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SYNTHESIS AND BIOLOGICAL ACTIVITY OF AMIDES OF 28-METHOXY-28-OXO-1-CYANO-2,3-*seco*-LUP-20(29)-EN-3-OIC ACID

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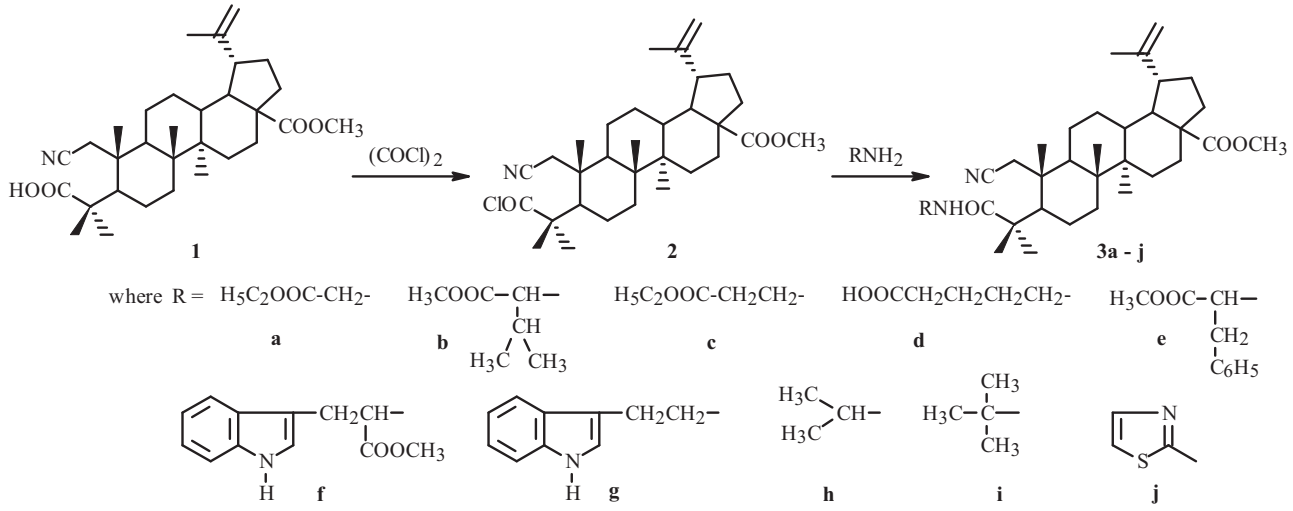
New A-seco-lupane C-3 amide conjugates were prepared by the reaction of 28-methoxy-28-oxo-1-cyano-2,3-seco-lup-20(29)-en-3-oic acid chloride with primary amines and synthetic and natural amino acids. Ethyl-N-*[28-methyl-28-oxo-1-cyano-2,3-seco-lup-20(29)-en-3-oyl]*-β-*alaninate, which was cytotoxic against rhabdomyosarcoma tumor cells (IC_{50} 47.5 μM), in addition to ethyl*-N-*[28-methoxy-28-oxo-1-cyano-2,3-seco-lup-20(29)-en-3-oyl]glycinate and* N-*[28-methyl-28-oxo-1-cyano-2,3-seco-lup-20(29)-en-3-oyl]-2-aminothiazole, which inhibited reproduction of herpes simplex virus type 1 (EC_{50} 35.1 and 30.9 μM, respectively), were selected.*

Keywords: A-*seco*-triterpenoids, betulin, betulonic acid, amides, amino acids, cytotoxic activity, antiviral activity, herpes simplex virus type 1.

A promising direction in the synthesis of biologically active compounds is the modification of the carbon framework of available triterpenoids. In this respect, targeted synthesis of A-*seco*-triterpenoids, natural analogs of which exhibit antitumor, antiviral, and bactericidal activity [1], is interesting. In particular, approaches to the synthesis of biologically active 2,3-*seco*-triterpenoids were proposed [2–6]. However, further functionalization of semi-synthetic 2,3-*seco*-triterpenoids is practically not discussed in the literature [2, 7, 8]. Nevertheless, it was shown that the cytotoxic activity of, for example, 1-cyano-2,3-*seco*-19β,28-epoxy-18α-olean-3-oic acid could be increased by modifying the C-3 carboxylic acid with the ethyl ester of β-alanine [9]. Herein we describe the synthesis and present results from a study of the effect of the C-3 substituent on the cytotoxic and antiviral activity of 28-methoxy-28-oxo-1-cyano-2,3-*seco*-lup-20(29)-en-3-oic acid amide conjugates.



