Betulinic Acid and Dihydrobetulinic Acid Derivatives as Potent Anti-HIV Agents[1]

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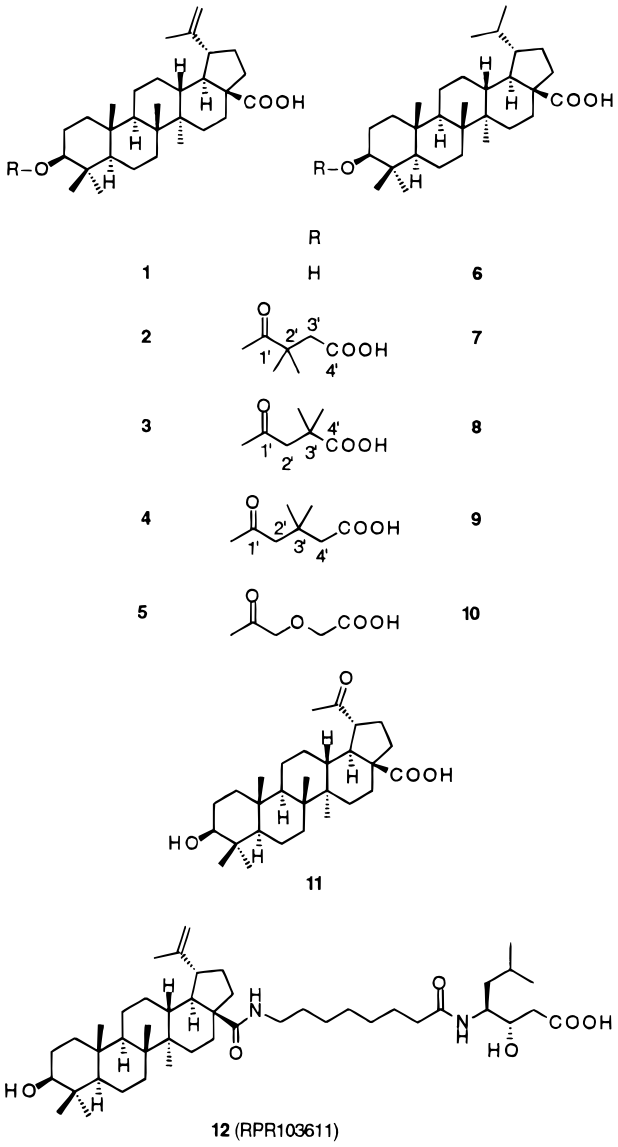
Various efforts are currently underway to develop therapeutic agents to arrest the replication of the human immunodeficiency virus (HIV). Although several nucleoside HIV-1 reverse transcriptase (RT) inhibitors, AZT, ddI, ddC, and D4T, are approved by the FDA and used clinically, adverse side effects and the development of drug resistant virus have been reported.[2,3] Therefore, compounds possessing potent anti-HIV activity with novel modes of action are urgently needed to add to the existing anti-HIV therapies. Currently, the development of new anti-HIV agents is focused on discovering diverse compounds with either novel structures or a new mechanism(s) of action.

Discovery of novel plant-derived natural products as potential new lead compounds for anti-HIV agents as well as the modification of these new lead compounds are continuing goals of our laboratory. Suitable structural modification of the initial lead structures may provide analogs with greatly enhanced activity. As a successful example, we isolated and identified suksdorfin as an anti-HIV principle from *Lomatim suksdorfii*.[4] Subsequent modification of suksdorfin has yielded 3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (DCK), which demonstrated extremely potent inhibitory activity against HIV replication in H9 lymphocyte cells with an EC_{50} value of 0.0004 μM and a therapeutic index (TI) value of 136 719.[5,6]

Previously, we isolated betulinic acid and platanic acid as anti-HIV principles from *Syzigium claviflorum*.[7] Betulinic acid (**1**) and platanic acid (**11**) exhibited inhibitory activity against HIV-1 replication in H9 lymphocyte cells with EC_{50} values of 1.4 and 6.5 μM, respectively, and TI values of 9.3 and 14, respectively. Hydrogenation of betulinic acid yielded dihydrobetulinic acid (**6**), which showed slightly more potent anti-HIV activity with an EC_{50} value of 0.9 and a TI value of 14. On the basis of these findings, modification of these lead compounds, betulinic acid and dihydrobetulinic acid, has been carried out and has led to the discovery of six potent anti-HIV agents. This paper describes the preparation of these betulinic acid and dehydrobetulinic acid derivatives and evaluation of their anti-HIV activities.



The C3 hydroxy, C17 carboxylic acid, and C20 *exo*-methylene groups in betulinic acid can be easily modified. The modification described in this paper was focused on the introduction of an acyl group at the C3 hydroxy groups of betulinic acid and dihydrobetulinic acid. Thus, betulinic acid and dihydrobetulinic acid were treated with 3,3-dimethylglutaric anhydride or diglycolic anhydride in pyridine in the presence of (dimethylamino)pyridine to furnish the corresponding 3-*O*-acyl derivatives (**4**, **5**, **9**, and **10**). In contrast, similar treatment of betulinic acid and dihydrobetulinic acid with dimethylsuccinic anhydride afforded a mixture of 3-*O*-(2′,2′-dimethylsuccinyl)- and 3-*O*-(3′,3′-dimethylsuccinyl)betulinic acid (**2** and **3**) and -dihydrobetulinic acid (**7** and **8**), respectively. The mixture was separated successfully by preparative scale HPLC yielding pure samples. The structures of these isomers were assigned by long-range $^1H-^{13}C$ COSY examinations.

