JOURNAL OF NATURAL PRODUCTS

© Copyright 2002 by the American Chemical Society and the American Society of Pharmacognosy

Volume 65, Number 8 August 2002

Full Papers

Durhamycin A, a Potent Inhibitor of HIV Tat Transactivation

Hiranthi Jayasuriya,*,† Russell B. Lingham,† Pia Graham,‡ Donette Quamina,† Lucia Herranz,§ Olga Genilloud,§ Magda Gagliardi,† Renee Danzeisen,‡ Joanne E. Tomassini,‡ Deborah L. Zink,† Ziqiang Guan,† and Sheo B. Singh†

Merck Research Laboratories, P.O. Box 2000, Rahway, New Jersey 07065, West Point, Pennsylvania 19486, and Merck Sharp & Dohme de Espana S. A. Josefa Valcarcel 38, 28027 Madrid, Spain

Received December 20, 2001

Tat is a small HIV protein essential for both viral replication and the progression of HIV disease. In our efforts to discover Tat inhibitors from natural product screening of microbial fermentation extracts, we discovered durhamycin A (**1**) as a potent inhibitor (IC_{50} = 4.8 nM) of Tat transactivation. Detailed NMR and MS/MS studies were utilized to elucidate the structure of **1** as a new member of the aureolic acid family of antibiotics. It consists of tetrasaccharide and disaccharide moieties attached to the aglycone, which is hitherto unknown in the aureolic acid family. Three other novel analogues, durhamycin B (**2**), compound (**3**), and the aglycone (**4**), were also discovered or chemically prepared that were less potent than durhamycin A.

HIV-1 Tat is a 14 kDa protein encoded by the HIV-1 genome and is located in the nucleus of HIV-1 infected cells.[1] The major function of Tat in HIV replication is to regulate viral gene expression at the level of transcription. Tat stimulates initiation of HIV transcription and stabilizes the elongation of HIV-1 messenger RNA transcripts.[2−5] Tat protein therefore represents another attractive target, in addition to HIV-1 reverse transcriptase, protease, and integrase, for the development of novel therapeutics for the treatment of HIV infection.

During our effort to identify novel natural product inhibitors of transcriptional activation by the HIV Tat, we discovered durhamycin A (**1**) as a potent inhibitor in both Tat-dependent in vitro transcription and Tat-dependent cell-based assays. The structure of durhamycin A is closely related to the antitumor antibiotic UCH9.[6] It contains a disaccharide [olivosyl-(1→3)-olivose] and a tetrasaccharide [olivosyl-(1→3)-olivosyl-(1→3)-4-acetyl-oliosyl-(1→3)-olivosyl-(1→3)-olivose] connected to the agylcone. The aglycone unit in UCH9 and durhamycin A is similar, and the differences between the two compounds are in the number, arrangement, and substitution pattern of the sugar moieties.

Results and Discussion

Isolation. A liquid fermentation of *Actinoplanes durhamensis* was extracted with methyl ethyl ketone. Gel permeation chromatography on sephadex LH20 followed by reversed-phase HPLC afforded durhamycin A (**1**, 6 mg/L) and minor amounts of durhamycin B (**2**, 0.4 mg/L) as homogeneous yellow powders.

Structure Elucidation. High-resolution FABMS analysis of durhamycin A (**1**) suggested a molecular formula $C_{62}H_{92}O_{28}$. The molecular formula was supported by 62 signals in the ^{13}C NMR spectrum (11 CH_3, 8 CH_2, 32 CH, and 11 quaternary carbons). The high level of oxygenation in the molecular formula hinted at the presence of sugars in the molecule. The six anomeric protons (δ 4.08−5.63) in the ^1H NMR spectrum and the corresponding ^{13}C signals (δ 98.3−101.8) in the ^{13}C NMR spectra revealed the presence of six sugars (Table 1). The $^1H-^1H$ connectivities of the sugars were determined by COSY and TOCSY

* To whom correspondence should be addressed. Tel: (732) 594-4808. Fax: (732)-594-6880. E-mail: hiranthi_jayasuriya@merck.com.
† Rahway.
‡ West Point.
§ Madrid.

