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Anti-AIDS Agents 54. A Potent Anti-HIV Chalcone and Flavonoids from Genus *Desmos*

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Abstract—Sixteen flavonoids and their derivatives isolated from *Desmos* spp. were evaluated for inhibition of HIV replication in H9 lymphocyte cells. 2-Methoxy-3-methyl-4,6-dihydroxy-5-(3′-hydroxy)cinnamoylbenzaldehyde (**12**) and lawinal (**6**) demonstrated potent anti-HIV activity with EC_{50} values of 0.022 and 2.30 μg/mL and therapeutic indexes of 489 and 45.2, respectively. Compound **12** appears to be an excellent lead for further anti-HIV drug development.

Desmos (Annonaceae) spp. are distributed in southern Asian countries and used as folk medicines in China as antimalarial, insecticidal, antirheumatic, antispasmodic, and analgesic agents.[1] The main constituents in this genus are flavonoids. This compound class is widespread in plants and exhibits many biological properties, including antitumor, anti-inflammatory, and antiviral activities. Acquired immunodeficiency syndrome (AIDS), which is caused by the human immunodeficiency virus (HIV), has been a life-threatening health problem since 1981,[2] and flavonoids have been investigated for anti-HIV activity.[3]

Our research approach is to discover novel plant-derived natural products as new lead compounds for potential anti-HIV agents, and to modify these compounds to find still more potent anti-HIV agents. As part of our screening, we examined the inhibitory effects of organic extracts of four *Desmos* Lour. species against HIV-1 replication in acutely infected H9 lymphocytic cells. *D. chinensis*, *D. grangifolius*, *D. dumosus*, and *D. yunnanensis* showed significant anti-HIV activity. Using bioactivity-directed fractionation, we have isolated and characterized 18 flavonoids and their derivatives from these four species.[4–13] We report herein the inhibitory effects of 16 selected flavonoids against HIV-1 replication in H9 lymphocytes.[14]

The tested flavonoids (**1**–**16**) are shown in Figure 1. They were categorized structurally into five groups, including flavones (**1**–**5**), flavanones (**6**–**10**), flavan (**11**), chalcones (**12**–**13**), and other flavonoid derivatives with unique structures (**14**–**16**). These flavonoids and their derivatives from geuns *Desmos* Lour have several unique structural features. All compounds have an unsubstituted B ring and, except for **1** and **2**, a completely substituted A ring. Several compounds contain an aldehyde group on the A ring, which is quite rare in natural products. Table 1 shows the anti-HIV activities of these compounds, with AZT included in the same experiment for comparison. 2-Methoxy-3-methyl-4,6-dihydroxy-5-(3′-hydroxy)cinnamoylbenzaldehyde (**12**) demonstrated potent anti-HIV activity (EC_{50} 0.022 μg/mL) with a good therapeutic index (TI) (489). Interestingly, **13**, which has an OH rather than an OMe group at C-2, was inactive. Thus, a C-2 methoxy group in the chalcone skeleton may be critical for anti-HIV activity. Lawinal (**6**) demonstrated potent anti-HIV activity (EC_{50} 2.3 μg/mL) with an adequate TI (45.2), while desmethoxyatteucinol (**8**) was less active with EC_{50} and TI values of 4.97 μg/mL and 4.18, respectively. This result indicated that an aldehyde is more favorable than a methyl group at the flavanone C-6 position. In contrast, comparing the data for **8** and **7** (no suppression) showed that a methyl group is more favorable than an aldehyde at the flavanone C-8 position. Desmosflavanone II (**10**) showed only weak anti-HIV activity (EC_{50} 11.0 μg/mL, TI 9.85), while the remaining compounds showed no anti-HIV activity.

