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ARTICLE

Antiviral potency of lupane and oleanane alkynyl-derivatives against human cytomegalovirus and papillomavirus

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Abstract

A library of 18 structurally diverse semisynthetic lupane, oleanane, and ursane types triterpenoids, including C19- or C28-(1,2,3-triazolyl)- and aminomethylated derivatives obtained by the «click» reaction with various aromatic and sugar azides or by Mannich reaction with secondary amines, were tested for antiviral activity against HCMV, HSV-1, and HPV-11 types. C28-Triazolyl-derivative with a benzyl substituent of 2,3-indolo-oleanolic acid was the most active against the HCMV virus with $EC_{50} < 0.05$ ($SI > 81$). Lupane 3,28-diacetoxy-triazolyl derivatives with phenyl- and fluorophenyl-fragments possess the highest activity among all screened compounds toward HPV-11 type virus with EC_{50} values of 2.97 μM and 1.20 μM, SI_{90} values of 28 and >125, respectively. One can see that modification of triterpenic alkynes to Mannich bases was more efficient in increasing an activity against HSV-1 than their conversion to triazoles.

Introduction

Natural products have played a leading role for many centuries as a rich source of biologically active compounds that can be employed in the development of new drugs [1–5]. Triterpenoids exhibit a variety of antiviral activities [6] mainly involving effects on DNA viruses [7–9]. Bevirimat [3-*O*-(3',3',-dimethylsuccinyl)-betulinic acid] has been shown to inhibit HIV-1 maturation by a previously described mechanism [7]. Betulin alone and in combination with acyclovir have been reported to inhibit HSV I and II [10]. Betulinic and betulonic acids are also active against HSV, as well as against influenza A and ECHO-6 picornavirus [11], and enveloped/non-enveloped viruses [12]. A series of triterpenoids were found to inhibit HPV-11 and HPV-16 [13–15]. The synergistic effect of rimantadine and betulin-derived compounds combinations against the reproduction of influenza virus types A (H1N1, H7N1, and H3N2) and B in vivo is established [16]. It was shown that betulin/betulinic acid and artesunic acid hybrids [17] and triazine derivatives of allobetulin and betulinic acid [18] were active against HCMV with an EC_{50} in the micromolar range. Recent data provide evidence for the sensitivity of RNA viruses, for example, the significant synergistic effects of betulin derivatives when combined with 3'-amino-3'-deoxy-adenosine against Semliki Forest virus were shown [19].

Triterpenoids with an alkynyl moiety at C2 [20–22], C19 [23], C3-, and C28 [24], have become one of the most actively developing areas of organic chemistry since they are used as key intermediates in the synthesis of biologically active compounds of Mannich and click reactions. 1,2,3-Triazole unit is known to decrease the overall lipophilicity of triterpenes, and to improve ADME parameters [24]. However, it was found that triterpenes with a triazole moiety had significantly lower cytotoxicity and, in some cases, even lower selectivity. In general, as summarized in the reference [25], the goals of becoming new promising anticancer leads have rarely been achieved, but there are also mentioned that in the field of antiviral-active compounds, triterpene-triazole hybrids showed slightly more potency. For example, oleanolic acid dimeric bis-triazole binds to the HCV envelope protein E2 and thus blocks the virus-host fusion with an IC_{50} 10.3 nM [26]. Some anti-HCV activity was observed for triazole-speared ursolic acid cyclodextrin conjugates [27, 28]. Compounds of significantly higher anti-HIV activity were

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obtained from ursolic acid holding a propargyl moiety at position C-3 that were used for click reactions with analogs of the T20 peptide [29]. The two most important structural elements of bevirimat, AZT (an inhibitor of reverse transcriptase) and LH55 (an inhibitor of HIV fusion), were combined via triazole by classical click reactions, but no biological data have been published. However, most of the studies report on the biological activity of targeted compounds, and there is no information about the activity of alkynyl-derivatives. A large number of novel substrates have been synthesized using the aminomethylation Mannich reaction and evaluated as potential treatments for a multitude of diseases [30].