10.1021/np010642f CCC: $22.00 © 2002 American Chemical Society and American Society of Pharmacognosy
Published on Web 07/04/2002

Table 1. ^{1}H and ^{13}C NMR Assignment of Durhamycin A (**1**) in C_5D_5N

position	δ ^{13}C	δ ^{1}H, J in Hz	HMBC (J_{CH} = 7 Hz) H → C	NOESY (mix = 0.3 and 0.6 s)
1	204.4			
2	78.3	4.94, d, 12.0	C-3, 4, 1′, S3: C−1	H-1′ (w), S3: H−1
3	42.3	3.42, brd, 11.5	C-2	H-6, 3′
4	28.6	3.28, dd, 16, 13	C-2, 3, 5, 6, 14	H-6, 1′(w)
		3.07, dd, 16, 3.5	C-2, 3, 5, 6, 14	H-6, 1′(w)
5	137.5			
6	117.5	6.57, s	C-4, 5, 7, 8, 12, 14	H-4α, 4*β*, 8
7	139.9			
8	102.5	7.03, s	C-6, 9, 10, 12	H-6, S1: H−1, 3, 5
9	161.3			
10	119.6			
11	157.7			
12	109.3			
13	166.4			
14	109.2			
1′	83.2	5.47, d, 1.5	C-2, 3, 4, 2′, 1′-OMe	H-2, 3, 4α, 4*β*, 3′, 4′, OMe
2′	213.6			
3′	81.2	4.69, d, 3	C-2′, 4′, 5′	H-3, 1′, 4′, 5′
4′	69.5	4.82, dq, 6.5, 2.5	C-3′, 5′	H-1′, 3′, 5′
5′	20.9	1.59, d, 6.5	C-3′, 4′	H-3′, 4′
1′−OMe	59.3	3.70, s	C-1′	H-1′
1”	32.3	3.84[a], m	C-9, 10, 11, 2″, 3″, 4″	H-3″, 4″
2”	28.6	2.25, m		H-1”
		1.90, m		
3”	13.9	0.99, t, 7.5	C-1″, 2″	
4”	19.9	1.56, d, 7.1	C-10, 1″, 2″	H-1”
S1		*β*-olivose		
1	99.1	5.63, dd, 10, 2	C-9, S1: C-1	H-8, S1: H-2e, 3, 5
2	38.1	2.77, dd, 10.5, 5.5	S1: C-1, 3, 4	S1: H-1
		2.31, t, 12	S1: C-1, 3	
3	80.5	4.24, ddd, 11.5, 8.5, 5	S1: C-4, S2: C-1	H-8, S1: H-1, 5, S2: H-1
4	76.0	3.63, t, 9	S1: C-3, 5, 6	
5	73.7	3.99, dq, 8, 6.5	S1: C-1, 3, 4, 6	S1: H-1, 3
6	19.3	1.70, d, 6.8	S1: C-4, 5	
S2		*β*-olivose		
1	99.7	5.05, dd, 10, 2.1	S1: C-3, S2: C-2	S1: H-3, S2: H-3, 5
2	41.4	2.65, ddd, 12.5, 5, 2	S2: C-1, 3, 4	
		2.15, t, 12	S2: C-1, 3	
3	72.3	4.14, ddd, 12, 9, 5.5	S2: C-4	S2: H-1, 5
4	78.6	3.58, t, 9	S2: C-3, 5, 6	
5	73.9	3.74, dd, 9.5, 6.5		S2: H-1, 3
6	19.0	1.59, d, 6.4	S2: C-4, 5	
S3		*β*-olivose		
1	101.8	5.38, dd, 10, 1.5	C-2, S3: C-2	H-2, S3: H-2e, 3, 5
2	38.4	2.95, dd, 10.5, 4.5	S3: C-1, 3, 4	S2: H1
		2.00, t, 10		
3	81.5	4.06, ddd, 13.5, 8.5, 5.5	S3: C-4, S4: C-1	S3: H-1, 5, S4: H-1
4	76.2	3.47, t, 8.7	S3: C-3, 5, 6	
5	73.5	3.65, m	S3: C-1, 3, 4, 6	S3: H-1, 3
6	19.2	1.65, d, 6.2	S3: C-4, 5	
S4		*β*-oliose		
1	99.8	4.85, dd, 10, 1.5	S3: C-3, S4: C-2	S3: H-3, S4: H-3, 5
2	33.8	2.40, ddd, 10.5, 4.5, 1.5	S4: C-1, 3, 4	
		2.24, t, 10	S4: C-1, 3	
3	73.4	4.43, td, 4.5, 12.5	broad signal, no correlations	S4: H-1,4, 5, S5: H-1
4	71.4	5.54, brd, 3.0	S4: C-2, 3, 4−CO	S4: H-3, 5, 6
5	71.0	3.85, brq, 6.5	S4: C-1, 4, 6	S4: H-1, 3, 4
6	17.5	1.29, d, 6.4	S4: C-4, 5	S4: H-4
4-CO_2	171.0			
CH_3	21.4	2.10, s	4-CO	
S5		*β*−olivose		
1	98.3	4.97, dd, 10, 2.0	S4: C-3, S5: C-2, 3	S4: H-3, S5: H-3, 5
2	38.4	2.46, ddd, 12, 4.5, 1.5	S5: C-1, 3, 4	
		1.93, t, 12		
3	80.5	4.05, ddd, 14, 9, 5.5	S5: C-4, S6: C-1	S5: H-1, 5, S6: H-1
4	76.1	3.47, t, 8.7	S5: C-3, 5, 6	
5	73.5	3.65, m	S5: C-1, 3, 4, 6	S5: H-1, 3
6	19.1	1.63, d, 6.2	S5: C-4, 5	

