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Isolation and Characterization of New Anti-HIV and Cytotoxic Leads from Plants, Marine, and Microbial Organisms[1]

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New cytotoxic isomalabaricane triterpenes have been isolated from a sponge *Stelletta* sp. (**1**−**7**); anti-HIV pterocarpans (**8** and **9**) and isoflavanoids (**12**−**16** and **18**) were elucidated from two tropical plants in the genus *Erythrina*; and anti-HIV enniatins (**20** and **22**−**23**) were characterized from fungi in the genera *Fusarium* and *Alternaria*. The enniatins were evaluated for *in vivo* anti-HIV activity in the hollow fiber assay.

The present research has focused upon the isolation and structure elucidation of metabolites from selected extracts found active in the National Cancer Institute's (NCI) primary anti-HIV or antitumor screens. Organisms of interest have included plants, marine invertebrates, fungi, micro- and macroalgae, cyanobacteria, and other microbes. Recent results described herein include the isolation of new cytotoxic isomalabaricane triterpenes **1**−**7** from *Stelletta* sp., pterocarpans **8** and **9** and isoflavanoids **12**−**16** and **18** from *Erythrina* spp., and cyclohexadepsipeptide enniatins **20** and **22**−**23** from *Fusarium* and *Alternaria* spp. The enniatins were evaluated for *in vivo* anti-HIV activity using the hollow-fiber assay.

Results and Discussion

A. Isomalabaricane Triterpenes from a *Stelletta* Species. Several groups have reported the isolation of isomalabaricane triterpenes from sponges of the genera *Jaspis*[2−6] and *Stelletta*.[7,8] We recently reported the isolation of four new members of this family, stellettins C (**1**), D (**2**), E (**3**), and F (**4**), along with three known isomalabaricanes, stellettins A (**5**), B (**6**), and G (**7**) (Chart 1).[9] These compounds were isolated from a *Stelletta* sp. (Stellettidae) collected under contract for the National Cancer Institute off the northern coast of Australia, near Cape Wilberforce, at −15 m. The sponge extract was selected for bioassay-guided fractionation based on the cytotoxicity profile of the crude organic extract in the NCI's 60-cell line antitumor assay.[10] Stellettins A−D (**5**, **6**, **1**, **2**, respectively) all contain the same tricyclic isomalabaricane ring system, which is substituted at C-13 with a 10-carbon polyene chain that terminates in a γ-pyrone. The stellettin A/B and C/D pairs are geometrical isomers at the C-13 olefin; stellettins A and C have an *E* configuration, while stellettins B and D have a 13(*Z*) configuration. Stellettins A and B have a C-3 keto group, which is reduced and acetylated in stelletins C and D. Stellettins E (**3**), F (**4**), and G (**7**) all lack the terminal γ-pyrone functionality, but instead terminate in a free carboxylic acid and are geometrical isomers at both the C-13 and C-24 olefins.

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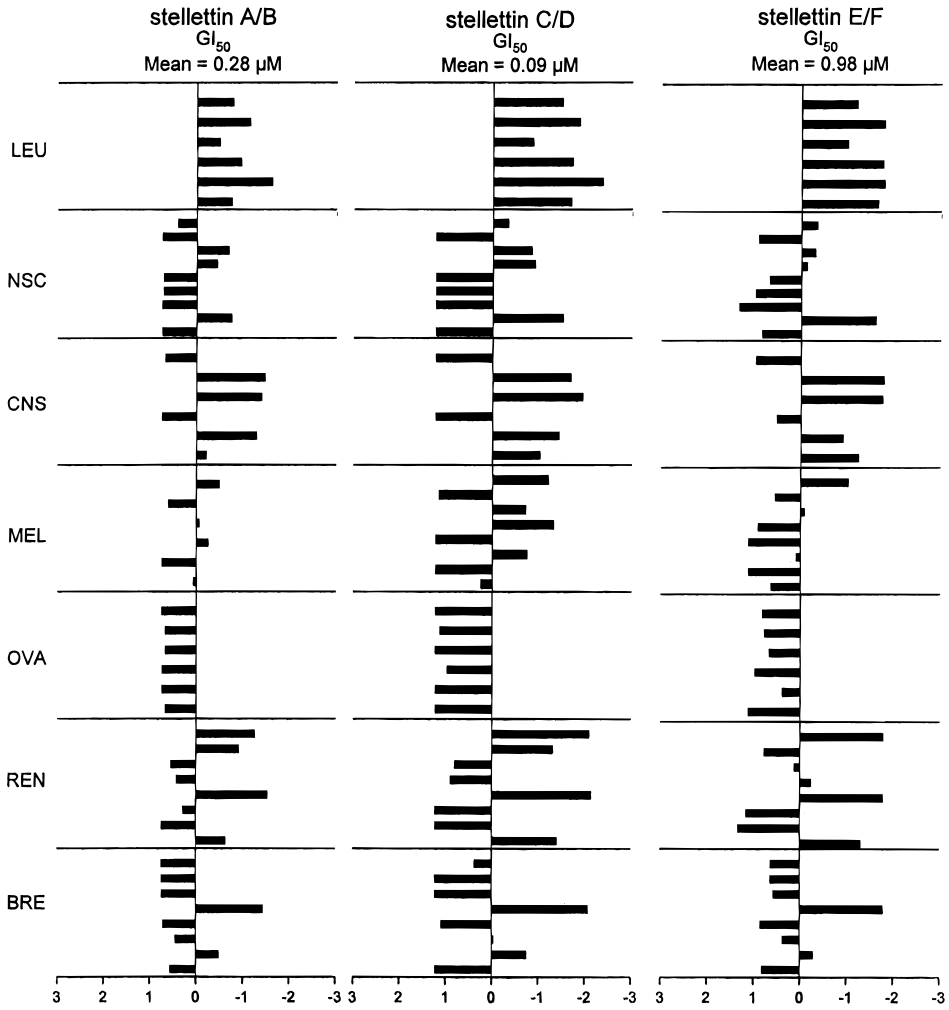
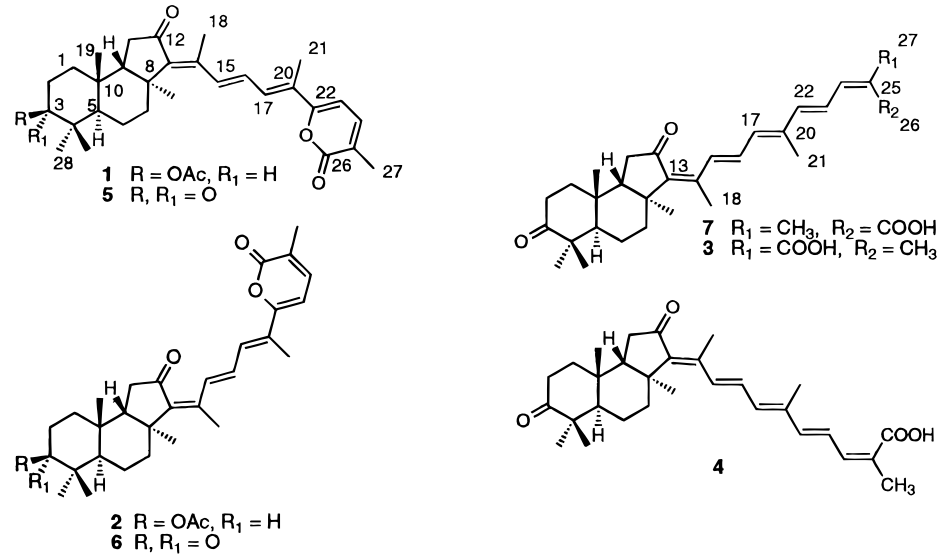


