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The Synthesis of Triterpenic Amides on the Basis of 2,3-*seco*-1-Cyano-19β,28-Epoxy-18α-Oleane-3-oic Acid

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Abstract—Novel 2,3-*seco*-triterpenic amides were prepared by the interaction of the chloride of 1-cyano-19β,28-epoxy-18α-oleane-3-oic acid with primary amines and synthetic and biogenic amino acids. A cytotoxic triterpenic conjugate with a residue of the ethyl ester of β-alanine was found among the synthesized nitrogen-containing derivatives. Treatment with this conjugate in a concentration of 100 μM resulted in the 45.5% survival of melanoma cells in the medium.

Key words: 2,3-seco-triterpenoids, betulin, allobetulon, amides, amino acids, cytotoxic activity, melanoma

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INTRODUCTION

Triterpenic 2,3-*seco* derivatives of the oleanane and lupane types were found in extracts of some medicinal plants that are traditionally used in folk medicines in Africa and Southeast Asia [1–3].[2] In recent years, the antivirus and antitumor properties of these compounds have attracted increased attention. For example, Chinese and Japanese scientists isolated 16β-hydroxy-2,3-*seco*-lup-20(29)-en-2,3-dioic acid [3] that exhibited anti-HIV-1 activity (IC_{50} 8.7 μg/ml) from stalks of the *Stauntonia obovatifoliola* Hayata subsp. *inermedia* plant. Its synthetic HIV-1-inhibiting (IC_{50} 3.9–25.4 μM) A-*seco* analogues were also prepared from triterpenoids of the oleanane, lupane, and dammarane types [4, 5]. Urban et al. [6] synthesized 2,3-*seco*-lup-20(29)-en-2,3,28-triol that was cytotoxic (IC_{50} 9–49 μM) towards cells of the CEM, HT 29, K562, K562 Tax, and PC-3 lines. Lupane and 19β,28-epoxy-18α-oleanane 2,3-*seco*-triterpenoids with pronounced immune activity were described [7, 8]. It was also reported that A-*seco* derivatives of betulonic acid inhibited the replication of the type-I herpes simplex virus and the influenza A virus (EC_{50} 1.9–21.3 μM) [9]. However, there is no data in published works on the synthesis and activity of functionalized 2,3-*seco*-triterpenoids, which could be promising as new pharmacologically active agents. The goal of our study is the synthesis and determination of the cytotoxic activity of amides of 2,3-*seco*-1-cyano-19β,28-epoxy-18α-oleane-3-oic acid.

RESULTS AND DISCUSSION

One of the basic 2,3-*seco*-triterpenoids, 2,3-*seco*-3-carbonic acids (**I**) (its preparation was described in [7, 9]) was used as a starting compound for the synthesis of 19β,28-epoxy-18α-oleanane amides (Scheme 1).

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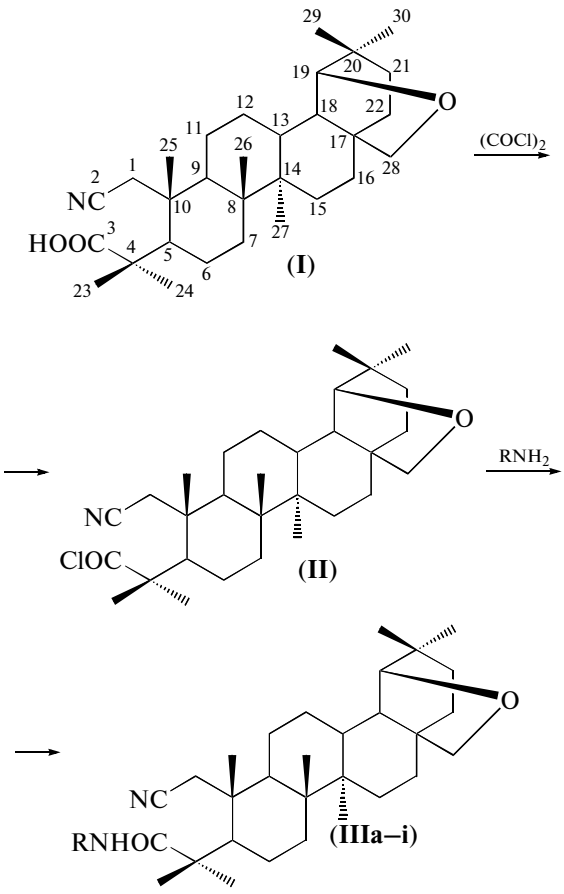
[2] Abbreviations: DMSO, dimethylsulfoxide; MTT, 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrasolium bromide.

