.0, 19.2, 20.1, 23.5, 26.9, 27.0, 27.3, 27.9, 29.3, 29.5, 33.8, 34.3, 36.4, 36.9, 37.9, 38.2, 40.7, 42.3, 46.5, 49.2, 49.9, 51.4, 55.2, 63.2 (C28), 82.0 (C3), 123.1 (C5'), 123.2 (C5"), 125.9 (C3'), 126.6 (C3"), 152.9 (C6'), 153.0 (C6"), 136.8 (C4'), 136.8 (C4"), 150.6 (C2'), 150.6 (C2"), 165.3 and 164.7 (OC=O), 211.1 (C20).

28-*ortho*-nicotinoyl-lupa-2(3),20(29)-diene (XVIII). $SOCl_2$ in an amount of 1 ml was added to a solution containing 0.55 g (1 mmol) of compound (**XII**) in 10 ml of pyridine and boiled for 2 h with a backflow

condenser. The reaction mixture was poured into 50 ml of water and the residue was filtered, washed by water, and dried. The residue was purified by column chromatography; the eluents were benzene and chloroform. Yield was 0.40 g (76%). R_f 0.42. T_m 112–114°C. $[\alpha]_D^{20}$ –30° (*c* 0.5, $CHCl_3$). Found, %: C 81.40; H 9.52; N 2.44. $C_{36}H_{51}NO_2$ (M_r 529.81). Calculated, %: C 81.61; H 9.70; N 2.64. ^{1}H NMR spectrum: 0.85, 0.85, 0.98, 1.02, 1.10 (15 H, 5 s, 5 CH_3), 1.10–2.10 (22 H, m, CH, CH_2), 1.71 (3 H, s, H30), 2.52 (1 H, dt, *J* 5.2, 10.3, H19), 4.12 (1 H, d, *J* 10.6, H28), 4.55–4.61 (2 H, m, H28, H29), 4.72 (1 H, s, H29), 5.21–5.40 (2 H, m, H2, H3), 7.41 (1 H, dd, *J* 8.0, 4.0, H5'), 8.31 (1 H, ddd, *J* 8.0, 1.7, 1.3, H4'), 8.75 (1 H, dd, *J* 4.0, 1.7, H6'), 9.25 (1 H, d, *J* 1.3, H2'). ^{13}C NMR spectrum: 14.6, 15.6, 16.2, 18.9, 19.3, 21.1, 21.9, 25.2, 27.0, 29.5, 29.8, 31.6, 33.2, 34.5, 36.2, 37.7, 37.9, 40.8, 41.1, 42.6, 46.1, 47.6, 48.7, 48.9, 51.9, 63.6 (C28), 109.8 (C29), 121.4 (C2), 123.1 (C5'), 126.2 (C3'), 136.8 (C4'), 137.8 (C3), 149.8 (C2'), 150.7 (C20), 153.2 (C6'), 165.3 (OC=O).

28-*ortho*-Nicotinoyl-20-oxo-29-norlup-2-ene (XIX). Compound (**XVIII**) in an amount of 1 mmol was obtained analogous to compound (**XIV**). Yield was 0.37 g (70%). R_f 0.48. T_m 89–91°C. $[\alpha]_D^{20}$ +16° (*c* 0.5 $CHCl_3$). Found, %: C 78.81; H 9.40; N 2.55. $C_{35}H_{49}NO_3$ (M_r 531.78). Calculated, %: C 79.05; H 9.29; N 2.63. ^{1}H NMR spectrum: 0.89, 0.99, 1.01, 1.04 (15 H, 5 s, 5 CH_3), 1.05–2.00 (22 H, m, CH, CH_2), 2.13 (3 H, s, H30), 2.70 (1 H, dt, *J* 5.3, 11.4, H19), 4.00 and 4.48 (2 H, both d, *J* 11, H28), 5.23–5.49 (2 H, m, H2, H3), 7.45 (1 H, dd, *J* 7.9, 4.8, H5'), 8.24 (1 H, ddd, *J* 7.9, 1.3, 1.6, H4'), 8.72 (1 H, dd, *J* 4.7, 1.6, H6'), 9.16 (1 H, s, H2'). ^{13}C NMR spectrum: 15.3, 15.6, 15.9, 16.2, 16.8, 17.6, 17.8, 19.0, 20.0, 20.8, 22.2, 26.6, 27.0, 29.1, 29.9, 31.3, 34.1, 35.9, 36.1, 37.7, 39.9, 40.4, 42.6, 51.6, 54.9, 63.0 (C28), 121.0 (C2), 123.0 (C5'), 126.5 (C3'), 136.7 (C4'), 137.5 (C3), 150.3 (C2'), 152.9 (C6'), 164.9 (OC=O), 210.9 (C20).