Figure 1. Comparison of the GI_{50} mean bar graphs for stellettins A−F (**1**−**6**). The center line of each graph is the arithmetically calculated mean for all cell lines tested. The log scale at the bottom of each graph indicates how each individual cell line compares to the mean. Those cell lines that fall to the right of the mean are more sensitive to the tested compound, while those that fall to the left of the mean are more resistant to the test agent. The cell line panels tested are identified by abbreviations on the left hand side of the graph as follows: LEU, leukemia; NSC, non-small cell lung; CNS, tumors of the central nervous system; MEL, melanomas; OVA, ovarian; REN, renal; BRE, breast.

Chart 1



The configuration of stellettin E is 13(*Z*),24(*E*), of stellettin F, 13(*E*), 24(*Z*), and of stellettin G, 13(*Z*), 24-(*Z*).

Exposure of the pure compounds to light resulted in rapid isomerization to their geometrical isomers. Consequently, the compounds were tested as isomeric pairs. The GI_{50} mean bar graphs[11] from testing of the three stellettin pairs in the NCI 60-cell screen are shown in

Chart 2

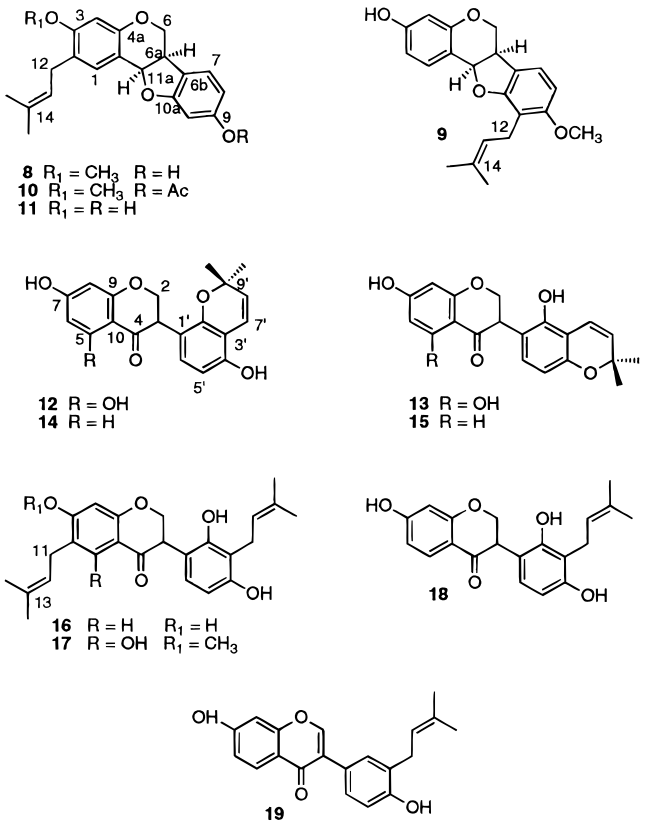


Figure 1. The stellettin C/D pair was the most potent, with a mean panel GI_{50}[11] of 0.09 μM. The stellettin E/F pair was nearly 10 times less potent (mean GI_{50} of 0.98 μM). The mean graph differential cytotoxicity profiles of the three stellettin pairs were virtually indistinguishable, as confirmed by COMPARE analyses,[11] but they did not show high COMPARE correlations (e.g., $\geq$0.8) to any profiles in the NCI standard agent database.[11]

B. HIV-Inhibitory Pterocarpans and Isoflavanoid Metabolites from the Genus *Erythrina*. Plants of the genus *Erythrina* (Leguminosae) are known to produce flavanoids,[12−14] isoflavanoids,[15−17] and pterocarpans.[18−22] An organic bark extract from a Guatemalan *Erythrina glauca* Willd. was selected for bioassay-guided fractionation to determine if the observed anti-HIV activity was attributable to new compound class(es) and/or members of the aforementioned class(es). Fractionation proceeded through solvent−solvent partitioning and Sephadex LH-20 size-exclusion chromatography, followed by reversed-phase HPLC to yield pterocarpans **8** and **9** as the active compounds (Chart 2).

A molecular formula of $C_{21}H_{22}O_4$ for **8** was provided by HREIMS (M^+, *m/z* 338.1502, calcd 338.1518). The ^{13}C-NMR spectrum confirmed the presence of 21 carbons, while a DEPT experiment accounted for 21 of the 22 protons (Table 1). A broad IR absorption band at 3388 cm^{-1} suggested the presence of a hydroxyl group; this was confirmed by acetylation, which yielded the monoacetate, **10**. Both the ^{1}H- and ^{13}C-NMR spectra of **8** closely resembled those of compounds having a pterocarpan skeleton.[23] NMR resonances indicative of a methoxyl and a γ,γ-dimethylallyl substituent were evident, while aromatic proton signals revealed the presence of a three-proton spin system (δ 7.05 d, J = 8.1 Hz; 6.31 dd, J = 8.1, 2.2 Hz; 6.24 d, J = 2.2 Hz) and two singlet aromatic protons (δ 7.14 s; 6.42 s). An ABMX spin system (δ 5.38 d, J = 6.5 Hz; 4.18 dd, J = 9.5, 3.7 Hz; 3.47 t, J = 9.5 Hz; 3.44 ddd, J = 9.5, 6.5, 3.7 Hz) was assigned to the fused heterocyclic ring protons in the pterocarpan skeleton. A *cis* orientation for H-6a and H-11a was supported by a 6.5 Hz vicinal coupling and NOE interactions observed between these two protons. HMQC and HMBC experiments allowed the complete assignment of the ^{1}H- and ^{13}C-NMR spectra of **8**. Correlations from the C-12 protons to C-1, C-2, and C-3 placed the isopentenyl substituent at C-2, while the position of the methoxyl group was defined by a correlation between the resonance of the OCH_3 protons and C-3. A closely related compound, calopocarpin (**11**), which lacks the C-3 methoxyl substituent, has been reported from a *Calopogonium* species.[24] Thus, we were able to assign the structure of compound **8** as 3-*O*-methylcalopocarpin. Although the absolute stereochemistry of **8** was not determined, its large negative optical rotation ($[\alpha]_D$ −235°) is consistent with other related pterocarpans that have been assigned 6a-(*R*) and 11a(*R*) stereochemistry.[21]

