Anti-AIDS Agents 38. Anti-HIV Activity of 3-*O*-Acyl Ursolic Acid Derivatives[1]

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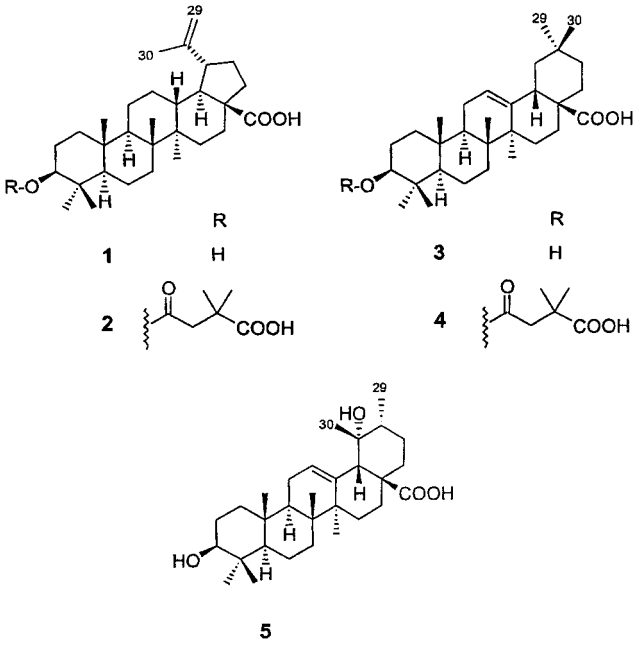
Based on our previous finding that 3-*O*-acyl-betulinic and -oleanolic acids, especially the 3-*O*-(3′,3′-dimethyl)-succinyl derivatives (**2** and **4**), demonstrated potent anti-HIV activity [EC_{50} < 0.00035 and 0.00086 μM; therapeutic index (TI) > 20 000 and 22 326, respectively], several 3-*O*-acyl-ursolic acids were prepared and evaluated for anti-HIV activity. Ursolic acid (**6**) was equipotent (EC_{50} 4.4 μM) with oleanolic acid (EC_{50} 3.7 μM), although it was slightly toxic (IC_{50} 14.3 μM, TI 3.3). 3-*O*-Diglycoryl-ursolic acid (**10**) demonstrated relatively potent anti-HIV activity with an EC_{50} of 0.31 μM and a TI of 155.5. In contrast, 3-*O*-(3′,3′-dimethylsuccinyl)-ursolic acid (**8**), which is analogous to the extremely potent anti-HIV betulinic acid and oleanolic acid derivatives **2** and **4**, displayed only weak anti-HIV activity (EC_{50} 2.1 μM, TI 23.6).

In our continuing search for potential anti-HIV natural products, betulinic acid (**1**), isolated from the leaves of *Syzygium claviflorum* (Myrtaceae), was identified as an anti-HIV agent with an EC_{50} value of 1.4 μM and therapeutic index (TI) of 9.3.[2] Subsequent modification of betulinic acid yielded 3-*O*-(3′,3′-dimethylsuccinyl)-betulinic acid (**2**), which demonstrated extremely potent anti-HIV activity with an EC_{50} value of < 0.00035 μM and TI of >20 000.[3] The related oleanolic acid (**3**) was isolated as an anti-HIV compound from several plants, including *Rosa woodsii* (Rosaceae), *Prosopis glandulosa* (Leguminosae), *Phoradendron juniperinum* (Loranthaceae), *Syzygium claviflorum* (Myrtaceae), *Hyptis capitata* (Lamiaceae), and *Ternstromia gymnanthera* (Theaceae), while pomolic acid (**5**) was identified as an anti-HIV agent from *Pr. glandulosa*, *Ph. juniperinum*, *S. claviflorum*, and *H. capitata*. Both compounds displayed weak anti-HIV activity, with EC_{50} values of 3.7 and 3.0 μM, respectively, and TI values of 12.8 and 16.3, respectively.[4] As with betulinic acid, similar modification of oleanolic acid also gave a potent anti-HIV active derivative, 3-*O*-(3′,3′-dimethylsuccinyl)-oleanolic acid (**4**) (EC_{50} 0.00086 μM; TI 22 400).[4]

With an EC_{50} value of 4.4 μM, ursolic acid (**6**), isolated from *Pr. glandulosa*, *Ph. juniperinum*, *S. claviflorum*, and *H. capitata*, showed a level of anti-HIV activity similar to that shown by oleanolic acid, although **6** was somewhat toxic to H9 cells as revealed by the small TI value of 3.3.[4] The structures of ursolic acid and oleanolic acid are almost identical, and differ only in the position of the C-29 methyl group in the E-ring. Therefore, as with oleanolic acid, modification of ursolic acid was expected to yield potent anti-HIV derivative(s). Based on these previous findings, we have prepared 3-*O*-acyl-ursolic acid derivatives and evaluated their anti-HIV activity.