[a] Resolved in the 600 MHz NMR.

Table 1 (Continued)

position	δ ^{13}C	δ ^{1}H, J in Hz	HMBC (J_{CH} = 7 Hz) H → C	NOESY (mix = 0.3 & 0.6 s)
S6		β-olivose		
1	99.4	4.91, dd, 10, 2	S5: C-3, S6: C-2	S5: H-3, S6: H-3, 5
2	41.4	2.53, ddd, 12.5, 5, 1.5	S6: C-1, 3, 4	
		2.08, t, 12		
3	72.3	4.08, ddd, 12, 8.5, 5	S6: C-4	S6: H-1, 5
4	78.6	3.53, t, 8.9	S6: C-3, 6	
5	73.8	3.68, m	S6: C-1, 3, 4, 6	S6: H-1, 3
6	19.0	1.59, d, 6.4	S6: C-4, 5	

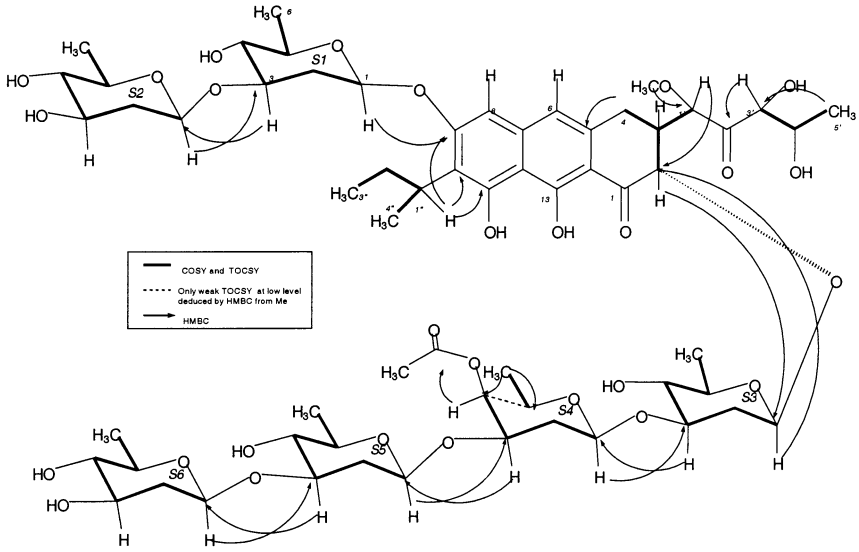


Figure 1. Selected HMBC correlations of durhamycin A (**1**).

experiments, which indicated that they are all 2,6-dideoxy sugars. Five of the six hexoses (S1, S2, S3, S5, and S6) revealed only large couplings (J > 7 Hz) between vicinal protons, indicating them to be β-olivoses. In contrast, the S4 sugar revealed large couplings (J > 7 Hz) between H1 and H2a, and H2a and H3, and small couplings (J = 3 Hz) between H3/H4 and H5, indicating it to be a β-oliose. This was further verified by NOESY correlations of the respective 1,3-diaxial protons (Table 1) H1 and H2a. These experiments also revealed an isobutyl unit, a methine coupled to two oxymethines, a methylene group, and an oxymethine attached to a deshielded oxymethine and a methyl group. HMBC correlations (Figure 1) of the two aromatic singlets at δ 7.03 and 6.57 to carbons at δ 161.3, 119.6, 109.3 and 102.5, 139.9, 109.3, respectively, indicated the positioning of the aromatic protons in a *peri*-relationship on a naphthalene core.