Table 1. NMR Data for 3-*O*-Methylcalocarpin (**8**)[a]

position	δ_C (mult)	δ_H [no., mult, *J* (Hz)]	HMBC correlations
1	132.1 (d)	7.14 (1 H, s)	C-3, C-4a, C-11a, C-12
1a	112.9 (s)		
2	124.8 (s)		
3	159.9 (s)		
4	100.0 (d)	6.42 (1 H, s)	C-1a, C-2, C-3, C-4a
4a	156.2 (s)		
6	67.7 (t)	4.18 (1 H, dd, 9.5, 3.7) 3.47 (1 H, t, 9.5)	C-4a, C-6a, C-6b, C-11a
6a	40.9 (d)	3.44 (1 H, ddd, 9.7, 6.5, 3.7)	C-6, C-6b, C-10a
6b	119.5 (s)		
7	126.0 (d)	7.05 (1 H, d, 8.1)	C-6a, C-9, C-10a
8	108.6 (d)	6.31 (1 H, dd, 8.1, 2.2)	C-6b, C-7[b], C-9[b], C-10
9	159.8 (s)		
10	98.8 (d)	6.24 (1 H, d, 2.2)	C-6b, C-8, C-9, C-10a
10a	161.9 (s)		
11a	79.9 (d)	5.38 (1 H, d, 6.5)	C-1, C-1a, C-4a, C-6
12	28.7 (t)	3.23 (2 H, d, 7.5)	C-1, C-2, C-3, C-13, C-14
13	123.9 (d)	5.26 (1 H, tq, 7.5, 1.4)	C-2, C-12, C-15, C-16
14	133.0 (s)		
15	26.0 (q)	1.74 (3 H, br s)	C-13, C-14, C-15
16	17.8 (q)	1.71 (3 H, br s)	C-13, C-14, C-15
OCH_3	55.9 (q)	3.77 (3 H, s)	C-3

[a] Spectra were acquired in CD_3OD and referenced to the residual solvent signal. [b] Correlation observed with the acetate derivative **10**.

Compounds **8** and **9** inhibited the cytopathic effects of *in vitro* HIV-1 infection in a human T-lymphoblastoid cell line (CEM-SS).[25] 3-*O*-Methylcalopocarpin (**8**) was cytoprotective over a modest concentration range (EC_{50} = 0.2 μg/mL; IC_{50} 3.0 μg/mL) with a maximum of 80−95% protection. Sandwicensin (**9**) was less effective (EC_{50} = 2 μg/mL; IC_{50} 7 μg/mL), with a maximum protection of only 50−60%.

A second organic extract, from the roots of *Erythrina lysistemon* Hutch. (Leguminosae), collected in Tanzania, was also found to be active in the NCI's primary anti-HIV screen. Solvent−solvent partitioning of a portion of the extract concentrated the activity in methyl *tert*-butyl ether (MeOtBU) and hexane fractions. A combi-

nation of size-exclusion chromatography (Sephadex LH-20), vacuum−liquid chromatography (VLC, Si gel), and finally, reversed-phase C_4 HPLC led to the isolation of six isoflavanoids.

The first two compounds (**12** and **13**) were identified as having an identical molecular formula of $C_{20}H_{18}O_6$ by HREIMS and nearly identical spectral characteristics. The ^{13}C-NMR spectrum of **12** contained signals for all 20 carbons in the molecule, including a ketone at δ 199.0, while COSY and HMQC experiments identified five spin systems that accounted for 15 of the 18 protons, suggesting the presence of three exchangeable protons. The five spin systems identified for **12** included a pair of *meta*-coupled aromatic protons (δ 5.88 d, J = 2.0 Hz; 5.86 d, J = 2.0 Hz), an oxygenated methylene (^{13}C δ 71.4; ^{1}H δ 4.50 dd, J = 11.2, 10.7 Hz; 4.34 dd, J = 10.7, 5.9 Hz) coupled to a methine (δ 4.08 dd, J = 11.2, 5.9 Hz), two olefins with *cis*-coupling (δ 6.79 d, 6.31 d, J = 8.3 Hz; and δ 6.62 d, 5.55 d, J = 10.0 Hz), and a *gem*-dimethyl group (δ 1.31 s, 1.30 s).

HMBC experiments optimized for 8.3 and 5.5 Hz provided correlations that identified compounds **12** and **13** as prenylated isoflavanones. The two structures differed only at the point of cyclization of the 3′-prenyl group, but 2′ vs 4′ cyclization could not be determined from the observed data. However, both compounds had been reported in the literature. Comparison of the published NMR chemical shifts with our compounds allowed the assignment of **12** as glyasperin F[26] (2′ cyclization), originally isolated from *Glycyrrhiza aspera*, and **13** as licoisoflavanone[27] (4′ cyclization), which was isolated as its triacetate derivative from *Glycyrrhiza* sp.

A pair of new compounds (**14** and **15**) was identified as having the molecular formula of $C_{20}H_{18}O_5$. The ^{13}C-NMR spectrum of **14** featured a ketone carbonyl at δ 194.4, four sp^2 carbons bearing oxygen (δ 166.3, 165.8, 154.2, and 152.8), and 10 additional sp^2 carbons between 131.5 and 103.7 ppm, which accounted for eight of the required 12 double bond equivalents and suggested the presence of four rings. HMQC and COSY experiments allowed the construction of five identical spin systems for both compounds. Indeed, four of the five spin systems were the same as those identified for compounds **12** and **13**. The difference was the replacement of the *meta*-coupled aromatic spin system in **12** and **13** with a three-proton aromatic system having a coupling pattern consistent with a 1,2,4-trisubstituted ring (δ 7.75 d, J = 8.5 Hz; 6.49 dd, J = 8.5, 2.2 Hz; 6.34 d, J = 2.2 Hz) in **14** and **15**. HMBC correlation experiments indicated that compounds **14** and **15** were the 5-deoxy derivatives of compounds **12** and **13**. As before, NMR experiments did not enable a distinction of the 2′ vs 4′ cyclization of the 3′-prenyl group; however, the structures were conclusively identified as 5-deoxyglyasperin F (**14**) and 5-deoxylicoisoflavanone (**15**) by direct comparison of their ^{1}H- and ^{13}C-NMR chemical shifts to those of **12** and **13**.