Here, we report the synthesis of new lupane, oleanane, and ursane types alkynyl-derivatives. Obtained compounds, as well as previously described, were evaluated for antiviral activity against human cytomegalovirus, herpes simplex virus, and human papillomavirus.

Results and discussion

Chemistry

The chemical synthesis and characterization of alkynes **1**, **2**, and **3** obtained from betulin by oxidation to methylketone fragment in cycle E with following dehydratation using $POCl_3$, alkynes **10** and **12**–**14** synthesized via acyl chloride method from the corresponding acid, C19-(1,2,3-triazolyl)-**6**–**9**, aminoalkyl- **4**, **11**, and **15**–**17** derivatives obtained using Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition or Mannich reaction screened in this study have been previously reported (chemical structures are presented in Fig. 1) [23, 31–33].

The 1,3-dipolar cycloaddition reaction of 3,28-diacetoxy-C19-alkynyl betulin **1** (for **5**) or 2,3-indolo-olean-12-en-28-propargyl amide **13** (for **18** and **19**) with 4-(azidomethyl)-2,3,5,6-tetramethylphenol, azidomethyl phenyl sulfide, or benzyl azide under standard conditions with $CuSO_4{\cdot}5H_2O$ and sodium ascorbate in CH_2Cl_2-H_2O allowed us to obtain new C19- and C28-1,2,3-triazoles **5**, **18**, and **19** with yields of 45%, 62% and of 56%, correspondingly (Figs. 1 and 2). The structures of the compounds were ascertained by combined use of spectroscopy (^{1}H NMR, ^{13}C NMR and MS) and elemental analyses. Thus, in the spectra of compounds **18** and **19** disappearance of the acetylene fragment signals at δ 2.21–2.23 ppm (NMR ^{1}H) and at δ 71.6–80.0 ppm (NMR ^{13}C) and formation of 1,2,3-triazole ring with signal of methine carbon atom as singlets at δ 7.52 ppm, δ 7.59 ppm (NMR ^{1}H) and at δ 122.1 ppm, δ 122.7 ppm (NMR ^{13}C) were characteristic. The ^{1}H NMR spectrum of compound **5** showed the characteristic signal of methylene group as multiplet at δ 5.59–5.69 ppm and of methine group of triazole fragment as singlet at δ 6.91 ppm, as well as the ^{13}C NMR spectrum of **5** showed the signals of aromatic carbons at δ 120.6–153.0 ppm (NMR ^{13}C) (the NMR spectra of compounds see in Supplementary Material Fig. S1–S6).

Antiviral activity

Our previous studies of triterpene oxidized indoles [15], azepanes [34], and l8αH,19βH-ursanes [35] have revealed their promising antiviral potency. For example, 19β,28-epoxy-18α-olean-28-oxo-2-nor-2,3-4'(1H)-quinolone was active against HPV-11 with EC_{50} 0.45 μM and SI_{50} 322 [15]. Azepanobetulin, azepanouvaol, and azepanoglycyrrhetol showed high potency toward HCMV (EC_{50} 0.15, 0.11, 0.11 μM) with selectivity indexes SI_{50} 115, 136, 172 respectively [34]. 3β-Acetoxy-21β-acetyl-20β,28-epoxy-18α,19βH-ursane showed moderate activity (EC_{50} 4.87 μM) toward the HCMV-resistant isolate (GDGr K17) compared to standard drug Cidofovir and was four times more potent than Ganciclovir [35].

Taking into account these data, lupane and oleanane alkynyl-derivatives **1**–**19** were evaluated against DNA viruses (human herpes simplex virus 1, cytomegalovirus, and papillomavirus 11) using the possibilities of Division of Microbiology and Infectious Diseases of the National Institutes of Allergy and Infectious Diseases (http://www.niaid-aacf.org/) program for antiviral assays. The detailed information regarding antiviral screening and methods can be found at http://www.niaid-aacf.org/ and were described in the literature [36, 37].

