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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and evaluation of antiviral activities of triterpenic conjugates with 2-aminobutan-1-ol as potent microbicidal agents

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Abstract

The effect of the synthetic modifications of the triterpenic A ring on the level of antiviral activity of triterpenic C3, C28 amides with a residue of racemic, (*S*), or (*R*)-enantiomeric 2-aminobutan-1-ol against herpes simplex viruses type I (HSV-I) and type II (HSV-II), as well as against human immunodeficiency virus type I (HIV-1) was investigated. The 2,3-secolupane racemic amide **5a** was selected as a potent microbicidal agent with the highest virus inhibitory (against HSV-I and HSV-II) and virucidal (against HSV-1 and HIV-1) actions, the antiviral activity of which was provided by the (*S*)-enantiomeric conjugate **5b**.

Keywords Betulinic acid · Amides · HIV · HSV Antiviral activity · Microbicides

Introduction

The worldwide Human Immunodeficiency Virus (HIV) epidemic has swept over 40 million people and the number of HIV-positive patients is constantly growing. Currently, more than 30 anti-retroviral (ARV) drugs and novel drug candidates represented by the inhibitors of integrase, reverse transcriptase, protease, as well as inhibitors of the HIV-I stages of fusion, penetration and maturation, are used in anti-HIV chemotherapy or are under clinical evaluation (Sosnik et al. 2009). In absence of cure or an effective vaccine, ARV agents remain the cornerstone of treatment and prevention of HIV infection. The introduction of highly active ARV therapy based on drug cocktails in 1996 significantly reduced the AIDS-related deaths. However, high long-term toxicity of anti-HIV drugs and the rapid development of HIV-I drug resistance remain as limiting factors of anti-HIV chemotherapy (Martinez et al. 2006; Chawla et al. 2018). To reduce the spread of HIV infection, an integrated approach including some preventive measures appears to be relevant. Currently, the development of prophylactic anti-HIV microbicides termed also "chemical contraceptives", as prophylactic measures to prevent the HIV infection, is meant as the most promising strategy to combat the HIV-AIDS epidemic.

The microbicides are topically administered drugs to protect against HIV infection by direct viral inactivation or blocking penetration of virus particles into cells. The analysis of the early and current microbicide generations showed the last researches to be focused on the development of microbicides containing the highly-specific ARV drugs used to treat HIV/AIDS (Das Neves and Sarmento. 2014). But the challenge posed by the virus mutation remains active: The ARV-based microbicides can exhibit a possible loss of their activity against transmission of viruses from drug-resistant HIV carriers (Martinez et al. 2006). In addition, the clinical trials' results of the most promising 1% gel of the nucleoside reverse transcriptase inhibitor Tenofovir proved to be disappointing (Pialoux et al. 2016).

The spectrum of novel effective anti-HIV agents is continuously updated. As a valuable reservoir for the discovery of new medicines, including ARV drugs and microbicides, plant-derived natural products are widely

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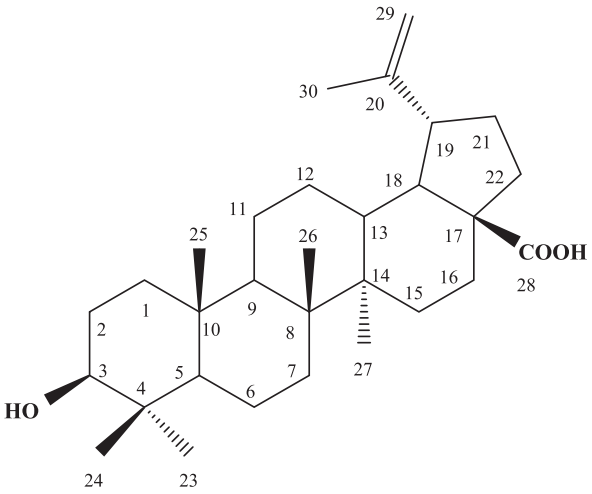


Fig. 1 Betulinic acid

used (Salehi et al. 2018). Among them, biologically active polycyclic triterpenoids without marked toxicity even at higher concentrations (more often lupane-, oleanane-, and ursane-type triterpenoids) are one of the most popular platforms for the drug development (Zhou et al. 2017), in particular, of antiviral agents (Xiao et al. 2018). In turn, the triterpenoid betulin extractable from the birch bark has a high synthetic potential to obtain agents with a broad antiviral spectrum, including that against HIV-I, HSV-I, etc. (Bednarczyk-Cwynar and Günther 2017; Pokorny et al. 2018; Xiao et al. 2018). Selective modifications of C3 and C28 atoms of the lupane skeleton enable to significantly enhance their antiviral properties (Fig. 1). For example, the amide and ester conjugates of betulinic acid (Fig. 1) proved to be highly active against HIV-I, with bevirimat and its derivatives being the most active anti-HIV agents (Kashiwada et al. 1996; Coric et al. 2013). Special attention was paid to the synthesis of antiviral agents with multitarget anti-HIV activity capable of inhibiting the different stages of the HIV life cycle. The C3 substituted betulinic acid derivatives having become known as capable of inhibiting the HIV-I maturation stage, and C28 substituted betulinic acid derivatives—as capable of blocking the HIV-I penetration stage, hybrid molecules with pharmacophore substituents at C3 and C28 atoms and the dual mode of action blocking both stages of the viral life cycle were synthesized (Evers et al. 1996; Soler et al. 1996; Sun et al. 1998; Lee 2010; Csuk 2014).

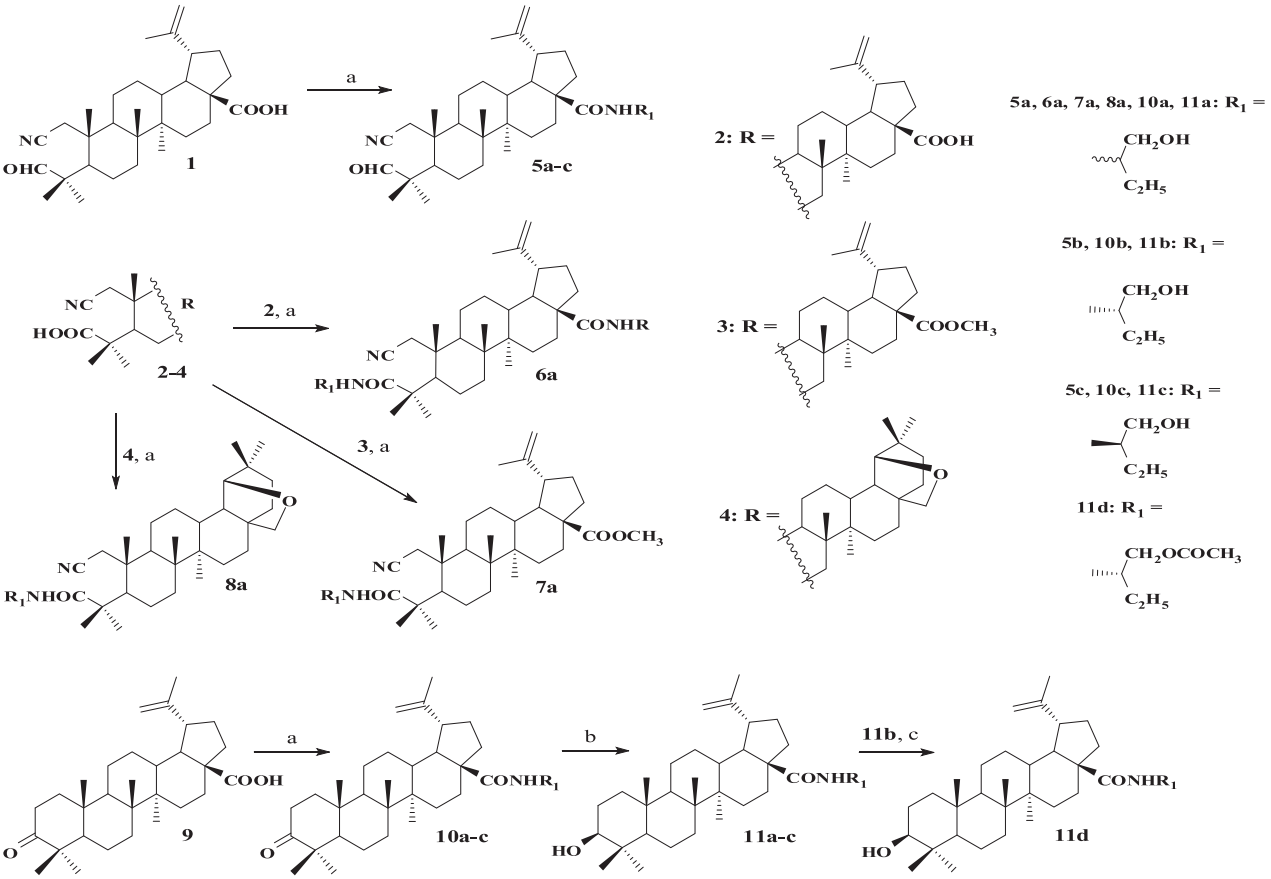
Earlier, we had demonstrated the 2,3-secotriterpenic derivatives synthesized from betulin as potentially capable of inhibiting HIV-I, herpes simplex virus type I (HSV-I), influenza A virus, and vesicular stomatitis virus (VSV) (Tolmacheva et al. 2009; Galayko et al. 2010; Tolmacheva et al. 2013; Tolmacheva et al. 2014; Grishko et al. 2014; Tolmacheva et al. 2017; Pereslavtseva et al. 2014; Konysheva et al. 2017; Galaiko et al. 2018). The virucidal nature of betulinic acid against the enveloped VSV (Kaminska et al. 2004) permits the assumption that 2,3-secotriterpenoids would be similarly active against other enveloped viruses, such as HIV and HSV. The active search for new effective bitarget anti-HIV and anti-HSV ARV agents, including microbicides (Chamoun-Emanuelli et al. 2014; Kizima et al. 2014; Gordts et al. 2015), was initiated by reason of the fact that HSV-II infection is an important risk factor facilitating the HIV-I transmission through mucosal membranes disrupted by HSV-I (Looker et al. 2017); at this point, both HSV-1 and HSV-2 are commonly regarded as sexually transmitted viruses (Jin et al. 2006).