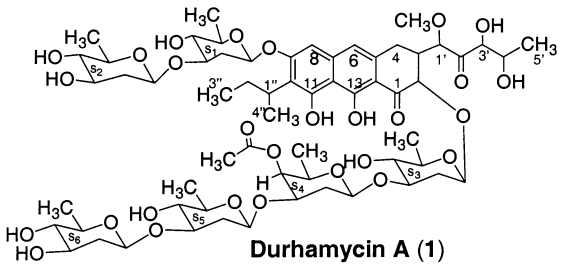
The connectivities obtained from the COSY and TOCSY experiments are depicted in Figure 1. The HMBC correlations obtained from the 4″ CH_3 group (δ 1.56) to the carbon at δ 119.6 (C-10) and from the 1″ methine proton (δ 3.84) to carbons at δ 119.6 (C-10), 157.7 (C-11), and 161.3 (C-9) allowed the placement of the *sec*-butyl group at position 10 of the aglycone. The HMBC correlations from the aromatic proton at δ 6.5 (H-6) to the carbon at δ 28.6 (C-4) and from the methine proton at δ 5.47 (H-1′) to carbons at δ 28.6 (C-4), 42.3 (C-3), and 78.3 (C-2) allowed the extension of the naphthalene ring system and the placement of the ketone-containing side chain at position 3 of the aglycone. Comparison of the structural features assembled by spectroscopic evidence and the chemical shifts of the carbons of the aglycone of compound **1** indicated that it was similar to that of the antitumor antibiotic UCH9.

The proton and the carbon spectral assignments of the sugar moieties were made possible by a combination of COSY, TOCSY, HMQC, and HMBC experiments. The linkages between the sugars were deduced by the HMBC correlation from the anomeric proton of one sugar to the carbon of the neighboring sugar at the position of linkage and vice versa (see Figure 1). This allowed the six sugars to be linked in two groups as disaccharide and tetrasaccharide moieties. The HMBC correlation from H-1 (δ 5.63) of the S1 sugar to the aromatic carbon at δ 161.3 allowed the placement of the disaccharide at C-9 of the aglycone. Similarly the HMBC correlations arising from H-1 of the S3 sugar to a carbon at δ 78.3 allowed the placement of the tetrasaccharide at C-2 of the aglycone. This was further corroborated by the HMBC correlation from H-2 (δ 4.94) of the aglycone to the C-1 (δ 101.8) of sugar S3.

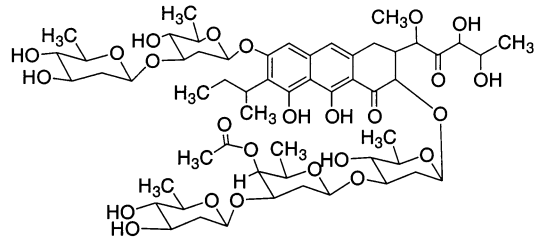
MS/MS studies further supported the proposed structure. The ESI mass spectrum of durhamycin A (**1**) showed its sodiated molecule at m/z 1307 $(M + Na)^+$. MS/MS of m/z 1307 $(M + Na)^+$ produced a product ion at m/z 1047 due to the loss of a disaccharide unit (260 Da) as a result of cleavage of the glycosidic bond at C-9. The presence of another product ion at m/z 585 arising from cleavage at C-2 indicated that the tetraglycosidic unit contains an acetyl group. In further MS/MS experiments, the m/z 585 ion sequentially lost two olivose units from both ends of the chain, indicating that the acetyl group is located at one of the inner sugars of the tetrasaccharide unit. The exact positioning of the acetyl group was confirmed at C-4 of S4 by the observed HMBC correlation from the H-4 (δ 5.54) of S4 to the carbonyl (δ 171.0) of the acetyl group. Absolute stereochemistry of the aglycone of UCH9 has not been determined. The strong J coupling (12 Hz) between H2 and H3 indicated a trans relationship for the relative stereochemistry.

