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Betulin and ursolic acid synthetic derivatives as inhibitors of Papilloma virus

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ABSTRACT

The synthesis of new betulin and ursolic acid derivatives and evaluation of their antiviral activity in vitro is reported. Betulin was modified at positions C-3, C-20 and C-28 to afford the derivatives with nicotinoyl-, methoxycynnamoyl-, alkyne and aminopropoxy-2-cyanoethyl-moieties. The two stage conversion of betulin to the new ursane-type triterpenoid by treatment of allobetulin with Ac_2O–$HClO_4$ is suggested. Cyanoethylation of ursonic acid oxime led to cyanoethyloximinoderivative. According to the results of antiviral screening against human papillomavirus type 11 the selectivity index for tested triterpenoids has a range from 10 to 35 with no cellular cytotoxicite, the most remarkable activity was found for 3β,28-di-*O*-nicotinoylbetulin. 3β,28-Dihydroxy-29-norlup-20(30)-yne was also active against HCV replicon (EC_{50} 1.32; EC_{90} 16.82; IC_{50} 12.41; IC_{90} >20; SI_{50} 9.4; SI_{90} >1.19). 28-*O*-Methoxycynnamoylbetulin was active against influenza type A virus (H1N1) (EC_{50} 2; IC_{50} >200; SI >100).

Betulin **1** and ursolic acid (UA) **2** belong to a large family of pentacyclic triterpenoids obtained biosynthetically by cyclic reactions from squalene. Betulin is a major constituent of the bark of white birches[1] and UA was found in various plants.[2] These triterpenoids exhibit a variety of biological activities including antitumor[3] and antiviral[4] activities. Reports on the antiviral properties of betulin, UA and their derivatives mainly involve effects on DNA viruses.[4–6] Bevirimat [3-*O*-(3′,3′-dimethylsuccinyl)-betulinic acid] has been shown to inhibit HIV-1 maturation by a previously undescribed mechanism.[4] Some C-28 substituted betulinic acid derivatives displayed selective anti-HIV-2 activity at nanomolar concentrations.[7] Betulin alone and in combination with aciclovir has been reported to inhibit Herpes simplex virus types I and II.[8] Betulinic and betulonic acids are also active against HSV, as well as against influenza A and ECHO-6 picornavirus.[9,10] The synergistic effect of combination of rimantadine and betulin-derived compounds against reproduction influenza virus type A (H1N1, H7N1, H3N2) and B in vivo is established.[11] Furthermore, 3-*epi*-betulinic acid 3-*O*-sulfates was recently demonstrated to inhibit HSV, influenza A, and respiratory syncytial virus (RSV).[12] The significant synergistic effects of betulin derivates when combined with 3′-amino-3′-deoxy-adenosine against Semliki Forest virus was shown.[13] Thus, recent data provide evidence for the sensitivity of RNA viruses toward betulin compounds.

Papillomaviruses are small, non-enveloped DNA viruses that infect and replicate in the cutaneous or mucosal epithelia of human and other mammals.[14] There are over 80 types of human papillomavirus (HPV), which cause conditions ranging from plantar (HPV1) and genital warts (HPV6 and -11) to cervical cancer (HPV16, - 18, and -31). HPV6 and -11 are also responsible for laryngeal papillomatosis, a rare but very serious infection of the respiratory tract. Antiviral agents capable of specifically inhibiting PV replication could play an important role in the treatment of these diseases, but unfortunately no such efficient antiviral agent exists at the present time.

It has been known that Birch (Betula species) contains betulin and betulinic acid, which have antiviral properties against warts. The antiproliferative and antiviral effects of ursolic acid and dexamethasone in (HPV)-associated cervical cancer cells were investigated.[15–17] Ursolic acid showed inhibitory effect on the growth of the HPV-negative cell lines, C33A, and the HPV-positive cell lines, SiHa (HPV-16), CaSki (HPV-16), and HeLa (HPV-18).[15] However, the abilities of semisynthetic triterpenoids of lupane and ursane types to inhibit human papillomavirus, especially HPV-11, have not been reported.