Earlier, 1-cyano-2,3-seco-2-norlup-20(29)-en-3-al-28-oic acid **1** (Tolmacheva et al. 2009) and its amide with 2-aminopropane-1,3-diol (Tolmacheva et al. 2014) were described by us as the most active agents inhibiting HSV-I replication. In the present investigation, we have synthesized a novel series of amide conjugates of 2,3-secotriterpenic acids with 2-aminobutan-1-ol as a new pharmacophore, and evaluated their antiviral properties against HSV-I, HSV-II, and HIV-I, as well as their microbicidal potential. In addition, the comparative antiviral screening of cyclic triterpenic analogues (amides of betulinic and betulonic acids with 2-aminobutan-1-ol and their derivatives with modified C3 and C20 atoms) has also been performed.

Results and discussion

The 2,3-secolupane and 2,3-seco-oleanane mono- and dicarboxylic acids **1**–**4** (Tolmacheva et al. 2008; Tolmacheva et al. 2009) treated with oxalyl chloride were transformed to corresponding acid chlorides which in reaction in situ with 2(*S*, *R*)-aminobutan-1-ol afforded the C3 and C28 mono- and diamides **5a**–**8a** in 23–42% yields (Scheme 1). The ^{1}H NMR spectra of the amides **5a**–**8a** contained the characteristic signals of the triterpenic fragment and those of the substituent at the nitrogen atom. As for the signal(s) of the amide proton(s), they appeared as one (**7a** and **8a**), two (**5a**), or three (**6a**) doublets in the range from 5.68 to 5.94 ppm. In the IR spectra, the absorption bands at 3301–3465 and 1639–1659 cm^{-1} were observed, therewith showing the evidence of availability of the amide bond(s).

The inhibitory effects of amides **5a, 6a, 7a** and **8a** on replication of HSV-I and HIV-1 were studied on the pre-infected cell cultures. The extracellular virucidal effects of the triterpenic derivatives were evaluated on the basis of the residual infectivity of the viruses after the virus-containing suspensions were treated with the test compounds for a preset period of time. When initially evaluated for inhibitory properties against HSV-I replication, the 2,3-secotriterpenic



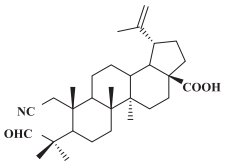
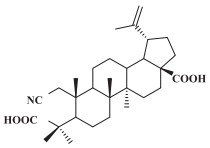
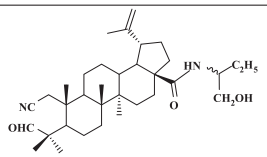
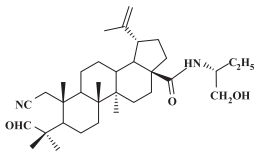
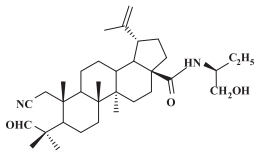
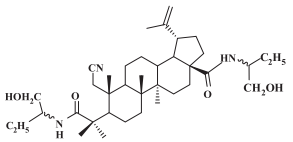
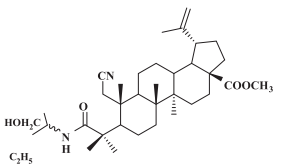
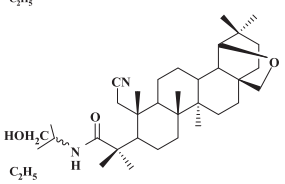
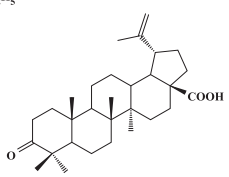
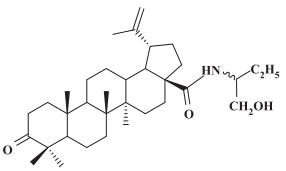
Scheme 1 The synthesis of triterpene-amide conjugates **5a–c, 6a–8a, 10a–c, 11a–d**. Reagents and conditions: **a** (1) $(COCl)_2$, CH_2Cl_2, room temperature, 6 h; (2) R_1-NH_2, $(C_2H_5)_3N$, CH_2Cl_2, room temperature, 4–6 h; **b** $NaHB_4$, CH_3OH, reflux, 5 min; **c** $(CH_3)_2O$, pyridine, room temperature, 20 h

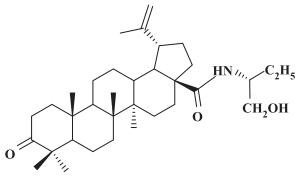
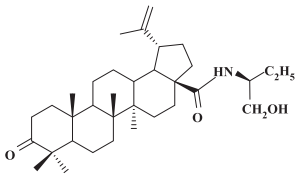
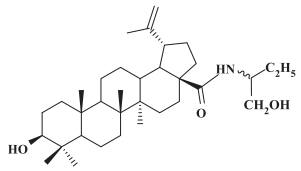
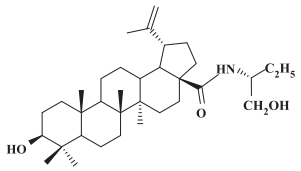
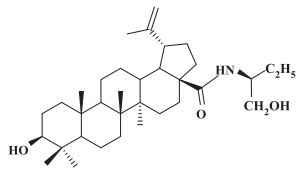
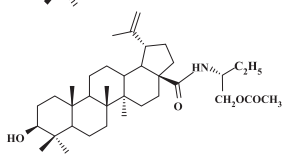
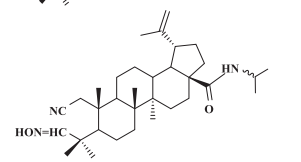
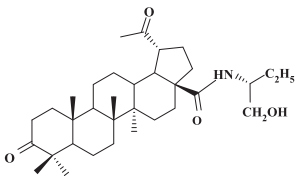
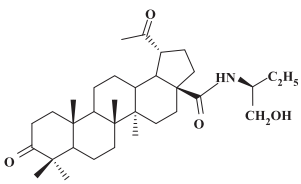
amides **5a, 6a, 7a** and **8a** showed mainly a lower level of activity (Table 1) as compared with the starting 2,3-seco-acids **1** and **2** (Tolmacheva et al. 2009). The most anti-HSV-I active amide **5a** (EC_{50} 20.7 µg/mL; MTC/EC_{50} 19.3) also showed a moderate anti-HSV-II effect (EC_{50} 45.3 µg/mL, MTC/EC_{50} 4.4) when studied to determine the replication-inhibiting activity against the HSV-II strain with a reduced sensitivity to acyclovir. According to the results of the HIV-1 replication inhibition assay, the amides **5a, 6a, 7a** and **8a** evinced inactivity against HIV-1. At the same time, the amide **5a** was the only compound that showed a pronounced virucidal action against both HSV-I (Table 2: EC_{50}15.6 µg/mL, ET_{50} 13.3 min) and HIV-1 (EC_{50} 0.6 µg/mL, ET_{50} 0.4 min).

