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3-*O*-Glutaryl-dihydrobetulin and related monoacyl derivatives as potent anti-HIV agents

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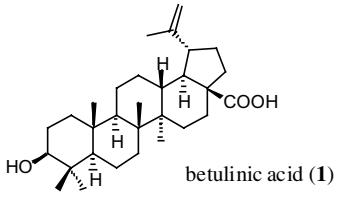
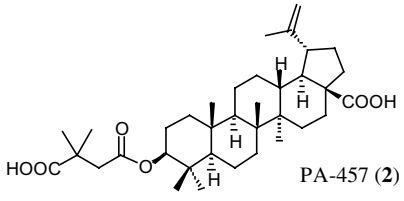
Abstract—3-*O*-Acyl-betulin and -dihydrobetulin derivatives were prepared and evaluated for anti-HIV activity. 3-*O*-Glutaryl-dihydrobetulin (**17**) demonstrated extremely potent anti-HIV activity with an EC_{50} value of 2×10^{-5} μM and a TI value of 1.12×10^{6}. 3-*O*-(3′,3′-Dimethylsuccinyl)- and 3-*O*-(3′,3′-dimethylglutaryl)-dihydrobetulins (**15**, **16**) were also potent anti-HIV compounds with EC_{50} values of 0.0017 and 0.0013 μM, respectively, and TI values of 16,160 and 19,530, respectively.

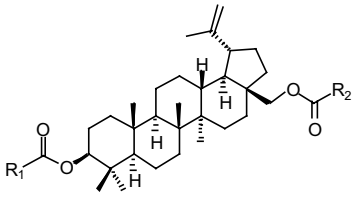
Our modification study on betulinic acid (**1**), which was identified as an anti-HIV principle [EC_{50} 1.4 μM, therapeutic index (TI) 9.3] from the leaves of *Syzygium claviflorum*,[1] has resulted in the design and discovery of 3-*O*-(3′,3′-dimethylsuccinyl)-betulinic acid (PA-457, **2**). PA-457 shows extremely potent anti-HIV activity with a reported EC_{50} value of <0.00035 μM and a therapeutic index (TI) value of >20,000.[2,3] Moreover, PA-457 was found to interfere with viral maturation/budding, the final step in the HIV life cycle, and thus, has a novel mechanism of action compared with the current AIDS drugs.[4,5] PA-457 is, therefore, the first compound known to target this step in virus replication and is undergoing clinical trials.[6]

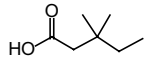


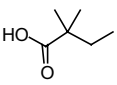
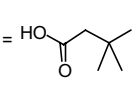
betulinic acid (**1**)



PA-457 (**2**)



3 $R_1 = R_2 =$ 

4 $R_1 =$  $R_2 =$ 

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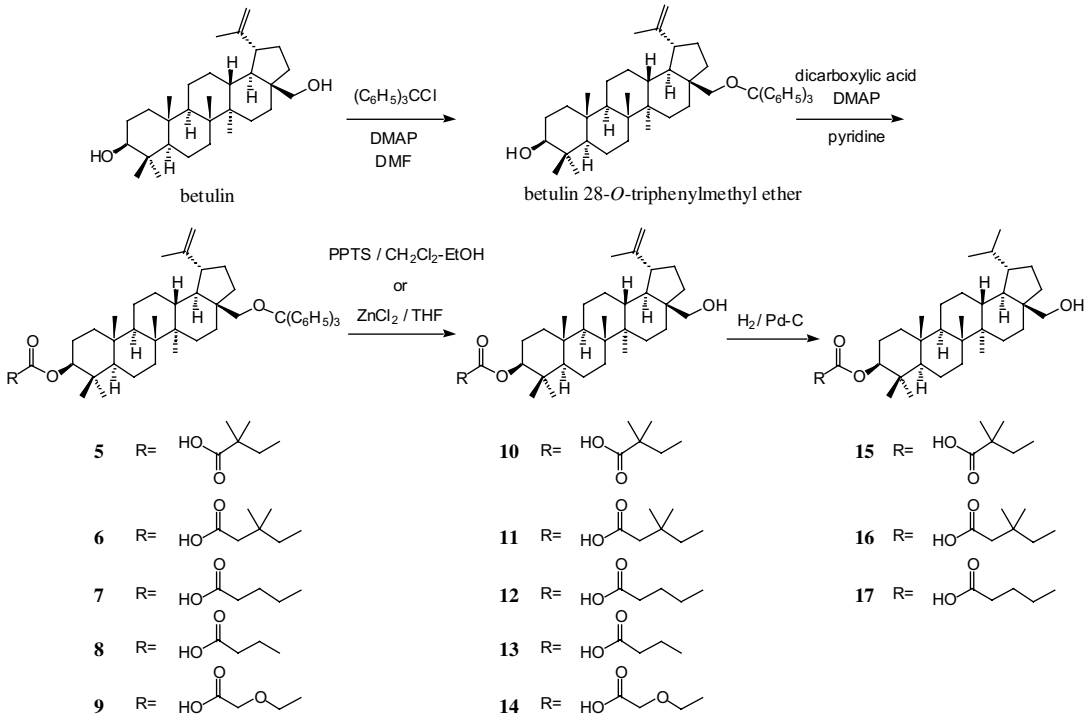
Various 3,28-di-*O*-acyl-betulins were also prepared as PA-457 related derivatives based on the structural similarity of betulin and betulinic acid. 3,28-Di-*O*-(3′,3′-dimethylglutaryl)-betulin (**3**) and 3-*O*-(3′,3′-dimethylsuccinyl)-28-*O*-(2″,2″-dimethylsuccinyl)-betulin (**4**) demonstrated potent anti-HIV activity with EC_{50} values of 0.00066 and 0.00087 μM, respectively, and TI values of 21,515 and 42,400, respectively.[7,8] In contrast, 28-*O*-dimethylsuccinyl-betulins, which were prepared in the course of the structure elucidation of 3,28-di-*O*-dimethylsuccinyl-betulin isomers, were not potent anti-HIV agents. These data suggested that a 3-*O*-acyl group was essential for potent anti-HIV activity.[8] In a continuing structure–activity relationship (SAR) study on PA-457 derivatives, we have now prepared 3-*O*-acyl-betulin and -dihydrobetulin derivatives, and evaluated their anti-HIV activity.