Scheme 1

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TABLE 1. Cytotoxic Activity of Amides **3a**–**c** and **3e**–**3j**

Compound	IC_{50}, μM			Compound	IC_{50}, μM		
	RD TE32	A549	MS		RD TE32	A549	MS
3a	170.5 ± 1.6	169.4 ± 17.6	161.8 ± 1.3	**3g**	> 200	174.8 ± 81.5	148.8 ± 7.8
3b	> 200	> 200	77.6 ± 2.5	**3h**	> 200	> 200	> 200
3c	47.5 ± 1.4	> 200	> 200	**3i**	125.5 ± 0.8	> 200	114.9 ± 7.4
3e	> 200	> 200	> 200	**3j**	111.4 ± 8.5	> 200	85.2 ± 2.2
3f	> 200	> 200	161.2 ± 18.4				

TABLE 2. Antiviral Activity Against Herpes Virus of Amides **3a**–**e**, **3h**, and **3j**

Compound	EC_{50} (I_{95}), μM	MTC/EC_{50} ratio	Compound	EC_{50} (I_{95}), μM	MTC/EC_{50} ratio
3a	35.1 (38.1 ÷ 32.2)	2.5	**3e**	> 1600	< 1
3b	> 400	< 1	**3h**	> 400	< 1
3c	> 50	< 1	**3j**	30.9 (33.5 ÷ 28.4)	2.9
3d	> 1000	< 1			

Amides **3a**–**j** were synthesized via the reaction of 28-methoxy-28-oxo-1-cyano-2,3-*seco*-lup-20(29)-en-3-oic acid chloride (**1**) [9] with primary amines and natural and synthetic amino acids. The yields of **3a**–**j** after purification using column chromatography over silica gel were 25–56% (Scheme 1).

The structures of **3a**–**j** were confirmed by IR and NMR spectroscopy. PMR spectra of **3a**-**j** had a characteristic resonance for the amide proton in the range 5.51–6.43 ppm. The resonance of the thiazole 3′-amine proton appeared at weak field (9.52 ppm) in the PMR spectrum amide **3j** because of amine–imine tautomerism (analogous to that described earlier for the oleanane analog [9]). The resonance of the C-3 atom in ^{13}C NMR spectra of **3a**–**j** was found in the range 176.59–179.24 ppm. The formation of an amide bond was confirmed by the presence in IR spectra of **3a**–**j** of characteristic absorption bands in the ranges 3182–3448 and 1629–1668 cm^{-1}.

Cytotoxic activity of **3a**–**j** against human tumor cell lines rhabdomyosarcoma RD TE32, lung carcinoma A549, and melanoma MS was estimated by the standard MTT test [10]. Table 1 shows that cell lines RD TE32 and MS were more sensitive to administration of the synthesized amides. However, the IC_{50} index was greater than 100 μM for the majority of the tested compounds. Amide **3c** showed the highest cytotoxic activity. Its IC_{50} level against cell line RD TE32 was 47.5 μM.

Antiviral activity of 2,3-*seco*-triterpene amides **3a**–**e**, **3h**, and **3j** was studied against herpes simplex virus type 1 (Table 2) and HIV-1. Compounds **3a** and **3j** were most active for suppressing reproduction of herpes virus. The EC_{50} indices of these were 35.1 and 30.9 μM, respectively. Inhibitors of HIV-1 were not found among **3a**–**e**, **3h**, and **3j**.

New A-*seco*--triterpene amides based on 28-methoxy-28-oxo-1-cyano-2,3-*seco*-lup-20(29)-en-3-oic acid were prepared. Although the synthesized compounds exhibited only moderate cytotoxic activity, this property diminished their attractiveness as antiviral agents. Thus, regardless of the pronounced inhibiting activity of the ethylglycinate or thiazole conjugates against herpes virus, the cytotoxic properties of these derivatives do not enable them to be regarded as promising therapeutic compounds.