The methods of the study of the antiviral activity of compounds (II), (IV), (VIII), (X), (XII), (XVII), and (XIX–XXII) are described at www.niaid-aacf.org.

The study of the antiviral activity of compounds (II), (IV) against the influenza A virus (H7N1) was conducted according to [21].

ACKNOWLEDGMENTS

This work was supported by the Program "Biomolecular and Medical Chemistry" of the Division of Chemistry and Materials Sciences, Russian Academy of Sciences. O.B. Kazakova is grateful to the Jozef Mianowski Fund (Poland). The authors are grateful to the National Institute of Allergy and Infectious Diseases (NIAID, United States, www.niaid-aacf.org) for the study of the antibacterial activity of compounds (**II**), (**IV**), (**VIII**), (**X**), (**XII**), (**XVII**), and (**XIX**)–(**XXII**) and E.I. Boreko and O.V. Savinova (Research Institute of Epidemiology and Microbiology of the Health Care Ministry of the Republic of Belarus) for the study of the activity of compounds (**II**), (**IV**) against the influenza A virus (H7N1).

REFERENCES

1. Iosinori, N., Obtaining Carbenoxolone and Its Disodium Salt, Jp. Appl. no. 61-165348, *Ref. J. Chem.*, 1987, 16O125P.
2. Lee, K.-H., Kashiwada, Y., Hashimoto, F., Cosentino, L.M., and Manak, M., Betulinic Acid and Dihydrobetulinic Acid Derivatives and Uses Therefore, US Patent no. 5 679 828, 1997.
3. Connor, A., Evans, P., Doto, J., Ellis, C., and Martin, D.E., *J. Clin. Pharmacol.*, 2009, vol. 49, no. 5, pp. 606–612.
4. Flekhter, O.B., Karachurina, L.T., Nigmatullina, L.R., Sapozhnikova, T.A., Baltina, L.A., Zarudii, F.S., Galin, F.Z., Spirikhin, L.V., Tolstikov, G.A., Plyasunova, O.A., and Pokrovskii, A.G., *Bioorg. Khim.*, 2002, vol. 28, pp. 543–550 [*Russ. J. Bioorg. Chem.*, 2002, vol. 28, pp. 494–500].
5. Flekhter, O.B., Karachurina, L.T., Plyasunova, O.A., Nigmatullina, L.R., Baltina, L.A., Pokrovskii, A.G., Davydova, V.A., Zarudii, F.S., Galin, F.Z., Shul'ts, E.E., and Tolstikov, G.A., RF Patent no. 2 174 982.
6. Karachurina, L.T., Agletdinov, E.F., Flekhter, O.B., and Zarudii, F.S., *Vopr. Biol. Med. Farm. Khim.*, 2009, no. 5, pp. 48–51.
7. Baltina, L.A., Glycyrrhizinic Acid Transformations: The Search for Novel Physiologically Active Compounds, *Doctoral (Chem.) Dissertation*, Ufa: IOKh UNTs RAN, 1995.
8. Plyasunova, O.A., Il'ina, T.V., Kiseleva, Ya.Yu., Fedyuk, N.V., Baltina, L.A., Tolstikov, G.A., and Pokrovskii, A.G., *Vestn. RAMN*, 2004, no. 11, pp. 42–46.
9. Mayaux, J.F., Bousseau, A., Pauwels, R., Huet, T., Henin, Y., Dereu, N., Evers, M., Soler, F., Poujade, C., and De Clercq, E., Le Pecq J.-B, *Proc. Natl. Acad. Sci. USA*, 1994, vol. 91, pp. 3564–3568.
10. Dang, Z., Lai, W., Qian, K., Ho, P., Lee, K.H., Chen, C.H., and Huang, L., *J. Med. Chem.,* 2009, vol. 52, no. 23, pp. 7887–7891.
11. Pavlova, N.I., Savinova, O.V., Nikolaeva, S.N., Boreko, E.I., and Flekhter, O.B., *Fitoterapia*, 2003, vol. 74, pp. 489–492.
12. Baltina, L.A., Flekhter, O.B., Nigmatullina, L.R., Boreko, E.I., Pavlova, N.I., Nikolaeva, S.N., Savinova, O.V., and Tolstikov, G.A., *Bioorg. Med. Chem. Lett.*, 2003, vol. 13, pp. 3549–3552.
13. Savinova, O.V., Pavlova, N.I., and Boreko, E.I., *Antibiot. Khimioter.*, 2009, vol. 54, nos. 5–6, pp. 16–20.
14. Bednarczyk-Cwynar, B., Synthesis of Lactam and Thiolactam Derivatives of Oleanolic Acid That Are Activators of Transdermal Transport, *Ph.D. Thesis,* Poznan: Poznan University of Medical Sciences, Pharmaceutical Faculty, 2007.