Results and Discussion

3-*O*-Acyl-ursolic acid derivatives were prepared by reacting ursolic acid with an anhydride or acid chloride in pyridine in the presence of 4-(dimethylamino)pyridine (DMAP) at reflux. As was found with betulinic acid,[3] the reaction of ursolic acid and 2,2-dimethylsuccinic anhydride gave a mixture of 2′,2′-dimethylsuccinyl- and 3′,3′-dimethylsuccinyl-ursolic acids (**7** and **8**, respectively), in which the latter isomer was the major product. The mixture was separated by semipreparative-scale HPLC, and the structures of these isomers were assigned by 2D NMR analyses, including $^1H-^{13}C$ COSY and long-range $^1H-^{13}C$ COSY.

3-*O*-(3′,3′-Dimethylsuccinyl)-ursolic acid (**8**) was expected to have potent anti-HIV activity because the corresponding derivatives of betulinic and oleanolic acids were extremely potent anti-HIV compounds. However, **8** displayed only weak anti-HIV activity (EC_{50} value of 2.1 μM), although it was less toxic than the parent acid to H9 cells (IC_{50} 49.5 μM). Among all 3-*O*-acyl-ursolic acid derivatives, 3-*O*-diglycoryl-ursolic acid (**10**) demonstrated relatively potent anti-HIV activity with an EC_{50} value of 0.31 μM and a TI of 155.5. However, **10** was less potent than the correspond-

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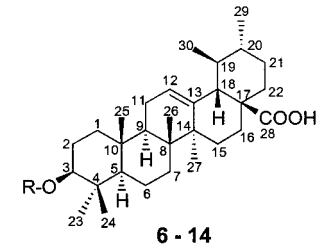
ing betulinic acid derivative (EC_{50} 0.01 μM, TI 1172).[3a] The other 3-*O*-acyl derivatives displayed weak or no anti-HIV activity, although again they were less toxic than ursolic acid against H9 cells. In contrast, the 3-oxo-derivative (**15**) was toxic; the same result was found with comparable betulinic and oleanolic acid derivatives. Ursolic acid and its potassium salt (**16**) had identical anti-HIV activity, while the potassium salt of oleanolic acid was more potent (EC_{50} 0.5 μM, TI 68.6)[4] than the weakly active parent acid.



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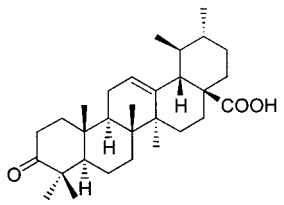
	R	R
6	H	
7		COOH
8		COOH
9		COOH
10		O COOH
11		COOH
12		COOH
13		
14		



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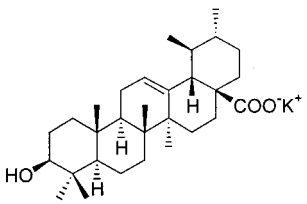

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Thus, although betulinic, oleanolic, and ursolic acids share many structural features, the 3-*O*-acyl-ursolic acid and oleanolic acid derivatives, except for **4**, were generally much less potent than the corresponding betulinic acid derivatives. These results suggest that partial structures in the triterpenoid moiety, together with the structure of the acyl group, are essential for potent anti-HIV activity. The structures of these three triterpenoids correlate closely in the A- through D-rings, but differ significantly in the E-ring, including the D/E-ring fusion. Betulinic acid has a five-membered E-ring, an isopropenyl substituent, and a trans D/E-ring fusion, while oleanolic acid and ursolic acid contain a six-membered E-ring, two methyl substituents, and a cis D/E-ring fusion. From these observations, the structure of the E-ring might play an important role in the anti-HIV potency. A detailed quantitative structure−activity relationship analysis is in progress.