EXPERIMENTAL

IR spectra were recorded in $CDCl_3$ solution on an IFS 66/S IR-Fourier spectrometer (Bruker, Germany). PMR and ^{13}C NMR spectra were recorded in $CDCl_3$ solution on a Mercury+ spectrometer (Varian, USA) at operating frequency 300 and 75.5 MHz, respectively (HMDS internal standard). Specific optical rotation was measured for $CHCl_3$ solutions on a 341 polarimeter (Perkin–Elmer, USA) at wavelength 589 nm. The threshold melting point at heating rate 1°C/min was determined on an OptiMelt MPA100 instrument (USA). Column chromatography was performed over silica gel (60–200 μm, Lancaster, Great Britain) with compound:sorbent ratio ~1:50 and hexane:EtOAc eluent (5:1). TLC used Sorbfil plates (Russia). Compounds were detected by phosphomolybdic acid solution (20%) in EtOH with subsequent heating at 100–120°C for 2–3 min.

Anhydrous solvents were prepared by standard methods [11]. 28-Methoxy-28-oxo-1-cyano-2,3-*seco*-lup-20(29)-en-3-oic acid (**1**) was prepared by the published method [12].

Preparation of 28-Methoxy-28-oxo-1-cyano-2,3-*seco*-lup-20(29)-en-3-oic Acid Amides (3a–j). A solution of **1** (1.1 mmol) in anhydrous CH_2Cl_2 (10 mL) under Ar was treated with oxalylchloride (0.2 mL, 2.2 mmol). The mixture was stirred at room temperature for 6 h. The solvent was distilled to dryness *in vacuo* (water aspirator) at water-bath temperature 30°C. The residue was dissolved in CH_2Cl_2 (10 mL). The solvent was distilled off. The procedure was repeated three times. The resulting suspension of acid chloride **2** in anhydrous CH_2Cl_2 (10 mL) under Ar was treated with amine (1.2 mmol) and Et_3N (0.17 mL, 1.2 mmol) [Commercially available amino-acid hydrochlorides (**a**–**f**) were converted beforehand to the free acids. A solution of amino-acid hydrochloride (1.2 mmol) in anhydrous CH_2Cl_2 (20 mL) under Ar was treated with Et_3N (0.17 mL, 1.2 mmol) and stirred for 1 h.] The mixture was stirred for 4–6 h at room temperature. The course of the reaction was monitored by TLC. The solvent was evaporated. The residue was purified by column chromatography.

Ethyl-*N*-[28-methoxy-28-oxo-1-cyano-2,3-*seco*-lup-20(29)-en-3-oyl]glycinate (3a), $C_{35}H_{54}N_2O_5$, yield 0.24 g (40%), R_f 0.3 ($CHCl_3$:EtOAc, 10:1), mp 65.4°C (hexane:EtOAc), $[\alpha]_D^{25}$ +3.5° (*c* 0.3, $CHCl_3$). IR spectrum (ν, cm^{-1}): 1651 (CONH), 1726 ($COOCH_3$, $COOC_2H_5$), 2239 (C≡N), 3388 (NH).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.92, 0.95, 1.01, 1.21, 1.24 (15H, 5s, $5CH_3$), 1.27 (3H, t, J = 7.2, $COOCH_2C\underline{H}_3$), 1.67 (3H, s, 3H-30), 2.40 and 2.50 (2H, 2d, J_{AB} = 18.3, 2H-1, AB-system), 2.96–3.01 (1H, m, H-19), 3.65 (3H, s, $COOCH_3$), 3.84 (1H, dd, J = 18.2, 3.8, $C\underline{H}_2NH$), 4.17–4.32 (3H, m, $COOC\underline{H}_2CH_3$, $C\underline{H}_2NH$), 4.59 and 4.72 (2H, 2s, 2H-29), 6.29 (1H, br.t, NH).

^{13}C NMR spectrum (75.5 MHz, $CDCl_3$, δ, ppm): 14.11, 14.65, 15.64, 18.40, 19.17, 20.19, 21.59, 22.05, 25.35, 28.25, 28.91, 29.68, 30.41, 31.88, 33.29, 36.81, 38.13, 40.53, 41.57, 42.34, 42.77, 44.99, 45.74, 46.88, 49.14, 51.29, 51.83, 56.47, 61.47, 109.85 (C-29), 118.90 (C-2), 150.24 (C-20), 169.97, 176.60 (C-28), 179.09 (C-3).