Judging from the test results of amides, the virucidal action of amide **5a** was of most interest (Tables 1, 2). Indeed, the findings obtained further suggested the virucidal anti-HSV-I activity for other lupane conjugates with the racemic, (*S*)-, or (*R*)-enantiomeric 2-aminobutan-1-ol residues including A-cyclic triterpenic amides. In this connection, the 2,3-secolupane amides **5b** and **5c** were synthesized with corresponding (*S*)- or (*R*)-enantiomeric-pure 2-aminobutan-1-ol residues, as well as their A-cyclic analogues **10a–c** and **11a–c** based on betulonic acid and betulinic acid, respectively (Scheme 1). It is worth noting that the monoacetate **11d** as a single reaction product was obtained during the mild acylation of (*S*) amide **11b** of betulinic acid (Scheme 1). In the ^{1}H NMR spectra of the synthesized amides **5b**,**c, 10a–c** and **11a–d**, the doublet signals of amide protons were recorded in the range of 5.68–5.85 ppm. The proton signals of the $-CH_2OH$ group of the amide substituent of compounds **5b**,**c**, **10a–c** and **11a–c** were registered as two doublets of doublets at 3.55–3.63 and 3.66–3.74 ppm, and in a weaker field of the ^{1}H NMR spectrum ($-CH_2$-$OCOCH_3$ at 4.07 and 4.17 ppm) in case of compound **11d**.

According to the estimation of the inhibitory activity of amides **5a–c, 6a–8a**, **10a–c**, **11a–d** against HSV-I (Table 1), among A-cyclic triterpenic amides **10a–c**, **11a–d**, the most significant results were obtained for the conjugates **10c** and **11c** with the (*R*)-amino alcohol, and for the conjugate **5b** with the (*S*)-amino alcohol in case of 2,3-secotriterpenic amides **5b,c**. A significant role of the chirality was also demonstrated by Dobrikov et al. (Dobrikov et al. 2012) on a

Table 1 The virus-inhibiting effect of the synthesized amides against HSV-I replication

Compound	Chemical structure	EC_{50}, μg/mL	MTC/EC_{50}
1*		4.1 μM	14.0
2*		44.0 μM	9.7
5a		20.7	19.3
5b		37.6	21.2
5c		117.8	1.7
6a		>50	<1
7a		>100	<1
8a		>50	<1
9**		2.5 μM	43.9
10a		10.7	18.7

Compound	Chemical structure	EC_{50}, μg/mL	MTC/EC_{50}
10b		10.3	4.8
10c		29.5	13.6
11a		20.5	9.8
11b		>50	<1
11c		22.7	8.8
11d		>100	<1
13a		>100	<1
17b		8.3	12.0
17c		126.1	1.6

[a]Data of Ref. (Tolmacheva et al. 2009)

[b]Data of Ref. (Baltina et al. 2003)

Table 2 The virucidal activity of synthesized amides against HSV-I

Compound	EC_{50}[a], μg/mL	ET_{50}, min
5a	15.6	13.3
5b	9.15	0.95
5c	>200	>30
10a	186.8	11.8
10b	36.8	10.1
10c	>200	>30
11a	505.3	0.13
11b	>200	>30
11c	417.0	0.53
13a	460.7	0.37
17b	>800	>60
17c	>800	>60

[a]Exposure time—15 min

series of (*R*)-2-amino-1-butanol derivatives, with some of which being more active than ethambutol, a (*S,S*)-configured bacteriostatic anti-tuberculosis drug. In case of triterpenic amides, the active (*S*)- or (*R*)-isomers mainly contributed to the high level of the antiviral activity of respective racemic amides **5a**, **10a**, and **11a**: The values of antiviral action of the triterpenic conjugates with the residue of the racemic amino alcohol **5a**, **10a**, and **11a** were comparable with the level of the individual active diastereomers **5b**, **10c**, and **11c**, respectively. The example of amide **11d** showed the protection of hydroxyl group in the amino alcohol residue to result in the loss of the virus-inhibitory activity.

In the study of the replication inhibitory and virucidal activities of the amides **5a–c**, **10a–c**, **11a–d** against HIV-I, the virucidal activity was demonstrated only by 2,3-seco-triterpenic amide **5a** (EC_{50} 0.6 μg/mL, ET_{50} 0.4 min) and by its (*S*)-enantiomer **5b** (EC_{50} 0.3 μg/mL, ET_{50} 0.9 min).

To evaluate the relationship between the structure and antiviral activity (SAR), the triterpenic conjugates **13a**, **15a**, **17b,c** with racemic 2-aminobutan-1-ol based on the known 3-hydroxyimino-1-cyano-2,3-seco-2-norlup-20(29)-ene-28-oic acid (**12**) (Grishko et al. 2014), betulonic acid oxime **14** (Flekhter et al. 2004), and 3,20-dioxo-30-norlupan-28-oic acid (**16**) (Samoshina et al. 2003) were synthesized (Scheme 2). In case of amide **15a**, the alternative methods for synthesis were used: (1) amidation of the oxime **14**, or (2) oximation of amide **10a** with the yields 27 and 61%, respectively. The individual (*S*) (**17b**) and (*R*) (**17c**) isomers were isolated by the chromatographic purification of the synthesized amide **17**. In the NMR ^{1}H spectra of the racemic conjugates **13a** and **15a**, the amide proton was recorded as two broad doublets at 5.69 and 5.73–5.74 ppm, while in case of enantiomers **17b** and **17c** it was registered as a doublet at 5.80 (**17b**) or 5.77 (**17c**) ppm. The reaction of 3,20-dioxo derivative **16** with hydroxylamine hydrochloride in pyridine led to new triterpenic 3-oxime **18** in 43% yields. We were unsuccessful in isolating the compound **18** in its pure individual form by column chromatography, so its structure was confirmed only by IR spectroscopy. The amide **19a** was synthesized by the reaction of corresponding oxime **18** with racemic 2-aminobutan-1-ol, this fact being confirmed by the detection of the amide proton as a broad doublet at 5.75 ppm in the NMR ^{1}H spectra.

According to the screening of the inhibitory and virucidal properties, the amides **13a**, **17b,c** and oxime **18** exhibited neither any anti-HIV-1 action, nor a noticeable antiviral activity against HSV-I as compared with amide **5a** and its (*S*)-isomer **5b**. At the same time, the synthesized A-cyclic amides **17b**,**c** were markedly inferior to their parent compound—betulonic acid **9** (EC_{50} 2.5 μM; MTC/EC_{50} 43.9)—in their anti-HSV-I inhibitory activity (Baltina et al. 2003).

Similarly, the inhibitory and virucidal properties against HSV and HIV-1 were most successfully combined when the triterpenic structure included the fragmented A-ring with the aldehyde group at C3 position and the amide bond between C28 atom and racemic or (*S*)-enantiomeric 2-aminobutan-1-ol.