The structure of durhamycin B (**2**) was elucidated by a careful comparison of the ^{1}H NMR spectrum with that of

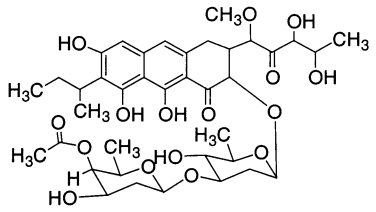
1 and MS/MS data. Durhamycin B produced a sodiated molecule at *m/z* 1177 $(M + Na)^+$. MS/MS of *m/z* 1177 $(M + Na)^+$ produced two product ions at *m/z* 917 (Na^+) and *m/z* 455 (Na^+). The fragment ion at *m/z* 917 (Na^+) indicated the loss of the disaccharide unit at C-9 of the aglycone. The product ion at *m/z* 455 (Na^+) in the mass spectrum confirmed the presence of an intact trisaccharide moiety consisting of one acetylated sugar. Hence durhamycin B (**2**) was elucidated to be a pentaglycoside with di- and trisaccharides side chains respectively at C-9 and C-2 of the aglycone.



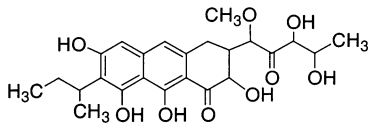
Durhamycin A (1)



Durhamycin B (2)



(3)



(4)

Acid Hydrolysis of Durhamycin A. Acid hydrolysis of durhamycin A in aqueous acetic acid produced compound **3** and the aglycone (**4**). Compound **3** produced a molecular ion at *m/z* 765 $(M + H)^+$, which indicated the cleavage of the disaccharide moiety at C-9 and the two outer olivoses in the tetrasacchride. This confirmed that **3** contained a disaccharide moiety at C-2 of the aglycone. The Proton NMR spectrum of **3** also supported the proposed structure. Compound **4** produced a molecular ion at 463 $(M + H)^+$ indicative of the aglycone portion of the durhamycins, and the proton NMR spectrum further supported the structure.

Biological Activity. All compounds were evaluated for their effect against Tat transactivation using a Jurkat-derived stable cell line (63A9), harboring a defective, integrated HIV provirus which is dependent upon the addition of exogenous Tat for activation. In parallel, cytotoxicity was measured using a quantitative MTS cell proliferation assay. Durhamycin A was the most potent compound of the series and inhibited Tat transactivation with an IC_{50} value of 4.8 nM in the absence of overt cytotoxicity at 25 μM in the MTS assay. It was found to be potent in Vertical, a single-cycle assay for HIV with an IC_{50} value of 11.6 nM with no cytotoxicity up to 5 μM. Durhamycin B (**2**) (IC_{50} = 48 nM) was less potent and the aglycone **4** was completely inactive in the Tat transactivation assay. Compound **3** was not tested due to its limited quantity.

In this paper we have described the isolation, structure elucidation, and the biological activity of durhamycins. Durhamycins closely resemble chromomycins,[7] oliviomycins,[8] and UCH9,[6] which are reported as being members of a distinct group of aureolic acid antibiotics. Durhamycin A differs from the others in the number and the substitution pattern of the sugar moieties. The aglycone of durhamycins and UCH9 differ from the others at the level of substitution at C-10; both contain a *sec*-butyl group, while chromomycin has a methyl substitution and oliviomycin is unsubstituted at C-10.

Experimental Section

General Experimental Procedures. All reagents were obtained from Sigma-Aldrich and were used without further purification. All solvent extracts were dried on anhydrous Na_2SO_4. NMR spectra were recorded on Varian Inova 400, 500, or 600 MHz instruments operating at 400, 500, and 600 MHz for ^1H and 100, 125, and 150 MHz for ^{13}C nuclei. A Hewlett-Packard HP1100 was used for analytical HPLC. LC-MS was performed on a Thermo Quest LCQ instrument using electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI). Mass spectra were recorded on JEOL SX-102A (electron impact, EI, 90 eV). High-resolution mass spectral analyses were performed on either a Thermo Quest FTMS using electrospray ionization or a JEOL SX-102A using a FAB probe with perfluorokerosenes (PFK) as internal standards.

Microorganism. The strain was isolated from a sawdust sample collected in a sawmill in Alajuela, Costa Rica. The strain was obtained by enrichment with the γ-collidine chemoattraction method[9] after plating on humic acid-based isolation agar.[10]

Identification. The strain produces vegetative sporangia directly borne from the substrate mycelium characteristic of the genus *Actinoplanes* when cultivated on mineral salts sporulation media. The strain has been confirmed to belong to the genus *Actinoplanes*. The phylogenetic analysis based on ribosomal DNA sequences shows that the strain appears closely associated to other strains of *Actinoplanes durhamensis*. This close relationship is highly supported by the bootstrapping value (92%) and suggests that the strain must represent another strain of the same species.