The effects of compounds **1**–**19** on antiviral activity against a normal laboratory HCMV strain, AD-169, and their cytotoxicity were evaluated on HFF cells using CellTiter-Glo (cytopathic effect/toxicity) assay (Table 1). The compounds **1**, **3**, **5**, **9**, **10**, **11**, **15**, **17**, **18** and **19** demonstrated activity against HCMV with EC_{50} values > 6 μM, while compounds **2** and **8** showed a weak activity with EC_{50} values > 30 μM, and derivative **6** was turned out to be inactive (EC_{50} > 150 μM). The derivatives **4**, **7**, **12**–**14**, and **16** have shown good viral inhibition toward HCMV (EC_{50} > 1.20 μM; EC_{90} > 1.20 μM) compared to standard drug Ganciclovir. The compound **19** was the most active with EC_{50} < 0.05 μM; EC_{90} < 0.05 μM, but at the same time it was cytotoxic with CC_{50} 3.90 (SI > 81).

The antiviral activity of compounds **1**–**19** against HSV-1 was studied on the E-377 strain of HFF cell line using the CellTiter-Glo (cytopathic effect/toxicity) assay (Table 1). Compounds **5**–**9** were not active while derivatives **1**, **2**, **10**, **13**, and **18** showed a weak anti-herpes activity (EC_{50} > 30 μM). The compounds **3**, **4**, **11**, **12**, **15**–**17**, and **19** showed a moderate potency (EC_{50} > 6 μM; EC_{90} > 6 μM). Compound **14** was the most active against HSV-1 with EC_{50} > 1.2 μM.

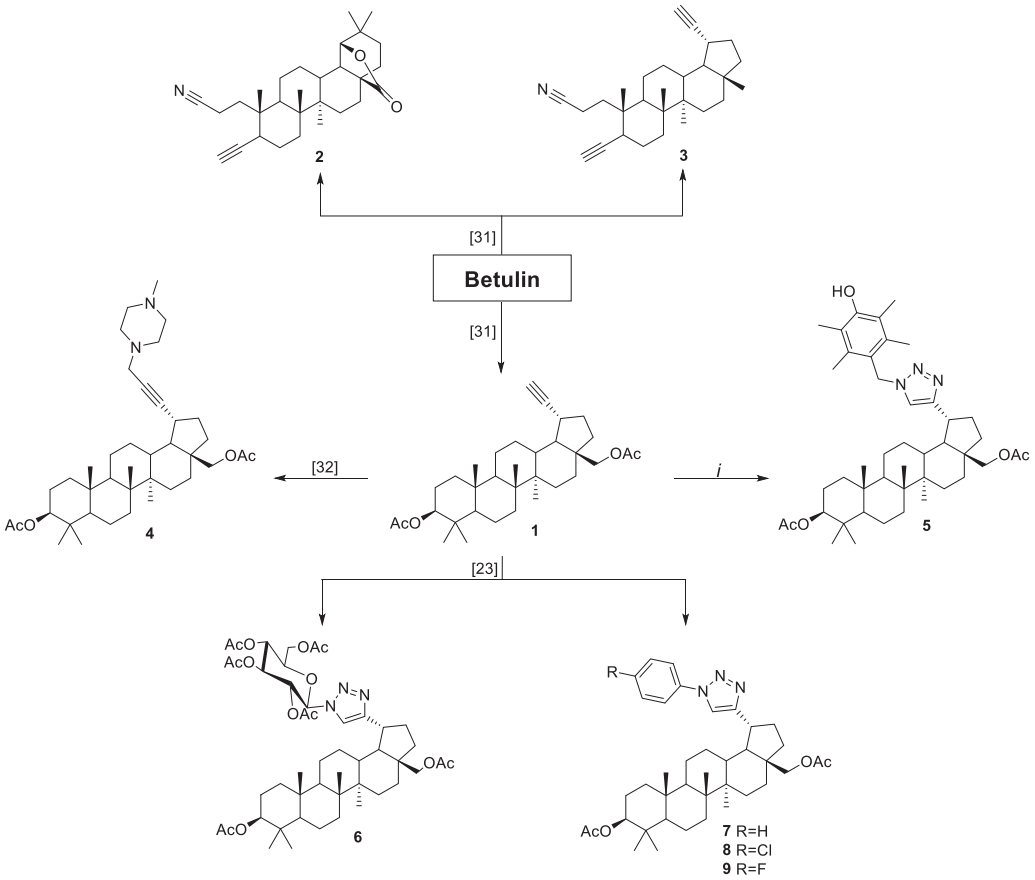

Fig. 1 Synthesis of betulin derivatives. Reagents and conditions: *i*. 4-(azidomethyl)-2,3,5,6-tetramethylphenol, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, CH_2Cl_2-H_2O, rt, 5 h

