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Synthesis of A-Secomethylenamino- and Substituted Amidoximotriterpenoids

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Abstract—Development of the functionalization of triterpenoids to A-secoamidoximes, A-secomethylenamines, and branched 3-(3-aminopropylamino)-3-(3-aminopropoxy)amidoximes is illustrated by the betulonic acid ketoxime. An effective way to get of the derivatives of 20,29-dihydrolupanes using diborane is suggested. The antiviral and antituberculosis activity data of some compounds are presented.

Keywords: triterpenoids, betulin, ketoximes, cyanoethylation, antiviral, antitubercular activity

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INTRODUCTION

Triterpene oximes are important substances both for chemical transformations and in the pursuit of valuable biological properties. Thus, the dosage forms of drugs for the treatment and prevention of influenza can be developed on the basis of the betulonic acid ketoxime [1, 2]. Acylation of the oximes of betulonic acid, its methyl ester, 20-oxoallobetulone, and ursolic acid by acetic, succinic, and phthalic anhydrides weakens the antiviral activity of the parent compounds [3–6]. Reduction of the oxime of ursolic acid to the 3-deoxy-3-amino derivative produces in the latter cytotoxicity against HL-60, Bel-7402, and Hela cells. It was also found that the 3β-amino isomer is 20 times more active than the 3α-isomer [7, 8]. The products of Beckmann rearrangements of betulonic acid oxime exhibit antibacterial activity against *Streptococcus faecalis* and *Staphylococcus aureus* at concentrations 25–50 mg/ml [9]. Derivatives of oleanolic acid can be used in cosmetology, because they transport biologically active compounds deep into tissue and make them more potent [10]. A-seconitrile lupeol exhibits antimalarial activity against *Plasmodium falciparum* at MIC > 50 mg/ml [11].

RESULTS AND DISCUSSION

In this paper, the new conversion of the oxime methyl ester of betulonic acid (**I**) was implemented. One of the directions was to use cyanoethylation reactions of triterpene oximes and amidoximes, examples of which are lacking in the literature. Cyanoethylation of oxime (**I**) by acrylonitrile led to a 2-cyanoethoxy imine (**II**) with 83% yield (scheme). NMR spectroscopy established that the characteristic proton signals of C(2')H_2- and C(1')H_2 groups appeared in the form of triplets in the δ 2.64–2.72 and δ 4.14–4.25 ppm, respectively. Aminopropoxyaminoderivative (**XIV**) was formed in 72% yield in the reduction of the nitrile group of compound (**II**) by diborane in an inert atmosphere as described in [12]. The signals of methylene of C3'H_2 and C1'H_2 in the ^{1}H NMR spectrum were observed in the field δ 2.79–3.06 and 3.29–3.41 ppm, respectively. In the ^{13}C NMR spectrum of the compound (**XIV**), the signal of the C3 atom appears at δ 58.6 ppm unlike that at δ 63.2 ppm for compound (**II**).

Along with the reduction of the nitrile group, the hydrogenation of the isopropenyl fragment occurred, as is evidenced by the absence of signals of a double bond of C20(29) in the NMR spectra. We note that preparation of 20,29-dihydrolupanes described in the literature is based on two methods: gaseous hydrogen reduction catalyzed by Ni-Raney [13], Pd/C [14], PtO_2 [15], Pt [16], and reduction by hydrogen produced *in situ* (application of amalgam of zinc in a mixture of acetic and hydrochloric acids [17]). Thus, we proposed an effective method of obtaining of 20,29-dihydrolupane derivatives using diborane.

Amidoxime (**III**), cyanoethylation of which by acrylonitrile led to 3-(2-cyanoethylamino)-3-(2-cyanoethoxy) amidoximes (**IV**) with a yield of 74%, was synthesized by interaction of compound (**II**) with hydroxylamine in boiling ethanol. Its structure was confirmed by the signals of two CN-groups at δ 113.3 and 114.3 ppm, as well as signals of C3, at δ 174.1 ppm in the spectrum of ^{13}C NMR. Because the amino group in amidoxime is electron deficient and therefore less reactive than the hydroxyl, cyanoethylation of only the hydroxyl group is most likely [18], but in the case of compound (**IV**) reaction was at the hydroxyl and amino groups at the same time. 3-[3-((*N*-(3-Ami-