In the process of further development of our research program dedicated to the investigation of pharmacological potency of plant metabolites[18,19] we describe synthesis of selected betulin and ursolic acid derivatives which demonstrated the most remarkable activity against human papillomavirus type 11 among some dozens of compounds (data not shown) tested at the National Institute of Allergy and Infectious Diseases (NIAID).[20]

The synthetic routes of betulin derivatives are outlined in Scheme 1. Betulin **1** was converted to 3β,28-di-*O*-nicotinoylbetulin **3**[21] and 28-*O*-methoxycynnamoylbetulin **4**[22] by interaction with nicotinic or *p*-methoxycynnamic acid chloride. Acylation of betulinic acid obtained in two-step synthesis from betulin with nicotinic acid chloride in similar conditions afford 3β-*O*-nicotinoylbetulinic acid **5**.[23] The synthesis of betulin derivative **6**[24] with alkyne moiety

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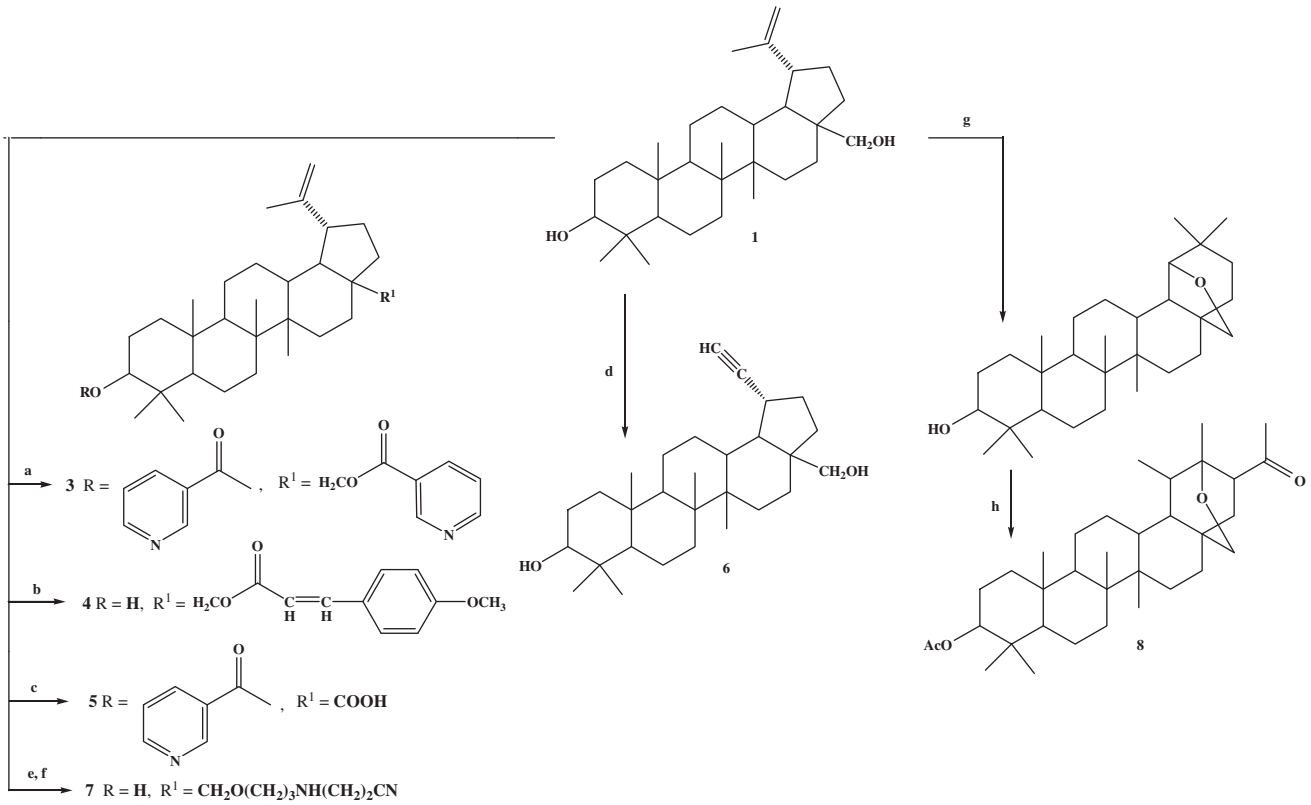


Scheme 1. Reagents and conditions: (a) nicotinic acid chloride, pyridine, DMAP, 115 °C, 4 h, 90%; (b) *p*-methoxycinnamic acid chloride, pyridine, DMAP, rt, 8 h, 73%; (c) (i) Jones' oxidation, 75%; (ii) $NaBH_4$, *i*-PrOH, rt, 2 h, 90%; (iii) nicotinic acid chloride, pyridine, DMAP, 115 °C, 4 h, 85%; (d) (i) Ac_2O, pyridine, reflux, 4 h, 92%; (ii) O_3, CH_2Cl_2, −40 °C, 85%; (iii) $POCl_3$, pyridine, 115 °C, 6 h, 80%; (iv) 5% KOH/EtOH, rt, 3 h, 90%; (e) (i) CH_2CHCN, $C_6H_5CH_2N(C_2H_5)_3Cl$, 40% KOH, 1,4-dioxane, rt, 19 h, column chromatography, 48%; (ii) H_2, Ni-Raney, MeOH, 80 °C, 100 atm, 12 h, 87%; (f) CH_2CHCN, $C_6H_5CH_2N(C_2H_5)_3Cl$, 40% KOH, 1,4-dioxane, rt, 16 h, column chromatography, 42%; (g) *p*-TsOH, $CHCl_3$, reflux, 2 h, 90%; (h) Ac_2O, few drops $HClO_4$, reflux, 8 h, 55%.