A fifth compound (**16**) isolated from this extract had a molecular formula of $C_{25}H_{28}O_5$. The ^{13}C-NMR spectrum of **16** contained signals for all 25 carbons, including signals for a ketone carbonyl δ 195.2, four oxygenated sp^2 carbons (δ 165.0, 164.4, 156.7, and 155.0), and 12 additional sp^2 carbons between 133.5 and 102.9 ppm. These carbon resonances accounted for nine of the 12 required double-bond equivalents, indicating the presence of only three rings. Analysis of COSY and HMQC experiments indicated the presence of two isopentenyl groups ((a) δ 3.21 2H, d, J = 7.7 Hz; 5.29 1H, tq, J = 7.7, 1.3 Hz; 1.69 3H, s; 1.74 3H, s; and (b) δ 3.29 2H, d, J = 7.6 Hz; 5.18 1H, tq, J = 7.6, 1.3 Hz; 1.64 3H, s; δ 1.75 3H, s), one pair of *ortho*-coupled aromatic protons (δ 6.82 1H, d, J = 8.2 Hz; 6.31 1H, d, J = 8.2 Hz), two additional singlet aromatic protons (δ 7.57, 6.31), and a characteristic oxygenated methylene (δ 4.62 1H, dd, J = 11.4, 8.2 Hz; 4.50 1H, dd, J = 11.4, 4.9 Hz) coupled to a methine proton (δ 4.03 1H, dd, J = 8.2, 4.9 Hz) of the isoflavanone system. The identified spin systems suggested the presence of an isoflavanone with two isopentenyl groups that, in this case, were not cyclized. Observed HMBC correlations between C-4 (δ 129.0) and the allylic methylene at δ 3.21 (H-11), which was also correlated to the carbon at 165.0 ppm (C-7), placed one isopentenyl group at C-6 (δ 125.1). This carbon (C-6) was also correlated to the methylene protons at C-11. The remaining isopentenyl group was placed at position C-3′ on the basis of correlations between its methylene group at δ 3.29 (H-7′) and carbons at δ 155.1 (C-2′), 117.9 (C-3′), and 156.7 (C-4′) to give the gross structure of compound **16**. This novel compound is related to licoricidin (**17**), previously isolated from *Glycyrrhiza uralensis*.[28,29]

The final compound isolated from *E. lysistemon* had a molecular formula of $C_{20}H_{20}O_5$ by HREIMS. Compared to the ^{1}H-NMR spectrum of **16**, the analogous data of this compound indicated the presence of only one of the characteristic olefinic protons (ca. δ 5.20 tq) seen in **16** and suggested the loss of one of the isopentenyl groups in **18**. This was supported by a difference of C_5H_8 in the molecular formula of **18**. The reappearance of the three aromatic proton coupling pattern (δ 7.78 1H, d, J = Hz; 6.51 1H, dd, J = Hz; 6.35 1H, d, J = Hz) seen in 5-deoxyglyasperin F (**14**) and 5-deoxylicoisoflavanone (**15**) suggested that the isopentenyl moiety at C-6 in **16** was not present in **18**. A conventional battery of NMR experiments, including COSY, HMQC, and HMBC, was performed to confirm the gross structure of compound **18** as shown. Compound **18** differs from the known compound neobavaisoflavone (**19**)[30] by the presence of an additional hydroxyl group at position C-2′ and reduction of the Δ^2 olefin; thus, we have named the new compound 2,3-dihydro-2′-hydroxyneobavaisoflavanone (**18**).

All six of the isolated isoflavanoids were tested for anti-HIV activity in the NCI's XTT-based primary screen.[31] Only two of the six compounds, 5-deoxyglyasperin F (**14**) and 2′-hydroxyneobavaisoflavanone (**18**), were modestly active (EC_{50} 11.5 and 7.6 μg/mL for **14** and **18**, respectively). A comparison of the planar structures of the compounds tested suggests that both a free 4′-hydroxyl group and a lack of substituents at positions C-5 and C-6 are necessary for even minimal *in vitro* anti-HIV activity.

C. Enniatins and the Hollow-Fiber *in Vivo* Assay. Further work has focused upon elucidation of anti-HIV constituents from culturable microorganisms. Anti-HIV bioassay-guided fractionation of an organic extract of *Alternaria kikuchiana* Tanaka (ATCC-11570) led to the isolation of a series of enniatins. The same type of compound was subsequently isolated from several species of *Fusarium*. Enniatins are well-known

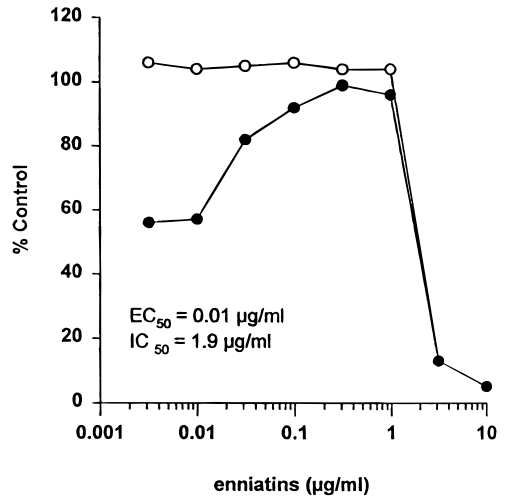
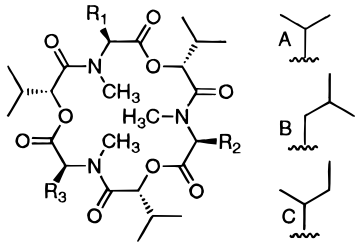


Figure 2. Effects of a range of concentrations of a mixture of enniatins B (**20**), B_1 (**22**), and A_1 (**23**) upon uninfected CEM-SS cells (○) and upon infected CEM-SS cells (●), as determined after 6 days in culture as assessed by the XTT assay.

fungal metabolites first isolated in 1947 by Gaumann *et al.* from *Fusarium orthoceras* var. *enniatinium*.[32,33] These compounds are cyclic hexadepsipeptides, which consist of alternating D-α-hydoxyvaleric and *N*-methyl-amino acids (*N*-methylvaline, *N*-methylleucine, or *N*-methylisoleucine). For example, enniatin B (**20**) contains three *N*-methylvalines, while enniatin C (**21**) contains three units of *N*-methylleucine. Enniatins were previously known to have antimycobacterial activity; however, anti-HIV properties had not been reported. The enniatins act as ionophores by forming dimeric "sandwich" structures that transport monovalent ions across membranes, particularly mitochondrial membranes, and uncouple oxidative phosphorylation.[34,35]



20 R_1,R_2,R_3 = A
21 R_1,R_2,R_3 = B
22 R_1,R_2 = A, R_3 = C
23 R_1 = A, R_2,R_3 = C

We have isolated three enniatins: enniatins B (**20**), B_1 (**22**), and A_1 (**23**). The anti-HIV activity of enniatin B was essentially indistinguishable from that of a purified mixture of the three isolated enniatins. The *in vitro* anti-HIV screening data from the mixture of enniatins B (**20**), B_1 (**22**), and A_1 (**23**) is shown in Figure 2. The compounds protected human lymphoblastoid cells from HIV-1 induced cell killing with an *in vitro* "therapeutic index" of approximately 200.