Cultivation Conditions. A seed culture was produced by inoculating 50 mL of seed medium[11] in a 250 mL triple baffled Erlenmeyer flask with vegetative mycelia. Flasks were incubated at 28 °C and shaken at 220 rpm for 7 days in order to obtain sufficient biomass to inoculate the production medium. The production medium was inoculated by aseptic transfer of 2 mL of seed culture to 44 mL of production medium in a 250 mL nonbaffled Erlenmeyer flask. The production flasks were incubated at 28 °C and shaken at 220 rpm for 13 days prior to harvest.

Extraction and Isolation. A bioassay-guided isolation procedure was followed to obtain the active component. The liquid fermentation broth (2 L) was extracted with methyl ethyl ketone (MEK) and was concentrated to dryness to produce a 500 mg residue. It was purified on a Sephadex LH20 column (1.5 L) eluting with methanol to produce a single

bioactive cut weighing 300 mg and eluting at 0.5 column volume. Fifty milligrams of this fraction was further purified by preparative HPLC [Zorbax RX C8 (21.2 mm i.d. × 25 cm), CH_3CN/water 40:60 containing 0.1% TFA at a flow rate of 8 mL/min] to produce durhamycin A (12 mg, t_R = 65 min) and durhamycin B (0.8 mg, t_R = 52 min).

Durhamycin A (1): yellow powder, $[\alpha]^{23}_D$ −41.9° (*c*, 0.58, methanol): UV (MeOH) λ_{max} (log ∈) 204 (4.0), 230 (4.3), 270 (4.4), 318 (3.7), 412 (3.8) nm; IR (neat) ν_{max} 3394, 2935, 1676, 1626, 1371, 1249, 1134, 1063 cm^{-1}; ^1H and ^{13}C NMR in Table 1; ESIMS *m/z* 1307 $(M + Na)^+$; ESIMS *m/z* 1155, 1025, 895, 765, 723, 593, 463, 401; HRFABMS *m/z* 1284.6129 (calcd for $C_{63}H_{96}O_{27}$, 1284.6139).

Durhamycin B (2): yellow powder, UV (MeOH) λ_{max} (log ∈) 204 (4.5), 230 (4.5), 278 (4.5), 434 (3.9) nm; IR (neat) ν_{max} 3394, 2934, 1681, 1591, 1371, 1206, 1134, 1063 cm^{-1}; ^1H NMR (500 MHz, C_5D_5N) δ 0.98 (3H, t, *J* = 7.5 Hz, H-3″), 1.56 (3H, d, *J* = 7.2 Hz, H-4″), 1.59 (3H, d, *J* = 6.5 Hz, H-5′), 2.26, 1.90 (2H, m, H-2″), 3.09, 3.28 (2H, m, H-4), 3.47 (1H, brd, *J* = 11.5 Hz, H-3), 3.70 (3H, s, 1′-OCH_3), 3.84 (1H, m, 1″), 4.69 (1H, d, *J* = 3.0 Hz, H-3′), 4.83 (1H, dq, *J* = 6.5, 2.5 Hz, H-4′), 4.95 (1H, d, *J* = 12.0 Hz, H-2), 5.48 (1H, brs, H-1′), 6.60 (1H, s, H-6), 7.05 (1H, s, H-8), sugar signals 1.30 (3H, d, *J* = 6.5 Hz), 1.59 (3H, d, *J* = 6.5 Hz), 1.62 (3H, d, *J* = 6.5 Hz), 1.68 (3H, d, *J* = 6.5 Hz), 1.70 (3H, d, *J* = 6.5 Hz), 1.98 (1H, t, *J* = 12.0 Hz), 2.01 (1H, t, *J* = 10.0 Hz), 2.08 (3H, s), 2.12 (1H, t, *J* = 12.0 Hz), 2.16 (1H, t, *J* = 12.0 Hz), 2.2 (1H, t, *J* = 10.0 Hz), 2.31 (1H, t, *J* = 12.0 Hz), 2.36 (d), 2.40 (1H, dd, *J* = 10.5, 4.5 Hz), 2.54 (1H, dd, *J* = 12.0, 5.0 Hz), 2.65 (1H, dd, *J* = 12.5, 5.0 Hz), 2.78 (1H, dd, *J* = 10.5, 5.5 Hz), 2.98 (1H, dd, *J* = 10.5, 4.5 Hz), 3.32 (d), 3.47 (2H, brt), 3.57 (1H, m), 3.63 (2H, m), 3.74 (1H, dd, *J* = 9.5, 6.5 Hz), 3.85 (1H, brq, *J* = 6.5 Hz), 4.01 (1H, m), 4.05 (1H, m), 4.12 (2H, m), 4.25 (1H, m), 4.42 (1H, td, *J* = 4.5, 12.5 Hz), 4.90 (1H, dd, *J* = 10.0, 1.5 Hz), 5.0 (1H, dd, *J* = 10.0, 2.0 Hz), 5.1 (dd). 5.37 (1H, brd, *J* = 10.0 Hz), 5.57 (1H, d, *J* = 3.0 Hz), 5.64 (1H, brd, *J* = 10.0 Hz); ESIMS *m/z* 1177 $[M + Na]^+$.