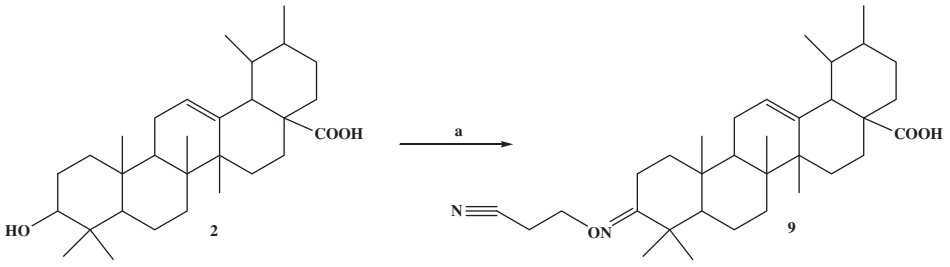
instead of isopropenyl group at C-20 was achieved by sequence of reactions by interaction of 3β,28-diacetoxy-20-oxo-29-norlupan with $POCl_3$ in pyridine under reflux. Cyanoethylation of betulin **1** with acrylonitrile followed by catalytic hydrogenation and again cyanoethylation led to triterpenoid **7**[25] bearing aminopropoxy-2-cyanoethylmoiety at C-28. The access to ursane-type triterpenoid **8**[26] in yield 55% was realized when betulin **1** was at first transformed to allobetulin and then treated by Ac_2O–$HClO_4$. Cyanoethylation of ursonic acid oxime led to cyanoethyloximinoderivative **9**[27] (Scheme 2).

Results of antiviral screening against HPV-11 for compounds **3**, **5**–**9** are shown in Table 1. Selectivity index has a range from 10 (compound **5**) with some cellular cytotoxicity to 35 (compound **3**) when cytotoxicity was not observed. Remarkably, that according to our previous data 3β,28-di-*O*-nicotinoylbetulin **3** has already shown hepatoprotective, anti-ulcer, anti-inflammatory, immunomodulatory activity in vivo and anti-HIV activity in vitro.[28]

Table 1
Antiviral activity of compounds **3**, **5**–**9** against HPV-11*

Compound	SI
3	35[a]
5	10[b]
6	30[a]
7	25[a]
8	34[a]
9	30[a]

SI—selectivity index.
For protocols and explanations of the test results see website (www.niaid-aacf.org).
* Screening Center: Penn State University, NIAID (National Institute of Allergy and Infectious Diseases).
[a] Comment from NIAID: excellent anti-viral activity at single dose tested; no cellular cytotoxicity.
[b] Comment from NIAID: excellent anti-viral activity at single dose tested; some cellular cytotoxicity.



Scheme 2. Reagents and conditions: (a) (i) Jones' oxidation, 80%; (ii) $NH_2OH{\cdot}HCl$, pyridine, 115 °C, 3 h, 89%; (iii) CH_2CHCN, 40% KOH, 1,4-dioxane, rt, 6 h, 86%.

Moreover, compound **6** was active against HCV replicon (EC_{50} 1.32; EC_{90} 16.82; IC_{50} 12.41; IC_{90} >20; SI_{50} 9.4; SI_{90} >1.19). Compound **6** has low toxicity (CC_{50} >100 μM) and no influence on HBV DNA replication at 10 μM concentration.

Compounds **3**, **5**–**9** were not active against viruses of respiratory panel (Adeno, Flu A (H1N1; H3N2; H5N1), Flu B, SARS, Rhinovirus Type 2) and Herpes simplex HSV-1, HSV-2. On the other hand, 28-*O*-methoxycynnamoylbetulin **4** was active against influenza type A virus (H1N1) (EC_{50} 2; IC_{50} >200; SI >100).

In conclusion, we have found new betulin and ursolic acid derivatives with promising antiviral activity against human papillomavirus type 11 and influenza virus type A (H1N1). Further synthesis and biological evaluation are currently in progress.