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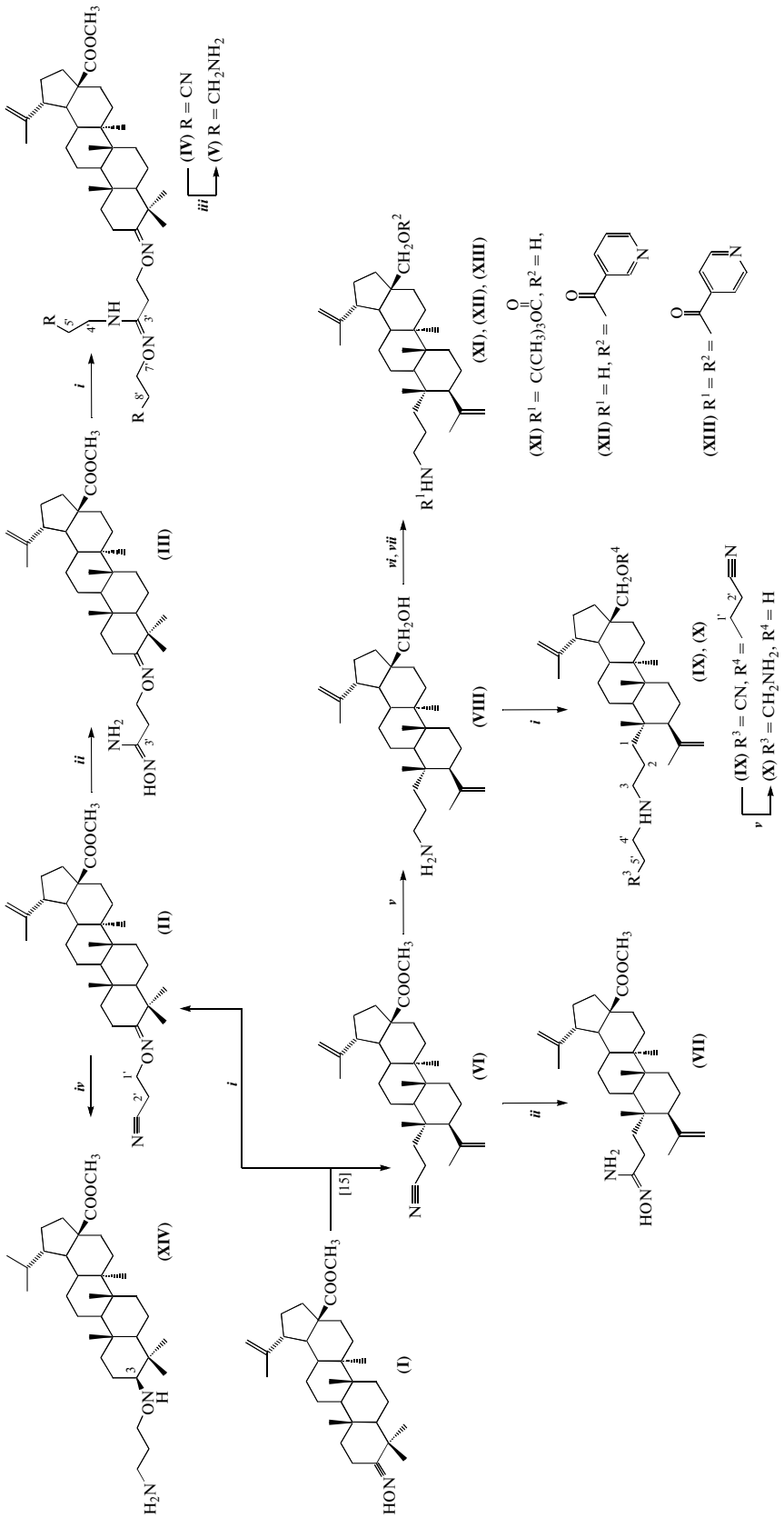

Reactions conditions: ***i***—CH_2=CHCN/1,4-dioxane; ***ii***—$NH_2OH \cdot HCl$, $NaHCO_3$/EtOH; ***iii***—H_2, Ni-Raney/MeOH; ***iv***—$NaBH_4$, BF_3-Et_2O/THF; ***v***—$LiAlH_4$/THF; ***vi***—Boc_2O/CH_2Cl_2; ***vii***—chloride of nicotinic acid (isonicotinic acid) chloranhydride/pyridine-DMAP.

Table 1. Antiviral activity of compounds (**II**), (**VI**), and (**VII**) against respiratory infections

Compound	Virus	Strain	EC_{50}, μg/ml	IC_{50}, μg/ml	SI
(II)	influenza A (H1N1)	California/07/2009	>0.84	0.84	0
	influenza (H3N2)	Brisbane/10/2007	>100	>100	0
	influenza (H5N1)	Vietnam/1203/2004H	>100	>100	0
	influenza type B	Florida/4/2006	>100	>100	0
(VI)	influenza A (H1N1)	California/07/2009	32	>100	>3.1
	influenza (H3N2)	Brisbane/10/2007	>100	>100	0
	influenza (H5N1)	Vietnam/1203/2004H	32	62	1.9
	influenza type B	Florida/4/2006	42	>100	>2.4
(VII)	influenza A (H1N1)	Vietnam/1203/2004H	3.2	3.5	1.1
	influenza type B	Florida/4/2006	3.2	3.9	1.2
	SARS	Urbani	3.2	3.2	1
	Adeno	65089/Chicago	>100	>100	0
	influenza A (H1N1)	California/07/2009	31	72	2.3
	influenza (H3N2)	Brisbane/10/2007	>100	>100	0
	rhinovirus type 2	HGP	>100	>100	0

Note: The study was performed in the departments of the National Institute of Allergy and Infectious Diseases (www.niaid-aacf.org).

nopropyl)-*O*-(3-aminopropyl)) amidoxime)propoxyimino]-20(29)-lupen-28-oic acid methyl ester (**V**) was obtained with a yield of 67% by catalytic hydrogenolysis in compound (**IV**).

The available oxime of methyl betulonate derivative, having the structure of a nitrile group, is 2-cyano-3,4-secolupa-20(29)-ene-28-oic acid methyl ester (**VI**). Previously, this was used for the synthesis of acid [19], amide, and amine [11], 4,20-diketone [20]. In this paper, we engaged the nitrile compound (**VI**) in the synthesis of amidoxime (**VII**) and 3-amino-3,4-secobetulin (**VIII**). Cyanoethylation of compound (**VIII**) by acrylonitrile proceeded to the hydroxyl and amino groups to formation of *O,N*-di-(2-cyanoethyl)-derivative (**IX**). Judging from the spectral data, the substitution of one hydrogen atom occurred in the amino group. Displacement of the signal of the C28 atom in the spectrum of the ^{13}C NMR compound (**IX**) reaches ~2 ppm compared with the spectrum of derivative (**VIII**) (shown at δ 69.7 ppm). In the spectrum of ^{1}H NMR of compound (**IX**), the characteristic signals of protons H2', H4', H5', and H1' are observed as a multiplet at δ 2.54–2.67 and 3.42–3.84 ppm, respectively.