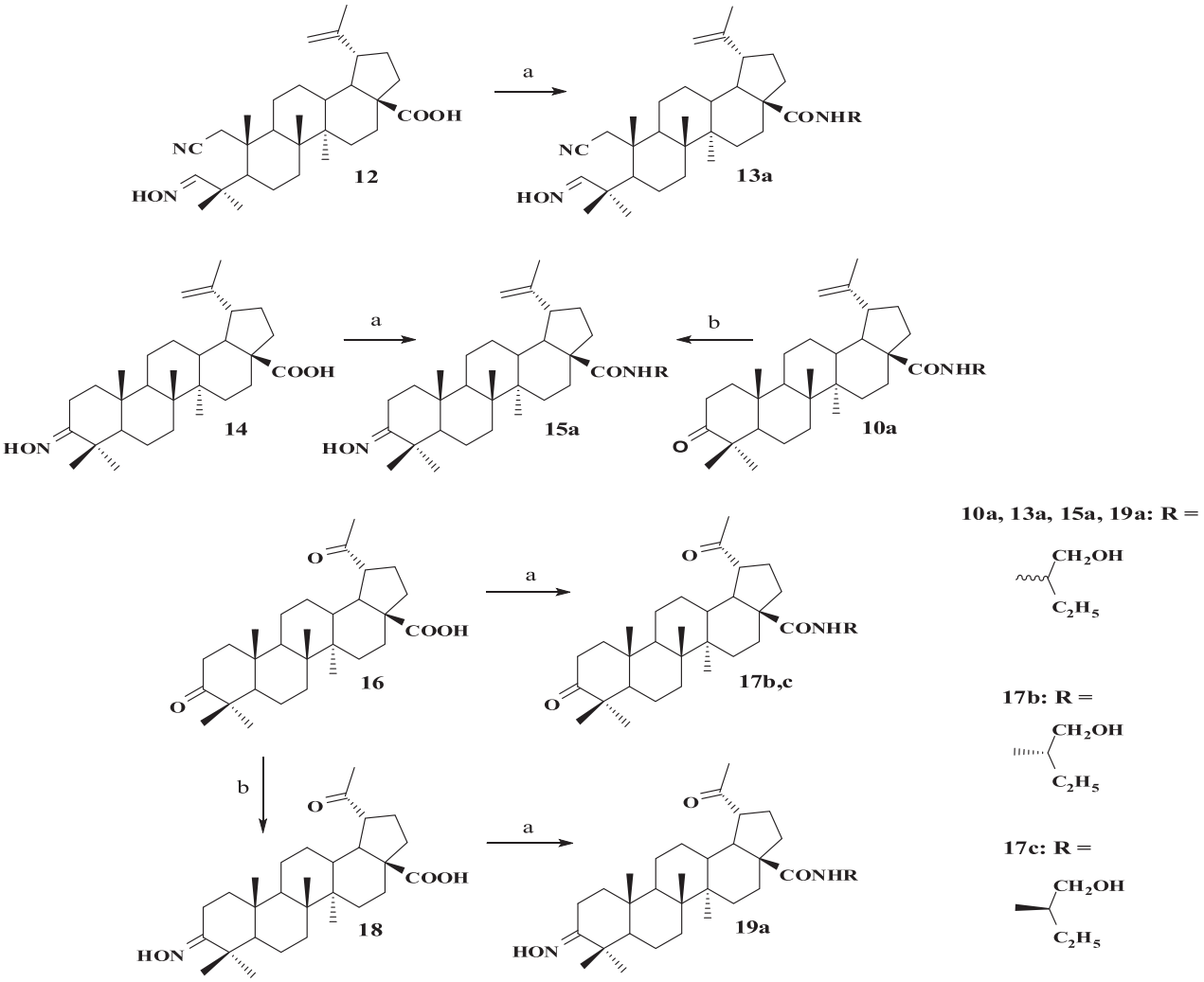
Conclusion

Thus, the new betulonic acid-based lupane C28 amides with the 2-aminobutan-1-ol residue and the modified A-ring, as well as those with the transformed isopropylene moiety, were synthesized. Configuration of the asymmetric center of the pharmacophore fragment of 2-aminobutan-1-ol was shown to play a determining role in manifestation of the virus-inhibiting and virucidal activities of the synthesized triterpenic amides against HSV-I: The (*R*)-isomeric amides were active among the A-cyclic conjugates, while the (*S*)-isomer was most active among the 2,3-secotriterpenic derivatives. Among the synthesized compounds, only amide **5a** and its (*S*)-isomer **5b** had anti-HIV virucidal activity. Taking into account the commercial accessibility of racemic 2-aminobutan-1-ol, contrary to its (*S*)-isomer, the synthesized triterpenic amide **5a** is to be considered as a more promising anti-HIV microbicidal agent with the anti-HSV properties.

Experimental

Chemistry

Melting points were determined on the OptiMelt MPA100 device at the heating rate of 1 °C/min. Optical rotation was measured with the Perkin-Elmer 341 polarimeter in $CHCl_3$ solution. The IR spectra were recorded from a thin film produced by evaporation of a $CHCl_3$ solution using the IFS



Scheme 2 The synthesis of triterpene-amide conjugates **13a**, **15a**, **17b**, **c**, **19a**. Reagents and conditions: **a** (1) $(COCl)_2$, CH_2Cl_2, room temperature, 6 h; (2) R_1-NH_2, $(C_2H_5)_3N$, CH_2Cl_2, room temperature, 4–6 h; **b** $NH_2OH{\cdot}HCl$, ethanol-pyridine, reflux, 2 h

66 ps IR-Fourier spectrometer. The ^1H and ^{13}C NMR spectra were recorded using the Varian Mercury plus 300 (^1H NMR: 300 MHz, ^{13}C NMR: 75.5 MHz) and the Bruker AVANCE (^1H NMR: 400 MHz, ^{13}C NMR: 100 MHz) spectrometers in $CDCl_3$ with tetramethylsilane as an internal standard. The Sorbfil plates were employed for the thin-layer chromatography (TLC). Elemental analyses were performed using the Vario El cube analyzer, and agreed with those calculated. Column chromatography was performed using the Macherey-Nagel 60 Silica standard adsorbent (0.063–0.2 mm). Mass spectra (MS) were determined on the Agilent Technologies 6890N with the capillary column HP-5ms (4000 × 0.25 mm, 0.25 μm) and the 5975B mass spectrometer. Triterpenic acids **1**–**4** (Tolmacheva et al. 2008, Tolmacheva et al. 2009), **9** (Kim et al. 1997), **12** (Grishko et al. 2014), **14** (Flekhter et al. 2004), **16** (Samoshina et al. 2003) were prepared according to the literature methods. We used chemical solvents and reagents of chemically pure, analytically pure, and high-purity grades (Russia) in addition to the commercially available reagents (2*R*,*S*)-aminobutan-1-ol, (2*S*)-aminobutan-1-ol, (2*R*)-aminobutan-1-ol (Alfa Aesar, USA).

General procedure for the preparation of 5a-c, 6a–8a, 10a-c, 13a, 15a, 17b,c, 19a

A solution of **1** (or **2**, or **3**, or **4**, or **9**, or **12**, or **14**, or **16**, or **18**, successively) (3.3 mmol) in anhydrous CH_2Cl_2 (20 mL) was treated with oxalyl chloride (6.6 mmol, 0.6 mL) and stirred at room temperature for 6 h; in case of acid **2**, 13.2 mmol (1.2 mL) of oxalyl chloride were added. The solvent was distilled under vacuum on a water bath at 30 °C to attain a dry state of the residue. The residue was treated with anhydrous CH_2Cl_2 (20 mL). The solvent was again distilled off. The procedure was reiterated thrice. A suspension of the triterpene acid chloride obtained in this way

in anhydrous CH_2Cl_2 (20 mL) was treated with (2*R*,*S*)-aminobutan-1-ol (or (2*S*)-aminobutan-1-ol, or (2*R*)-aminobutan-1-ol) (3.6 mmol) and Et_3N (3.6 mmol,) and stirred for 4–6 h at room temperature (7.2 mmol of (2*R*,*S*)-aminobutan-1-ol and 7.2 mmol of Et_3N were added in case of acid **2**). The reaction was monitored by TLC. The solvent was evaporated. The residue was purified by column chromatography.

***N*-[1-cyano-2,3-seco-2-norlup-20(29)-en-3-al-28-oyl]-(2*R*,*S*)-aminobutan-1-ol (5a)** The crude product was purified by column chromatography on silica gel ($CHCl_3$:MeOH 20:1) to afford **5a**. Yield 42%; m.p. 166.2 °C; $[\alpha]_D^{21} + 14.6$ ($c = 0.34$; $CHCl_3$); IR γ_{max} (KBr) cm^{-1}: 1640 (CONH), 1719 (CHO), 2242 (C≡N), 3376 (OH, NH); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 0.89, 0.96, 1.03, 1.08, 1.13 (5 × 3H, 5br s, $5H_3$), 0.94 and 0.95 (2 × 1.5 H, 2t, $J = 7.5$ Hz, $-CH_2\underline{CH_3}$), 1.68 (3H, br s, H_3-30), 2.22 and 2.60 (2H, 2br d, $J = 18.3$ Hz, H_2-1), 3.06–3.15 (1H, m, H-19), 3.53–3.59 and 3.65–3.69 (2 × 0.5 H, 2 m, $-\underline{CH_2}$-OH), 3.80–3.90 (1H, m, -CH-NH−), 4.59 and 4.72 (2H, 2s, H_2-29), 5.68 and 5.74 (2 × 0.5H, 2 br d, NH), 9.66 (1H, s, H-3).). GC-MS (*m/z, I*): 520.3 $[M - H_2O]^+$. Anal. Calcd for $C_{34}H_{54}N_2O_3$ (%): C, 75.79; H, 10.10; N, 5.20. Found: C, 75.85; H, 10.15; N, 5.22.

***N*-[1-cyano-2,3-seco-2-norlup-20(29)-en-3-al-28-oyl]-(2*S*)-aminobutan-1-ol (5b)** The crude product was purified by column chromatography on silica gel ($CHCl_3$:MeOH 20:1) to afford **5b**. Yield 49%; m.p. 212.2 °C; $[\alpha]_D^{25}$0.0 ($c = 0.5$; $CHCl_3$); IR γ_{max} (KBr) cm^{-1}: 1629 (CONH), 1720 (CHO), 2244 (C≡N), 3328 (OH, NH); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 0.89, 0.95, 1.03, 1.08, 1.13 (5 × 3H, 5s, $5H_3$), 0.95 (3H, t, $J = 7.5$, Hz, $\underline{CH_3}CH_2$), 1.67 (3H, s, H_3-30), 2.22 and 2.59 (2H, 2d, $J = 18.3$ Hz, H_2-1), 3.10 (1H, td, $J = 11.1$, 4.4 Hz, H-19), 3.58 (1H, 1dd, $J = 5.8$, 10.9 Hz, $\underline{CH_2}$-OH), 3.67 (1H, 1dd, $J = 3.5$, 10.9 Hz, $\underline{CH_2}$-OH), 3.80–3.87 (1H, m, -CH-NH-), 4.60 and 4.72 (2H, 2s, H_2-29), 5.70 (1H, br d, NH), 9.66 (1H, s, H-3); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 10.73, 14.55, 15.92, 18.76, 19.39, 19.67, 20.23, 21.95, 23.37, 24.17, 25.60, 29.41, 29.70, 30.79, 33.32, 33.70, 37.74, 38.51, 40.69, 42.27, 42.93, 44.74, 46.60, 49.11, 49.88, 50.77, 53.01, 55.77, 65.84, 109.64 (C-29), 118.04 (C-2), 150.52 (C-20), 177.33 (C-28), 206.01 (C-3). GC-MS (*m/z*, *I*): 520.5 $[M - H_2O]^+$. Anal. Calcd for $C_{34}H_{54}N_2O_3$ (%): C, 75.79; H, 10.10; N, 5.20. Found: C, 75.87; H, 10.13; N, 5.23.