The 3-*O*-acyl-betulin derivatives were prepared as shown in Scheme 1. Protection of the 28-hydroxyl group of betulin with triphenylmethyl chloride yielded betulin 28-*O*-triphenylmethyl ether, whose solution in pyridine was further treated with an appropriate dicarboxylic acid in the presence of dimethylamino pyridine at reflux to give 3-*O*-acyl-betulin 28-*O*-triphenylmethyl ether derivatives. The C-28 protecting group was subsequently removed by refluxing with pyridinium *p*-toluenesulfonate (PPTS) in CH_2Cl_2–EtOH, and the product was purified by silica gel chromatography to give the 3-*O*-acyl-betulin derivatives. However, although the reaction of dimethylsuccinic acid and betulin 28-*O*-triphenylmethyl ether yielded mainly the 3-*O*-(3′,3′-dimethylsuccinyl)-betulin derivative, the undesired 2′,2′-dimethyl isomer was also formed, as was seen previously in 3-*O*-acyl-betulinic acid derivatives.[3] After removing the 28-protective group of the products using the conditions described above, the desired 3′,3′-dimethylsuccinyl-betulin could be obtained by crystallization from EtOH. On the other hand, removing the 28-*O*-triphenylmethyl ether of 3-*O*-succinyl and 3-*O*-glutaryl derivatives with PPTS in CH_2Cl_2–EtOH yielded mainly the corresponding ethyl ester derivatives, together with the desired product. Alternatively, the desired 3-*O*-succinyl- and 3-*O*-glutaryl-betulins were obtained by refluxing with $ZnCl_2$ in 95% THF. Initially, we attempted to prepare the 3-*O*-acyl-dihydrobetulin derivatives directly from the 3-*O*-acyl-betulin 28-*O*-triphenylmethyl ethers by catalytic reduction with Pd–C/H_2, but the 28-*O*-triphenylmethyl ether group was not removed under these conditions. Therefore, the 3-*O*-acyl-dihydrobetulin derivatives[10] were prepared from the 3-*O*-acyl-betulin derivatives by treatment with Pd–C/H_2.

Anti-HIV activities for 3-*O*-acyl-betulin and -dihydrobetulin derivatives are summarized in Table 1. Among these derivatives, 3-*O*-glutaryl-dihydrobetulin (**17**) demonstrated extremely potent anti-HIV activity in acutely infected H9 cells with an EC_{50} value of 2×10^{-5} μM and inhibited uninfected H9 cell growth with an IC_{50} value of 23.59 μM. Its calculated TI value was 1.12×10^6. Compound **17** was more potent than AZT in this assay, and this analog appears to show the greatest anti-HIV activity among the betulinic acid, dihydrobetulinic acid, betulin, and dihydrobetulin derivatives evaluated thus far. 3-*O*-(3′,3′-Dimethylsuccinyl)- and 3-*O*-(3′,3′-dimethylglutaryl)-dihydrobetulins (**15**, **16**) were also potent anti-HIV compounds with EC_{50} values of 0.0017 and 0.0013 μM, respectively, and TI values



Scheme 1. Preparation of 3-*O*-acyl-betulin and -dihydrobetulin derivatives.