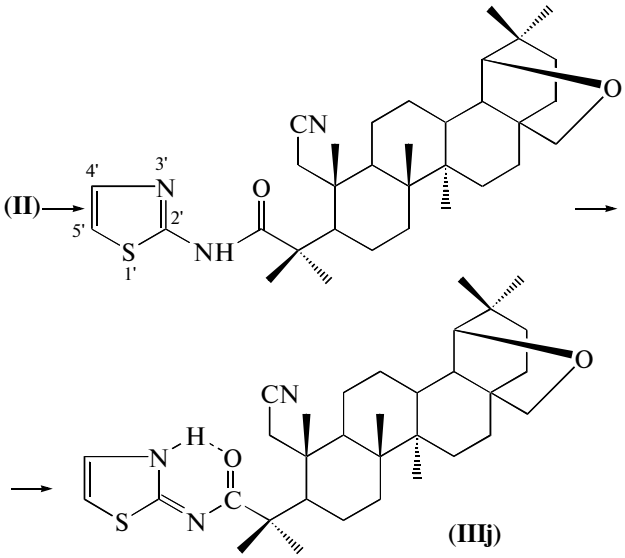


R = (a); (b); (c); (d); (e); (f); (g); (h); (i); (j)

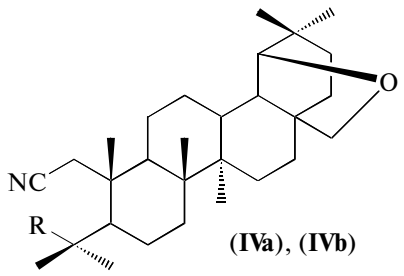
Scheme 1.

The chloride of 2,3-*seco*-1-cyano-19,28-epoxy-18α-oleane-3-oic acid (**II**) was prepared in situ by the treatment of acid (**I**) with oxalyl chloride in dichloromethane. Amides (**IIIa**)–(**IIIj**) were synthesized by the interaction of compound (**II**) with primary amines and biogenic or synthetic amino acids in the presence of triethylamine (Scheme 1). Compounds (**IIIa**)–(**IIIj**) were purified by column chromatography on silica gel and obtained with yields of 28–54%. A comparison of the ^{1}H NMR spectra of acid (**I**) and amides (**IIIa**)–(**IIIi**) confirmed the introduction of the amide fragment. Resonance from the amide proton and characteristic resonances from protons of the substitute at the nitrogen atom were observed in the area of 5.52–6.61 ppm of the spectra of compounds (**IIIa**)–(**IIIi**). Resonance from the C3 atom appeared in the area of 177.04–179.07 of the ^{13}C NMR spectra of compounds (**IIIa**)–(**IIIj**), whereas this resonance was found at 184.13 ppm in the spectrum of compound (**I**) [7]. Absorption bands in the area of 3207–3408 and 1629–1670 cm^{-1} were observed in the IR spectra of amides (**IIIa**)–(**IIIi**) and confirmed the formation of the amide bond.

The proton migration from the amide group to the nitrogen atom in position 3 of the thiazole fragment (Scheme 2) as a result of amino–imino tautomerism was observed for product (**IIIj**) that was prepared by the interaction of chloride (**II**) with 2-aminothiazole. This fact was confirmed by the presence of resonance from the N3' proton of the thiazole fragment in the low-field area (11.98 ppm) of the ^{1}H NMR spectrum that was recorded for the solution of compound (**IIIj**) in DMSO-d_6. The observed shift of the band of stretching vibrations of the *N*3' proton in the low-frequency area of the IR spectrum (3184 cm^{-1}) indicated the formation of an intramolecular hydrogen bond of the H-chelate type between the *N*3' proton and the amide carbonyl group of compound (**IIIj**).

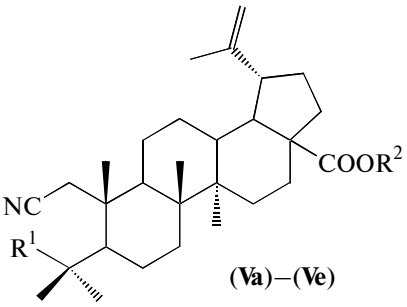


Scheme 2.



(IVa), **(IVb)**

(IVa) R = CHO;
(IVb) R = $COOCH_3$



(Va)–**(Ve)**

(Va) R^1 = CHO, R^2 = H; **(Vb)** R^1 = COOH, R^2 = H;
(Vc) R^1 = CHO, R^2 = CH_3; **(Vd)** R^1 = COOH, R^2 = CH_3;
(Ve) R^1 = $COOCH_3$, R^2 = CH_3

The preliminary selection of the tumor cell line sensitive to 2,3-*seco*-triterpenoids was performed by the MTT test [10, 11] using the basic 2,3-*seco*-compounds **(I)**, **(IVa)**, **(IVb)**, and **(Va)**–**(Ve)** [7]. The studied 2,3-*seco*-triterpenoids were found to be ineffective towards cells of the JurkatE61 line of human lymphoblast leucosis in a concentration of 100 μM (table). The cells of Mel. P. human melanoma proved to be more sensitive to the 2,3-*seco*-triterpenoids. However, the survival of the melanoma cells of lower than 50% was revealed only in the presence of lupane 2,3-*seco*-aldehyde acid **(Va)**. This fact is in good agreement with the published data [12–14] on the selective inhibiting activity of derivatives of betulinic acid towards melanoma cells.