***N*-[1-cyano-2,3-seco-2-norlup-20(29)-en-3-al-28-oyl]-(2*R*)-aminobutan-1-ol (5c)** The crude product was purified by column chromatography on silica gel ($CHCl_3$:MeOH 20:1) to afford **5c**. Yield 38%; m.p. 140.0 °C; $[\alpha]_D^{25} + 29.0$ ($c = 0.1$; $CHCl_3$); IR γ_{max} (KBr) cm^{-1}: 1638 (CONH), 1718 (CHO), 2241 (C≡N), 3381 (OH, NH); ^1H NMR ($CDCl_3$): δ 0.88, 0.96, 1.03, 1.08, 1.13 (5 × 3H, 5s, $5H_3$), 0.94 (3H, t, $J = 7.4$ Hz, $\underline{CH_3}CH_2$), 1.67 (3H, s, H_3-30), 2.22 and 2.58 (2H, 2d, $J = 18.3$ Hz, H_2-1), 3.10 (1H, td, $J = 11.1$, 4.5 Hz, H-19), 3.55 (1H, dd, $J = 6.2$, 10.8 Hz, $\underline{CH_2}$-OH), 3.68 (1H, dd, $J = 3.5$, 10.8 Hz, $\underline{CH_2}$-OH), 3.82–3.91 (1H, m, -CH-NH), 4.59 and 4.72 (2H, 2s, H_2-29), 5.63 (1H, br d, NH), 9.65 (1H, s, H-3); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 10.66, 14.58, 15.81, 18.77, 19.40, 19.65, 20.20, 21.94, 23.41, 24.24, 25.58, 29.40, 29.68, 30.77, 33.28, 33.80, 37.62, 38.50, 40.71, 42.29, 42.95, 44.75, 46.61, 49.14, 49.94, 50.79, 53.11, 55.71, 66.35, 109.69 (C-29), 118.07 (C-2), 150.51 (C-20), 177.58 (C-28), 206.05 (C-3). Anal. Calcd for $C_{34}H_{54}N_2O_3$ (%): C, 75.79; H, 10.10; N, 5.20. Found: C, 75.78; H, 10.14; N, 5.25.

***N*,*N*′-[1-cyano-2,3-seco-2-norlup-20(29)-en-3,28-dioyl]-di-(2*R*,*S*)-aminobutan-1-ol (6a).** The crude product was purified by column chromatography on silica gel ($CHCl_3$: MeOH 20:1) to afford **6a**. Yield 32%; m.p. 203.7 °C; $[\alpha]_D^{21} + 9.5$ ($c = 0.07$; $CHCl_3$); IR γ_{max} (KBr) cm^{-1}: 1639 (CONH), 2244 (C≡N), 3371 (OH, NH); ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 0.93 (2 × 3H, br t, $J = 7.2$ Hz, 2−$CH_2\underline{CH_3}$), 0.95 (6H, s, $2H_3$), 1.01, 1.22, 1.25 (3 × 3H, 3s, $3H_3$), 1.67 (3H, s, H_3-30), 2.47 and 2.64 (2H, 2d, $J = 18.3$ Hz, H_2-1), 3.05–3.16 (3H, m, H-19), 3.53–3.92 (6H, m, 2−$\underline{CH_2}$OH and 2−$\underline{CH}$NH−), 4.59 and 4.72 (2H, 2s, H_2-29), 5.71 and 5.77 (2 × 0.5H, 2br d, NH), 5.94 (1H, br d, NH). Anal. Calcd for $C_{38}H_{63}N_3O_4$ (%): C, 72.92; H, 10.15; N, 6.71. Found: C, 73.00; H, 10.21; N, 6.81.

***N*-[28-metoxy-28-oxo-1-cyano-2,3-seco-2-norlup-20(29)-en-3-oyl]-(2*R*,*S*)-aminobutan-1-ol (7a)** The crude product was purified by column chromatography on silica gel ($CHCl_3$: MeOH 20:1) to afford **7a**. Yield 35%; m.p. 152.0 °C; $[\alpha]_D^{21} + 8.8$ ($c = 0.38$; $CHCl_3$); IR γ_{max} (KBr) cm^{-1}: 1641 (CONH), 1725 ($COOCH_3$), 2243 (C≡N), 3301, 3465 (OH, NH); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 0.92, 0.93, 0.97, 0.98, 1.23, 1.25 (6 × 1.5H, 6s, $3H_3$), 1.01, 1.26 (2 × 3H, 2s, $2H_3$), 0.94 and 0.96 (2 × 1.5, 2t, $J = 7.4$ Hz, $-CH_2\underline{CH_3}$), 1.67 (3H, s, H_3-30), 2.46, 2.49, 2.55 and 2.66 (4 × 0.5 H, 4d, $J = 18.0$ Hz, H_2-1), 2.95–3.01 (1H, m, H-19), 3.56–3.64 and 3.70–3.80 (2H, 2m, $-\underline{CH_2}$-OH), 3.66 (3H, c, $COO\underline{CH_3}$), 3.83–3.91 (1H, m, $-\underline{CH}$-NH), 4.60 and 4.72 (2H, 2s, H_2-29), 5.89 and 5.91 (2 × 0.5H, 2br d, NH). GC-MS (*m/z, I*): 568.4 $[M^+]$. Anal. Calcd for $C_{35}H_{56}N_2O_4$ (%): C, 73.90; H, 9.92; N, 4.92. Found: C, 74.13; H, 10.17; N, 4.86.

***N*-[1-cyano-2,3-seco-19β,28-epoxy-2-nor-18α-olean-3-oyl]-(2*R*,*S*)-aminobutan-1-ol (8a)** The crude product was purified by column chromatography on silica gel ($CHCl_3$: MeOH 20:1) to afford **8a**. Yield 23%; m.p. 93.3 °C;

$[\alpha]_D^{21} + 4.2$ ($c = 0.42$; $CHCl_3$); IR γ_{max} (KBr) cm^{-1}: 1659 (CONH), 2239 (C≡N), 3421 (OH, NH); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 0.79, 0.93, 0.96, 0.98, 1.01, 1.23, 1.27 ($7 \times 3H$, 7s, $7H_3$), 0.97 (3H, br t, $J = 7.5$ Hz, $-CH_2\underline{CH_3}$), 2.50 and 2.70 (2H, 2d, $J = 18.1$ Hz, H_2-1), 3.44 (1H, d, $J = 7.8$ Hz, H_2-28), 3.52 (1H, s, H-19), 3.64 (1H, dd, $J = 5.5$, 11.3 Hz, $-\underline{CH_2}OH$), 3.75–3.79 (2H, m, H_2-28 and $-\underline{CH_2}OH$), 3.84–3.92 (1H, m, $-\underline{CH}NH-$), 5.90 (1H, br d, NH). Anal. Calcd for $C_{34}H_{56}N_2O_3$ (%): C, 75.51; H, 10.44; N, 5.18. Found: C, 75.57; H, 10.39; N, 5.11.

***N*-[3-oxolup-20(29)-en-28-oyl]-(2*R*,*S*)-aminobutan-1-ol (10a)** The crude product was purified by column chromatography on silica gel ($CHCl_3$:MeOH 20:1) to afford **10a**. Yield 53%; m.p. 121.4 °C; $[\alpha]_D^{25} + 30.2$ ($c = 0{,}4$; $CHCl_3$); IR γ_{max} (KBr) cm^{-1}: 1638 (CONH), 1702 (C=O), 3372 (OH, NH); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 0.94, 0.99, 1.00, 1.03, 1.08 ($5 \times 3H$, 5br s, $5H_3$), 0.98 and 0.99 ($2 \times 1.5H$, 2t, $J = 7.2$ Hz, $\underline{CH_3}CH_2$), 1.70 (3H, s, H_3-30), 3.10–3.16 (1H, m, H-19), 3.57–3.62 (1H, m, $\underline{CH_2}$-OH), 3.69–3.76 (1H, m, $\underline{CH_2}$-OH), 3.84–3.96 (1H, m, -$\underline{CH}$-NH-), 4.62 and 4.75 (2H, 2s, H_2-29), 5.72 and 5.77 ($2 \times 0.5H$, 2br d, NH). Anal. Calcd for $C_{34}H_{55}NO_3$ (%): C, 77.66; H, 10.54; N, 2.66. Found: C, 77.63; H, 10.58; N, 2.71.