Table 1. Anti-HIV Activity for Betulinic Acid (**1**), Oleanolic Acid (**3**), and Their Derivatives (**2** and **4**), Pomolic Acid (**5**), and Ursolic Acid (**6**) and Its Derivatives (**7**−**16**)

compound	IC_{50} (μM)[a]	EC_{50} (μM)[b]	TI[c]
1	12.9	1.4	9.2
2	7.0	<0.000 35	>20 000
3	47.8	3.7	12.9
4	19.2	0.000 86	22 326
5	48.9	3.0	16.3
6	14.3	4.4	3.3
7	30.7	N.S.[d]	
8	49.5	2.1	23.6
9	7	N.S.[d]	
10	48.2	0.31	155.5
11	44.9	15.3	2.9
12	30.7	6.4	4.8
13	>18.5	N.S.[d]	
14	>18.0	N.S.[d]	
16	1.8	N.S.[d]	
AZT	1871	0.045 ± 0.056[e]	41 667

[a] The agent concentration that inhibited H9 cell growth by 50%. [b] The agent concentration that inhibited viral replication in H9 cell by 50%. [c] In vitro TI ratio: IC_{50}/EC_{50}. [d] N.S. = no suppression. [e] This EC_{50} value represents the mean and standard deviation of 65 EC_{50} values for AZT.

Experimental Section

General Experimental Procedures. Melting points were measured on a Yanako micromelting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-1000 or Perkin-Elmer 241 polarimeter. MS were determined on a JEOL HX-110 spectrometer. ^{1}H and ^{13}C NMR spectra were measured on JEOL A-400 and JEOL A-500 spectrometers with TMS as an internal standard. Column chromatography was carried out with Kiesel gel 60 (70−230 mesh, Merck). HPLC was performed on a Waters 600E solvent delivery system equipped with a Waters R401 differential refractometer and a Capcell PAK C_{18} (SG120, 5 μm) (Shiseido) column (4 mm i.d. × 250 mm and 20 mm i.d. × 250 mm for analytical and semipreparative scale, respectively).

General Procedure for Preparation of 3-*O*-Acyl Ursolic Acids. A mixture of ursolic acid and appropriate acid anhydride (2.5−10 molar equiv) and (DMAP) (1 molar equiv) in pyridine (5−10 mL) was refluxed overnight. The reaction mixture was diluted with ice water and extracted with $CHCl_3$. The organic layer was washed with H_2O, dried over $MgSO_4$, and concentrated under reduced pressure. For **9** and **10**, crystallization (diluted MeOH) of the $CHCl_3$-soluble portion yielded pure samples, whereas for **7** and **8**, the reaction of ursolic acid and 2,2-dimethylsuccinic anhydride gave a crystalline mixture, which was purified by semipreparative HPLC (95% CH_3CN−0.2% TFA). Compounds **11**−**14** were purified by Si gel column chromatography eluting with C_6H_6-EtOAc (1:1, 3:1, and 40:1 for **11**, **12**, and **13**, respectively) and $CHCl_3$ for **14**.

3-*O*-(2′,2′-Dimethylsuccinyl)-ursolic acid (7): a white crystalline powder (from MeOH−EtOAc); mp 276−278 °C; $[\alpha]^{27}_D$ +50.0° [*c* 0.7, MeOH + $CHCl_3$ (1:1)]; ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 3; positive HRFABMS *m*/*z* 607.3975 ([M + Na]$^+$), $C_{36}H_{56}NaO_6$ requires 607.3974.

3-*O*-(3′,3′-Dimethylsuccinyl)-ursolic acid (8): colorless rods (from MeOH−EtOAc); mp 273−274 °C; $[\alpha]^{26}_D$ +50.5° [*c* 0.6, MeOH + $CHCl_3$ (1:1)]; ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 3; positive HRFABMS *m*/*z* 607.3970 ([M + Na]$^+$), $C_{36}H_{56}NaO_6$ requires 607.3974.

3-*O*-(3′,3′-Dimethylglutaryl)-ursolic acid (9): colorless needles (from MeOH), mp 230−232 °C; $[\alpha]^{16}_D$ +56.9 ° (*c* 0.58, $CHCl_3$); ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 3; positive HRFABMS *m*/*z* 597.4161 ([M + H]$^+$), $C_{37}H_{57}O_6$ requires 597.4155.