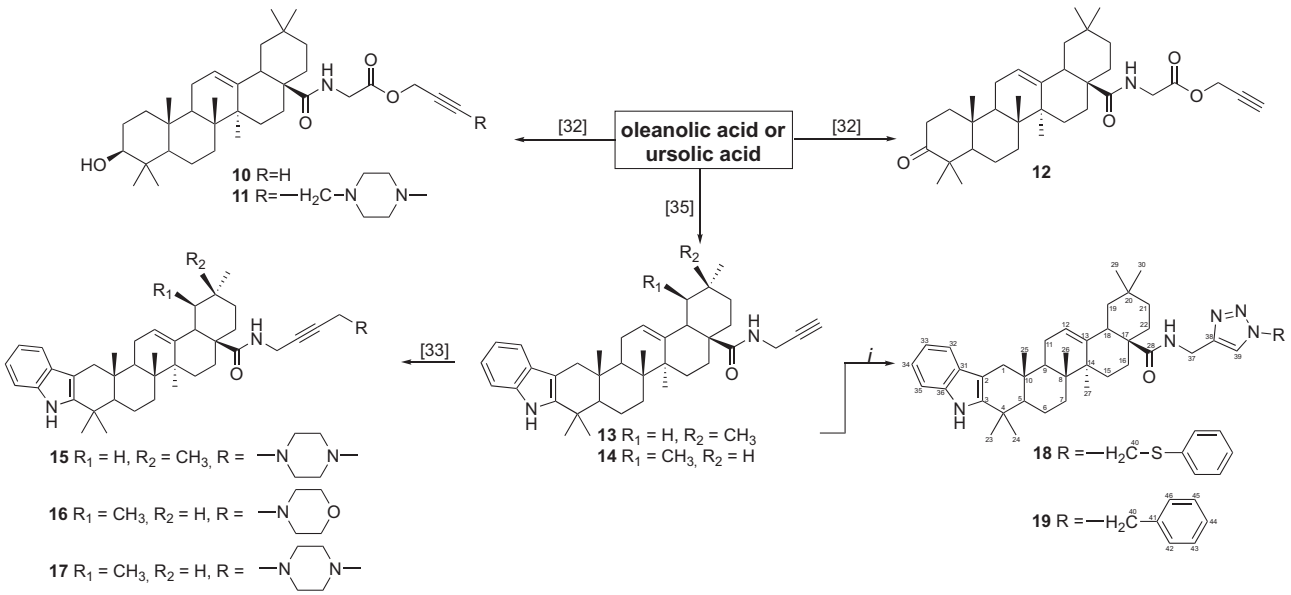

Fig. 2 Synthesis of oleanolic and ursolic acids derivatives. Reagents and conditions: *i*. Azidomethyl phenyl sulfide or benzyl azide, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, CH_2Cl_2-H_2O, rt, 5 h