Hydrolysis of Durhamycin A (1). A solution of durhamycin A (10 mg) in 50% aqueous acetic acid (0.5 mL) was heated at 60 °C. After complete disappearance of the starting material (4 h) the reaction was diluted with water and extracted with EtOAc. Ethyl acetate was evaporated under reduced pressure, and the product was purified by preparative HPLC (Zorbax RX C8 21.2 X 250 mm, 45:55 acetonitrile/water containing 0.5% TFA acid) to produce glycoside **3** (0.3 mg) and the aglycone **4** (1.0 mg).

Compound (3): yellow powder, UV (MeOH) λ_{max} (log ∈) 206 (4.0), 224 (4.0), 284 (4.0), 402 (3.3), 488 (2.9) nm; IR (neat) ν_{max} 3420, 2932, 1687, 1597, 1369, 1206, 1138, 1064 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 0.86 (3H, t, *J* = 7.5 Hz), 1.12 (2H, d, *J* = 6.0 Hz), 1.24 (3H, d, *J* = 6.5 Hz), 1.28 (3H, d, *J* = 6.5 Hz), 1.29 (3H, brs,), 1.37 (3H−d, *J* = 7.0 Hz), 1.44 (1H, m), 1.52 (1H, m), 1.75 (1-H, m), 1.95 (1H,m), 2.0 (1H, m), 2.0 (3H, s), 2.52 (1H, dd, *J* = 16.5, 2.5 Hz), 2.63 (1H, brt), 2.75 (1H, t, *J* = 9.5 Hz), 2.99 (2H, m), 3.22 (1H, m), 3.3 (1H, m), 3.43 (3H, s), 3.75 (1H, m), 4.16 (1H, d, *J* = 3 Hz), 4.21 (2H, m), 4.51 (1H, d, *J* = 12.5 Hz), 4.77 (1H, m), 4.91 (1H, brs), 6.17 (1H, s), 6.23 (1H, s); ESIMS *m/z* 765 $[M + H]^+$.

Aglycone (4): yellow gum, IR (neat) ν_{max} 3393, 2929, 1751, 1615, 1433, 1286, 1061 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 0.86 (3H, t, *J* = 7.5 Hz, H-3″), 1.24 (3H, d, *J* = 6.5 Hz), 1.32 (3H, d, *J* = 7.0 Hz), 1.68 (1H, m), 1.98 (1H, m), 2.64 (1H, dd, *J* = 16.0, 3.5 Hz), 2.74 (1H, brt, *J* = 12.5 Hz), 2.92 (1H, brt, *J* = 12.5 Hz), 3.43 (3H, s), 4.2 (1H, d, *J* = 2.5 Hz), 4.25 (2H, m), 4.45 (1H, d, *J* = 12.0 Hz), 6.44 (1H, s), 6.66 (1H, s); ESIMS *m/z* 463 $[M + H]^+$.

Bioassay. Cells. 63A9 is a stable Jurkat-derived cell line containing a defective, integrated HIV provirus (HXB2) utilizing luciferase reporter gene expression, which is dependent upon an exogenous source of Tat. These cells are grown in complete RPMI 1640 (GIbco/BRL) containing 10% heat-inactivated calf serum, 2 mM L-glutamine, and penicillin/streptomycin. These cells were maintained in complete RPMI 1640 supplemented with 600 ug/mL Geneticin (G418) (Gibco/BRL).