Because of their ionophoric nature, we felt that further biological activity information would be essential to determine the priority, if any, of further pursuit of the enniatins as an anti-HIV lead. The similarity of the activity of the pure compounds and the enniatin mixture suggested we could obtain the desired biological evaluation using the mixture of enniatins. Extensive searching of the scientific literature indicated that no *in vivo* studies on the enniatins had been published. Therefore, we subjected the enniatin mixture to *in vivo* anti-HIV activity screening in the "hollow-fiber" assay model recently developed by Hollingshead and co-workers.[36] This assay provides a relatively low-cost and high-throughput screen for the preliminary evaluation of the *in vivo* efficacy of potential anti-HIV compounds.[36]

The hollow-fiber assay uses polyvinylidene fibers filled with HIV-infected or uninfected control human cells, with a 500 kDa molecular weight cutoff, cut into 2 cm lengths and inserted into SCID (severe combined immune deficient) mice for the 6-day assay period. Use of the fibers in this model, unlike the other small animal HIV models,[37] allows for the recovery of the cells and the use of a variety of quantitative assay endpoints. The fibers can be filled with either acutely or chronically infected human cells or with uninfected human cells as toxicity controls. Six fibers are placed in each mouse, three intraperitoneally and three subcutaneously. The test compounds are routinely injected intraperitoneally at 8-h intervals for a total of 6 days. Endpoint measurements may include the following: (1) MTT-tetrazolium determination of cytoprotection and/or cellular viability; (2) RT (reverse transcriptase) or p24 (core protein) levels in serum and/or intraperitoneal cavity wash; (3) histopathological evaluations of fiber cross-sections; or (4) visual observations of recovered cells.[36]

The top three doses of the enniatin mixture (40, 20, 10 mg/kg) tested in the hollow-fiber assay were toxic to all mice in the test groups. With the top dose, most deaths occurred between days 2 and 3, while for the 20 and 10 mg/kg dose groups the deaths occurred between days 4 and 5. For all surviving groups, there was a dose-dependent weight loss. These toxic effects indicated that a maximally tolerated dose for the enniatins was achieved within the tested dose range. However, no significant reduction of p24 was found in the serum of any dose group when compared to controls. In addition, only one of three mice of the 5 mg/kg dose group had measurable suppression of intraperitoneal p24 levels.

In some respects, these *in vivo* assay results were disappointing in that there was no significant *in vivo* activity. The studies nonetheless illustrated the potential utility of the hollow-fiber assay to provide rapid, cost effective, *in vivo* information to aid in the timely assignment of an appropriate priority (or lack thereof) for further pursuit of new potential anti-HIV leads. In the present instance, the hollow-fiber assay results discouraged further pursuit of the enniatins.

Experimental Section

General Experimental Procedures. Size-exclusion chromatography was carried out using Sephadex LH-20 (Pharmacia). HPLC was performed on either a Rainin or Waters system utilizing Rainin Dynamax columns. Mass spectra were measured on VG Micromass ZAB or Finnigan MAT 90 spectrometers. IR spectra were obtained using a Perkin-Elmer 1600 FTIR spectrometer. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. NMR spectra were recorded on a Varian VXR 500S spectrometer. ^1H and ^{13}C chemical shifts are reported in ppm relative to the residual undeuterated solvent. The bioassay utilized for HIV leads is the XTT-based *in vitro* assay developed at

the National Cancer Institute and used as the primary screen, details of which have been previously reported.[25]

Isomalabaricanes from the Sponge *Stelletta* species. The experimental details of this work are described elsewhere.[9]

Pterocarpans and Isoflavanoids from *Erythrina* sp. A. Pterocarpans from *E. glauca*. Plant Material. The stem bark of an *E. glauca* Willd. (Leguminosae) was collected in 1988 from a secondary forest in the Puerto Barrios region of Guatemala under contract by New York Botanical Gardens (Q65V-903), where a voucher specimen from this collection is maintained. The plant was identified by J. J. Castillo. A second voucher specimen has been deposited at the Smithsonian Institution.

Extraction and Isolation. The air-dried plant was ground to a coarse powder (417 g) and sequentially extracted with MeOH−CH_2Cl_2 (1:1) and MeOH. The combined organic extracts were evaporated under reduced pressure to give 11.1 g of a dark brown gum. A 10.6 g portion of the extract was separated in a four-step solvent−solvent partitioning protocol[38] that concentrated the anti-HIV activity in the hexane-soluble (1.62 g) and CCl_4 (2.05 g) fractions. Size exclusion of the combined active material on Sephadex LH-20 with hexane−CH_2Cl_2−MeOH (2:5:1) provided three late-eluting active fractions. The first of these fractions (300 mg) was virtually pure 3-*O*-methylcalopocarpin (**8**), the second fraction was a 1:1 mixture of **8** and sandwicensin (**9**), and the third fraction contained primarily **9**. A 20 mg aliquot of the third fraction was separated by C_{18} (Rainin Dynamax 1 × 25 cm) HPLC with MeOH−H_2O (4:1, 4 mL/min, UV at 205 nm) to give 10 mg of pure **9**.

3-*O*-Methylcalopocarpin (8): white amorphous solid; $[\alpha]_D$ −235° (*c* 0.28, $CHCl_3$); UV (MeOH) λ_{max}214 (log ϵ 4.59), 230 sh (4.15), 287 (3.88); IR (film) ν_{max} 3388, 2933, 1621, 1510, 1485, 1465, 1446, 1351, 1265, 1082 cm^{-1}; ^1H- and ^{13}C-NMR data, see Table 1; HREIMS *m/z* 338.1502 (M^+, $C_{21}H_{24}O_4$, calcd 338.1518).