As is seen in the table, only the product of the attachment of the ethyl ester of β-alanine to the C3 carboxylic group demonstrated significant cytotoxic activity towards the human Mel. P. cells among all of the synthesized amides **(IIIa)**–**(IIIj)** of 2,3-*seco*-1-cyano-19,28-epoxy-18α-oleane-3-oic acid **(I)**. Amide **(IIId)** in a concentration of 100 μM exhibited cytotoxic activity that corresponded to the 45.5% survival of the cells.

Cytotoxic activity of 2,3-*seco*-triterpenoids **(I)**, **(IVa)**, **(IVb)**, **(IIIa)**–**(IIIj)**, and **(Va)**–**(Ve)** towards the JurkatE6.1 and Mel. P. human tumor cells

Compound	Survival of the cells, %		Compound	Survival of the cells, %
	JurkatE6.1	Mel. P		Mel. P
(I)	90.4	80.0	**(IIIa)**, **(IIIc)**	77.7
(IVa)	100.4	65.7	**(IIIb)**	101.1
(IVb)	89.9	72.3	**(IIId)**	45.5
(Va)	100.8	49.1	**(IIIe)**	92.2
(Vb)	87.5	86.8	**(IIIf)**	105.5
(Vc)	90.4	84.2	**(IIIg)**, **(IIIh)**	103.3
(Vd)	93.4	87.1	**(IIIi)**	106.7
(Ve)	93.4	77.5	**(IIIj)**	79.4

EXPERIMENTAL

The IR spectra (ν, cm^{-1}) were recorded on an IFS 66/S IR-Fourier spectrometer (Bruker, Germany) in a paste with Vaseline oil. The ^1H and ^{13}C NMR spectra were recorded on a Varian Mercury+ spectrometer (United States) in a $CDCl_3$ solution at a working frequency of 300 and 75.5 MHz, respectively (δ, ppm; *J*, Hz). Hexamethyldisiloxane was used as an internal standard. The specific optical rotation was determined on a Perkin-Elmer 341 polarimeter (United States) in solutions of $CHCl_3$ at a wavelength of 589 nm. The melting points were measured on a PTP device for the melting point determination (Russia). Column chromatography was performed on 60–200 μm silica gel (Lancaster, Great Britain) at a substance–sorbent ratio of approximately 1 : 50 in a mixture of hexane and ethyl acetate (5 : 1). TLC was carried out on Sorbfil plates (Russia). The substances were detected by treatment with a solution of phosphomolybdic acid in ethanol with the subsequent heating to 100–120°C for 2–3 min. The anhydrous solvents were prepared according to the standard procedures [15]. The syntheses of 2,3-*seco*-1-cyano-19,28-epoxy-18α-oleane-3-oic acid **(I)** and compounds **(IV)**, **(IVa)**, **(IVb)**, and **(Va)**–**(Ve)** were described in [7, 8].

Procedure of the preparation of the amides of 2,3-*seco*-1-cyano-19β,28-epoxy-18α-oleane-3-oic acid (IIIa)–(IIIk). Oxalyl chloride (2.2 mmol, 0.2 ml) was added to a solution of 2,3-*seco*-acid **(I)** (1.1 mmol) in anhydrous methylene chloride (10 ml) in an argon atmosphere. The reaction mixture was stirred at room temperature for 6 h. The solvent was removed to dryness in a vacuum of a water jet pump at a temperature of the water bath of 30°C. Anhydrous methylene chloride (10 ml) was added to the residue, and the solvent was evaporated. This procedure was repeated three times. Anhydrous dichloromethane (10 ml), the corresponding amine (1.2 mmol), and triethylamine (1.2 mmol, 0.17 ml) were added to the prepared chloride of 2,3-*seco*-1-cyano-19β,28-epoxy-18α-oleane-3-oic acid **(II)** in an argon atmosphere. The commercially available hydrochlorides of esters of glycine, valine, β-alanine, phenylalanine, and tryptophan were preliminary converted into free acids: the hydrochloride of an amino acid ester (1.2 mmol) was treated

with triethylamine (1.2 mmol, 0.17 ml) in a solution of anhydrous dichloromethane (20 ml) in an argon atmosphere for 1 h. The reaction mixture was stirred for 4–6 h at room temperature with TLC monitoring. The solvent was evaporated, and the residue was purified by column chromatography.