Methyl-*N*-[28-methoxy-28-oxo-1-cyano-2,3-*seco*-lup-20(29)-en-3-oyl]-L-valinate (3b), $C_{37}H_{58}N_2O_5$, yield 0.27 g (44%), R_f 0.7 ($CHCl_3$:EtOAc, 10:1), mp 171.8°C (hexane:EtOAc), $[\alpha]_D^{25}$ +11.1° (*c* 0.4, $CHCl_3$). IR spectrum (ν, cm^{-1}): 1659 (CONH), 1723, 1732 ($2COOCH_3$), 2246 (C≡N), 3356 (NH).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.92, 0.95, 1.02, 1.27, 1.29 (15H, 5s, $5CH_3$), 0.93 and 0.94 (6H, 2d, J = 6.9, $2CH_3$), 1.67 (3H, s, 3H-30), 2.40 and 2.53 (2H, 2d, J_{AB} = 18.5, 2H-1, AB-system), 2.99 (1H, td, J = 10.5, 5.1, H-19), 3.65 and 3.73 (6H, 2s, $2COOCH_3$), 4.49 (1H, dd, J = 8.1, 4.8, $C\underline{H}NH$), 4.60 and 4.72 (2H, 2s, 2H-29), 6.17 (1H, br.d, NH).

^{13}C NMR spectrum (75.5 MHz, $CDCl_3$, δ, ppm): 14.63, 15.68, 18.04, 18.51, 18.85, 19.17, 20.87, 21.71, 24.08, 25.43, 27.10, 29.26, 29.60, 30.44, 30.73, 31.88, 33.20, 36.81, 38.22, 40.52, 42.13, 42.77, 45.14, 46.69, 46.89, 49.16, 50.56, 51.25, 52.06, 56.50, 57.34, 109.82 (C-29), 118.90 (C-2), 150.25 (C-20), 172.41, 176.60 (C-28), 178.37 (C-3).

Ethyl-*N*-[28-methoxy-28-oxo-1-cyano-2,3-*seco*-lup-20(29)-en-3-oyl]-*β*-alaninate (3c), $C_{36}H_{54}N_2O_5$, yield 0.29 g (49%), R_f 0.3 ($CHCl_3$:EtOAc, 10:1), mp 74.0°C (hexane:EtOAc), $[\alpha]_D^{25}$ –9.0° (*c* 0.4, $CHCl_3$). IR spectrum (ν, cm^{-1}): 1640 (CONH), 1723 ($COOC_2H_5$), 2237 (C≡N), 3384 (NH).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.92, 0.94, 1.01, 1.17, 1.21 (15H, 5s, $5CH_3$), 1.25 (3H, t, J = 7.2, $COOCH_2C\underline{H}_3$), 1.67 (3H, s, 3H-30), 2.38 and 2.51 (2H, 2d, J_{AB} = 18.2, 2H-1, AB-system), 2.56 (2H, t, J = 6.2, $CH_2C\underline{H}_2COOC_2H_5$), 2.93–3.02 (1H, m, H-19), 3.37–3.46 (2H, m, $CONHC\underline{H}_2$), 3.65 (3H, s, $COOCH_3$), 4.14 (2H, q, J = 7.2, $COOC\underline{H}_2CH_3$), 4.60 and 4.72 (2H, 2s, 2H-29), 6.43 (1H, br.t, NH).

^{13}C NMR spectrum (75.5 MHz, $CDCl_3$, δ, ppm): 14.18, 14.64, 15.64, 18.15, 19.19, 20.28, 21.65, 22.04, 25.40, 28.17, 29.08, 29.60, 30.44, 31.89, 33.26, 33.56, 35.37, 36.82, 38.17, 40.54, 42.37, 42.79, 44.95, 45.63, 46.89, 49.18, 51.26, 51.88, 56.50, 60.70, 109.84 (C-29), 119.01 (C-2), 150.23 (C-20), 172.53, 176.59 (C-28), 178.98 (C-3).