3-*O*-Diglyoryl-ursolic acid (10): colorless needles (from MeOH); mp 263−265 °C; $[\alpha]^{16}_D$ +60.1 ° (*c* 0.45, pyridine); ^{1}H

Table 2. ^{1}H NMR Data (δ, *J* in Hz) for Compounds **7**−**14**

	compound							
proton	**7**[a]	**8**[b]	**9**[b]	**10**[c]	**11**[d]	**12**[d]	**13**[d]	**14**[d]
H-3	4.76 (dd, 4.5, 11.5)	4.76 (dd, 4.5, 11.5)	4.75 (dd, 4.5, 11.5)	4.63 (dd, 6.5, 9.5)	4.78 (dd, 5, 11)	4.74 (dd, 5, 11)	4.73 (dd, 4.5, 11.5)	4.71 (dd, 4.5, 11)
H-12	5.45 (t, 3.5)	5.46 (t, 3)	5.48 (t, 3)	5.25 (t, 3.5)	5.46 (t, 3)	5.48 (t, 3.5)	5.49 (t, 3.5)	5.48 (t, 3.5)
H-18	2.62 (d, 11.5)	2.63 (d, 11.5)	2.64 (d, 11.5)	2.21 (d, 11.5)	2.63 (d, 12)	ca. 2.65[e]	2.65 (d, 12)	2.65 (d, 11.5)
H-23	1.04 (s)	1.01 (s)	1.03 (s)	0.98 (s)	1.02 (s)	1.03 (s)	1.04 (s)	1.04 (s)
H-24	0.94 (s)	0.94 (s)	0.93 (s)	0.88 (s)	0.93 (s)	0.90 (s)	0.92 (s)	0.92 (s)
H-25	0.82 (s)	0.81 (s)	0.81 (s)	0.82 (s)	0.83 (s)	0.83 (s)	0.85 (s)	0.85 (s)
H-26	1.02 (s)	0.99 (s)	0.98 (s)	0.90 (s)	0.99 (s)	0.95 (s)	0.95 (s)	0.98 (s)
H-27	1.22 (s)	1.21 (s)	1.23 (s)	1.12 (s)	1.22 (s)	1.24 (s)	1.24 (s)	1.24 (s)
H-29	1.01 (d, 6)	1.00 (d, 6)	1.02 (d, 7)	0.96 (d, 6)	1.01 (d, 6)	1.02 (d, 6)	1.02 (d, 6)	1.02 (d, 6.5)
H-30	0.97 (d, 6)	0.97 (d, 6)	0.96 (d, 6)	0.88 (d, 6)	0.97 (d, 6)	0.98 (d, 6)	0.98 (d, 6)	0.98 (d, 6)
acyl moiety								
$-CH_2-$	2.90, 2.94 (d,16)	2.88, 2.95 (d, 15.5)	2.75, 2.82 (d, 14.8) 2.78 (2H, s)	3.35 (4H, m)	2.89, 2.93 (each 2H, m)	2.24 (2H, m) 2.64 (4H, m)	2.27 (d, 6.5)	2.24, 2.31 (d, 13)
$-CH_3$	1.48 (6H, s)	1.54 (6H, s)	1.38, 1.39 (each 3H, s)				0.96 (d, 6.5)	1.09 (9H, s)

[a] Measured at 500 MHz in pyridine-d_5. [b] Measured at 300 MHz in pyridine-d_5. [d] Measured at 400 MHz in $CDCl_3$ + CD_3OD (1:1). [d] Measured at 400 MHz in pyridine-d_5. [e] The coupling constant could not be determined due to overlap with glutaryl methylene signals.

