JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Saquayamycins G–K, Cytotoxic Angucyclines from *Streptomyces* sp. Including Two Analogues Bearing the Aminosugar Rednose

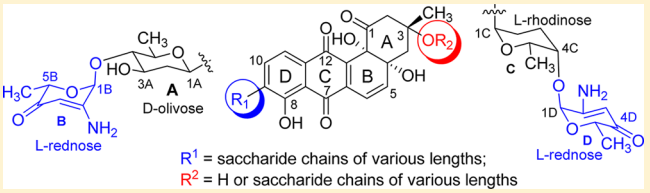
Khaled A. Shaaban,[‡] Tamer A. Ahmed,[†] Markos Leggas,[†,‡] and Jürgen Rohr*[,†,‡]

[†]Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, 789 South Limestone Street, Lexington, Kentucky 40536-0596, United States

[‡]Markey Cancer Center, University of Kentucky, 800 Rose Street, Lexington, Kentucky 40536-0293, United States

Supporting Information

ABSTRACT: *Streptomyces* sp. KY40-1, a strain isolated from the Kentucky Appalachian foothills, is the producer of moromycins A (**18**) and B (**19**). Further investigations of this strain led to the isolation and structure elucidation of the five new saquayamycins G–K (**1**–**5**), along with known compounds. Two of the new compounds bear the unusual aminosugar rednose, which was found here for the first time in angucyclines. The different attachment positions of this aminosugar in these two compounds indicate a high acceptor substrate flexibility of the responsible glycosyl transferase or alternatively the involvement of multiple glycosyl transferases. The cytotoxic activity of the isolated compounds was determined using human prostate cancer (PC-3) and non-small-cell lung cancer (H460) cell lines. Cell viability assays showed that saquayamycins J (**4**), K (**5**), A (**7**), and B (**8**) were most active in PC3 cells, with saquayamycin B (**8**) showing the highest activity (GI_{50} = 0.0075 μM). The aminosugar-containing saquayamycins H (**2**) and saquayamycin B (**8**) showed the highest activity against H460 cells, with a GI_{50} of 3.3 and 3.9 μM, respectively. The results presented here provide more insights into the structure–activity relationship of saquayamycins with respect to the nature, number, and linkage of sugar residues.