2-*N*-[2,3-*seco*-1-cyano-19β,28-epoxy-18α-oleane-3-oyl]aminopropane (IIIa). Yield 0.22 g (39%); R_f 0.4 (hexane–ethyl acetate, 7 : 3); mp 110–112°C (hexane–ethyl acetate); $[\alpha]_D^{20}$ + 29.20° (*c* 0.4, $CHCl_3$); IR: 1660 (CONH), 2240 (C≡N), 3384, (NH); ^{1}H NMR: 0.79, 0.92, 0.96, 0.98, 1.00, 1.19, 1.23 (21 H, 7 s, $7CH_3$), 1.17 and 1.23 (6 H, 2 d, *J* 6.6, $2CH_3$), 2.45 and 2.60 (2 H, 2 d, J_{AB} 17.9, H1, AB system), 3.44 and 3.75 (2 H, 2 d, J_{AB} 7.7, H28, AB system), 3.52 (1 H, s, H19), 4.00 (1 H, g, *J* 6.6, $\underline{CH}(CH_3)_2$), 5.56 (1 H, d, *J* 6.9, NH); ^{13}C NMR: 13.49, 15.53, 18.28, 20.47, 21.83, 21.94, 22.56, 22.63, 24.51, 26.13, 26.34, 26.41, 28.73, 28.76, 28.94, 32.66, 32.77, 34.30, 36.25, 36.63, 40.42, 41.12, 41.45, 42.10, 42.40, 45.44, 45.76, 46.58, 51.74, 71.25 (C28), 87.84 (C19), 119.45 (C2), 178.10 (C3).

Ethyl-*N*-[2,3-*seco*-1-cyano-19β,28-epoxy-18α-oleane-3-oyl]glycinate (IIIb). Yield 0.26 g (42%); mp 90–92°C (chloroform–ethyl acetate); R_f 0.3 (chloroform–ethyl acetate, 10 : 1); $[\alpha]_D^{20}$ + 21.46° (*c* 0.4, $CHCl_3$); IR: 1652 (CONH), 1748 ($COOC_2H_5$), 2237 (C≡N), 3376 (NH); ^{1}H NMR: 0.78, 0.92, 0.97, 1.22, 1.30 (15 H, 5 s, $5CH_3$), 0.97 (6 H, s, 2 CH_3), 1.28 (3 H, t, *J* 6.9, $COOCH_2\underline{CH_3}$); 2.44and 2.53 (2 H, 2 d, J_{AB} 18.3, H1, AB system), 3.44 and 3.75 (2 H, 2 d, J_{AB} 7.8, H28, AB system), 3.52 (1 H, s, H19), 3.83, 3.84 and 4.24, 4.26 (2 H, 4 d, J_{AB} 18.3, (*D*,*L*) $CONH\underline{CH_2}COO$, AB system), 4.21 (2 H, dd, *J* 14.1, 6.9, $COO\underline{CH_2}CH_3$), 6.31 (1 H, t, *J* 6.2, NH); ^{13}C NMR: 13.51, 14.10, 15.52, 18.68, 20.14, 21.68, 22.03, 24.48, 26.11, 26.28, 26.40, 28.42, 28.74, 28.92, 32.64, 32.85, 34.22, 36.23, 36.61, 40.46, 41.11, 41.43, 41.57, 42.39, 45.50, 45.75, 46.57, 51.93, 61.46, 71.23 (C28), 87.83 (C19), 118.88 (C2), 169.94 ($COOC_2H_5$), 179.07 (C3).

Methyl-*N*-[2,3-*seco*-1-cyano-19β,28-epoxy-18α-oleane-3-oyl]-*L*-valinate (IIIb). Yield 0.18 g (28%); mp 85–87°C (chloroform–ethyl acetate); R_f 0.4 (chloroform–ethyl acetate, 10 : 1); $[\alpha]_D^{20}$ + 40.88° (*c* 0.4 $CHCl_3$); IR: 1665 (CONH), 1735 ($COOCH_3$), 2237 (C≡N), 3393 (NH); ^{1}H NMR: 0.79, 0.92, 0.94, 1.28, 1.30 (15 H, 5 s, 5 CH_3), 0.94 and 0.97 (6 H, 2 d, *J* 6.9, $2CH_3$), 0.97 (6 H, s, $2CH_3$), 2.16–2.26 (1 H, m, $\underline{CH}(CH_3)_2$); 2.46 and 2.53 (2 H, 2 d, J_{AB} 18.6, H1, AB system), 3.44 and 3.75 (2 H, 2 d, J_{AB} 7.9, H28, AB system), 3.52 (1 H, s, H19), 3.74 (3 H, c, $COO\underline{CH_3}$); 4.49 (1 H, dd, *J* 7.9, 4.8, $CONH\underline{CH}$), 6.18 (1 H, d, *J* 8.1, NH); ^{13}C NMR: 13.46, 15.54, 18.06, 18.79, 18.84, 20.74, 21.77, 23.89, 24.48, 26.10, 26.29, 26.39, 27.27, 28.74, 29.37, 30.70, 32.63, 32.72, 34.27, 36.22, 36.60, 40.41, 41.08, 41.42, 42.16, 45.59, 46.55, 46.62, 50.63, 52.06, 57.35, 71.23 (C28), 87.82 (C19), 118.86 (C2), 172.40 ($COOCH_3$), 178.37 (C3).