***N*-[3-oxolup-20(29)-en-28-oyl]-(2*S*)-aminobutan-1-ol (10b)** The crude product was purified by column chromatography on silica gel ($CHCl_3$:MeOH 20:1) to afford **10b**. Yield 50%; m.p. 166.0 °C; $[\alpha]_D^{25} + 8.9$ ($c = 0.35$; $CHCl_3$); IR γ_{max} (KBr) cm^{-1}: 1642 (CONH), 1706 (C=O), 3386 (OH, NH); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 0.95, 1.00, 1.01, 1.04, 1.09 ($5 \times 3H$, 5s, $5H_3$), 0.99 (3H, t, $J = 7.4$ Hz, $\underline{CH_3}CH_2$), 1.71 (3H, s, H_3-30), 3.13 (1H, td, $J = 11.1$, 4.4 Hz, H-19), 3.61 and 3.71 (2H, 2dd, $J = 4.0$, 10.9 Hz, $\underline{CH_2}$-OH), 3.84–3.92 (1H, m, –$\underline{CH}$-NH–), 4.62 and 4.76 (2H, 2s, H_2-29), 5.73 (1H, br d, NH); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 10.23, 14.07, 15.41, 15.44, 19.05, 19.16, 20.50, 21.02, 23.69, 25.23, 26.14, 28.97, 30.44, 33.27, 33.36, 33.62, 36.47, 37.44, 38.08, 39.17, 40.28, 42.10, 46.21, 46.81, 49.57, 49.61, 52.55, 54.61, 55.37, 65.39, 108.87 (C-29), 150.28 (C-20), 176.84 (C-28), 217.43 (C-3). GC-MS (*m/z, I*): 525.3 [M^+]. Anal. Calcd for $C_{34}H_{55}NO_3$ (%): C, 77.66; H, 10.54; N, 2.66. Found: C, 77.69; H, 10.51; N, 2.68.

***N*-[3-oxolup-20(29)-en-28-oyl]-(2*R*)-aminobutan-1-ol (10c)** The crude product was purified by column chromatography on silica gel ($CHCl_3$:MeOH 20:1) to afford **10c**. Yield 43%; m.p. 140.1 °C; $[\alpha]_D^{25} + 46.0$ ($c = 0.36$; $CHCl_3$); IR γ_{max} (KBr) cm^{-1}: 1638 (CONH), 1701 (C=O), 3380 (OH, NH); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 0.94, 1.00, 1.01, 1.03, 1.08 ($5 \times 3H$, 5s, $5H_3$), 0.98 (3H, t, $J = 7.5$ Hz, $\underline{CH_3}CH_2$), 1.71 (3H, s, H_3-30), 3.13 (1H, td, $J = 11.1$, 4.5 Hz, H-19), 3.61 and 3.72 (2H, 2dd, $J = 4.0$, 10.9 Hz, $\underline{CH_2}$-OH), 3.86–3.94 (1H, m, –$\underline{CH}$-NH-), 4.62 and 4.76 (2H, 2s, H_2-29), 5.85 (1H, br d, NH); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 10.70, 14.58, 15.84, 15.92, 19.53, 19.61, 21.02, 21.46, 24.22, 25.65, 26.57, 29.42, 30.87, 33.67, 33.90, 34.15, 36.94, 37.76, 38.58, 39.66, 40.74, 42.56, 46.67, 47.34, 50.03, 50.08, 53.13, 55.08, 55.75, 66.26, 109.43 (C-29), 150.74 (C-20), 177.56 (C-28), 218.09 (C-3). GC-MS (*m/z, I*): 525.3 [M^+]. Anal. Calcd for $C_{34}H_{55}NO_3$ (%): C, 77.66; H, 10.54; N, 2.66. Found: C, 77.71; H, 10.49; N, 2.70.

***N*-[1-cyano-3-hydroxymino-2,3-seco-2-norlup-20(29)-en-28-oyl]-(2*S*,*R*)-aminobutan-1-ol (13a)** The crude product was purified by column chromatography on silica gel ($CHCl_3$:MeOH 20:1) to afford **13a**. Yield 37%; m.p. 188.8 °C; $[\alpha]_D^{25} + 23.86$ ($c = 0.3$; $CHCl_3$); IR γ_{max} (KBr) cm^{-1}: 1636 (CONH), 1715 (COOH), 2238 (C≡N), 3366 (OH); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 0.92 and 0.93 ($2 \times 1.5H$, 2s, H_3), 0.97 and 0.98 ($2 \times 1.5H$, 2t, $J = 7.4$ Hz, $\underline{CH_3}CH_2-$), 0.99, 1.04, 1.17 and 1.22 ($4 \times 3H$, 4s, $4H_3$), 1.70 (3H, s, H_3-30), 2.48 and 2.59 (2H, 2d, $J = 18.2$ Hz, H_2-1), 3.10–3.20 (1H, m, H-19), 3.55–3.62 and 3.67–3.72 ($2 \times 1H$, 2m, $-\underline{CH_2}$-OH), 3.84–3.93 (1H, m, -$\underline{CH}$-NH-), 4.63 and 4.75 (2H, 2s, H_2-29), 5.69 and 5.74 ($2 \times 0.5H$, 2br d, NH), 7.48 (1H, br s, NOH), 7.55 (1H, s, H-3). GC-MS (*m/z, I*): 535.3 [$M - NH_2OH]^+$. Anal. Calcd for $C_{34}H_{55}N_3O_3$ (%): C, 73.74; H, 10.01; N, 7.59. Found: C, 73.79; H, 10.07; N, 7.64.

***N*-[3-hydroxyminolup-20(29)-en-28-oyl]-(2*S*,*R*)-aminobutan-1-ol (15a)** The crude product was purified by column chromatography on silica gel ($CHCl_3$:MeOH 20:1) to afford **15a**. Yield 27%; m.p. 154.8 °C; $[\alpha]_D^{25}$-8.0 ($c = 0.4$; $CHCl_3$); IR γ_{max} (KBr) cm^{-1}: 1637 (CONH), 3345 (NH); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 0.93 and 0.94 ($2 \times 1.5H$, 2s, H_3), 0.98, 1.00, 1.06, 1.16 ($4 \times 3H$, 4br s, $4H_3$), 0.98 and 0.99 ($2 \times 1.5H$, 2t, $J = 7.3$ Hz, $\underline{CH_3}CH_2-$), 1.71 (3H, c, H_3-30), 3.10–3.16 (1H, m, H-19), 3.58–3.62 and 3.68–3.73 ($2 \times 1H$, 2m, $-\underline{CH_2}OH$), 3.85–3.94 (1H, m, -$\underline{CH}$-NH-), 4.62 and 4.76 (2H, 2s, H_2-29), 5.69 and 5.73 ($2 \times 0.5H$, 2br d, NH). Anal. Calcd for $C_{30}H_{47}N_2O_2$ (%): C, 77.04; H, 10.13; N, 5.99. Found: C, 77.00; H, 10.05; N, 6.15.

***N*-[3,20-dioxo-29-norlup-20(29)-en-28-oyl]-(2*S*)-aminobutan-1-ol (17b)** The crude product was purified by column chromatography on silica gel ($CHCl_3$:MeOH 20:1) to afford **17b**. Yield 36%; m.p. 227.0 °C; $[\alpha]_D^{25}$-6.4 ($c = 0.4$; $CHCl_3$); IR γ_{max} (KBr) cm^{-1}: 1641 (CONH), 1703 (C=O), 3380 (NH); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 0.93, 0.98, 1.02, 1.03, 1.08 ($5 \times 3H$, 5s, $5H_3$), 0.99 (3H, t, $J = 7.5$ Hz, $\underline{CH_3}CH_2-$), 2.18 (3H, s, H_3-30), 3.45 (1H, td, $J = 11.2$, 4.6 Hz, H-19), 3.60 (1H, dd, $J = 5.8$, 10.9 Hz, $-\underline{CH_2}OH$),

3.70 (1H, dd, $J = 3.6$, 10.9 Hz, $-\underline{CH_2}OH$), 3.83–3.91 (1H, m, -CH-NH-), 5.79 (1H, br d, NH); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 10.24, 14.13, 15.36, 15.43, 19.15, 20.46, 21.00, 23.66, 26.25, 26.74, 28.04, 29.01, 29.65, 32.59, 33.13, 33.54, 36.44, 36.49, 37.65, 39.07, 40.17, 40.86, 46.73, 49.33, 49.39, 50.56, 52.49, 54.41, 55.18, 65.18, 176.59 (C-28), 212.07 (C-3), 217.39 (C-20). GC-MS (*m/z, I*): 527.4 [M^+]. Anal. Calcd for $C_{33}H_{53}NO_4$ (%): C, 75.10; H, 10.12; N, 2.65. Found: C, 75.15; H, 10.17; N, 2.61.