Table 3. ^{13}C NMR Data for Compounds **7**−**14**

	compound							
carbon	**7**[a]	**8**[a]	**9**[b]	**10**[c]	**11**[d]	**12**[d]	**13**[d]	**14**[d]
1	38.3	38.4	38.4	39.5	38.3	38.3	38.3	38.4
2	23.9	24.0	24.1	24.7	23.9	24.0	24.0	24.1
3	80.6	81.0	80.7	84.3	80.9	80.7	80.6	80.6
4	38.1	37.9	37.8	39.0	37.9	37.9	37.9	37.8
5	55.7	55.6	55.6	56.7	55.6	55.5	55.6	55.6
6	18.4	18.5	18.5	19.4	18.4	18.5	18.5	18.5
7	33.4	33.4	33.4	34.2	33.3	33.4	33.4	33.4
8	39.9	39.9	40.0	40.8	39.9	39.9	39.9	39.9
9	47.8	47.8	47.8	48.8	47.8	47.8	47.8	47.8
10	37.1	37.1	37.1	38.1	37.0	37.1	37.1	37.1
11	23.5	23.6	23.6	24.5	23.5	23.6	23.6	23.6
12	125.5	125.5	122.5	126.6	125.4	125.5	125.5	125.5
13	139.3	139.3	139.3	139.6	139.2	139.3	139.3	139.3
14	42.5	42.5	42.5	43.4	42.4	42.5	42.5	42.5
15	28.7	28.7	28.7	29.3	28.6	28.7	28.7	28.7
16	24.9	24.9	24.9	25.5	24.8	24.9	24.9	24.9
17	48.1	48.1	48.1	49.1	48.0	48.1	48.1	48.1
18	53.6	53.6	53.6	54.2	53.5	53.6	53.5	53.6
19	39.5	39.5	39.5	40.4	39.4	39.5	39.5	39.5
20	39.4	39.4	39.4	40.3	39.3	39.4	39.4	39.4
21	31.1	31.1	32.7	31.9	31.0	31.1	31.1	31.1
22	37.4	37.5	37.5	38.1	37.4	37.4	37.5	37.5
23	28.3	28.3	28.3	29.2	28.2	28.3	28.3	28.3
24	17.1	17.1	17.2	17.7	17.0	17.1	17.1	17.1
25	15.5	15.5	15.5	16.6	15.4	15.5	15.5	15.5
26	17.4	17.4	17.4	18.0	17.3	17.4	17.4	17.4
27	23.6	23.9	23.9	24.7	23.8	23.9	17.5	17.5
28	179.9	179.9	179.9	181.8	179.8	179.9	179.9	179.9
29	17.5	17.5	17.5	18.1	17.4	17.5	23.9	23.9
30	21.4	21.4	21.4	22.1	21.3	21.4	30.0	21.4
acyl moiety								
1′	173.9	171.5	172.0	172.3	174.7	173.0	172.6	171.9
2′	44.8	45.3	45.9	69.3[e]	29.9[e]	30.0[e]	43.9	48.4
3′	41.0	40.9	31.1		30.3[e]	21.3	26.0	30.7
4′	176.6	179.3	45.9	69.5[e]	172.4	34.2[e]		
5′			174.5	174.0		175.5		
CH_3	25.8	25.9	28.0				22.4	29.8
	25.9	26.3					22.5	(3C)

[a] Measured at 125 MHz in pyridine-d_5. [b] Measured at 75.5 MHz in pyridine-d_5. [d] Measured at 100 MHz in $CDCl_3$ + CD_3OD (1:1). [d] Measured at 100 MHz in pyridine-d_5. [e] Assignments may be interchanged in each column.

NMR, see Table 2; ^{13}C NMR, see Table 3; negative HRFABMS *m*/*z* 571.3634 ($[M - H]^-$), $C_{34}H_{51}O_7$ requires 571.3635.

3-*O*-Succinyl-ursolic acid (11): colorless needles from C_6H_6, mp 248−249°; $[\alpha]^{23}_D$ +37.3° (*c* 0.23, MeOH); ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 3; negative HRFABMS *m*/*z* 555.3690 $[M - H]^-$, $C_{34}H_{51}O_6$ requires 555.3685.

3-*O*-Glutaryl-ursolic acid (12): colorless needles from CH_3OH, mp 212−214 °C; $[\alpha]^{20}_D$ +35.9° (*c* 1.03, pyridine); ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 3; positive HRFABMS *m*/*z* 593.3815 ($[M + Na]^+$), $C_{35}H_{54}NaO_6$ requires 593.3818.

3-*O*-Isovalaryl-ursolic acid (13): colorless needles from CH_3OH, mp 279−281 °C; $[\alpha]^{15}_D$ +57.6° (*c* 0.92, $CHCl_3$); ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 3; positive HRFABMS *m*/*z* 563.4070 ($[M + Na]^+$), $C_{35}H_{56}NaO_4$ requires 563.4076.

3-*O*-*tert*-Butylacetyl-ursolic acid (14): colorless needles from hexane, mp 265−266 °C; $[\alpha]^{24}_D$ +55.8° (*c* 0.86, $CHCl_3$);

^{1}H NMR, see Table 2; ^{13}C NMR, see Table 3; positive HR-FABMS *m/z* 577.4227 ([M + Na]$^+$), $C_{36}H_{58}NaO_4$ requires 577.4233.