Ethyl-*N*-[2,3-*seco*-1-cyano-19β,28-epoxy-18α-oleane-3-oyl]-β-alaninate (IIId). Yield 0.33 g (53%); mp 110–112°C (chloroform–ethyl acetate); R_f 0.3 (chloroform–ethyl acetate, 10 : 1); $[\alpha]_D^{20}$ + 12.89° (*c* 0.4, $CHCl_3$); IR: 1668 (CONH), 1726 ($COOC_2H_5$), 2238 (C≡N), 3207 (NH); ^{1}H NMR: 0.79, 0.92, 1.18, 1.23 (12 H, 4 s, $4CH_3$), 0.97 (9 H, s, $3CH_3$), 1.26 (3 H, t, *J* 7.2, $COOCH_2\underline{CH_3}$), 2.43 and 2.53 (2 H, 2 d, J_{AB} 18.2, H1, AB system), 2.57 (2 H, t, *J* 6.0, $CH_2\underline{CH_2}COOCH_2CH_3$), 3.37–3.47 (1 H, m, $CONH\underline{CH_2}CH_2$), 3.45 and 3.76 (2 H, 2 d, J_{AB} 7.7, H28, AB system), 3.53 (1 H, s, H19), 3.60–3.70 (1 H, m, $CONH\underline{CH_2}$), 4.15 (2 H, dd, *J* 14.3, 7.2, $COO\underline{CH_2}CH_3$), 6.50 (1 H, t, *J* 6.0, NH); ^{13}C NMR: 13.48, 14.16, 15.50, 18.44, 20.19, 21.71, 22.00, 24.48, 26.09, 26.28, 26.38, 28.31, 28.72, 29.09, 32.63, 32.80, 33.54, 34.21, 35.37, 36.22, 36.59, 40.44, 41.10, 41.42, 42.41, 45.43, 45.62, 46.55, 51.95, 60.72, 71.20 (C28), 87.84 (C19), 119.02 (C2), 172.58 ($COOC_2H_5$), 179.08 (C3).

***5*-*N*-[2,3-*seco*-1-cyano-19β,28-epoxy-18α-oleane-3-oyl]amino valerate (IIIe).** Yield 0.17 g (30%); mp 116–118°C (hexane–ethyl acetate); R_f 0.3 (hexane–ethyl acetate, 5 : 1); $[\alpha]_D^{20}$ + 13.70°(*c* 0.4, $CHCl_3$); IR: 1634 (CONH), 1707 (COOH), 2242 (C≡N), 3371 (NH); ^{1}H NMR: 0.79, 0.92, 0.96, 0.97, 0.98, 1.18, 1.25 (21 H, 7 s, $7CH_3$), 2.38 (2 H, t, *J* 6.8, $HOOC\underline{CH_2}(CH_2)_3NHCO$), 2.43 and 2.53 (2 H, 2 d, J_{AB} 18.0, H1, AB system), 3.02–3.13 (2 H, m, $HOOCCH_2(CH_2)_2\underline{CH_2}NHCO$), 3.45 and 3.77 (2 H, 2 d, J_{AB} 7.8, H28, AB system), 3.54 (1 H, s, H19), 6.00 (1 H, t, *J* 5.4, NH); ^{13}C NMR: 13.47, 15.53, 18.32, 20.18, 21.74, 21.77, 21.93, 24.49, 26.10, 26.31, 26.38, 28.21, 28.56, 28.73, 29.41, 32.63, 32.83, 33.34, 34.21, 36.22, 36.60, 39.37, 40.46, 41.10, 41.43, 42.48, 45.41, 45.56, 46.55, 52.24, 71.19 (C28), 87.84 (C19), 119.27 (C2), 177.73 (COOH), 179.25 (C3).