Cell-Based Tat Transactivation. 63A9 cells (described above) were transfected as described previously[12] with a Tat expression plasmid (pUCD5 Tat) DNA or with a control plasmid DNA lacking Tat (pUCD5). Cells (1×10^6 cells/mL) in complete RPMI were transfected with DNA (0.5 μg/1.25×10^6 cells) by a modified DEAE/Dextran method. Cells were plated into 96-well plates at 6.25×10^4 cells/well and incubated for 20−24 h at 37 °C and 5% CO_2. Test compounds at various levels with a final solvent (DMSO) concentration of 1% were added to replicate plates, which were incubated for 24 h. Luciferase activity was assayed by the Promega luciferase assay system using modified lysis buffer, and activity was detected with a Dynatech ML3000 96 well plate luminometer (Dynatech, Chantilly, VA). The percentage of inhibition was determined relative to the luminescence units obtained in Tat transfected control cells minus the units obtained in Tat control cells transfected with pUCD5 DNA. All assays were performed in duplicate.

Cytotoxicity Assay. The effect of compound upon cell proliferation was measured using a nonradioactive cell proliferation assay kit, CellTiter 96 AQueous (Promega, Madison,-WI), which is a quantitative MTS {3-(4,5-dimethylthiazol-2-yl)-5-3-carboxymethoxyphenyl)-2-4-sulfophenyl)-2H-tetrazolium inner salt)} colorimetric assay. The assay was performed in replicate plates in parallel with the cell-based HIV Tat transactivation assay. Briefly, test compound or solvent was added to the cells in 96-well plates as described above. At 2 h prior to scheduled harvest, 20 μL of 333 μg/mL MTS/25 mM PMS (phenazine methosulfate) was added to wells containing 100 μL of media. Cells were incubated at 37 °C, 5% CO_2, for 2 h, and the absorbance at 490 nM was read using a Molecular Dynamics (UV max) plate reader (Molecular Dynamics, Sunnyvale, CA). The percentage of inhibition for compound-treated cells was determined relative to solvent-treated control cells minus the background of reagent added to media alone. The assay was linear for the number of cells (trypan blue) and absorbance at 490 nM.

Vertical Assay. P4/P5 Hela cells were plated at $2.5. \times 10^3$ cells per well in a 96-well plate in DMEM + 10% FBS and incubated for 24 h at 37 °C/5% CO_2. Compound and HIV H9IIIb were added, and incubation continued for 48 h. Tropix β-gal substrate was added, incubated in the dark at 37 °C for 1 h, and read in a luminometer. Toxicity was measured in the absence of added HIV. The IC_{50} of the small peptide inhibitor used as the positive control was 1 nM. All assays were performed in duplicate.

References and Notes

(1) Arya, S. K.; Guo, C.; Josephs, S. F.; Wong-Staal, F. *Science* **1985**, *229*, 69−73.
(2) Berkhout, B. B.; Gatignol, A.; Rabson, A. B.; Jeang, K. *Cell* **1990**, *62*, 757−767.
(3) Feinberg, M. B.; Baltimore, D.; Frankel, A. D. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 4045−4049.
(4) Laspia, M. F.; Rice, A. P.; Mathews, M. B. *Cell* **1989**, *59*, 283−292.
(5) Sharp, P. A.; Marciniak, R. A. *Cell* **1989**, *59*, 229−230.
(6) Ogawa, H.; Yamashita, Y.; Katahira, R.; Chiba, S.; Iwasaki, T.; Ashizawa, T.; Nakano, H. *J. Antibiotics* **1998**, *51*, 261−266.
(7) Miyamoto, M.; Kawamatsu, Y.; Kawashima, K.; Shinohara, M.; Tanaka, K.; Tatsuoka, S. *Tetrahedron* **1967**, *23*, 421−437.
(8) Koneuma, M.; Uchida, N.; Yamaguchi, K.; Kawamura, Y.; Matsumoto, K. *J. Antibiot.* **1988**, *XL1*, 45−52.
(9) Hayakawa, M.; Nonomura, H. *Actinomycetologica* **1989**, *3*, 95−104.
(10) Hayakawa, M.; Tamura, T.; Nonomura, H. *J. Ferm. Bioeng.* **1991**, *72*, 426−432.
(11) Lingham, R. B.; Hsu, A. H. M.; O'Brien, J. A.; Sigmund, J. M.; Sanchez, M.; Gagliardi, M. M.; Heimbuch, B. K.; Genilloud, O.; Martin, I.; Diez, M. T.; Hirsch, C. F.; Nallin-Omstead, M.; Zink, D. L.; Liesch, J. M.; Koch, G. E.; Gartner, S. E.; Garrity, G. M.; Tsou, N. N.; Salituro, G. M. *J. Antibiot.* **1996**, *49*, 253−259.
(12) Byrnes, V.; Hazuda. D. *Methods Enzymol.* **1996**, *275*, 348−361.

NP010642F