Table 1. Anti-HIV activities[9] for betulinic acid (**1**), PA-457 (**2**), 3,28-di-*O*-acyl-betulin derivatives (**3, 4**), and 3-*O*-acyl-betulin (**10**–**14**) and -dihydrobetulin (**15**–**17**) derivatives

Compd	IC_{50}[a]	EC_{50}[b]	TI[c]
1	12.9	1.4	9.2
2	7.0	<0.00035	>20,000
3	14.2	0.00066	21,515
4	36.9	0.00087	42,400
10	33.46	0.0056	5974
11	28.9	0.0044	6568
12	44.36	0.0662	670
13	25.98	N.S.[d]	—
14	>44.75	0.0246	>1819
15	26.99	0.0017	16,160
16	25.69	0.0013	19,530
17	23.59	2×10^{-5}	1.12×10^{6}
AZT	1873	0.0062	302,096

[a] Concentration (μM) that is toxic to 50% of mock-infected H9 cells.
[b] Concentration (μM) that inhibits HIV-1 replication by 50%.
[c] TI (therapeutic index) is defined by IC_{50}/EC_{50}.
[d] No suppression at concentrations up to 100 μg/mL.

of 16,160 and 19,530, respectively. 3-*O*-Acyl-betulin derivatives (**10**, **11**, **12**) also showed relatively potent anti-HIV activities with EC_{50} values ranging from 0.0044 to 0.0662 μM and TI values ranging from 670 to 6568, but they were less active than the corresponding dihydrobetulin derivatives. Comparison of the anti-HIV activities for 3-*O*-acyl-betulin and -dihydrobetulin derivatives suggested that hydrogenation of the betulin Δ^{29} double bond increased anti-HIV potency. 3-*O*-Diglycolyl-betulin (**14**) also displayed significant anti-HIV activity, which might be improved by hydrogenation.

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9. The HIV-1 (IIIB isolate) growth inhibition assay in the H9 cells was performed according to the procedure described in Refs. 1–3 and 7.
10. All new compounds gave satisfactory analytical and spectroscopic data. Selected spectroscopic data for 3-*O*-glutaryl dihydrobetulin (**17**): Colorless needles (from MeOH); mp 230–231 °C; $[\alpha]_D$ −6.6 (*c* 0.47, pyridine); ^{1}H NMR (500 MHz, pyridine-d_5) δ: 0.83, 0.89 (each 3H, d, J = 6.7 Hz, CH_3-29 and 30), 0.81, 0.90, 0.91, 0.97, 1.01 (each 3H, s, CH_3-23, 24, 25, 26, 27), 2.23 (2H, m, glutaryl H_2-3′), 2.59–2.66 (4H, m, glutaryl H_2-2′ and 4′), 3.60, 4.06 (each 1H, d, J = 10.5 Hz, H_2-28), 4.74 (1H, dd, J = 4.5, 11.5 Hz, H-3); positive FABHRMS *m/z* 581.4174 ($[M+Na]^+$), $C_{35}H_{58}O_5Na$ requires 581.4182. 3-*O*-(3′,3′-dimethylglutaryl)-dihydrobetulin (**16**): Colorless needles (from MeOH); mp 224–225 °C; $[\alpha]_D$ −12.5 (*c* 0.4, pyridine); ^{1}H NMR (500 MHz, pyridine-d_5) δ: 0.83, 0.90 (each 3H, d, J = 7.0 Hz, CH_3-29 and 30), 0.81, 0.93, 0.95, 0.97, 1.00 (each 3H, s, CH_3-23, 24, 25, 26, 27), 1.37, 1.38 (each 3H, s, dimethylglutaryl CH_3), 2.75, 2.81 (each 1H, d, J = 14 Hz, dimethylglutaryl H_2-2′), 2.69 (2H, s, dimethylglutaryl H_2-4′), 3.60, 4.05 (each 1H, d, J = 11 Hz, H_2-28), 4.75 (1H, dd, J = 4.5, 11.5 Hz, H-3); positive FABHRMS *m/z* 609.4502 ($[M+Na]^+$), $C_{37}H_{62}O_5Na$ requires 609.4495. 3-*O*-(3′,3′-dimethylsuccinyl)-dihydrobetulin (**15**): Colorless needles (from MeOH); mp 273–274 °C; $[\alpha]_D$ −15.6 (*c* 0.5, pyridine); ^{1}H NMR (pyridine-d_5): 0.84, 0.90 (each 3H, d, J = 7.0 Hz, CH_3-29 and 30), 0.79, 0.95, 0.97, 0.97, 0.99 (each 3H, s, CH_3-23, 24, 25, 26, 27), 1.54 (6H, s, dimethylsuccinyl CH_3), 2.88, 2.95 (each 1H, d, J = 15.5 Hz, dimethylsuccinyl H_2-2′), 3.59, 4.04 (each 1H, d, J = 10.5 Hz, H_2-28), 4.76 (1H, dd, J = 4.5, 11.8 Hz, H-3); Positive FABHRMS *m/z* 595.4326 ($[M+Na]^+$), $C_{36}H_{60}O_5Na$ requires 595.4338.