Methyl-*N*-[2,3-*seco*-1-cyano-19β,28-epoxy-18α-oleane-3-oyl]-*L*-phenylalaninate (IIIe). Yield 0.34 g (51%); mp 91–93°C (hexane–ethyl acetate); R_f 0.4 (chloroform–ethyl acetate, 10 : 1); $[\alpha]_D^{20}$ + 51.89° (*c* 0.4, $CHCl_3$); IR: 1659 (CONH), 1740 ($COOCH_3$), 2238 (C≡N), 3382 (NH); ^{1}H NMR: 0.78, 0.88, 0.92, 0.93, 0.95, 1.12, 1.14 (21 H, 7 s, $7CH_3$), 2.46 (2 H, s, H1), 3.16 (2 H, t, *J* 6.9, $C_6H_5\underline{CH_2}$), 3.44), 3.44 and 3.75 (2 H, 2 d, J_{AB} 8.0, H28, AB system), 3.52 (1 H, s, H19), 3.70 (3 H, c, $COO\underline{CH_3}$), 4.79 (1 H, dd, *J* 13.5, 6.3, $C_6H_5CH_2\underline{CH}$), 6.08 (1 H, d, *J* 7.5, NH), 7.13–7.33 (5 H, m, $\underline{C_6H_5}$); ^{13}C NMR: 13.52, 15.52, 18.76, 20.42, 21.73, 23.02, 24.49, 26.11, 26.29, 26.40, 27.73,

28.75, 29.10, 32.64, 32.76, 34.25, 36.23, 36.61, 37.39, 40.41, 41.09, 41.43, 42.18, 45.58, 46.13, 46.56, 50.98, 52.23, 53.20, 71.23 (C28), 87.84 (C19), 118.99 (C2), 127.17, 128.65 (2C), 129.16 (2C), 135.84, 172.17 ($\underline{C}OOCH_3$), 178.20 (C3).

Ethyl-{4-*N*-[2,3-*seco*-1-cyano-19β,28-epoxy-18α-oleane-3-oyl]amino}benzoate (IIIg). Yield 0.31 g (48%) as a yellow caramel; R_f 0.3 (hexane–ethyl acetate, 5 : 1); $[\alpha]_D^{20}$ + 1.68° (*c* 0.4, $CHCl_3$); IR: 1629 (CONH), 1715 ($COOC_2H_5$), 2237 (C≡N), 3371 (NH); ^{1}H NMR: 0.78, 0.92, 0.96, 0.99, 1.02, 1.31, 1.32 (21 H, 7 s, $7CH_3$), 1.37 (3 H, t, *J* 7.2, $\underline{CH_3}CH_2OOC$), 2.41 and 2.49 (2 H, 2 d, J_{AB} 18.3, H1, AB system), 3.44 and 3.75 (2 H, 2 d, J_{AB} 7.5, H28, AB system), 3.51 (1 H, s, H19), 4.30 (2 H, dd, *J* 14.4, 6.9, $CH_3\underline{CH_2}OOC$), 6.61 (1 H, broadened s, NH), 7.69 (2 H, d, *J* 8.0, aromatic protons), 8.00 (2 H, d, *J* 8.7, aromatic protons); ^{13}C NMR: 13.43, 14.30 (2C), 14.37, 15.55, 18.11, 20.47, 21.83, 22.14, 24.47, 26.07, 26.29, 26.36, 28.61, 28.72, 32.66, 34.24, 36.20, 36.56, 40.44, 41.09, 41.40, 42.58, 45.25, 46.50, 47.04, 51.97, 60.23, 71.18 (C28), 87.78 (C19), 119.01, 119.98(C2), 126.04, 130.69 (2C), 141.74, 150.74, 166.06 ($\underline{C}OOC_2H_5$), 177.39 (C3).

***N*-[2,3-*seco*-1-cyano-19β,28-epoxy-18α-oleane-3-oyl]tryptamine (IIIh).** Yield 0.30 g (45%); mp 228–230°C (hexane–ethyl acetate), R_f 0.4 (hexane–ethyl acetate, 1 : 1); $[\alpha]_D^{20}$ + 21.90° (*c* 0.4, $CHCl_3$); IR: 1649 (CONH), 2235 (C≡N), 3408 (NH); ^{1}H NMR: 0.78, 1.11, 1.13 (9 H, 3 s, $3CH_3$); 0.92 and 0.94 (12 H, 2 s, $4CH_3$), 2.36 and 2.51 (2 H, 2 d, J_{AB} 18.3, H1, AB system), 3.03 (2 H, t, *J* 6.9, $\underline{CH_2}CH_2NHCO$), 3.44 and 3.75 (2 H, 2 d, J_{AB} 7.7, H28, AB system), 3.52 (1 H, s, H19), 3.37–3.48 and 3.74–3.82 (2 H, 2 m, $CH_2\underline{CH_2}NHCO$), 5.89 (1 H, t, *J* 5.1, NH), resonances from the protons of the indole fragment: 7.06 (1 H, d, *J* 2.5), 7.10 (0.5 H, d, *J* 6.9), 7.12 (0.5 H, d, *J* 8.1), 7.18 (0.5 H, d, *J* 8.1), 7.20 (0.5 H, d, *J* 6.9), 7.36 (1 H, d, *J* 7.8), 7.64 (1 H, d, *J* 7.2), 8.16 (1 H, broadened s, NH); ^{13}C NMR: 13.48, 15.51, 18.29, 20.18, 21.71, 21.98, 24.49, 24.80, 26.11, 26.31, 26.37, 28.24, 28.76, 29.08, 32.65, 32.78, 34.23, 36.24, 36.61, 40.15, 40.42, 41.09, 41.44, 42.37, 45.40, 45.56, 46.57, 52.12, 71.23 (C28), 87.84 (C19), 111.16, 113.00, 118.82, 119.22 (C2), 119.44, 121.98, 122.13, 127.39, 136.38, 178.93 (C3).