Sandwicensin (9):[22] clear glass; $[\alpha]_D$ −116° (*c* 0.24, $CHCl_3$); ^{13}C-NMR ($CDCl_3$) δ 132.4 (C-1), 113.1 (C-1a or C-10), 109.6 (C-2), 158.5 (C-3 or C-10a), 103.0 (C-4), 156.8 (C-4a or C-9), 66.5 (C-6), 39.9 (C-6a), 119.3 (C-6b), 121.6 (C-7), 103.5 (C-8), 156.6 (C-9 or C-4a), 113.3 (C-10 or C-1a), 158.6 (C-10a or C-3), 77.8 (C-11a), 22.9 (C-12), 122.2 (C-13), 131.6 (C-14), 25.8 (C-15), 17.8 (C-16), 55.9 (OMe).

3-*O*-Methylcalopocarpin Acetate (10). 3-*O*-Methylcalopocarpin (10 mg) was dissolved in pyridine (0.2 mL) and acetic anhydride (0.4 mL). The reaction mixture was stirred for 12 h at 24 °C and then evaporated under a stream of N_2 to give the monoacetate (**10**): ^1H NMR (CD_3OD) δ 7.15 (1H, s, H-1), 6.43 (1H, s, H-4), 4.26 (1H, dd, *J* = 9.5, 3.7 Hz, H-6α) 3.60 (1H, m, H-6β), 3.60 (1H, m, H-6a), 7.26 (1H, d, *J* = 8.1 Hz, H-7), 6.60 (1H, dd, *J* = 8.1, 2.2 Hz, H-8), 6.54 (1H, d, *J* = 2.2 Hz, H-10), 5.52 (1H, d, *J* = 6.5 Hz, H-11a), 3.23 (2H, d, *J* = 7.5 Hz, H-12), 5.27 (1H, br t, *J* = 7.5 Hz, H-13), 1.74 (3H, br s, H-15), 1.71 (3H, br s, H-16), 3.77 (3H, s, OMe), 2.23 (3H, s, OAc); ^{13}C NMR (CD_3OD) δ 17.8, 20.9, 26.0, 28.7, 41.2, 55.6, 67.3, 80.4, 100.1, 105.2, 112.6, 114.9, 123.9, 125.0, 126.0, 126.6, 132.1, 133.1, 153.0, 156.3, 159.7, 171.2; EIMS *m/z* 380 (M^+, appropriate for $C_{23}H_{24}O_5$).

B. Isoflavanoids from *E. lysistemon*. Plant Material. The tree *E. lysistemon* Hutch. was collected (100 g dry wt) under contract by R. Gereau and J. Lovett of the Missouri Botanical Garden in the Iringa region of the Mufundi District of Tanzania in November, 1988. Voucher specimens of the collection (Q65T-194) are deposited at the Smithsonian Institution and the Missouri Botanical Garden. The sample was air-dried prior to shipment to the NCI. Upon arrival the plant specimens were stored at −20 °C until extraction.

Extraction and Isolation. The specimen was ground to a course powder and sequentially extracted with 1:1 MeOH/CH_2Cl_2 followed by MeOH. The organic extracts were combined, and the solvent was removed under reduced pressure to generate the organic extract (4.1 g). A 4 g portion of the organic extract was put through the following solvent−solvent partitioning scheme: The organic extract was dissolved in a minimum volume of 90% MeOH (300 mL) and partitioned with hexane (3 × 300 mL). The MeOH concentration was adjusted with H_2O to 30% and partitioned with MeOtBu−hexane (9:1; 3 × 300 mL). The solvent from the resulting three fractions was removed under reduced pressure. A portion (700 mg) of the active MeOtBu-soluble materials was permeated through Sephadex LH-20 (1:1 MeOH−CH_2Cl_2, 2 × 90 cm). A late-eluting fraction (310 mg) was further separated by flash Si gel chromatography (step gradient, hexane−EtOAc). Fraction C (8:2 hexane−EtOAc, 31.9 mg) was enriched with **15**. Fraction D (7:3 hexane−EtOAc, 21.8 mg) was enriched with compound **16**. Fraction E (6:4 hexane−EtOAc, 41.2 mg) was enriched with **12**−**14**. Purified **14** (9.4 mg, 0.09%) was obtained by precipitation upon standing in the solvent system. The other compounds were purified by repetitive HPLC (Rainin Dynamax C_4 column, 1 × 25 cm, 6:4 MeOH−H_2O, 3 mL/min, UV monitored at 290 nm) to yield **12** (6.1 mg, 0.06%), **13** (6.6 mg, 0.07%), **15** (7.0 mg, 0.07%), **16** (1.9 mg, 0.02%), and **18** (2.1 mg, 0.02%).

Glyasperin F (12): white amorphous powder; $[\alpha]_D$ −4.2° (*c* 1.0, MeOH); UV (MeOH) λ_{max} 226 nm (log ϵ 3.94), 286 (3.86); IR (film) ν_{max} 3672−3015 (br), 2974, 2933, 1642, 1595, 1503, 1477, 1441, 1390, 1364, 1297, 1256, 1159, 1123, 1097, 1051, 1030, 892, 733 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; EIMS *m/z* 354 (M^+, 22), 339 (100), 323 (30), 187 (95), 149 (30), 125 (35); HREIMS *m/z* M^+ 354.1118 ($C_{20}H_{18}O_6$, calcd 354.1133).

Licoisoflavanone (13): pale yellow amorphous powder; $[\alpha]_D$ −2.6° (*c* 0.19, MeOH); UV (MeOH) λ_{max} 224 nm (log ϵ 4.40), 286 (4.13); IR (film) ν_{max} 3610−3015 (br), 2985, 2933, 1636, 1600, 1477, 1446, 1385, 1364, 1287, 1262, 1159, 1118, 1097, 1051 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; EIMS *m/z* 354 (M^+, 35), 339 (100), 213 (30), 187 (55); HREIMS *m/z* M^+ 354.1122 ($C_{20}H_{18}O_6$, calcd 354.1133).