Table 1 In vitro antiviral activity of compounds **1**–**19**, μM

Compound	EC_{50}	EC_{90}	CC_{50}	SI_{50}	SI_{90}
Human cytomegalovirus[a]					
1	>6	>6	18.87	<3	<3
Ganciclovir	0.8	>150	>150	>187	1
2	>30	>30	53.01	<2	<2
3	>6	>6	11.45	<2	<2
Ganciclovir	0.24	1.08	>150	>625	>139
4	>1.2	>1.2	4.63	<4	<4
5	>6	>6	25.71	<4	<4
6	>150	>150	>150	1	1
7	>1.2	>1.2	5.16	<4	<4
8	>30	>30	55.18	<2	<2
9	>6	>6	27.85	<5	<5
10	>6	>6	20.85	<3	<3
11	>6	>6	9.99	<2	<2
Ganciclovir	0.4	0.88	>150	>377	<170
12	>1.2	>1.2	5.49	<5	<5
13	>1.2	>1.2	4.27	<4	<4
14	>1.2	>1.2	3.64	<3	<3
15	>6	>6	13.31	<2	<2
16	>1.2	>1.2	2.99	<2	<2
17	>6	>6	10.44	<2	<2
18	>6	>6	18.43	<3	<3
19	<0.05	<0.05	3.90	>81	>81
Ganciclovir	0.8	>150	>150	>187	1
Herpes simplex virus 1[b]					
1	>30	>30	72.70	<2	<2
Acyclovir	0.87	>150	>150	>172	1
2	>30	>30	89.22	<3	<3
3	>6	>6	29.35	<5	<5
Acyclovir	0.83	>150	>150	>181	1
4	>6	>6	6.84	<1	<1
5	>150	>150	>150	1	1
6	>150	>150	>150	1	1
7	>150	>150	>150	1	1
8	>150	>150	>150	1	1
9	>150	>150	>150	1	1
10	>30	>30	63.92	<2	<2
11	>6	>6	15.39	<3	<3
Acyclovir	0.61	1.14	>150	>246	>132
12	>6	>6	19.38	<3	<3
13	>30	>30	32.03	<1	<1
14	>1.2	>1.2	5.14	<4	<4
15	>6	>6	9.36	<2	<2
16	>6	>6	7.36	<1	<1
17	>6	>6	9.85	<2	<2
18	>30	>30	142.15	<5	<5
19	>6	>6	9.66	<2	<2
Acyclovir	0.87	>150	>150	>172	1
Human papillomavirus 11[c]					
1	>30	>30	90.58	<3	<3
2	87.15	140.81	>150	>2	>1
3	12.28	14.84	63.10	5	4
9-[2-(Phosphonomethoxy) ethyl]guanine	0.68	100.62	>150	>221	>1
4	>1.20	>1.20	4.41	<4	<4
5	17.14	>30	109.18	6	<5
6	>150	>150	>150	1	1
7	2.97	18.27	82.79	28	5
8	8.41	23.40	71.64	9	3
9	1.20	20.07	>150	>125	>7
10	>30	>30	133.93	<4	<4
11	>6	>6	12.64	<2	<2
12	7.79	16.50	46.94	6	3
14	>150	>150	>150	1	1
16	4.25	>30	133.71	31	<4
17	>1.2	>1.2	3.23	<3	<3
9-[2-(Phosphonomethoxy) ethyl]guanine	0.89	102.22	>150	>168	>1
13	>150	>150	>150	1	1
15	>1.2	>1.2	3.32	<3	<3
18	110.32	149.54	>150	>1	>1
19	>6	>6	10.12	<2	<2
9-[2-(Phosphonomethoxy) ethyl]guanine	0.68	100.62	>150	>221	>1

EC_{50}—compound concentration that reduced viral replication by 50%; EC_{90}—compound concentration that reduced viral replication by 90%; CC_{50}—compound concentration that reduced cell viability by 50%; SI_{50}—Selectivity index (CC_{50}/EC_{50})

[a]Virus strain: AD169; cell line: HFF; vehicle: DMSO; drug conc. range: 0.048–150 μM; control conc. range: 0.048–150 μM; control assay order: primary; control assay name: CellTiter-Glo (cytopathic effect/toxicity)

[b]Virus strain: E-377; cell line: HFF; vehicle: DMSO; drug conc. range: 0.048–150 μM; control conc. range: 0.048–150 μM; control assay name: CellTiter-Glo (cytopathic effect/toxicity)

[c]Virus strain: HE611260.1; cell line: C-33 A; vehicle: DMSO; drug conc. range: 0.048–150 μM; control conc. range: 0.048–150 μM; control assay name: Nano-Glo Luciferase (Nanoluc)/CellTiter-Glo (toxicity)