Methyl-*N*-[2,3-*seco*-1-cyano-19β,28-epoxy-18α-oleane-3-oyl]-*L*-tryptophanate (IIIi). Yield 0.35 g (54%); mp 133–135°C (hexane–ethyl acetate); R_f 0.4 (hexane–ethyl acetate, 1 : 1); $[\alpha]_D^{20}$ + 45.25° (*c* 0.4, $CHCl_3$); IR: 1647 (CONH), 1739 ($COOCH_3$), 2237 (C≡N), 3316 (NH); ^{1}H NMR: 0.79, 0.91, 0.92, 0.94, 0.97, 1.06, 1.08 (21 H, 7 s, $7CH_3$), 2.42 and 2.52 (2 H, 2 d, J_{AB} 18.0 18.0, H1, AB system), 3.37 *J* 5.7, $\underline{CH_2}CHCOOCH_3$); 3.44 and 3.75 (2 H, 2 d, J_{AB} 7.7, H28, AB system), 3.52 (1 H, s, H19), 4.82 (1 H, dd, *J* 12.9, 5.7, $-CH_2\underline{CH}COOCH_3$); 6.21 (1 H, d, *J* 7.5, NH), resonances from the protons of the indole fragment: 7.08 (1 H, d, *J* 2.1), 7.11 (0.5 H, d, *J* 7.8), 7.13 (0.5 H, d, *J* 6.9), 7.18 (0.5 H, d, *J* 6.6), 7.20 (0.5 H, d, *J* 6.9), 7.35 (1 H, d, *J* 7.8), 7.61 (1 H, d, *J* 7.5), 8.23 (1 H, broadened s, NH); ^{13}C NMR: 13.54, 15.50, 18.80, 20.29, 21.71, 22.79, 24.50, 26.13, 26.30, 26.42, 26.97, 27.88, 28.76, 28.89, 32.67, 32.80, 34.27, 36.25, 36.63, 40.41, 41.11, 41.45, 42.23, 45.62, 46.02, 46.60, 51.27, 52.24, 53.01, 71.24 (C28), 87.87 (C19), 109, 96, 111.28, 118.63, 119.12 (C2), 119.72, 122.34, 122.89, 127.53, 136.20, 172.53 ($\underline{C}OOCH_3$), 178.57 (C3).

2-*N*-[2,3-*seco*-1-cyano-19β,28-epoxy-18α-oleane-3-oyl]-aminothiazole (IIIj). Yield 0.35 g (54%); mp 130–133°C (hexane–ethyl acetate); R_f 0.2 (hexane–ethyl acetate, 5 : 1); $[\alpha]_D^{20}$ + 19.10° (*c* 0.4, $CHCl_3$); IR: 1533, 1670, (CON=C), 2237 (C≡N), 3184 (NH); ^{1}H NMR: 0.78, 0.92, 0.97, 0.98, 1.00, 1.34, 1.42 (21 H, 7 s, $7CH_3$), 2.25 and 2.44 (2 H, 2 d, J_{AB} 18.5 18.5, H1, AB system), 3.44 and 3.75 (2 H, 2 d, J_{AB} 8.0, H28, AB system), 3.51 (1 H, s, H19), 6.96 (1 H, d, *J* 3.5, thiazolyl), 7.46 (1 H, d, *J* 3.5, thiazolyl); ^{1}H NMR (DMSO-d_6; δ, ppm): 11.98 (1 H, broadened s, NH); ^{13}C NMR: 13.50, 15.54, 18.83, 20.32, 21.67, 22.37, 24.50, 26.12, 26.24, 26.43, 28.12, 28.56, 28.75, 32.65, 32.86, 34.23, 36.25, 36.62, 40.48, 41.15, 41.45, 42.45, 45.49, 46.41, 46.58, 51.45, 71.24 (C28), 87.85 (C19), 113.57, 117.97 (C2), 137.14, 158.53, 177.04 (C3).