Retro-Michaelis dissociation on the C28 with the formation of 3-aminopropylamino-3,4-secobetulin (**X**) occurred in the reduction of compound (**IX**) with lithium aluminum hydride. Compound (**VIII**) is characterized as a *tert*-butoxycarbonate (**XI**) and *N*-acylates (**XII**), (**XIII**), containing fragments of 3- and 4-pyridinecarboxylic acids.

The investigation of the activity of several compounds obtained was carried out in units of the National Institute of Allergy and Infectious Diseases (NIAID, United States, www.niaid-aacf.org). According to the study of antiviral activity against respiratory infections, the selectivity index SI for compounds (**II**), (**VI**), and (**VII**) had a range from 0 to > 3.1 (Table 1). For compound (**VII**) the parameters of activity against hepatitis B virus (HBV) are CC_{50}[2] equal to 23 μmol/ml, EC_{90}[3] > 10 μmol/ml. With regard to the hepatitis C virus (HCV), the degree of suppression of replication of viral nucleic acid by compound (**VII**) was 98.6%, and the cytotoxicity (percentage of cells remaining alive) was 8.9%, SI < 1. Thus, promising new antiviral agents among the studied group of betulin derivatives were not revealed.

A percentage growth inhibition of the virulent *Micobacterium tuberculosis* (strain H37Rv) by betulin and its derivatives (**I**), (**II**), (**VI**), (**VII**), and (**XI**) in the studied concentration > 10 mcmol/ml amounted from –14.29 to 4.67%, indicating their low activity (Table 2). Oxime of betulonic acid and the compound (**VI**) inhibit the growth of the microbacteria by 77.55 and 60.06%; a sufficiently high activity may be associated with the aggregation of these substances with the bacteria.

[2] CC_{50} is the cytotoxic concentration 50%.
[3] EC_{90} is the effective concentration.

Table 2. Antituberculosis activity of triterpenoids against *Mycobacterium tuberculosis* (strain H37Rv)

Compound	Solubility in DMSO, mg/ml	Growth inhibition,%
Betulin	>10	2.51
Betulonic acid methyl ester	>10	–5.47
Oxime of betulonic acid	>10	77.55
(I)	>10	4.67
(II)	>10	–9.70
(VI)	>10	60.06
(VII)	>10	2.35
(XI)	>10	–14.29

Note: The study was performed in the departments of the National Institute of Allergy and Infectious Diseases (www.niaid-aacf.org).

EXPERIMENTAL

^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker AM-300 spectrometer (Germany, 300 and 75.5 MHz, respectively, δ, ppm, SSCC Hz) with a TMS internal standard. The melting points were determined on a Boetius microstage. Optical absorption was measured on a Perkin-Elmer 241 MC polarimeter (Germany) in a 1-dm tube. TLC was performed on Sorbfil plates (ZAO Sorbpolimer, Russia) using solvent system chloroform–ethyl acetate 40 : 1. Column chromatography was performed on neutral Al_2O_3 (Reachim). Compounds **(I)**, **(VI)** were obtained according to [7, 19]. Chlorides of nicotinic and isonicotinic acid were obtained as described [21, 22].

3-[2-(cyanoethoxy)imino]-20(29)-lupen-28-oic acid methyl ester (II). A mixture of 0.5 g (1 mmol) of compound **(I)**, 4 ml of acrylonitrile, 0.3 ml of 40% KOH in 15 ml of dioxane was stirred for 4 h at 20°C in an argon atmosphere, then the reaction mixture was poured onto 40 g of ice and 5 ml of HCl. The precipitate was filtered, washed with water and dried. The yield was 0.44 g (83%), R_f 0.82, mp 165–167°C. $[\alpha]_D^{20}$ –0.5° (*c* 1.07, $CHCl_3$). Anal. calcd. for $C_{34}H_{52}N_2O_3$ (MW 536.795): C, 76.08; H, 9.76; and N, 5.22. Found: C, 75.88; H, 9.42; and N, 5.01. ^{1}H NMR spectrum: 0.86, 0.92, 0.96, 1.04, 1.13 (15 H, 5 s, $5CH_3$), 1.20–2.00 (21 H, m, CH_2, CH), 1.69 (3 H, s, H30), 2.35–2.56 (3 H, m, H13, H16), 2.64–2.72 (2 H, t, *J* 6.3,H2'), 3.03–3.17 (1 H, m, H19), 3.65 (3 H, s, OCH_3), 4.14–4.25 (2 H, t, *J* 6.3, H1'), 4.62, and 4.75 (2 H, br. s, H29). ^{13}C NMR spectrum: 14.5, 15.7, 15.8, 17.9,18.3, 18.9 (C2'), 19.3, 21.1, 22.9, 25.4, 27.4, 29.5, 30.5, 32.0, 33.8, 36.8, 37.0, 38.2, 38.6, 40.2, 40.6, 42.3, 46.7, 49.3, 50.0, 51.1, 55.4, 56.4, 67.2 (C1'), 109.5 (C29), 117.8 (C3'), 150.3 (C20), 168.1 (C3), and 176.5 (C28).