Compounds **1**–**19** were also evaluated against a HPV-11 strain HE611260.1 on C-33A cells using Nano-Glo Luciferase (NanoLuc)/CellTiter-Glo (toxicity) assay. Compounds **6**, **13**, **14**, and **18** were not active against the studied virus strain, whereas compounds **1**–**3**, **5**, **8**, **10**–**12**, and **19** showed moderate or weak activity. The derivatives **4**, **15**, and **17** displayed good activity with $EC_{50} > 1.20$ μM and $EC_{90} > 1.20$ μM. Compounds **7** and **16** showed moderate activity against HPV-11 with EC_{50} 2.97 μM and 4.25 μM, and low values of cytotoxicity (CC_{50} 82.79 and 133.71 respectively) as well. Compound **9** showed activity toward HPV-11 (EC_{50} 1.20 μM; EC_{90} 20.07 μM) with a good selectivity index ($SI_{50} > 125$; $SI_{90} > 7$) compared to standard drug 9-[2-(phosphonomethoxy)ethyl] guanine (EC_{50} 0.89 μM; EC_{90} 102.22 μM; $SI_{50} > 168$; $SI_{90} > 1$). Generally it seems that modification of triterpenic alkynes such as **1**, **13** or **14** to Mannich bases was more efficient in increasing an activity against HSV-1 than their conversion to triazoles, such as **5**–**8**, **18**.

Based on the differences in activity between the derivatives, the following structure-activity relationships could be

observed. In the case of HCMV, C19-triazols with different phenyl substitutes **5**, **7–9** were comparable with a starting alkyne **1**, while the introduction of a sugar moiety **6** led to a complete loss of activity. The compound **7** with phenyl fragment was found to be more effective and at the same time more cytotoxic. The modification of C28-alkynyl amides had a positive influence on EC_{50} or CC_{50} value. The introduction of a benzyl fragment had the most favorable effect on activity (EC_{50} 0.05 μM). The activity of Mannich bases **4**, **11**, **15–17** was comparable or better than an activity of parent compounds, but triterpenoids **4** and **16** were more cytotoxic.

In the case of HSV-1, modification of C19-alkyne **1** to triazoles **5–9** led to the loss of activity. For compounds **1–3** the beneficial effect of acetylene groups was observed. Modification of both C19- **1** and C28-alkynyl derivatives **13**, **14** by Mannich reaction improved the values of activity, as well as against HPV-11. The exception was compound **18**, which activity was comparable to the parent alkyne.

In addition, increased activity against HPV-11 was displayed by all triterpenic triazoles with aromatic fragment **5** and **7–9**, except sugar triazole **6**. Thus, compounds **7** and **9** with phenyl- and fluoro-phenyl-fragments possess the highest activity among all screened compounds with EC_{50} values of 2.97 μM and 1.20 μM, SI_{50} values of 28 and >125, respectively.

Experimental

General

The spectra were recorded at the Center for the Collective Use "Chemistry" of the Ufa Institute of Chemistry of the UFRC RAS and RCCU "Agidel" of the UFRC RAS. ^{1}H and ^{13}C NMR spectra were recorded on a "Bruker *Avance*-III" (Bruker, Billerica, MA, USA, 500 and 125.5 MHz respectively, δ, ppm, Hz) in $CDCl_3$, internal standard—tetramethylsilane. Mass spectra were obtained on a liquid chromatograph–mass spectrometer LCMS-2010 EV (Shimadzu, Kyoto, Japan). Melting points were detected on a microtable «Rapido PHMK05» (Nagema, Dresden, Germany). Optical rotations were measured on a polarimeter Perkin-Elmer 241 MC (PerkinElmer, Waltham, MA, USA) in a tube length of 1 dm. Elemental analysis was performed on a Euro EA-3000 CHNS analyzer (Eurovector, Milan, Italy), the main standard is acetanilide. Thin-layer chromatography analyzes were performed on Sorbfil plates (Sorbpolimer, Krasnodar, Russia), using the solvent system chloroform–ethyl acetate, 40:1. Substances were detected by a 10% solution of sulfuric acid solution with subsequent heating at 100–120 °C for 2–3 min. All chemicals were of reagent grade (Sigma-Aldrich). Compounds **1**, **2** and **3** [31], **4**, **10–12** [32], **6–9** [23], **13–17** [33] were obtained according to the methods described previously.