***N*-[28-Methyl-28-oxo-1-cyano-2,3-*seco*-lup-20(29)-en-3-oyl]-5-aminovalerate (3d),** $C_{36}H_{56}N_2O_5$, yield 0.15 g (25%), R_f 0.3 ($CHCl_3$:EtOAc, 10:1), mp 87.6°C (hexane:EtOAc), $[\alpha]_D^{20}$ +17.2° (*c* 0.3, $CHCl_3$). IR spectrum (ν, cm^{-1}): 1639 (CONH), 1704 (COOH), 1723 ($COOCH_3$), 2234 (C≡N), 3364 (NH).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.92, 0.95, 1.01, 1.18, 1.25 (15H, 5s, $5CH_3$), 1.67 (3H, s, 3H-30), 2.38 (2H, t, J = 6.5, $HOOCCH_2$), 2.40 and 2.50 (2H, 2d, J_{AB} = 18.3, 2H-1, AB-system), 2.96–3.10 (1H, m, H-19), 3.46–3.55 (2H, m, $C\underline{H}_2NHCO$), 3.66 (3H, s, $COOCH_3$), 4.60 and 4.72 (2H, 2s, 2H-29), 5.95 (1H, br.t, NH).

^{13}C NMR spectrum (75.5 MHz, $CDCl_3$, δ, ppm): 14.09, 14.63, 15.67, 18.04, 19.23, 21.91, 22.67, 25.45, 28.11, 28.60, 29.33, 29.67, 29.68 (2C), 30.47, 31.91, 33.30, 36.84, 38.20, 39.30, 40.56, 40.58, 42.45, 42.81, 44.95, 45.63, 46.90, 49.21, 51.29, 52.16, 56.52, 109.86 (C-29), 119.28 (C-2), 150.24 (C-20), 176.61 (C-28), 179.24 (C-3).

Methyl-*N*-[28-methyl-28-oxo-1-cyano-2,3-*seco*--lup-20(29)-en-3-oyl]-L-phenylalaninate (3e), $C_{41}H_{58}N_2O_5$, yield 0.34 g (51%), R_f 0.3 ($CHCl_3$:EtOAc, 10:1), mp 158.6°C (hexane:EtOAc), $[\alpha]_D^{25}$ +24.0° (*c* 0.4, $CHCl_3$). IR spectrum (ν, cm^{-1}): 1650 (CONH), 1718, 1740 ($2COOCH_3$), 2246 (C≡N), 3361 (NH).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.89, 0.90, 1.01, 1.11, 1.13 (15H, 5s, $5CH_3$), 1.67 (3H, s, 3H-30), 2.42 (2H, s, 2H-1), 2.98 (1H, td, J = 10.5, 5.4, H-19), 3.12 and 3.20 (2H, 2dd, J = 6.4, $C_6H_5C\underline{H}_2$), 3.65 and 3.70 (6H, 2s, $2COOC\underline{H}_3$), 4.60 and 4.72 (2H, 2s, 2H-29), 4.79 (1H, dd, J = 13.7, 6.4, $C_6H_5CH_2C\underline{H}$), 6.07 (1H, br.d, NH), 7.13–7.31 (5H, m, C_6H_5).

^{13}C NMR spectrum (75.5 MHz, $CDCl_3$, δ, ppm): 14.66, 15.64, 18.46, 19.18, 20.52, 21.66, 23.17, 25.40, 27.58, 28.95, 29.60, 30.44, 31.89, 33.23, 36.81, 37.39, 38.19, 40.51, 42.14, 42.77, 45.11, 46.18, 46.89, 49.17, 50.90, 51.24, 52.20, 53.19, 56.49, 109.82 (C-29), 118.98 (C-2), 127.14, 128.63 (2C), 129.16 (2C), 135.86, 150.23 (C-20), 172.16, 176.58 (C-28), 178.19 (C-3).