5-Deoxyglyasperin F (14): white amorphous powder; $[\alpha]_D$ −3.8° (*c* 0.4, MeOH); UV (MeOH) λ_{max} 274 nm (log ϵ 3.01), 307 (2.74); IR (film) ν_{max} 3651−3159 (br), 2984, 2933, 1656, 1641, 1610, 1579, 1477, 1451, 1369, 1344, 1282, 1256, 1220, 1210, 1164, 1118, 1067, 1056 cm^{-1}; ^1H NMR (acetone-d_6) δ 7.78 (1H, d, *J* = 8.3 Hz, H-4), 6.80 (1H, d, *J* = 8.3 Hz, H-6′), 6.67 (1H, d, *J* = 9.8 Hz, H-7′), 6.60 (1H, dd, *J* = 8.8, 2.4 Hz, H-5), 6.40 (1H, d, *J* = 2.4 Hz, H-7), 6.37 (1H, d, *J* = 8.3 Hz, H-5′), 5.62 (1H, d, *J* = 9.8 Hz, H-8′), 4.57 (1H, dd, *J* = 11.2, 11.0 Hz, H-2A), 4.43 (1H, dd, *J* = 10.8, 5.4 Hz, H-2B), 4.07

Table 2. ^{1}H NMR Data for Compounds **12**−**16** and **18**[a]

position	**12** (CD_3OD) δ_H [no., mult, *J* (Hz)]	**13** ($CDCl_3$) δ_H [no., mult, *J* (Hz)]	**14** (CD_3OD) δ_H [no., mult, *J* (Hz)]	**15** (CD_3OD) δ_H [no., mult, *J* (Hz)]	**16** (CD_3OD) δ_H [no., mult, *J* (Hz)]	**18** (CD_3OD) δ_H [no., mult, *J* (Hz)]
2A	4.50 (1H, dd, 11.2, 10.7)	4.89 (1H, dd 12.2, 2.3)	4.60 (1H, dd, 11.4, 11.0)	4.62 (1H, dd, 9.8, 11.2)	4.62 (1H, dd, 11.4, 8.2)	4.65 (1H, dd, 11.2, 8.8)
B	4.34 (1H, dd, 10.7, 5.9)	4.77 (1H, dd, 11.7, 4.4)	4.40 (1H, dd, 11.0, 5.7)	4.48 (1H, dd, 11.2, 5.4)	4.50 (1H, dd, 11.4, 4.9)	4.35 (1H, dd, 11.2, 5.4)
3	4.09 (1H, dd, 11.2, 5.9)	3.86 (1H, dd, 4.4, 2.3)	4.00 (1H, dd, 11.4, 5.7)	4.09 (1H, dd, 9.8, 5.4)	4.03 (1H, dd, 8.2, 4.9)	4.08 (1H, dd, 8.8, 5.4)
5			7.75 (1H, d, 8.5)	7.77 (1H, d, 8.8)	7.57 (1H, s)	7.78 (1H, d, 8.8)
6	5.86 (1H, d, 2.0)	5.95 (1H, br d, 2.4)	6.49 (1H, dd, 8.5, 2.2)	6.50 (1H, dd, 8.8, 2.4)		6.51 (1H, dd, 8.8, 2.4)
7						
8	5.88 (1H, d, 2.0)	5.95 (1H, br d, 2.4)	6.34 (1H, d, 2.2)	6.33 (1H, d, 2.4)	6.31 (1H, s)	6.33 (1H, d, 2.4)
9					3.21 (2H, d, 7.6)	
10					5.29 (1H, tq, 7.6, 1.3)	
12					1.69 (3H, br s)	
13					1.74 (3H, br s)	
5′	6.31 (1H, d, 8.3)	6.39 (1H, d, 8.4)	6.28 (1H, d, 8.4)	6.27 (1H, d, 8.3)	6.31 (1H, d, 8.2)	6.33 (1H, d, 8.3)
6′	6.79 (1H, d, 8.3)	7.24 (1H, d, 8.4)	6.75 (1H, d, 8.4)	6.87 (1H, d, 8.3)	6.82 (1H, d, 8.2)	6.80 (1H, d, 8.3)
7′	6.62 (1H, d, 10.0)	6.71 (1H, d, 10.1)	6.61 (1H, d, 9.7)	6.64 (1H, d, 10.1)	3.29 (2H, d, 7.6)	3.31 (2H, br d, 6.8)
8′	5.55 (1H, d, 10.0)	5.59 (1H, d, 10.1)	5.54 (1H, d, 9.7)	5.62 (1H, d, 10.1)	5.18 (1H, tq, 7.6, 1.3)	5.91 (1H, tq, 6.8, 1.5)
10′	1.30 (3H, s)	1.40 (3H, s)	1.30 (3H, s)	1.38 (3H, s)	1.64 (3H, br s)	1.80 (3H, s)
11′	1.31 (3H, s)	1.35 (3H, s)	1.29 (3H, s)	1.38 (3H, s)	1.75 (3H, br s)	1.65 (3H, s)

[a] All spectra referenced to residual solvent signal.

Table 3. ^{13}C NMR Data for Compounds **12**−**16** and **18** in CD_3OD and 13 in $CDCl_3$[a]

position	**12** δ_C (mult)	**13** δ_C (mult)	**14** δ_C (mult)	**15** δ_C (mult)	**16** δ_C (mult)	**18** δ_C (mult)
2	71.4 (t)	69.4 (t)	72.1 (t)	71.9 (t)	72.1 (t)	72.0 (t)
3	48.3 (d)	44.6 (d)	48.4 (d)	48.8 (d)	48.3 (d)	48.2 (d)
4	199.0 (s)	196.9 (s)	194.6 (s)	194.6 (s)	195.2 (s)	195.2 (s)
5	165.7 (s)	165.3 (s)	130.3 (d)	130.3 (d)	129.0 (d)	131.9 (d)
6	97.2 (d)	95.5 (d)	111.7 (d)	111.8 (d)	125.1 (s)	111.7 (d)
7	168.6 (s)	165.5 (s)	165.8 (d)	166.6 (s)	165.0 (s)	155.5 (s)
8	96.1 (d)	97.0 (d)	103.7 (d)	103.6 (d)	108.6 (d)	103.4 (d)
9	165.1 (s)	162.8 (s)	166.3 (s)	165.5 (s)	164.4 (s)	165.6 (s)
10	103.6 (s)	101.4 (s)	115.9 (s)	115.2 (s)	114.4 (s)	114.9 (s)
11					28.4 (t)	
12					123.3 (d)	
13					133.5 (s)	
14					25.9 (q)	
15					18.0 (q)	
1′	114.8 (s)	115.0 (s)	115.4 (s)	117.3 (s)	116.3 (s)	116.0 (s)
2′	152.6 (s)	151.1 (s)	152.8 (s)	152.1 (s)	155.1 (s)	156.7 (s)
3′	110.8 (s)	111.5 (s)	110.9 (s)	112.4 (s)	117.9 (s)	117.8 (s)
4′	154.2 (s)	154.0 (s)	154.2 (s)	154.3 (s)	156.7 (s)	155.0 (s)
5′	108.4 (d)	109.4 (d)	108.2 (d)	109.8 (d)	102.9 (d)	108.5 (d)
6′	131.4 (d)	126.2 (d)	131.4 (d)	130.3 (d)	127.3 (d)	127.3 (d)
7′	118.0 (d)	116.9 (d)	118.1 (d)	118.0 (d)	23.5 (t)	23.4 (t)
8′	129.2 (d)	129.5 (d)	128.3 (d)	130.2 (d)	124.2 (d)	124.1 (d)
9′	77.4 (s)	75.8 (s)	77.4 (s)	76.5 (s)	131.9 (s)	130.5 (s)
10′	27.7 (q)	27.7 (q)	27.7 (q)	27.8 (q)	17.8 (q)	17.9 (q)
11′	28.2 (q)	28.0 (q)	28.2 (q)	27.9 (q)	25.9 (q)	25.8 (q)

[a] All spectra referenced to residual solvent signals (MeOH or $CHCl_3$).