Acknowledgements

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21. *3β,28-Di-O-nicotinoyl-lupa-20(29)-ene* **3**: mp 117 °C; $[\alpha]_D^{20}$ +28 (*c* 0.1, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 0.82, 0.84, 0.91, 0.94, 1.00 (15H, 5s), 1.00–2.00 (28H, m), 1.63 (3H, s), 2.48 (1H, *J* = 5.7, 10.6, 11.6 Hz ddd,), 4.05 (1H, *J* = 11 Hz, d), 4.45–4.53 (2H, m), 4.60–4.67 (2H, m), 7.40 (1H, *J* = 3.8, 4.7 Hz, dd), 8.24 (1H, *J* = 4.7, 1.6, 1.7 Hz, ddd), 8.70 (1H, *J* = 4.1 Hz, t), 9.18 (1H, *J* = 1.8, 5.9 Hz, dd); ^{13}C NMR (300 MHz, $CDCl_3$) δ 14.7, 16.0, 16.1, 16.7, 18.1, 19.1, 20.8, 23.7, 25.1, 27.0, 28.0, 29.5, 29.8, 34.0, 34.6, 37.0, 37.6, 38.1, 38.3, 40.9, 42.7, 46.6, 47.7, 48.9, 50.2, 55.6, 63.7, 82.2, 110.0, 123.2, 123.2, 126.2, 126.6, 137.0, 137.0, 149.8, 149.8, 150.8, 153.1, 153.3, 164.8, 165.8. Anal. Calcd for $C_{42}H_{56}N_2O_4$: C, 77.26; H, 8.64; N, 4.29. Found: C, 77.03; H, 8.41; N, 4.05.
22. *28-p-Methoxycynnamoyl-lupa-20(29)-ene* **4**: mp 183 °C; $[\alpha]_D^{20}$ +44 (*c* 0.1, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 0.74, 0.81, 0.95, 0.97, 1.03 (15H, 5s), 1.00–2.00 (26H, m), 1.68 (3H, s), 2.40–2.47 (1H, m), 3.81 (3H, s), 3.96 and 4.37 (2H, *J* = 11 Hz, both d), 4.58 and 4.69 (2H, both s), 6.31 (1H, *J* = 16 Hz, d), 6.88 and 7.46 (4H, *J* = 8.4 Hz, both d), 7.63 (1H, *J* = 16 Hz, d); ^{13}C NMR (300 MHz, $CDCl_3$) δ 14.8, 15.5, 16.0, 16.2, 18.3, 19.1, 20.8, 25.2, 25.2, 27.0, 27.4, 29.1, 29.7, 34.0, 34.2, 37.2, 37.3, 38.5, 38.7, 38.8, 40.9, 47.6, 47.7, 48.8, 50.4, 55.1, 55.3, 62.6, 78.8, 109.8, 114.3, 114.4, 115.7, 127.1, 128.3, 129.7, 144.3, 150.1, 161.3, 167.8. Anal. Calcd for $C_{40}H_{58}O_4$: C, 79.69; H, 9.70. Found: C, 79.28; H, 9.84.
23. *3β-O-Nicotinoyl-lupa-20(29)-ene-28-oic acid* **5**: mp 156 °C; $[\alpha]_D^{20}$ +26 (*c* 0.5, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 0.82, 0.89, 0.91, 0.98, 0.99 (15H, 5s), 1.10–2.00 (25H, m), 1.67 (3H, s), 3.03–3.17 (1H, m), 4.61–4.81 (3H, m), 7.42 (1H, *J* = 7.8, 4.9 Hz, dd), 8.32 (1H, *J* = 7.8, 1.1, 1.3 Hz, ddd), 8.80 (1H, *J* = 4.1, 1.1 Hz, dd), 9.21 (1H, *J* = 1.3 Hz, d); ^{13}C NMR (300 MHz, $CDCl_3$) δ 14.6, 16.0, 16.1, 16.7, 18.2, 19.4, 20.9, 23.7, 25.4, 28.1, 29.7, 30.6, 32.2, 34.2, 37.1, 37.2, 38.1, 38.2, 38.4, 40.7, 42.4, 46.6, 49.3, 50.4, 55.5, 56.3, 82.4, 109.6, 123.4, 126.9, 137.3, 150.4, 150.5, 152.8, 164.8, 181.3. Anal. Calcd for $C_{36}H_{51}NO_4$: C, 76.97; H, 9.15; N, 2.49. Found: C, 77.08; H, 9.03; N, 2.29.