Methyl-*N*-[28-methyl-28-oxo-1-cyano-2,3-*seco*--lup-20(29)-en-3-oyl]tryptophanate (3f), $C_{43}H_{59}N_3O_5$, yield 0.32 g (45%), R_f 0.5 ($CHCl_3$:EtOAc, 10:1), mp 124.5°C (hexane:EtOAc), $[\alpha]_D^{20}$ –9.7° (*c* 0.4, $CHCl_3$). IR spectrum (ν, cm^{-1}): 1649 (CONH), 1728 ($2COOCH_3$), 2239 (C≡N), 3406 (NH).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.88 (6H, s, $2CH_3$), 1.02, 1.05, 1.06 (9H, 3s, $3CH_3$), 1.67 (3H, s, 3H-30), 2.39 and 2.48 (2H, 2d, J_{AB} = 18.0, 2H-1, AB-system), 2.98 (1H, td, J = 10.4, 5.4, H-19), 3.37 (2H, d, J = 5.4, $C\underline{H}_2CHCOOCH_3$), 3.65 and 3.66 (6H, 2s, $2COOCH_3$), 4.60 and 4.72 (2H, 2s, 2H-29), 4.77–4.84 (1H, m, $CH_2C\underline{H}NH$), 6.20 (1H, d, J = 7.5, NH), indole protons: 7.06 (1H, d, J = 2.4), 7.10 (0.5H, d, J = 7.2), 7.13 (0.5H, d, J = 8.1), 7.17 (0.5H, d, J = 7.8), 7.20 (0.5H, d, J = 6.9), 7.35 (1H, d, J = 8.1), 7.61 (1H, d, J = 7.2), 8.27 (1H, br.s., NH).

^{13}C NMR spectrum (75.5 MHz, $CDCl_3$, δ, ppm): 14.66, 15.61, 18.49, 19.19, 20.35, 21.61, 22.87, 25.39, 26.94, 27.77, 28.71, 29.60, 30.44, 31.89, 33.24, 36.81, 38.18, 40.49, 42.17, 42.76, 45.13, 46.02, 46.90, 49.18, 51.18, 51.25, 52.22, 53.00, 56.50, 109.88 (2C, C-29), 111.28, 118.58, 119.12 (C-2), 119.68, 122.29, 122.89, 127.50, 136.18, 150.23 (C-20), 172.51, 176.61 (C- 28), 178.57 (C-3).

***N*-[28-Methyl-28-oxo-1-cyano-2,3-*seco*--lup-20(29)-en-3-oyl]tryptamine (3g)**, $C_{41}H_{57}N_3O_3$, yield 0.30 g (47%), R_f 0.35 ($CHCl_3$:EtOAc, 10:1), mp 126.1°C (hexane:EtOAc), $[\alpha]_D^{25}$ +12.4° (*c* 0.5, $CHCl_3$). IR spectrum (ν, cm^{-1}): 1639 (CONH), 1728 ($COOCH_3$), 2240 (C≡N), 3384 (NH).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.89 (6H, s, $2CH_3$), 0.98, 1.10, 1.12 (9H, 3s, $3CH_3$), 1.66 (3H, s, 3H-30), 2.33 and 2.47 (2H, 2d, J_{AB} = 18.2, 2H-1, AB-system), 2.95–3.05 (1H, m, H-19), 3.03 (2H, t, J = 6.9, $CONHCH_2C\underline{H}_2$), 3.36–3.45 and 3.73–3.84 (2H, 2m, $CONHC\underline{H}_2CH_2$), 3.65 (3H, s, $COOCH_3$), 4.59 and 4.71 (2H, 2s, 2H-29), 5.87 (1H, br.t, NH), indole protons: 7.06 (1H, s), 7.09 (0.5H, d, J = 7.5), 7.12 (0.5H, d, J = 7.2), 7.17 (0.5H, d, J = 8.1), 7.20 (0.5H, d, J = 7.2), 7.35 (1H, d, J = 7.8), 7.61 (1H, d, J = 7.5), 8.14 (1H, br.s, NH).

^{13}C NMR spectrum (75.5 MHz, $CDCl_3$, δ, ppm): 14.61, 15.62, 18.00, 19.19, 20.25, 21.63, 22.04, 24.80, 25.40, 28.08, 28.98, 29.56, 29.66, 30.43, 31.88, 33.22, 36.81, 38.16, 40.50, 42.32, 42.76, 44.90, 45.58, 46.88, 49.16, 51.27, 51.99, 56.49, 109.84 (C-29), 111.14, 113.01, 118.81, 119.21 (C-2), 119.43, 121.97, 122.13, 127.38, 136.36, 150.23 (C-20), 176.59 (C-28), 178.91 (C-3).