(1H, dd, *J* = 11.2, 5.4 Hz, H-3), 1.33 (3H, s, H-10′), 1.32 (3H, s, H-11′); FABMS (noba) *m*/*z* 339 (MH^+, 100), 323 (35), 152 (50), 135 (75), 119 (95), 85 (90); HREIMS *m*/*z* M^+ 338.1152 ($C_{20}H_{18}O_5$, calcd 354.1158).

5-Deoxylicoisoflavanone (15): white amorphous powder; $[\alpha]_D$ −0.6° (*c* 0.8, MeOH); UV (MeOH) λ_{max} 278 nm (log ϵ 3.81), 309, (3.55); IR (film) ν_{max} 3682−3015 (br), 2974, 2933, 1630, 1600, 1482, 1451, 1384, 1343, 1261, 1215, 1159, 1117, 1056 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 7.75 (1H, d, *J* = 8.8 Hz, H-5), 7.09 (1H, d, *J* = 8.8 Hz, H-6′), 6.71 (1H, d, *J* = 9.8 Hz, H-7′), 6.58 (1H, dd, *J* = 8.8, 2.5 Hz, H-6), 6.42 (1H, d, *J* = 2.5 Hz, H-8), 6.30 (1H, d, *J* = 8.3 Hz, H-5′), 5.66 (1H, d, *J* = 10.3 Hz, H-8′), 4.83 (1H, dd, *J* = 11.7, 5.9 Hz, H-2A), 4.73 (1H, dd, *J* = 11.7, 4.9 Hz, H-2B), 4.04 (1H, dd, *J* = 5.9, 4.9 Hz, H-3), 1.35 (3H, s, H-10′), 1.34 (3H, s, H-11′); ^{13}C NMR (acetone-d_6) δ 192.8 (s, C-4), 166.0 (s, C-7), 164.8 (s, C-9), 154.3 (C-4′), 152.1 (s, C-2′), 130.5 (d, C-6′), 130.0 (d, C-5), 128.6 (d, C-8′), 117.6 (d, C-7′), 116.7 (s, C-1′), 113.9 (s, C-10), 111.8 (d, C-6), 111.5 (s, C-3′), 103.4 (d, C-8), 76.1 (s, C-9′), 71.1 (t, C-2), 46.9 (t, C-3), 28.0 (q, C-10′), 27.8 (q, C-11′); EIMS *m*/*z* 338 (30), 323 (100), 213 (20), 187 (83); HREIMS *m*/*z* M^+ 338.1156 ($C_{20}H_{18}O_5$, calcd 354.1158).

Compound 16: white amorphous powder; $[\alpha]_D$ +5.0° (*c* 0.04, MeOH); UV (MeOH) λ_{max} 279 nm (log ϵ 4.19), 322 (3.95); IR (film) ν_{max} 3672−3015 (br), 2975, 2932, 1641, 1507, 1483, 1385, 1267, 1174, 1154, 1051 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; EIMS *m*/*z* 408 (M^+, 45), 205 (100), 149 (25); HREIMS *m*/*z* M^+ 408.1926 ($C_{25}H_{28}O_5$, calcd 408.1916).

5-Hydroxyneobavaisoflavanone (18): pale yellow amorphous powder; $[\alpha]_D$ +2.6° (*c* 0.4, MeOH); UV (MeOH) λ_{max} 276 nm (log ϵ 4.68), 305 (4.47); IR (film) ν_{max} 3682−3005 (br), 2974, 2923, 1656, 1600, 1579, 1477, 1451, 1385, 1344, 1272, 1246, 1164, 1123, 1036 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; EIMS *m*/*z* 340 (M^+, 80), 204 (45), 148 (40), 137 (100); HREIMS *m*/*z* M^+ 340.1314 ($C_{20}H_{20}O_5$, calcd 340.1317).

C. Isolation of the Enniatins and Evaluation in the *in Vivo* Hollow-Fiber Assay. Culture and Extraction Conditions. The culture (USDA-123091) was obtained from the USDA (Frederick, MD), where an archive strain of the material is maintained. It was grown in SGSM media (40 g of glucose, 10 g of soluble starch, 10 g of bactopeptone, 5 g of $CaCO_3$, 1 mL of Tween 80 in 1 L of distilled H_2O) with agitation (250 rpm) and constant temperature (25 °C) and was harvested after 7 days. The mycelial mat was sequentially extracted with MeOH followed by MeOH−CH_2Cl_2 (1:1). The organic solvents were combined, and the solvent was removed under reduced pressure to obtain the organic extract.

Isolation of a Mixture of Enniatins A_1 (23), B (20), and B_1 (22). A portion (1 g) of the organic extract of *Alternaria kikuchiana* (USDA-123091) was subjected to a solvent−solvent partitioning scheme that concentrated the anti-HIV activity in the MeOtBu soluble

materials (332 mg). This material was then eluted through a Sephadex LH-20 column (2 × 89 cm; 1:1 MeOH−CH_2Cl_2). The active, mid-range eluting material comprised a pure mixture of enniatins (317 mg, 31.7%), which was used in the hollow-fiber assay without further purification.

Hollow-Fiber Assay. The general procedure for the hollow-fiber has been described in detail.[36] Changes to the described procedure include dosing at 8 h rather than 12 h intervals. Doses used in the assay (based on average weight of each group) were 40, 20, 10, 5, 2.5, 1.25 mg/kg. Test groups consisted of three mice each; the control group contained four mice. The body weight of each mouse was monitored daily and reported as total group weight. Dose solutions were prepared in DMSO and injected interperitoneally at 8 h intervals. After 6 days, the anti-HIV activity was evaluated by analyzing p24 levels in the mouse total serum and in an intraperitoneal washout. The endpoint p24 antigen assay was performed using commercially available kits (Coulter Corp, Hialeah, FL) and conducted as described by the manufacturer.

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