24. *3β,28-Dihydroxy-29-norlup-20(30)-yne* **6**: mp 216 °C; $[\alpha]_D^{20}$ −58 (*c* 2.60, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 0.65, 0.75, 0.85, 0.87, 0.92 (15H, 5s), 1.05–1.85 (27H, m), 1.98 (1H, s), 2.95–3.05 (1H, m), 3.40 and 3.55 (2H, *J* = 7 Hz, both d); ^{13}C NMR (300 MHz, $CDCl_3$) δ 14.3, 15.1, 15.4, 15.6, 15.9, 20.5, 24.5, 26.6, 26.9, 27.7, 28.0, 28.7, 29.6, 30.9, 33.2, 34.0, 36.7, 36.9, 38.6, 38.7, 40.6, 42.4, 47.2, 50.2, 55.1, 59.7, 60.2, 70.5, 90.4. Anal. Calcd for $C_{29}H_{46}O_2$: C, 81.63; H, 10.87. Found: C, 81.44; H, 10.62.
25. *3β-Hydroxy-28-O-(3-(aminopropyl)-(2-cyanoethyl))-lupa-20(29)-ene* **7**: mp 175 °C; $[\alpha]_D^{20}$ +10 (*c* 0.53, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 0.76, 0.81, 0.92, 0.93, 1.01 (15H, 5s), 1.07–2.08 (22H, m), 1.69 (3H, s), 2.33–2.46 (4H, m), 2.52–2.67 (4H, m), 2.78–2.87 (3H, m), 3.12–3.21 (2H, m), 3.54–3.62 (1H, *J* = 9.0 Hz, d), 3.62–3.68 (2H, *J* = 6.3 Hz, t), 3.73–3.88 (2H, m), 4.57 and 4.73 (2H, both s); ^{13}C NMR (300 MHz, $CDCl_3$) δ 14.7, 15.3, 15.9, 16.0, 18.2, 18.8, 19.0, 20.7, 21.2, 22.9, 25.1, 27.1, 27.3, 28.0, 29.8, 34.1 34.6, 37.1, 37.5, 38.6, 38.8, 39.9, 40.9, 42.6, 47.3, 47.9, 48.7, 50.3, 55.2, 65.8, 66.1, 69.8, 78.8, 109.6, 118.1, 150.4. Anal. Calcd for $C_{36}H_{60}N_2O_2$: C, 78.21; H, 10.94; N, 5.07. Found: C, 78.02; H, 10.76; N, 4.83.
26. *3β-Acetoxy-21-acetyl-20,28-epoxy-18α,19βH-ursane* **8**: mp 308 °C; $[\alpha]_D^{20}$ +35 (*c* 0.1, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 0.81, 0.85, 0.87, 0.90, 0.96, 1.03 (18H, 6s), 1.10–1.77 (26H, m), 2.00 (3H, s), 2.22 (3H, s), 2.83–2.89 (1H, m), 3.41 and 4.12 (2H, *J* = 8.5 Hz, both d), 4.42–4.47 (1H, m); ^{13}C NMR (300 MHz, $CDCl_3$) δ 14.1, 15.7, 16.4, 16.5, 18.1, 19.7, 21.2, 21.6, 23.1, 23.6, 25.7, 26.3, 27.9, 29.7, 29.8, 31.0, 33.8, 37.0, 37.2, 37.7, 38.5, 39.8, 40.6, 41.2, 44.2, 46.2, 50.4, 50.5, 55.4, 68.9, 72.6, 80.8, 170.9, 211.4. Anal. Calcd for $C_{34}H_{54}O_4$: C, 77.52; H, 10.33. Found: C, 77.31; H, 10.14.
27. *3-Deoxy-(2-cyanoethyl)-oximino-urs-12-en-28-oic acid* **9**: mp 180 °C; $[\alpha]_D^{20}$ +32 (*c* 0.65, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 0.78, 0.87, 0.93, 1.01, 1.03, 1.11, 1.14 (21H, 7s), 1.22–2.37 (24H, m), 2.63–2.71 (2H, m), 4.14–4.28 (2H, m), 5.22–5.31 (1H, s); ^{13}C NMR (300 MHz, $CDCl_3$) δ 14.7, 17.0, 17.8, 18.3, 18.9, 22.7, 23.3, 23.4, 25.8, 27.3, 27.6, 29.6, 30.5, 32.2, 32.3, 32.9, 33.7, 37.0, 38.1, 39.2, 40.1, 40.8, 41.6, 45.7, 46.5, 47.0, 55.6, 67.3, 117.9, 122.5, 143.5, 168.0, 183.5. Anal. Calcd for $C_{33}H_{50}N_2O_3$: C, 75.82; H, 9.52; N, 5.36. Found: C, 75.41; H, 9.06; N, 4.84.
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