Cytotoxic activity of compounds (I), (IIIa)–(IIIj), (IVa), (IVb), and (Va)–(Ve). The JurkatE6.1 (human lymphoblast leucosis) and Mel.P (human melanoma) cellular lines were used in this study. The cells were cultured on an RPMI-1640 culture medium with the addition of 10% fetal calf serum at 37°Cin an atmosphere of 5% CO_2. At the beginning of the experiment, the cells were placed in 96-well plates (15×10^3 cells per one well). The tested compounds were added to the wells 24 h later in a DMSO solution at a final concentration of 100 μM. Four parallel samples corresponded to each compound. The total volume of the incubation medium in a well was 200 μl. The DMSO content in the culture medium was no higher than 0.01%. The subsequent incubation of the cells with the studied compounds was performed for 48 h at 37°C under the same conditions. When the incubation was finished, MTT was introduced into the wells in the final concentration of 0.5 mg/ml, and the plates were incubated for 2 h at 37°C in an atmosphere of 5% CO_2. The medium was removed, and DMSO (100 μl in each well) was added to dissolve the blue formazan crystals that formed as a result of the MTT reduction by the dehydrogenases of the living cells. The optical absorption of the formazan-colored solutions in the wells was measured on a Titertek Multiscan MCC-340 scanning spectrometer (Great Britain) at 570 nm. The results

were expressed as a survival index (%) that was determined as the relative value $A_o/A_k \times 100\%$, where A_o and A_k were optical absorptions of the experimental and control samples, respectively. The compound was considered to be active if the cellular survival was ≤50% at a concentration of 100 μM. The experimental error was no higher than 5%.

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REFERENCES

1. Lontsi, D., Sondengam, B.L., and Ayafor, J.F., *J. Nat. Prod.*, 1989, vol. 52, pp. 52–56.
2. Chen, I.-H., Du, Y.-Ch., Lu, M.-Ch., Lin, A.-Sh., Hsieh, P.-W., Wu, Ch.-Ch., Chen, Sh.-L., Yen, H.-F., Chang, F.-R., and Wu, Y.-Ch., *J. Nat. Prod.*, 2008, vol. 71, pp. 1352–1357.
3. Wei, Y., Ma, Ch.-M., Chen, D.-Yu., and Hattori, M., *Phytochemistry,* 2008, vol. 69, pp. 1875–1879.
4. Wei, Y., Ma, Ch.-M., and Hattori, M., *Bioorg. Med. Chem.*, 2009, vol. 17, pp. 3003–3010.
5. Wei, Y., Ma, Ch.-M., and Hattori, M., *Eur. J. Med. Chem*, 2009, DOI: 10.1016/j.ejmech.2009.05.002.
6. Urban, M., Sarek, J., Klinot, J., Korinkova, G., and Hajduch, M., *J. Nat. Prod.*, 2004, vol. 67, pp. 1100–1105.
7. Tolmacheva, I.A., Nazarov, A.V., Maiorova, O.A., and Grishko, V.V., *Khim. Prirod. Soed.*, 2008, no. 5, pp. 491–494.
8. Tolmacheva, I.A., Grishko, V.V., Boreko, E.I., Savinova, O.V., and Pavlova, N.I., *Khim. Prirod. Soed.*, 2009, no. 5 (in press).
9. Anikina, L.V., Tolmacheva, I.A., Vikharev, Yu.V., and Grishko, V.V., *Bioorg. Khim.*, 2010, vol. 36, pp. 259–264 [*Russ. J. Bioorg. Chem.* (Engl. Transl.), 2010, vol. 36 (in press)].
10. Alley, M.C., Scudiero, D.A., Monks, A., Hursey, M.L., Czerwinski, M.J., Fine, D.L., Abbott, B.J., Mayo, J.G., Shoemaker, R.H., and Boyd, M.R., *Cancer Res.*, 1988, vol. 48, pp. 589–601.
11. Kim, J.Y., Koo, H.-M., and Kim, D.S.H.L., *Bioorg. Med. Chem. Lett.*, 2001, vol. 11, pp. 2405–2408.
12. Cichewicz, R.H. and Kouzi, S.A., *Med. Res. Rev.*, 2004, vol. 24, pp. 90–114.
13. Alakurtti, S., Mäkelä, T., Koskimies, S., and Yli-Kauhluoma, J., *Eur. J. Pharm. Sci.*, 2006, vol. 29, pp. 1–13.
14. Fulda, S., *Int. J. Mol. Sci.*, 2008, no. 9, pp. 1096-1107.
15. *Laboratornaya tekhnika organicheskoi khimii* (Laboratory Equipment in Organic Chemistry), Keil, B., Ed., Moscow: Mir, 1966, p. 596.