***N*-[28-Methoxy-28-oxo-1-cyano-2,3-*seco*--lup-20(29)-en-3-oyl]-2-aminopropane (3h)**, $C_{34}H_{54}N_2O_3$, yield 0.22 g (43%), R_f 0.35 ($CHCl_3$:EtOAc, 10:1), mp 183.7°C (hexane:EtOAc), $[\alpha]_D^{25}$ –3.5° (*c* 0.55, $CHCl_3$). IR spectrum (ν, cm^{-1}): 1650 (CONH), 1723 ($COOCH_3$), 2232 (C≡N), 3292, 3448 (NH).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.92, 0.97, 1.01, 1.19, 1.21 (15H, 5s, $5CH_3$), 1.16 and 1.18 (6H, 2d, J = 6.6, $2CH_3$), 1.67 (3H, s, 3H-30), 2.42 and 2.57 (2H, 2d, J_{AB} = 18.0, 2H-1, AB-system), 2.99 (1H, td, J = 10.5, 5.1, H-19), 3.66 (3H, s, $COOCH_3$), 3.92–4.06 [1H, m, $C\underline{H}(CH_3)_2$], 4.60 and 4.72 (2H, 2s, 2H-29), 5.55 (1H, br.d, NH).

^{13}C NMR spectrum (75.5 MHz, $CDCl_3$, δ, ppm): 14.63, 15.63, 18.00, 19.20, 20.55, 21.76, 21.95, 22.32, 22.62, 25.45, 28.59, 28.76, 29.60, 30.44, 31.88, 33.23, 36.81, 38.22, 40.52, 42.06, 42.33, 42.78, 44.95, 45.80, 46.88, 49.17, 51.26, 51.59, 56.51, 109.83 (C-29), 119.44 (C-2), 150.24 (C-20), 176.59 (C-28), 178.10 (C-3).

***N*-[28-Methoxy-28-oxo-1-cyano-2,3-*seco*--lup-20(29)-en-3-oyl]-2-amino-2-methylpropane (3i)**, $C_{35}H_{56}N_2O_3$, yield 0.16 g (28%), R_f 0.3 (hexane:EtOAc, 5:1), mp 102.9°C (hexane:EtOAc), $[\alpha]_D^{25}$ +3.0° (*c* 0.4, $CHCl_3$). IR spectrum (ν, cm^{-1}): 1663 (CONH), 1728 ($COOC_2H_5$), 2234 (C≡N), 3397 (NH).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.92, 0.95, 1.01, 1.16, 1.18 (15H, 5s, $5CH_3$), 1.37 (9H, s, $3CH_3$), 1.67 (3H, s, 3H-30), 2.44 and 2.58 (2H, 2d, J_{AB} = 18.0, 2H-1, AB-system), 2.94–3.04 (1H, m, H-19), 3.66 (3H, s, $COOCH_3$), 4.60 and 4.72 (2H, 2s, 2H-29), 5.51 (1H, br.s, NH).

^{13}C NMR spectrum (75.5 MHz, $CDCl_3$, δ, ppm): 14.65, 15.61, 18.23, 19.22, 20.50, 21.76, 22.55, 25.45, 28.22 (3C), 29.13, 29.36, 29.61, 30.47, 31.89, 33.25, 36.82, 38.23, 40.53, 42.36, 42.78, 45.00, 46.44, 46.89, 49.18, 51.26, 51.37, 51.51, 56.52, 109.82 (C-29), 119.56 (C-2), 150.26 (C-20), 176.59 (C-28), 178.16 (C-3).

***N*-[28-Methyl-28-oxo-1-cyano-2,3-*seco*--lup-20(29)-en-3-oyl]-2-aminothiazole (3j)**, $C_{34}H_{49}N_3SO_3$, yield 0.58 g (56%), R_f 0.65 ($CHCl_3$:EtOAc, 10:1), mp 128.9°C (hexane:EtOAc), $[\alpha]_D^{25}$ –10.8° (*c* 0.6, $CHCl_3$). IR spectrum (ν, cm^{-1}): 1668 (CONH), 1723 ($COOCH_3$), 2237 (C≡N), 3182 (NH).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.92, 0.97, 1.00, 1.34, 1.39 (15H, 5s, $5CH_3$), 1.67 (3H, s, 3H-30), 2.25 and 2.43 (2H, 2d, J_{AB} = 18.3, 2H-1, AB-system), 2.90–3.04 (1H, m, H-19), 4.59 and 4.71 (2H, 2s, 2H-29), 6.98 (1H, d, J = 3.6, thiazole), 7.49 (1H, d, J = 3.6, thiazole), 9.52 (1H, br.s, NH).