***N*-[3,20-dioxo-29-norlup-20(29)-en-28-oyl]-(2*R*)-aminobutan-1-ol (17c)** The crude product was purified by column chromatography on silica gel ($CHCl_3$:MeOH 20:1) to afford **17c**. Yield 42%; m.p. 217.8 °C; $[\alpha]_D^{25} + 19.3$ ($c = 0.4$; $CHCl_3$); IR γ_{max} (KBr) cm^{-1}: 1642 (CONH), 1703 (C=O), 3382 (NH); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 0.92, 0.98, 1.08 (9H, 3s, $3H_3$), 0.97 (3H, t, $J = 7.5$ Hz, $\underline{CH_3}CH_2-$), 1.02 (6H, s, $2H_3$), 2.18 (3H, s, H_3-30), 3.44 (1H, td, $J =$ 11.1, 4.5 Hz, H-19), 3.61 (1H, dd, $J = 5.8$, 10.9 Hz, $-\underline{CH_2}OH$), 3.70 (1H, dd, $J = 3.5$, 10.9 Hz, $-\underline{CH_2}OH$), 3.84–3.92 (1H, m, -CH-NH-), 5.77 (1H, dr d, NH); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 10.11, 14.13, 15.24, 15.40, 19.13, 20.47, 20.98, 23.72, 26.21, 26.73, 28.04, 28.97, 29.67, 32.62, 33.08, 33.55, 36.39, 36.43, 37.63, 39.08, 40.17, 40.86, 46.74, 49.38 (2C), 50.56, 52.44, 54.44, 55.12, 65.35, 176.53 (C-28), 212.07 (C-3), 217.39 (C-20). GC-MS (*m/z, I*): 527.3 [M^+]. Anal. Calcd for $C_{33}H_{53}NO_4$ (%): C, 75.10; H, 10.12; N, 2.65. Found: C, 75.18; H, 10.20; N, 2.57.

***N*-[3-hydroxymino-20-oxo-29-norlup-28-oyl]-(2*S,R*)-aminobutan-1-ol (19a)** The crude product was purified by column chromatography on silica gel ($CHCl_3$:MeOH 10:1) to afford **19a**. Yield 54%; m.p. 153.9 °C; $[\alpha]_D^{25} + 96.9$ ($c =$ 0.4; $CHCl_3$); IR γ_{max} (KBr) cm^{-1}: 1652 (CONH, C=N), 1697 (C=O), 3277 (NH, OH); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 0.95, 1.00, 1.01, 1.04, 1.10 ($5 \times 3H$, 5br s, $5H_3$), 1.00 (3H, br t, $J = 7.5$ Hz, $\underline{CH_3}CH_2-$), 1.90 (3H, s, H_3-30), 3.16–3.23 (1H, m, H-19), 3.67–3.71 and 3.75–3.78 (2H, 2m, $-\underline{CH_2}OH$), 3.84–3.92 (1H, m, -CH-NH-), 5.75 (1H, br d, NH), 7.62 (1H, br s, NOH). Anal. Calcd for $C_{33}H_{54}N_2O_4$ (%): C, 73.02; H, 10.03; N, 5.16. Found: C, 72.98; H, 10.16; N, 5.11.

General procedure for the preparation of 11a-c from 10a-c

Compound **10a** (or **10b**, or **10c**) (0.58 g, 1.1 mmol) was dissolved in CH_3OH (50 mL) and treated in portions with $NaBH_4$ (0.42 g, 11 mmol) under stirring. The reaction mixture was stirred for 40 min at room temperature, and then refluxed for 5 min. The solvent was evaporated. The resulting solid was dissolved in HCl (100 mL, 10%). The products were extracted with ethylacetate (2×30 mL). The organic layer was separated and dried over anhydrous $MgSO_4$. The solvent was evaporated. The residue was purified by column chromatography.

***N*-[3-hydroxylup-20(29)-en-28-oyl]-(2*R,S*)-aminobutan-1-ol (11a)** The crude product was purified by column chromatography on silica gel ($CHCl_3$:MeOH 20:1) to afford **11a**. Yield 52%; m.p. 209.0 °C; $[\alpha]_D^{25} + 7.6$ ($c = 0{,}24$; $CHCl_3$); IR γ_{max} (KBr) cm^{-1}: 1641 (CONH), 3467 (OH, NH); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 0.77, 0.84, 0.97, 0.98, 0.99 (15H, 5br s, $5H_3$); 0.99 (3H, br т, $J = 7.3$ Hz, $\underline{CH_3}CH_2$), 1.70 (3H, s, H_3-30), 3.12 (1H, td, $J = 11.2$, 4.4 Hz, H-19), 3.20 (1H, dd, $J = 5.4$, 11.0 Hz, H-3), 3.57–3.62 and 3.67–3.72 ($2 \times 1H$, 2m, $-\underline{CH_2}$-OH), 3.83–3.91 (1H, m, -CH-NH-), 4.61 and 4.75 (2H, 2s, H_2-29), 5.69 and 5.73 ($2 \times 0.5H$, 2br d, NH). Anal. Calcd for $C_{34}H_{57}NO_3$ (%): C, 77.37; H, 10.88; N, 2.65. Found: C, 77.31; H, 10.99; N, 2.63.

***N*-[3-hydroxylup-20(29)-en-28-oyl]-(2*S*)-aminobutan-1-ol (11b)** The crude product was purified by column chromatography on silica gel (CH_3Cl:MeOH 20:1) to afford **11b**. Yield 28%; m.p. 202.2 °C; $[\alpha]_D^{25}$-13.5 ($c = 0.34$; $CHCl_3$); IR γ_{max} (KBr) cm^{-1}: 1631 (CONH), 3335 (OH, NH); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 0.78, 0.85, 0.98, 0.99, 1.00 (15H, 5s, $5H_3$), 0.99 (3H, t, $J = 7.3$ Hz, $\underline{CH_3}CH_2$), 1.71 (3H, s, H_3-30), 3.13 (1H, td, $J = 11.0$, 4.7 Hz, H-19), 3.20 (1H, dd, $J = 5.4$, 11.2 Hz, H-3), 3.60 (1H, dd, $J = 5.9$, 10.9 Hz, $-\underline{CH_2}$-OH), 3.70 (1H, dd, $J =$ 3.6, 10.9 Hz, $-\underline{CH_2}$-OH), 3.84–3.92 (1H, m, –CH-NH–), 4.62 and 4.75 (2H, 2s, H_2-29), 5.72 (1H, br d, NH); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 10.23, 14.16, 14.82, 15.63, 17.81, 19.05, 20.49, 21.19, 23.69, 25.23, 26.96, 27.49, 28.99, 30.47, 33.42, 33.98, 36.77, 37.40, 38.11, 38.30, 38.37, 40.35, 42.06, 46.28, 49.67, 50.23, 52.59, 54.98, 55.41, 65.46, 78.51 (C-3), 108.82 (C-29), 150.35 (C-20), 176.89 (C-28). GC-MS (*m/z, I*): 527.4 [M^+]. Anal. Calcd for $C_{34}H_{57}NO_3$ (%): C, 77.37; H, 10.88; N, 2.65. Found: C, 77.45; H, 10.94; N, 2.61.

***N*-[3-hydroxylup-20(29)-en-28-oyl]-(2*R*)-aminobutan-1-ol (11c)** The crude product was purified by column chromatography on silica gel ($CHCl_3$:MeOH 20:1) to afford **11c**. Yield 30%; m.p. 166.4 °C; $[\alpha]_D^{25} + 36.0$ ($c = 0.2$; $CHCl_3$); IR γ_{max} (KBr) cm^{-1}: 1633 (CONH), 3353 (OH, NH); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 0.77, 0.84, 0.97, 0.98, 1.00 (15H, 5s, $5H_3$), 0.98 (3H, t, $J = 7.6$ Hz, $\underline{CH_3}CH_2$), 1.71 (3H, s, H_3-30), 3.13 (1H, td, $J = 11.0$, 4.5 Hz, H-19), 3.20 (1H, dd, $J = 5.2$, 11.1 Hz, H-3), 3.60 (1H, dd, $J = 6.2$, 11.0 Hz, $\underline{CH_2}$-OH), 3.72 (1H, dd, $J = 3.2$, 11.0 Hz, $\underline{CH_2}$-OH), 3.85–3.92 (1H, m, –CH-NH-), 4.62 and 4.76 (2H, 2s, H_2-29), 5.69 (1H, br d, NH); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 10.20, 14.17, 14.84, 15.53, 15.63, 17.78, 18.92, 19.04, 20.45, 23.74, 26.94, 27.49, 28.96, 29.20, 30.41,

33.48, 33.78, 36.73, 37.22, 38.26, 38.37, 40.31, 42.03, 46.25, 49.65, 50.18, 52.72, 54.93, 55.31, 65.87, 78.54 (C-3), 108.90 (C-29), 150.33 (C-20), 177.17 (C-28). GC-MS (*m/z, I*): 527.3 [M^+]. Anal. Calcd for $C_{34}H_{57}NO_3$ (%): C, 77.37; H, 10.88; N, 2.65. Found: C, 77.33; H, 10.98; N, 2.71.