3-[3-(amidoxime)propoxy)imino]-20(29)-lupen-28-oic acid methyl ester (III). 0.17 g of $NaHCO_3$ and a solution of 0.5 g (1 mmol) of compound **(II)** in 20 ml of ethanol were added to a solution of 0.14 g of $NH_2OH \cdot HCl$ in 0.5 ml of water. The reaction mass was refluxed for 6 h, and the solvent was evaporated under vacuum. The residue was dissolved in diethyl ether and dried over $CaCl_2$. The solvent was again evaporated in a vacuum. The residue was chromatographed on a column, and elution was carried out with benzene–chloroform. The yield was 0.39 g (67%), R_f 0.32, mp 210–212°C, and $[\alpha]_D^{20}$ +5° (*c* 0.29, $CHCl_3$). Anal. calcd. for $C_{34}H_{55}N_3O_4$ (MW 569.825): C, 71.67; H, 9.73; and N, 7.37. Found: C, 71.26; H, 9.56; and N, 6.94. ^{1}H NMR spectrum: 0.86, 0.92, 0.96, 1.04, 1.13 (15 H, 5 s $5CH_3$), 1.20–2.00 (21 H, m, CH_2, CH), 1.69 (3 H, s, H30), 2.35–2.56 (3 H, m, H13, H16), 2.67–2.78 (2 H, m, H2'), 3.03–3.17 (1 H, m, H19), 3.65 (3 H, s, OCH_3), 4.12–4.28 (2 H, m, H1'), 4.62 (2 H, br. s, H29), 5.19–5.28 (3 H, m, OH, NH_2). ^{13}C NMR spectrum: 14.5, 15.7, 15.8, 17.9, 18.3, 18.9 (C2'), 19.1, 19.3, 21.1, 22.9, 25.4, 27.4, 29.5, 30.5, 32.0, 33.8, 36.8, 37.0, 38.2, 38.6, 40.6, 42.3, 46.7, 49.3, 50.0, 51.1, 55.4, 56.4, 67.2 (C1'), 109.5 (C29), 150.5 (C20), 167.2 (C3), 174.2 (C3'), 176.5 (C28).

3-[3-((*N*-(2-cyanoethyl)-O-(2-cyanoethyl))amidoxime)propoxyimino]-20(29)-lupen-28-oic acid methyl ester (IV). A mixture of 1 mmol of compound **(III)**, 12 ml of acrylonitrile, and 0.6 ml of 40% KOH in 15 ml of dioxane was stirred for 20 hours at room temperature under argon, and then the reaction mixture was poured on 40 g of ice and 5 ml of HCl. The precipitate was filtered, washed with water, and dried. The residue was chromatographed on a column; the eluents were benzene and chloroform. The yield was 0.47 g (70%), R_f 0.35, mp 72–74°C, and $[\alpha]_D^{20}$ +38° (*c* 0.03, $CHCl_3$). Anal. calcd. for $C_{40}H_{61}N_5O_4$ (MW 675.951): C, 71.08; H, 9.10; and N, 10.36. Found: C, 70.83; H, 8.92; and N, 10.04. ^{1}H NMR spectrum: 0.86, 0.92, 0.96, 1.04, 1.13 (15 H, 5 s, $5CH_3$), 1.20–2.00 (21 H, m, CH_2, CH), 1.69 (3 H, s,

H30), 2.35–2.56 (5 H, m, H13, H16, H8'), 2.64–2.73 (4 H, m, H2', H5'), 3.03–3.17 (1 H, m, H19), 3.34–3.58 (2 H, m, H4'), 3.65 (3 H, s, OCH_3), 4.14–4.25 (3 H, m, H1', NH), 4.35–4.52 (2 H, m, H7'), 4.62 (2 H, br. s, H29). ^{13}C NMR spectrum: 14.5, 15.7, 15.8, 17.9, 18.3, 18.9 (C2'), 19.1 (C8'), 19.3, 21.1, 22.9, 23.4 (C5'), 25.4, 27.4, 29.5, 30.5, 32.0, 33.8, 36.8, 37.0, 38.2, 38.6, 40.2, 40.6, 41.2 (C4'), 42.3, 46.7, 49.3, 50.0, 51.1, 55.4, 56.4, 67.2 (C1'), 68.4 (C7'); 109.5 (C29), 113.8 (C9'), 114.5 (C6'), 150.4 (C20), 167.3 (C3), 174.1 (C3'), and 176.5 (C28).

3-[3-((*N*-(3-aminopropyl)-O-(3-aminopropyl))amidoxime)propoxyimino]-20(29)-lupen-28-oic acid methyl ester (V). Hydrogen was passed in an autoclave for 8 h at 100 atm through a solution of 0.67 g (1 mmol) of compound (**IV**) in 30 ml of methanol containing 0.14 g of the Ni-Raney catalyst. The catalyst was filtered, and the mixture was poured into 100 ml of water. The precipitate was washed with water and dried. The yield was 0.46 g (67%), R_f 0.22, mp 106–108°C, and $[\alpha]_D^{20}$ +41° (*c* 0.09, CH_3OH). Anal. calcd. for $C_{40}H_{69}N_5O_4$ (MW 684.015): C, 70.24; H, 10.17; and N, 10.24. Found: C, 70.03; H, 9.96; and N, 10.07. ^1H NMR spectrum: 0.86, 0.92, 0.96, 1.04, 1.13 (15 H, 5 s, $5CH_3$), 1.20–2.00 (30 H, m, CH_2), CH, H5', H8', NH, $2NH_2$), 1.69 (3 H, s, H30), 2.35–2.56 (3 H, m, H13, H16), 2.64–2.88 (6 H, m, H2', H6', H9'), 3.03–3.34 (3 H, m, H19, H4'), 3.65 (3 H, s, OCH_3), 4.14–4.25 (4 H, m, H1', H7'), 4.62 (2 H, br. s, H29). ^{13}C NMR spectrum: 14.5, 15.7, 15.8, 17.9, 18.3, 18.9, 19.1, 19.3, 21.1, 22.9, 23.4, 25.4, 27.4, 29.5, 30.5, 32.0, 33.8, 36.8, 37.0, 38.2, 38.6, 39.8, 40.2, 40.6, 41.2, 42.3, 43.2, 46.7, 49.3, 50.0, 51.1, 55.4, 56.4, 67.2 (C1'), 68.4 (C7'), 109.5 (C29), 150.3 (C20), 168.1 (C3), 174.2 (C3'), and 176.6 (C28).