15. Baltina, L.A., Somov, N.A., Serdyuk, N.G., Murinov, Yu.I., Flekhter, O.B., Krasnova, L.V., and Tolstikov, G.A., RF Patent Application no. 93 040 899, 1995.
16. Shevtsov, S.A., Raldugin, V.A., and Shchukin, G.I., RF Patent no. 1816346, 1995.
17. Flekhter, O.B., Nigmatullina, L.R., Baltina, L.A., Karachurina, L.T., Galin, F.Z., Zarudii, F.S., Tolstikov, G.A., Boreko, E.I., Pavlova, N.I., Nikolaeva, S.N., and Savinova, O.V., *Khim.-Farm. Zh.*, 2002, vol. 36, pp. 26–28.
18. Naumova, B.S., Chekmareva, I.B., Zhdanovich, E.S., and Preobrazhenskii, N.A., *Khim.-Farm. Zh.*, 1969, vol. 3, pp. 11–12.
19. Flekhter, O.B., Medvedeva, N.I., Tolstikov, G.A., Galin, F.Z., Yunusov, M.S., Huong Nguen Thi Mai, Le Viet Tien, Savinova, I.V., Boreko, E.I., Titov, L.P., and Glukhov, I.V., *Bioorg. Khim.*, 2009, vol. 35, pp. 253–259 [*Russ. J. Bioorg. Chem.*, 2009, vol. 35, pp. 233–239].
20. Flekhter, O.B., Karachurina, L.T., Poroikov, V.V., Nigmatullina, L.R., Baltina, L.A., Zarudii, F.S., Davydova, V.A., Spirikhin, L.V., Baikova, I.P., Galin, F.Z., and Tolstikov, G.A, *Bioorg. Khim.*, 2000, vol. 26, pp. 215–223 [*Russ. J. Bioorg. Chem.*, 2000, vol. 26, pp. 192–200].
21. Flekhter, O.B., Medvedeva, N.I., Kukovinets, O.S., Spirikhin, L.V., Galkin, E.G., Galin, F.Z., Golovanov, D.G., Pavlova, N.I., Savinova, O.V., Boreko, E.I., and Tolstikov, G.A., *Bioorg. Khim.,* 2007, vol. 33, pp. 629–634 [*Russ. J. Bioorg. Chem.*, 2007, vol. 33, pp. 584–588].