Chemistry

Synthesis of compounds 5, 18, and 19

To a solution of compound **1** (0.51 g, 1 mmol) or **13** (0.56 g, 1 mmol), $CuSO_4{\cdot}5H_2O$ (0.04 g, 0.2 mmol) in CH_2Cl_2-H_2O (1:1, 5 ml), 4-(azidomethyl)-2,3,5,6-tetramethylphenol (0.21 mg, 1 mmol), azidomethyl phenyl sulfide (0.14 ml, 1 mmol), or benzyl azide (0.13 ml, 1 mmol) were added. The reaction mixture was stirred for 1 h at 20 °C, then Na-L-Asc (2 mg, 0.01 mmol) was added and stirred at 50 °C for 24 h. The reaction mixture was poured into H_2O/H^+, the precipitate was filtered off, washed with water until neutral pH, dried in air. The resulting material was chromatographed on SiO_2 using chloroform as an eluent.

3β,28-Diacetoxy-19-{1-(4-hydroxy-2,3,5,6-tetramethylbenzyl)-1*H*-1,2,3-triazol-4yl}-20,29,30-trinor-betulin (5)

Yield 0.32 g (45%). m.p. 192–194 °C; $[\alpha]_D^{20} + 21.00$ (*c* 0.1, $CHCl_3$); δ_H (500.13 MHz, $CDCl_3$) 0.81, 0.82, 0.83, 0.91, 0.98 (5 s, 15H, $5CH_3$), 1.01–1.99 (m, 25H, CH, CH_2), 2.01 and 2.09 (2 s, 6H, $2COCH_3$), 2.19 and 2.21 (2 s, 12H, $4CH_3$-arom), 3.22 (m, 1H, H-19), 3.81 and 4.25 (both d, $J = 11.0$ Hz, H-28), 4.45 (dd, 1H, $J = 10.8$ Hz, $J = 5.4$ Hz, H-3), 5.59–5.69 (m, 2H, CH_2), 6.91 (s, 1H, H-triazol); δ_C (125.76 MHz, $CDCl_3$) 12.6, 12.6, 14.6, 16.0, 16.1, 16.5, 16.5, 18.1, 20.6, 21.1, 21.2, 21.3, 23.7, 26.8, 26.9, 27.9, 29.6, 32.1, 34.0, 35.4, 36.0, 37.0, 37.1, 37.8, 38.4, 40.8, 42.7, 46.6, 49.9, 50.6, 53.5, 55.3, 62.5 (C-3), 80.9 (C-28), 120.4 (CH-triazol), 120.6 (C-arom), 120.6 (C-arom), 121.1 (C-arom), 134.9 (C-arom), 134.9 (C-arom), 152.1 (C-triazol), 153.0 (C-arom), 171.0, 171.5. MS: *m/z* 716.49 $[M + H]^+$; Anal. Calcd for $C_{44}H_{65}N_3O_5$: C, 73.81; H, 9.15; N, 5.87. Found: C, 73.80; H, 9.14; N, 5.86.

[3,2b]-Indolo-*N*-[1-((phenylthio)methyl)-1*H*-1,2,3-triazol-4-yl)methyl)]-olean-12(13)-en-28-carboxamide (18)