3,4-seco-3-amidoxime-4(23),20(29)-lupadien-28-oic acid methyl ester (VII) was prepared from 0.7 g (1 mmol) of compound (**VI**) analogously to compound (**III**). The yield was 0.40 g (80%), R_f 0.25, mp 138–140°C, and $[\alpha]_D^{20}$ +31° (*c* 0.13, $CHCl_3$). Anal. calcd. for $C_{31}H_{50}N_2O_3$ (MW 498.746): C, 74.66; H, 10.10; and N, 5.62. Found: C, 74.44; H, 9.93; and N, 5.41. ^1H NMR spectrum: 0.72, 0.92, 0.98 (9 H, 3 s, $3CH_3$), 1.20–2.00 (21 H, m, CH_2, CH), 1.63 (3 H, s, H24), 1.67 (3 H, s, H30), 2.19–2.48 (3 H, m, H13, H16), 2.79–3.07 (1 H, m, H19), 3.65 (3 H, s, OCH_3), 4.71 (2 H, d, *J* 7.3, H23), 4.59 and 4.81 (2 H, br. s, H29), 5.21–5.33 (3 H, m, OH, NH_2). ^{13}C NMR spectrum: 14.7, 15.0, 16.0, 19.3, 20.4, 22.5, 24.4, 25.0, 25.9, 29.6, 30.5, 32.0, 32.7, 36.7, 37.4, 38.2, 39.2, 40.4, 40.7, 42.7, 46.9, 49.4, 50.7, 51.1, 53.3, 109.6 (C29), 113.1 (C23), 148.4 (C4), 150.4 (C20), 154.4 (C3), and 176.5 (C28).

3,4-seco-3-amino-28-hydroxy-4(23),20(29)-lupadien (VIII). To a solution of 0.5 g (1 mmol) of compound (**VI**) in 50 ml dry THF under vigorous stirring was added 467.4 mg (12.3 mmol) $LiAlH_4$. The mixture was refluxed for 6 h. To the reaction mixture was added 3 ml of H_2O, and the precipitate $Al(OH)_3$ was filtered. The aqueous layer was extracted with chloroform (3 × 60 ml), and dried over $CaCl_2$. The solvent was evaporated under vacuum. The residue was chromatographed on a column; the eluents were chloroform and chloroform–methanol (50 : 1). The yield was 0.36 g (82%), R_f 0.17, mp 210–212°C, and $[\alpha]_D^{20}$ +47.8° (*c* 0.45, $CHCl_3$). Anal. calcd. for $C_{30}H_{51}NO$ (MW 441.739): C, 81.57; H, 11.64; and N, 3.17. Found: C, 81.14; H, 11.29; and N, 2.63. ^1H NMR spectrum: 0.72, 0.92, 0.98 (9 H, 3s, $3CH_3$), 1.20–2.00 (22 H, m, CH_2, CH), 1.63 (3 H, s, H24), 1.67 (3 H, s, H30), 2.19–2.63 (5 H, m, H13, H16, H3), 3.23–3.29 (1 H, m, H28b), 3.32–3.47 (1 H, m, H19), 3.61–3.79 (3 H, m, H28a, NH_2), 4.53 (2 H, d, *J* 18.4, H23), and 4.63 (2H, br. s, H29). ^{13}C NMR spectrum: 14.6, 15.0,16.0,19.1, 20.4, 21.4, 23.1, 24.6, 25.1, 27.0, 29.1, 29.8, 32.7, 33.9, 36.7, 37.4, 39.1, 40.5, 40.6, 42.8, 43.1 (C3), 47.7, 48.7, 50.4, 60.3 (C5), 67.9 (C28), 109.6 (C29), 112.8 (C23), 148.2 (C4), and 150.4 (C20).