General procedure for the preparation of 11d from 11b

Ac_2O (0.14 mL, 1.5 mmol,) was added to a solution of **11b** (0.26 g, 0.5 mmol,) in dry pyridine (10 mL), then the solution was stirred under Ar at room temperature. The course of the reaction was monitored by TLC. 20H later, the reaction mixture was washed with HCl (20%, 3 × 20 mL) and extracted with ethyl acetate (3 × 20 mL). The organic layer was dried over anhydrous $MgSO_4$. The solvent was evaporated under vacuum. The crude product was purified by column chromatography on silica gel ($CHCl_3$−MeOH 20:1) to afford compound **11d**.

***N*-[3-hydroxylup-20(29)-en-28-oyl]-(2*S*)-amino-1-acetoxybutane (11d)** Yield 27%; m.p. 102.7 °C; $[\alpha]_D^{25}$-18.0 ($c = 0.8$; $CHCl_3$); IR γ_{max} (KBr) cm^{-1}: 1640 (CONH), 1730 ($COOCH_3$), 3366 (NH); ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 0.78, 0.85, 0.97, 0.99, 1.00 (15H, 5s, $5H_3$), 0.99 (3H, t, $J = 7.5$ Hz, $\underline{CH_3}CH_2-$), 1.71 (3H, s, H_3-30), 2.08 (3H, s, $\underline{CH_3}CO-$), 3.14 (1H, td, $J = 11.1$, 4.3 Hz, H-19), 3.20 (1H, dd, $J = 5.2$, 11.0 Hz, H-3), 4.07 (1H, dd, $J = 3.3$, 10.3 Hz, $-\underline{CH_2}$-$OCOCH_3$), 4.17 (1H, dd, $J = 5.6$, 10.3 Hz, $-\underline{CH_2}$-$OCOCH_3$), 4.08–4.18 (1H, м, –CH-NH-), 4.61 and 4.75 (2H, 2s, H_2-29), 5.61 (1H, br d, NH); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 9.90, 14.15, 14.81, 15.63, 15.66, 17.81, 19.02, 20.24, 20.49, 24.22, 25.23, 26.97, 27.49, 28.99, 30.41, 33.30, 34.01, 36.77, 37.29, 37.88, 38.30, 38.37, 40.35, 42.04, 46.25, 49.02, 49.65, 50.25, 54.98, 55.33, 65.26, 78.50 (C-3), 108.80 (C-29), 150.40 (C-20), 170.63, 175.35 (C-28). GC-MS (*m/z, I*): 569.3 [M^+]. Anal. Calcd for $C_{36}H_{59}NO_4$ (%): C, 75.88; H, 10.44; N, 2.46. Found: C, 75.93; H, 10.38; N, 2.1.

General procedure for the preparation of 15a, 18

Hydroxylamine hydrochloride (2.0 mmol) was added to a solution of **10a** or **16** (0.67 mmol) in a mixture of ethanol-pyridine 5:1 (20 mL). The reaction mixture was refluxed for 2–3 h. The reaction mixture was treated with aqueous HCl until being weakly acidic and then extracted with ethyl acetate (2 × 30 mL). The organic layer was dried over anhydrous $MgSO_4$; after that, the solvent was evaporated.

***N*-[3-hydroxyminolup-20(29)-en-28-oyl]-(2*S*,*R*)-aminobutan-1-ol (15a).** The crude product was purified by column chromatography on silica gel ($CHCl_3$:MeOH 20:1) to afford **15a**. Yield 27%; mp 154.8 °C; $[\alpha]_D^{25}$ -8.0° ($c = 0.4$, $CHCl_3$); IR γ_{max} (KBr) cm^{-1}: 1637 (CONH), 3345 (NH); ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 0.94, 0.98, 1.00, 1.06, 1.16 (15H, 5br s, $5H_3$), 0.98 and 0.99 (2 × 1.5H, 2t, $J = 7.4$ Hz, $\underline{CH_3}CH_2-$), 1.71 (3H, c, H_3-30), 3.10–3.16 (1H, m, H-19), 3.58–3.62 and 3.68–3.73 (2 × 1H, 2m, $-\underline{CH_2}OH$), 3.85–3.94 (1H, m, –CH-NH–), 4.62 and 4.76 (2H, 2s, H_2-29), 5.69 and 5.73 (2 × 0.5H, 2br d, NH). GC-MS (*m/z, I*): 507.3 [$M–NH_2OH]^+$. Anal. Calcd for $C_{30}H_{47}N_2O_2$ (%):C, 77.04; H, 10.13; N, 5.99. Found: C, 77.00; H, 10.05; N, 6.15.

3-Hydroxymino-20-oxo-29-norlup-28-oic acid (18) The crude product was purified by column chromatography on silica gel (CH_3Cl:MeOH 20:1) to afford compound **18**. Yield 43%; IR γ_{max} (KBr) cm^{-1}: 1637 (C=N), 1702 (C=O, COOH), 3299 (NOH).

Biological study

Viruses and cells

Herpes simplex viruses types I and II (HSV-I, strain 1C; HSV-II, strain US), human immunodeficiency virus type I (HIV-I, strain HIV-Izmb), human rhabdomyosarcoma (RD) cell line, and MT-4 culture of human T-lymphoblastoid cells were used. The DMEM and RPMI-1640 maintenance media were used for vital activity of the cell cultures, respectively.

HSV replication inhibition assay

The anti-HSV-I and anti-HSV-II virus-inhibiting properties of the compounds were studied in the human rhabdomyosarcoma (RD) cell culture experiments. The test substances were predissolved in 10% ethanol and then diluted to attain needful concentrations in the maintenance medium (DMEM).

The monolayer cell culture grown in the flasks was infected with 0.01–0.001 $TCID_{50}$ (50% tissue cytopathogenic infective dose) virus per cell. The cells were covered with the maintenance medium containing various concentrations of the test substances. Morphological changes in the cell monolayer (cytopathic effect of the virus) were recorded after 72 h incubation at 37 °C. The virus titer in the presence of the test substance and that of the untreated control were calculated as $\log_{10}$ $TCID_{50}$. The difference in the virus titer in comparison with the control served as a criterion of antiviral action.

The concentration at which 50% suppression of virus replication in the presence of the test substance (EC_{50}) occurred was calculated from the data obtained. The maximum tolerant concentration (MTC)/EC_{50} ratio was used as a

range of active non-toxic concentrations of the substance. The MTC was defined as maximum concentration of the substance not affecting morphology of the non-stained cell culture.

The extracellular *anti-HSV virucidal properties* of the compounds were determined with use of residual infectivity of the virus. A suspension of the test substance was mixed with an undiluted virus suspension and kept at room temperature for a pre-set period of time. When the exposure time elapsed, serial 10-fold dilutions of the mixture were prepared to infect a new monolayer cell culture (grown in flasks or in well plates). After completion of incubation period, morphological changes in the cell monolayer were recorded and the virus titer was calculated. The difference in the virus titer in comparison with the non-exposed group (point "0") served as the criterion of virucidal action.

The time needful to attain the 50% virucidal effect (ET_{50}) was calculated. The EC_{50} value of virucidal action was calculated from the results obtained during the study of the substance at various concentrations.

HIV-1 replication inhibition assay

A suspended culture of the T-lymphoblastoid line of human MT-4 cells and the RPMI-1640 maintenance medium were used to study the inhibitory activity against replication of HIV-I. The study was performed in 96-well plates; the test substances were pre-dissolved in 10% ethanol and then diluted with a support medium to attain needful concentrations. The cell culture was infected with the virus dosed 6.0 log_{10} per monolayer. The infected cells were incubated in 5% CO_2 atmosphere at 37 °C for 72 h. After completion of incubation, the results were recorded by adding the MTT (3-(4,5-dimethyl-thiazole-2) −2,5-diphenol-tetrazolium bromide) reagent to the wells at 7.5 µg/mL concentration. After having been kept at 37 °C for 3 h, the supernatant was removed from the wells, and the formed formazan product was dissolved in dimethylsulfoxide. After that, intensity of the developed color was measured on the spectrophotometer at wavelength 550/630 nm. Based on the values obtained, the percentage of the viable cells was determined with subsequent calculation of the EC_{50} value of the virus-inhibitory effect of the test substance and of the MTC/EC_{50} ratio characterizing the range of its non-toxic effective concentration.

The study of *anti-HIV-I virucidal activity* of the synthesized compounds was performed in 96-well plates using a suspended culture of the T-lymphoblastoid cell line of human MT-4 cells and the RPMI-1640 maintenance medium in the sequence outlined for the herpes simplex virus. After the exposure of the virus-containing suspension with dilutions of the test compound, serial 10-fold dilutions of this mixture were prepared to infect a new culture of MT-4 cells. The percentage of the viable cells was determined using the formazan test in the MTT variant. The EC_{50} and ET_{50} values were calculated to determine the virucidal effect of the test substance.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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