^{13}C NMR spectrum (75.5 MHz, $CDCl_3$, δ, ppm): 14.62, 15.65, 18.43, 19.16, 20.55, 21.61, 22.70, 25.30, 27.86, 28.51, 29.59, 30.41, 31.85, 33.23, 36.79, 38.09, 40.52, 42.39, 42.78, 44.95, 46.45, 46.87, 49.11, 51.27, 51.50, 56.46, 109.86 (C-29), 113.74, 118.16 (C-2), 137.76, 150.19 (C-20), 157.90, 176.56 (C-28), 176.59 (C- 3).

Cytotoxic Activity of 2,3-*seco*-Triterpene Amides (3a–j). We used cell lines rhabdomyosarcoma RD TE32, lung carcinoma A549, and melanoma MS that were obtained from the Research Institute of Experimental Tumor Diagnostics and Therapy, N. N. Blokhin Russian Oncological Center, RAMS (Moscow). Cells were inoculated into 96-well plates and cultivated in DMEM growth medium (for RD TE32 and A549 cells) or RPMI 1640 (for MS cells) with added fetal calf serum (10%) and glutamine (0.3%) at 37°C in an Isotemp Barnstead CO_2 incubator (USA) with 5% CO_2. The tested compounds at a concentration of 100 μM in DMSO solution with subsequent serial dilution to 1.56 μM were added to the plate wells after 24 h. The DMSO concentration in a plate well was <0.1%. Survival of cells was evaluated after incubation for 72 h with the tested compounds using the MTT test [10] on a FLUOstar Optima spectrophotometer (BMG Labtech GmbH, Germany). The IC_{50} index corresponding to the concentration of tested compound causing death of 50% of the cells was used as the quantitative criterion of cytotoxicity. Survival of cells incubated in the appropriate growth media with added DMSO (0.1%) was taken as 100% (control). The experiments were carried out in triplicate.

Antiviral Properties of 2,3-*seco*-Triterpene Amides (3a–j). Antiviral activity was determined in experiments on passaged cell culture of human rhabdomyosarcoma (RD TE32) with herpes simplex virus type 1 (HSV, strain 1C) by estimating the cytopathic effect [13]. Compounds were dissolved beforehand in EtOH (10%) (stock solution concentration 5.0 mg/mL) and then in support medium to the required concentrations. The criterion for antiviral activity was a reduction of the virus titer in the presence of the tested compounds compared with a control. The concentration of the compounds that suppressed virus replication by 50% (mean effective concentration, EC_{50}) was determined using the computer program of Fung [14] that was based on probability analysis and weighted linear regression. The ratio of maximum tolerated concentration (MTC) of the compound to the EC_{50} was also calculated. The MTC of the compounds was defined as the maximum concentration of the compound that did not affect the morphology of the uninfected cell culture after incubation for 72 h.

Antiretroviral activity of the synthesized compounds was tested on passaged T-lymphoblastoids of human MT-4 cell line with an inoculated dose of $4–5\times10^5$ cells/mL. We used highly replicating strain HIV-1_{zmb} [15] with 6.0 log CPE_{50} titer in the experiments. Alcohol solutions of the compounds of concentration 5.0 mg/mL were titrated in five-fold increments of complete RPMI-1640 growth medium (Sigma, FRG). The reaction was carried out in 96-well Costar culture panels (USA) with a final volume of the reaction mixture of 200 μL/well and incubation at 37°C in a 5% CO_2 atmosphere. The results were calculated on the fourth day on a DAS A3 Plate Reader spectrophotometer (Italy) at wavelength 550/630 nm. Tests were performed according to the therapeutic protocol providing for addition of the infectious agent to the cell cultures immediately after adding the tested compounds. The positive control for anti-HIV activity in each series of experiments was a commercial azidothymidine preparation. The antiretroviral activity of the compounds was estimated using the MTT test with a local modification [16]. The cell protection index was calculated using the published formula [17]. The antiviral properties of the compounds were assessed from the chemotherapeutic index (CTI), i.e., the ratio of the MTC and the minimum active concentration (MAC) of the drugs.

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