3,4-seco-3-(2-cyanoethylamino)-28-(2-cyanoethoxy)-4(23),20(29)-lupadien (IX) was prepared from 0.4 g (1 mmol) of compound (**VIII**) analogously to compound (**IV**). Yield 0.44 g (81%), R_f 0.73, mp 132–134°C. $[\alpha]_D^{20}$ +27.3° (*c* 0.35, $CHCl_3$). Anal. calcd. for $C_{36}H_{57}N_3O$ (MW 547.865): C, 78.92; H, 10.49; and N, 7.67. Found: C, 78.61; H, 10.06; and N, 7.20. ^1H NMR spectrum: 0.72, 0.92, 0.98 (9 H, 3s, $3CH_3$), 1.20–2.00 (21 H, m, CH_2, CH), 1.63 (3 H, s, H24), 1.69 (3 H, s, H30), 2.21–2.46 (3 H, m, H13, H16), 2.51–2.64 (8 H, m, H3, H2', H3', H4'), 3.26 (1 H, d, *J* 10.6, H28b), 3.40–3.78 (5 H, m, H19, H28a, H1', NH), 4.53 (2 H, d, *J* 18.6, H23), 4.63 (2 H, br. s, H29). ^{13}C NMR spectrum: 14.7, 15.0, 15.1, 18.7 (C2', C5'), 19.0, 20.1, 21.4, 22.4, 24.8, 27.1, 29.7, 29.8, 32.4, 34.5, 36.1, 37.4, 39.1 (C2), 39.3, 40.5, 40.7., 42.6, 42.9, 47.2 (C4'), 47.9, 48.3, 48.5 (C3), 50.8, 66.0 (C1'), 69.7 (C28), 109.6 (C29), 113.5 (C23), 116.2 (C3'), 117.8 (C6'), 147.6 (C4), and 150.2 (C20).

3,4-seco-3-(3-aminopropylamino)-28-hydroxy-4 (23), 20(29)-lupadien (X) was prepared from 0.5 g (1 mmol) of compound (**IX**) analogously to compound (**VIII**). The yield was 0.37 g (74%). R_f 0.16, mp 173–175°C, and $[\alpha]_D^{20}$ +23° (*c* 0.21, CH_3OH). Anal. calcd. for $C_{33}H_{58}N_2O$ (MW 498.834): C, 79.46; H, 11.72; and N, 5.62. Found: C, 79.18; H, 11.36; and N, 5.23. ^1H NMR spectrum: 0.72, 0.92, 1.01 (9 H, 3 s, $3CH_3$), 1.20–2.00 (24 H, m, CH_2, CH, H2'), 1.58 (3 H, s, H24), 1.61 (3 H, s, H30), 2.23–2.38 (3 H, m, H13, H16), 2.51–2.69 (6 H, m, H3, H1', H2'), 3.16–3.24 (1 H, d, *J* 9.1, H28b), 3.29–3.57 (4 H, m, H19, NH, NH_2), 3.64–3.73 (1 H, d, *J* 9.3, H28a), 4.46–4.52 (2 H, d, *J* 18.9, H23), 4.61 and 4.75 (2 H, br. s, H29).

^{13}C NMR spectrum: 14.6, 15.0,16.0, 19.1, 20.4, 21.4, 23.1, 24.6, 25.1, 27.0, 28.4, 29.1, 29.8, 30.6, 32.7, 33.9, 35.3, 36.7, 37.4, 39.1, 40.5, 40.6, 42.8, 43.1 (C3), 47.7, 48.7, 50.4, 60.3 (C5), 67.9 (C28), 109.6 (C29), 112.8 (C23), 148.2 (C4), and 150.4 (C20).

3,4-seco-3-*tert*-butoxycarbonylamino-28-hydroxy-4(23),20(29)-lupadien (XI). To a solution of 0.44 g (1 mmol) of compound (**VIII**) in 20 ml CH_2Cl_2 was added 350 mg (1.6 mmol) of Boc_2O. The mixture was stirred at room temperature for 12 h. The solvent was evaporated under vacuum. The residue was chromatographed on a column; the eluent was benzene. The yield was 0.43 g (80%). R_f 0.45, mp 98–100°C, and $[\alpha]_D^{20}$ −35° (*c* 0.17, $CHCl_3$). Anal. calcd. for $C_{35}H_{59}NO_3$ (MW 541.855): C, 77.58; H, 10.97; and N, 2.58. Found: C, 76.92; H, 10.22; and N, 1.93. ^{1}H NMR spectrum: 0.72, 0.92, 0.98 (9 H, 3 s, $3CH_3$), 1.20–2.00 (21 H, m, CH_2, CH), 1.45 and 1.48 (9 H, two s, $3CH_3$), 1.63 (3 H, s, H24), 1.67 (3 H, s, H30), 2.19–2.48 (3H, m, H13, H16), 2.48–2.63 (2 H, m, H3), 3.23–3.29 (1 H, m, H28b), 3.32–3.47 (2 H, m, H19, OH), 3.61–3.79 (1 H, m, H28a), 4.53 (2 H, d, *J* 18.8, H23), 4.58 and 4.69 (2 H, br. s, H29), 5.31 (1 H, m, NH). ^{13}C NMR spectrum: 14.6, 15.0, 16.0, 19.1, 20.4, 21.4, 23.1, 24.6, 25.1, 27.0. 3 × 28.4 ($3CH_3$), 29.1, 29.8, 32.7, 33.9, 36.7, 37.4, 39.1, 40.5, 40.6, 42.8, 43.1 (C3), 47.7, 48.7, 50.4, 60.3 (C5), 67.9 (C28), 78.4 (C2'), 109.6 (C29), 112.8 (C23), 148.2 (C4), 150.4 (C20), and 155.8 (C1').

Methods of synthesis of compounds (XII), (XIII). To a solution of 0.44 g (1 mmol) of compound (**VIII**) in 15 ml of anhydrous pyridine was added 1.5 mmol of freshly prepared chloride of nicotinic or isonicotinic acid, 0.1 mg of dimethylaminopyridine, and refluxed for 4 h. The reaction mixture was poured into 200 ml of 5% solution of HCl. The precipitate filtered, washed with water, dried and the residue was purified by column chromatography; the eluent was chloroform.