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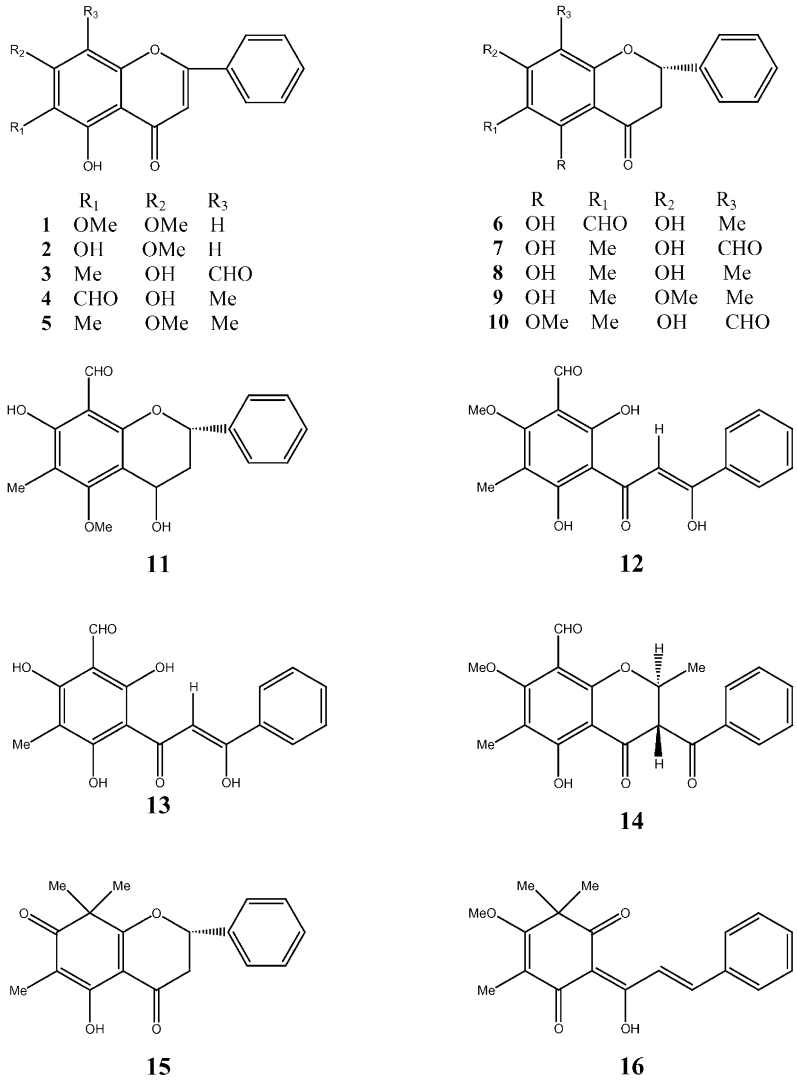

Figure 1. Structures of **1**–**16**.

Table 1. Inhibition of HIV-1 replication in H9 lymphocytic cells by flavonoids

Compd	IC_{50} (μg/mL)[a]	EC_{50} (μg/mL)[b]	Therapeutic index[c]
1	20.2	NS[d]	—
2	11.5	NS[d]	—
3	Did not dissolve		
4	Did not dissolve		
5	19.8	NS[d]	—
6	104	2.30	45.2
7	2.15	NS[d]	—
8	20.8	4.97	4.18
9	1.77	NS[d]	—
10	108	11.0	9.85
11	24.2	NS[d]	—
12	10.7	0.022	489
13	18.8	NS[d]	—
14	1.89	NS[d]	—
15	1.89	NS[d]	—
16	2.29	NS[d]	—
AZT	500	0.0221	22,600

[a]Concentration that inhibits uninfected H9 cell growth by 50%.
[b]Concentration that inhibits viral replication by 50%.
[c]TI = Therapeutic index IC_{50}/EC_{50}.
[d]NS = No suppression.

It should be noted that the flavanones **6**, **8**, and **10** did exhibit anti-HIV activity. Thus, these result suggest that the flavanone C_2–C_3 double bond may not be necessary for antiviral activity, as was postulated in a previous study.[11]

The most potent compound **12** is a chalcone. Chalcones have a wide range of pharmacological effects, including antimalarial, antiinflammatory, and antileishmanial activities.[15] However, our data are the first report that chalcones possess anti-HIV activity. 2-Methoxy-3-methyl-4,6-dihydroxy-5-(3′-hydroxy)cinnamoylbenzaldehyde (**12**) is an excellent lead compound for further anti-HIV drug development. The results from this and other studies will be used to further modify **12** to improve its pharmacological properties. Both the aromatic substituents and the keto-enol functionality are targets for the SAR studies in progress.

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14. Anti-HIV assay method: The T cell line, H9, was maintained in continuous culture with complete medium (RPMI 1640 with 10% fetal calf serum (FCS) supplemented with L-glutamine at 5% CO_2 and 37 °C. Aliquots of this cell line were only used in experiments when in log-phase of growth. Test samples were first dissolved in dimethyl sulfoxide (DMSO). The following were the final drug concentrations routinely used for screening: 100, 20, 4 and 0.8 μ/mL, but for active agents, additional dilutions were prepared for subsequent testing so that an accurate EC_{50} value could be achieved. As the test samples were being prepared, an aliquot of the T cell line, H9, was infected with HIV-1 (IIIB isolate) while another aliquot was mock-infected with complete medium. The mock-infected aliquot was used for toxicity determinations (IC_{50}). The stock virus used for these studies typically had a $TCID_{50}$ value of 10^4 Infectious Units/mL. The appropriate amount of virus for a multiplicity of infection (moi) between 0.1 and 0.01 infectious units/cell was added to the first aliquot of H9 cells. The other aliquot of H9 cells only received culture medium and then was incubated under identical conditions as the HIV-infected H9 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 drug control). In addition, AZT was also assayed during each experiment as a positive drug control. The plates were incubated at 37 °C and 5% CO_2 for 4 days. Cell-free supernatants were collected on day 4 for use in our 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 by a Coulter Counter on the mock-infected H9 cells, which had either received culture medium (no toxicity) or test sample or AZT.
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