Yield 0.45 g (62%); m.p. 157 °C; $[\alpha]_D^{20} + 29°$ (*c* 0.05, $CHCl_3$); δ_H (500.13 MHz, $CDCl_3$) 0.68, 0.92, 0.93, 0.94, 0.96, 1.22, 1.32 (7 s, 21H, $7CH_3$), 1.35–2.82 (m, 22H, CH, CH_2), 4.34–4.57 (m, 2H, H-37), 5.51 (s, 1H, H-12), 5.58 (s, 1H, H-40), 6.68 (br. s., 1H, NH), 7.06–7.47 (m, 9H, H-arom), 7.59 (s, 1H, CH-triazol), 8.13 (br. s., 1H, NH); δ_C (125.76 MHz, $CDCl_3$) 15.6, 16.4, 19.3, 23.3, 23.5, 23.6, 23.9, 25.6, 27.3, 30.7, 30.9, 31.9, 32.5, 33.0, 34.0, 34.1, 35.1, 36.8, 37.9, 39.5, 42.1, 46.2, 46.3, 46.6, 53.1, 53.8, 106.6 (C-2), 110.4 (C-arom), 117.9 (C-arom), 118.8 (C-arom), 120.9 (C-arom), 122.1 (CH- triazol), 123.5 (C-12), 128.2 (C-arom), 128.6 (C-triazol), 129.5 (2C, C-arom), 131.9 (2C, C-arom), 132.1 (C-arom), 136.2 (C-arom), 140.9 (C-arom), 144.1 (C-13), 145.3 (C-3), 178.4 (C-28); MS

(APCI) *m/z* 730.50 $[M]^+$, (calcd for $C_{46}H_{59}N_5OS$, 730.07). Anal. Calcd for $C_{46}H_{59}N_5OS$: C, 75.68; H, 8.15; N, 9.59; S, 4.39. Found: C, 75.75; H, 8.23; N, 9.46; S, 4.29.

[3,2b]-Indolo-*N*-[1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]-olean-12(13)-en-28-carboxamide (19)

Yield 0.39 g (56%); m.p. 164 °C; $[\alpha]_D^{20} + 14°$ (*c* 0.05, $CHCl_3$); δ_H (500.13 MHz, $CDCl_3$) 0.62, 0.86, 0.87, 1.15, 1.17, 1.21, 1.30 (7s, 21H, $7CH_3$), 1.32-2.90 (m, 22H, CH, CH_2), 5.13-5.20 (m, 2H, H-37), 5.34 (s, 1H, H-12), 5.46–5.51 (m, 1H, H-40), 7.03–7.44 (m, 9H, H-arom), 7.52 (s, 1H, H-triazol), 7.91 (br. s., 1H, NH); δ_C (125.76 MHz, $CDCl_3$) 15.6, 16.6, 19.4, 23.1, 23.3, 23.4, 23.6, 25.7, 27.7, 30.7, 31.0, 32.2, 32.3, 33.1, 33.9, 34.0, 36.8, 38.1, 39.4, 41.5, 41.9, 45.9, 46.3, 46.8, 53.2, 54.2, 57.6 (C-40), 106.8 (C-2), 110.4 (C-arom), 117.9 (C-arom), 118.9 (C-arom), 120.9 (C-arom), 121.9 (C-arom), 122.7 (CH- triazol), 124.5 (C-12), 128.1 (2C, C-arom), 128.3 (C-arom), 128.9 (C-triazol), 129.2 (2C, C-arom), 134.4 (C-arom), 136.2 (C-arom), 140.9 (C-3), 143.4 (C13), 177.8 (C28); MS (APCI) *m/z* 699.5 $[M + H]^+$ (calcd for $C_{46}H_{59}N_5O$, 698.01). Anal. Calcd for $C_{46}H_{59}N_5O$: C, 79.15; H, 8.52; N, 10.03. Found: C, 79.21; H, 8.45; N, 10.12.

Antiviral screening

All biology experimental procedures and molecular modeling methods are described in the Supplementary Materials.

Conclusions

By Cu(I)-catalyzed azide-alkyne cycloaddition and Mannich reaction 18 lupane, oleanane and ursane alkynyl-triterpenoids were synthesized and evaluated for antiviral activity against HCMV, HSV-1, and HPV-11. Among tested compounds, oleanane C28-1,2,3-triazole with a benzyl substituent **19** was active against HCMV with SI > 81, while lupane 3,28-diacetoxy-triazoles with phenyl-**7** and fluorophenyl-**9** fragments demonstrated strong HPV-11 antiviral activity. This approach was not effective toward HSV-1 type virus, while Mannich bases were more active than the parent alkyne. To sum up, of special interest are derivatives of 2,3-indolo-oleanolic acid and nor-lupane triazoles with promising potency against human cytomegalovirus and papillomavirus.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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