Oxidation of Ursolic Acid (15). A mixture of ursolic acid (**6**) (145 mg) and pyridinium chlorochromate (150 mg) in CH_2Cl_2 (45 mL) was stirred at room temperature for 25 h. The solid was filtered using Celite and discarded, and the filtrate was evaporated. The reaction product was purified by Si gel column chromatography using $CHCl_3$ as eluent to give ursolic acid (3-oxo-urs-12-en-28-oic acid) (**15**) (95 mg): colorless prisms (from *n*-hexane−EtOAc), mp 282−285 °C; $[\alpha]^{25}_D$ +85.7° (*c* 0.90, MeOH); HREIMS *m/z* 454.3429 [M]$^+$, $C_{30}H_{46}O_3$ requires 454.3447.

Preparation of Potassium Salts of Ursolic Acid (16). A solution of ursolic acid (95 mg) was treated with 2% KOH in Me_2CO-H_2O (1:1) at room temperature for 30 min. After removal of Me_2CO by evaporation, the resulting aqueous solution was passed through an MCI-gel CHP20P column, and washed with H_2O to remove excess KOH. Subsequent elution with MeOH gave potassium ursoate (**16**) (85 mg) as a white powder; mp 255−258 °C; FABMS *m/z* 495 [M + H]$^+$, 533 [M + K]$^+$.

Anti-HIV Assay. The T cell line, H9, was maintained in continuous culture with complete medium (RPMI 1640 with 10% fetal calf serum supplemented with L-glutamine) at 5% CO_2 and 37 °C. Aliquots of this cell line were used in experiments only when in log-phase of growth.

Test samples were first dissolved in dimethyl sulfoxide. The following are the final drug concentrations routinely used for screening: 100, 20, 4, and 0.8 μg/mL. For active agents, additional dilutions are prepared for subsequent testing so that an accurate EC_{50} value (see definition below) could be achieved.

As the test samples were being prepared, an aliquot of H9 cells was infected with HIV-1 (IIIB isolate), while another aliquot was mock-infected with complete medium. The mock-infected cells were used for toxicity determinations (IC_{50}, see definition below). The stock virus used for these studies typically had a $TCID_{50}$ value of 10^4 infectious units (IU)/mL. The appropriate amount of virus for a multiplicity of infection between 0.1 and 0.01 IU/cell was added to the first aliquot of cells. The other aliquot of cells received only culture medium and was then incubated under identical conditions to the HIV-infected cells. After a 4-h incubation at 37 °C and 5% CO_2, both cell populations were washed three times with fresh medium and then added to the appropriate wells of a 24-well plate containing the various concentrations of the test drug or culture medium (positive-infected control/negative-control drug). In addition, AZT was also assayed during each experiment as a positive-control drug. The plates were incubated at 37 °C and 5% CO_2 for 4 days. Cell-free supernatants were collected on day 4 and tested by an in-house p24 antigen ELISA assay. p24 Antigen is a core protein of HIV and, therefore, is an indirect measure of virus present in the supernatants. Toxicity was determined by performing cell counts via a Coulter counter on the mock-infected cells, which had either received culture medium (no toxicity) or test sample or AZT. If a test sample had suppressive capability and was not toxic, its effects are reported in the following terms: IC_{50}, the concentration of test sample that was toxic to 50% of the mock-infected cells; EC_{50}, the concentration of the test sample that was able to suppress HIV replication by 50%; and TI, the ratio of IC_{50} to EC_{50}.

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References and Notes

(1) For part 37, see: Xie, L.; Takeuchi, Y.; Cosentino, L. M.; Lee, K. H. *J. Med. Chem*. **1999**, *42*, 2662−2672.
(2) Fujioka, T.; Kashiwada, Y.; Kilkuskie, R. E.; Cosentino, L. M.; Ballas, L. M.; Jiang, J. B.; Janzen, W. P.; Chen, I. S.; Lee, K. H. *J. Nat. Prod*. **1994**, *57*, 243−247.
(3) (a) Kashiwada, Y.; Hashimoto, F.; Cosentino, L. M.; Chen, C. H.; Garrett, P. E.; Lee, K. H. *J. Med. Chem*. **1996**, *39*, 1016−1017. (b) Hashimoto, F.; Kashiwada, Y.; Cosentino, L. M.; Chen, C. H.; Garrett, P. E.; Lee, K. H. *Bioorg. Med. Chem*. **1997**, *5*, 2133−2143.
(4) Kashiwada, Y.; Wang, H. K.; Nagao, T.; Kitanaka, S.; Yasuda, I.; Fujioka, T.; Yamagishi, T.; Cosentino, L. M.; Kozuka, M.; Okabe, H.; Ikeshiro, Y.; Hu, C. Q.; Yeh, E.; Lee, K. H. *J. Nat. Prod*. **1998**, *61*, 1090−1095.

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