3,4-seco-3-amino-28-nicotinoiloxy-4(23),20(29)-lupadien (XII). The yield was 0.41 g (75%). R_f 0.15, mp 120–122°C, and $[\alpha]_D^{20}$ +41.5° (*c* 0.03, $CHCl_3$). Anal. calcd. for $C_{36}H_{54}N_2O_2$ (MW 546.834): C, 79.07; H, 9.95; and N, 5.12. Found: C, 78.64; H, 9.73; and N, 4.86. ^{1}H NMR spectrum: 0.72, 0.92, 0.98 (9 H, 3s, $3CH_3$), 1.20–2.00 (23 H, m, CH_2, CH, NH_2), 1.63 (3 H, s, H24), 1.67 (3 H, s, H30), 2.19–2.63 (5 H, m, H3, H13, H16), 3.32–3.47 (1 H, m, H19), 4.11 (1 H, d, *J* 11.1, H28b), 4.51 (1 H, d, *J* 10.9, H28a), 4.59 (2 H, d, *J* 18.9, H23), 4.60 and 4.77 (2 H, br. s, H29), 7.46 (1 H, dd, *J* 3.8, 4.7, H5'), 8.28 (1 H, ddd, *J* 4.7, 1.6, 1.7, H4'), 8.71 (1 H, t, *J* 4.1, H6'), and 9.22 (1 H, dd, *J* 1.8, 5.9, H2"). ^{13}C NMR spectrum: 14.7, 16.0, 19.1, 20.4, 21.4, 22.8, 24.4, 25.1, 27.1, 29.6, 29.8, 32.6, 34.6, 36.7, 37.8, 39.2, 40.6, 40.7, 42.8, 43.1 (C3), 47.8, 48.8, 50.7, 60.3 (C5), 63.7 (C28), 110.0 (C29), 113.0 (C23), 123.4 (C5'), 126.3 (C3'), 136.9 (C4'), 147.7 (C2'), 149.9 (C4), 150.8 (C20), 151.9,153.3 (C6'), and 165.7 (C31).

3,4-seco-3-isonicotinoilamino-28-isonicotinoiloxy-4(23),20(29)-lupadien (XIII). The yield was 0.44 g (80%). R_f 0.15, mp 105–107°C, and $[\alpha]_D^{20}$ +21.4° (*c* 0.86, $CHCl_3$). Anal. calcd. for $C_{42}H_{57}N_3O_3$ (*MW* 546.834): C, 77.38; H, 8.81; and N, 6.45. Found: C, 76.94; H, 8.32; and N, 6.03. ^{1}H NMR spectrum: 0.72, 0.92, 0.98 (9 H, 3s, $3CH_3$), 1.20–2.00 (21 H, m, CH_2, CH), 1.63 (3 H, s, H24), 1.67 (3 H, s, H30), 2.19–2.48 (3 H, m, H13, H16), 2.48–2.63 (2 H, m, H3), 3.21–3.48 (1 H, m, H19), 4.02–4.12 (1 H, d, *J* 11, H28b), 4.34–4.42 (1 H, br. s, NH), 4.48–4.55 (1 H, d, *J* 11, H28a), 4.59 (2 H, m, H23), 4.71 (2 H, br. s, H29), 7.59 (2 H, dd, *J* 5.9, H2', H2"), 7.79 (2 H, d, *J* 5.9, H4', H4"), 8.65 (2 H, dd, *J* 1.4, 3.1, H1', H5'), 8.74 (2 H, dd, *J* 1.4, 3.1, H1", H5"). ^{13}C NMR spectrum: 14.8,16.1,19.2, 20.5, 21.4, 22.7, 22.8, 24.4, 25.1, 27.1, 29.6, 29.8, 32.6, 34.6, 36.7, 37.8, 39.2, 40.6, 40.7, 43.1 (C3), 46.7, 47.7, 48.8, 50.6. 53.5, 64.1 (C5), 110.1 (C29), 113.1 (C23), 120.9 (C3', C5'), 122.8 (C3", C5"), 137.6 (C4'), 141.9 (C4"), 148.2 (C4), 149.9 (C20), 150.4 (C2', C6'), 150.6 (C2", C6"), 165.4 (C31'), and 165.5 (C31").

3β-(3-aminopropoxyamino)-lupan-28-oic acid methyl ester (XIV). To a solution of 0.5 g (1 mmol) of compound (**II**) and 0.26 g (12.5 mmol) of $NaBH_4$ in 20 ml dry THF was added 7 ml of $BF_3 \cdot Et_2O$ in 10 ml of THF under vigorous stirring. Then the solution was refluxed for 5 h in an argon atmosphere. The reaction mass was poured into 100 ml of 2M NaOH solution. The precipitate was filtered, washed with water, and dried. The yield was 0.39 g (72%). R_f 0.21, mp 198–200°C, and $[\alpha]_D^{20}$ −10° (*c* 0.06, CH_3OH). Anal. calcd. for $C_{34}H_{60}N_2O_3$ (MW 544.858): C, 74.95; H, 11.10; and N, 5.14. Found: C, 74.63; H, 10.84; N, 4.91. ^{1}H NMR spectrum: 0.59–0.68 (3 H, m, N<u>H</u>, N<u>H</u>$_2$), 0.78, 0.81, 0.86, 0.92, 0.96,1.04, 1.13 (21 H, 7s, $7CH_3$), 1.20–2.00 (24 H, m, CH_2, CH, H2'), 2.39–2.56 (3 H, m, H13, H16), 2.79–3.06 (4 H, m, H3, H19, H3'), 3.29–3.41 (2 H, m, H1'), 3.61 (3 H, s, OCH_3). ^{13}C NMR spectrum: 14.3,15.0,15.6,17.8, 18.0, 18.4, 20.6, 21.3, 26.0 (C2'), 26.6, 26.9, 27.8, 28.3, 29.4, 32.3, 34.1, 37.1, 37.8, 38.0, 39.0, 40.4 (C3'), 40.6, 42.3, 45.0, 48.3, 48.8, 50.2, 50.9, 55.7, 56.9, 59.2, 63.2 (C3), 70.3 (C1'), and 176.8 (C28).