The anti-HIV assay indicated that 3-*O*-(3′,3′-dimethylsuccinyl)betulinic acid (**3**) and -dihydrobetulinic acid (**8**) both demonstrated extremely potent anti-HIV activity in acutely infected H9 lymphocytes with EC_{50} values of $<3.5 \times 10^{-4}$ μM. They exhibited remarkable TI values of >20 000 and >14 000, respectively. In contrast, compounds **2** and **7**, the 2′,2′-dimethyl isomers,

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Table 1. Anti-HIV, HIV-RT, and Fusion Assay Data for Betulinic Acid and Dihydrobetulinic Acid Derivatives

	anti-HIV assay			HIV-RT assay	fusion assay
compd	IC_{50} (μM)[a]	EC_{50} (μM)[b]	TI[c]	IC_{50} (μM)[d]	IC_{100} (μM)[e]
1	13.0	1.4	9.3	>219	>219
2	15.9	2.7	5.9	>171	34
3	7.0	$<3.5 \times 10^{-4}$ [f]	>20000	>171	34
4	4.5	2.3×10^{-3}	1974	>167	50
5	11.7	0.01	1172	>175	70
6	12.6	0.9	14	>218	>218
7	7.7	0.56	13.8	NT[g]	NT[g]
8	4.9	$<3.5 \times 10^{-4}$ [f]	>14000	>171	34
9	5.8	5.7×10^{-3}	1,017	>167	33
10	13.1	5.6×10^{-3}	2344	>174	70
12	2.7	0.03[h]	>90[h]	no inhibition[h]	3[h]
AZT	1,875	0.15	12500		

[a] Concentration which is toxic to 50% of mock-infected H9 cells. [b] Concentration which inhibits viral replication by 50%. [c] IC_{50} value divided by EC_{50} value. [d] Concentration required to inhibit 50% of HIV-1 RT activity. [e] Concentration required to completely inhibit HIV-1-induced syncytia. [f] These EC_{50} values are the most conservative estimations under our experimental conditions due to an unusual plateau effect. [g] NT = not tested. [h] Data from ref 8.

showed anti-HIV activities with EC_{50} values of 2.7 and 0.56 μM, respectively, and TI values of 5.9 and 13.8, respectively, which were significantly lower than those of **3** and **8**. Compounds **4**, **5**, **9**, and **10** also exhibited potent anti-HIV activities with EC_{50} values ranging from 0.01 to 2.3×10^{-3} μM and TI values from 1017 to 2344.

The C3 acyl groups of the more active compounds have dimethyl groups or oxygen at the C3′ position. Since the lone pairs of the oxygen in the diglycoyl group might correspond to the dimethyl groups at C3′ in the dimethylsuccinyl or dimethylglutaryl groups, these three acyl groups are structurally similar to one another. This observation suggested that this type of acyl group might be important to the enhanced anti-HIV activity.

The inhibitory activities of **8** and **9** against HIV-1 replication in PHA-stimulated peripheral blood mononuclear cells (PBMCs) were also evaluated. Preliminary results demonstrate that these two agents also display potent inhibitory activity but their therapeutic indexes are 4−6-fold lower than those found in acute HIV-1 infection of H9 cells (*data not provided*). Studies are planned to evaluate these two agents with other HIV-infected target cells.

As a mechanism(s) of action study, the inhibitory activity of compounds **1**−**6** and **8**−**10** against HIV-1 RT was investigated. The tested compounds did not inhibit HIV-RT activity in a concentration range of 167−219 μM. In the same experiment, ddCTP, a known HIV-RT inhibitor, inhibited RT activity by 50% at 18 μM. Recently, other betulinic acid derivatives, such as RPR103611 (**12**), were reported as anti-HIV agents.[8] They were shown to inhibit HIV-induced membrane fusion. Therefore, compounds **1**−**6** and **8**−**10** were also evaluated for inhibitory activity against HIV-induced membrane fusion. Compounds **2**−**5** and **8**−**10** inhibited syncytia formation in a concentration range of 33−70 μM, suggesting that an inhibitory effect against HIV-induced membrane fusion could be involved in their mechanism(s) of action. However, the anti-HIV activity of **3** is 7700 times greater than that of **2**, although these compounds inhibited syncytia formation at the same concentration. This observation indicated that another mechanism(s) of action other than inhibition of syncytia formation could be involved in the anti-HIV activity shown by these compounds. Details of our continuing mechanism(s) of action study will be reported in a future paper.

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Supporting Information Available: Experimental procedures for the preparation of compounds **2**−**5** and **7**−**10** as well as for the biological assays (4 pages). Ordering information is given on any current masthead page.

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