Methods of studying the antiviral and antituberculous activity of betulin, betulonic acid methyl ester, oxime of betulonic acid, compounds (**I**), (**II**), (**VI**), (**VII**), and (**XI**) are given at www.niaid-aacf.org.

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REFERENCES

1. Boreko, E.I., Pavlova, N.I., Savinova, O.V., Flekhter, O.B., Nigmatullina, L.R., Baltina, L.A., Galin, F.Z., and Tolstikov, G.A., BY Patent No. 7811, 2005.
2. Savinova, O.V., Pavlova, N.I., and Boreko, E.I., *Antibiot. Khimioter.*, 2009, vol. 54, pp. 16–20.
3. Flekhter, O.B., Boreko, E.I., Nigmatullina, L.R., Pavlova, N.I., Medvedeva, N.I., Nikolaeva, S.N., Tret'yakova, E.V., Savinova, O.V., Baltina, L.A., Karachurina, L.T., Galin, F.Z., Zarudii, F.S., and Tolstikov, G.A., *Khim.-Farm. Zh.,* 2004, vol. 38, pp. 31–34.
4. White, A., Horsington, E.J., Nedjar, N., and Peakman, T.M., *Tetrahedron Lett.*, 1998, vol. 39, pp. 3031–3034.
5. Sun, I.-C., Wang, H.-K., Kashiwada, Y., Shen, J.-K., Cosentino, L.M., Chen, C.-H., Yang, L.-M., and Lee, K.-H., *J. Med. Chem.*, 1998, vol. 41, pp. 4648–4657.
6. Peakman, T.M., Haven, H.L., and Rullkotter, J., *Tetrahedron*, 1991, vol. 47, pp. 3779–3786.
7. Ma, C.-M., Cai, S.-Q., Cui, J.-R., Wang, R.-Q., Tu, P.-F., Hattori, M., and Daneshtalab, M., *Eur. J. Med. Chem.,* 2005, vol. 40, pp. 582–589.
8. Ma, C.-M., Wu, X.-H., Hattori, M., Wang, X.-J., and Kano, Y., *J. Pharm. Pharmac. Sci.*, 2009, vol. 12, pp. 243–248.
9. Valterova, I., Klinot, J., Sumanova, V., and Vystrcil, A., *Collect. Czech. Chem. Commun.,* 1983, vol. 48, pp. 649–661.
10. Zaprutko, L., Partyka, D., and Bednarczyk-Cwynar, B., *Bioorg. Med. Chem. Lett.*, 2004, vol. 14, pp. 4723–4726.
11. Kumar, S., Misra, N., Raj, K., Srivastava, K., and Puri, S.K., *Nat. Prod. Res.*, 2008, vol. 22, pp. 305–319.
12. Dunkelblum, E., *Tetrahedron*, 1972, vol. 28, pp. 3879–3883.
13. Monato, S.B., Banerjee, S.K., and Chakavarti, R.N., *Bull. Calcutta Sch. Trop. Med.*, 1968, vol. 16, pp. 122–125.
14. Evers, M., Poujade, C., Soler, F., Ribeill, Y., James, C., Lelievre, Y., Gueguen, J.-C., Reisdorf, D., Morize, I., Pauwels, R., De Clercq, E., Henin, Y., Bousseau, A., and Mayaux, J.-F., Le Pecq J.-B., Dereu N, *J. Med. Chem.*, 1996, vol. 39, pp. 1056–1068.
15. Suokas, E. and Hase, T., *Acta Chem. Scand., Ser. B,* 1974, vol. 28, pp. 793–796.
16. Semmler, F.W., Jonas, K.G., and Richter, W., *Ber. Dtsch. Chem. Ges.,* 1918, vol. 51, pp. 417–424.
17. Bilham, P., Kon, G.A.R., and Ross, W.C.J., *J. Chem. Soc.*, 1942, vol. 36, pp. 35–42.
18. Ouattara, M., Wein, S., Denoyelle, S., Ortial, S., Durand, T., Escale, R., Vial, H., and Vo-Hoang, Y., *Bioorg. Med. Chem. Lett.*, 2009, vol. 19, pp. 624–626.
19. Klinot, J., Sumanova, V., and Vystrcil, K., *Collect. Czech. Chem. Commun.*, 1972, vol. 37, pp. 603–609.
20. Flekhter, O.B., Medvedeva, N.I., and Suponitsky, K.Yu., *Acta Crystallogr.*, 2007, vol. E63, p. o2603.
21. Naumova, B.S., Chekmareva, I.B., Zhdanovich, E.S., and Preobrazhenskii, N.A., *Khim.-Farm. Zh.*, 1969, vol. 3, pp. 11–12.
22. Meyer, H. and Graf, R., *Berichte der Deutschen Chemischen Gesllschaft LXI*, 1928, vol. 2, pp. 2202–2215.