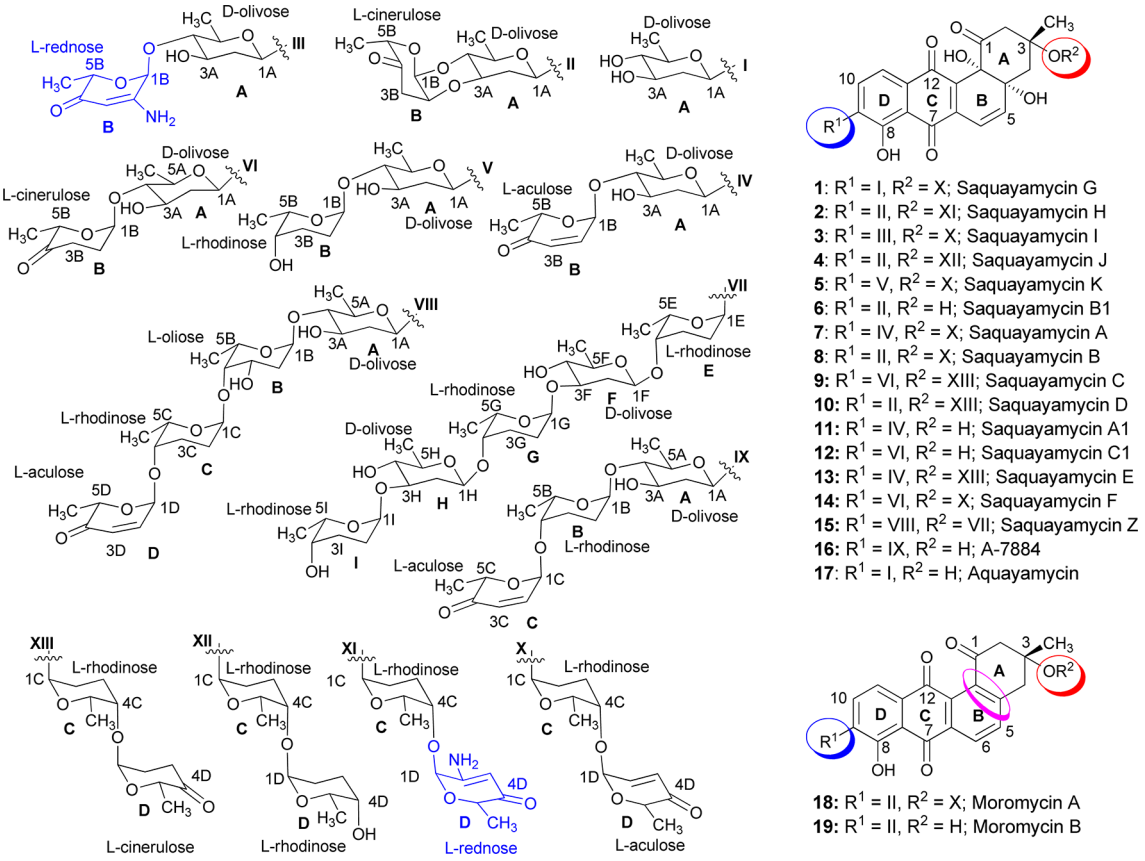


The angucycline group of antibiotics is one of the largest groups of polycyclic aromatic polyketides, rich in chemical scaffolds and biological activities, predominantly anticancer and antibacterial.[1−3] Saquayamycins,[4−8] urdamycins,[9−17] and landomycins[18−24] are well-known angucycline antitumor antibiotics. The structures of both saquayamycins and urdamycins contain the same aquayamycin (**17**)[25−27] as aglycone with the *C*-glycosidic sugar D-olivose attached at C-9 of the angucycline chromophore. So far, seven saquayamycin analogues were reported from *Streptomyces* spp. Saquayamycins differ from urdamycins by their saccharide patterns, which are attached at C-9 and C-3 positions in the saquayamycins, but at C-9 and C-12b positions in urdamycins. Saquayamycins A–D (**7**–**10**) were first isolated from *Streptomyces nodosus* MH190-16F3 and were reported as platelet aggregation inhibitors.[4] Saquayamycins A and B (**7** and **8**) contain three different *O*-glycosidically linked deoxysugars, L-rhodinose, L-aculose, and L-cinerulose (only in saquayamycin B). Saquayamycin A (**7**) was reported to be unstable to acid, and even contact with silica gel led to its conversion to saquayamycin B (**8**). Saquayamycins A1 (**11**), B1 (**6**), and C1 (**12**) were generated by partial acid hydrolysis of saquayamycins A–C (**7**–**9**), respectively.[4] The two isomers saquayamycins E (**13**) and F (**14**) were produced by *Actinomyces* strain MK290-AF1 and reported to inhibit the FPTase from bovine brain with IC_{50} values of 1.8 and 2.0 μM, respectively.[5] They differ slightly from the saquayamycins A (**7**) and C (**9**) with respect to their sugar moieties. The saquayamycin analogue A-7884 (**16**) was isolated from the *Streptomyces* sp. #AM1699; it has a trisaccharide side chain connected at C-9, which contains an L-rhodinose sugar moiety between the first sugar, a *C*-glycosidic bound D-olivose, and L-aculose of saquayamycin A1 (**11**).[28] Compound **16** showed inhibitory activity in the inducible nitric oxide synthase assay with IC_{50} values of 43.5 μM, better than saquayamycin A1 (**11**; IC_{50} value of 101.2 μM).[28] Recently, the largest saquayamycin analogue, saquayamycin Z (**15**), was reported from *Micromonospora* sp. strain Tü6368.[6] Saquayamycin Z (**15**) contains tetra- and pentasaccharide side chains linked at C-3 and C-9 positions of the benz[*a*]anthracene core. The tetrasaccharide side chain of saquayamycin Z (**15**) is striking due to the presence of an L-oliose, which is not a usual sugar constituent of the angucyclines.[6,29] Very recently, Ren et al. reported three novel members of the angucycline family, named N05WA963 A, B, and D, with the same aglycones as in the moromycins A (**18**) and B (**19**), except for an additional methoxy group attached at C-5.[30] N05WA963 A, B, and D were reported to have antiproliferative effects on a panel of cancer cell lines including SW620 (colon cancer), K-562 (chronic myelogeneous leukemia), MDA-MB-231 (estrogen receptor negative breast cancer), YES-4 (esophageal cancer), and T-98 and U251SP (both glioblastomae).

To study the structure–activity relationship of this saquayamycin group of antibiotics, we looked for further

Received: May 4, 2012
Published: July 3, 2012

Chart 1



saquayamycin analogues produced by *Streptomyces* sp. K40-1, the strain earlier reported as the producer of moromycins A and B (**18** and **19**).[7] The saccharide attachments in moromycins A (**18**) and B (**19**) are like those of saquayamycins B (**8**) and B1 (**6**), respectively; however, their tetracyclic angucyclinone core has an aromatic ring B and, thus, no angular hydroxy groups at the C-4a and C-12b positions.

We found five new metabolites, designated as saquayamycins G–K (**1**–**5**), produced by repeated fermentations of the same strain, along with saquayamycin B1 (**6**), which was previously not described as a natural product, and known saquayamycins. Two of the new angucyclines, saquayamycins H (**2**) and I (**3**), bear the unusual aminosugar rednose, which was found for the first time in an angucycline compound. Aminosugar-containing angucyclines are very rare, and previously only three examples had been reported, namely, the marmycins A and B[31,32] and mayamycin.[33] The marmycins contain an unusual branched and doubly (*C*- and *N*)-linked aminosugar, 3-epi-,4-epi-vancosamine, and mayamycin contains a unique *C*-glycosidically linked aminosugar, *N*-demethylangolosamine, attached at the C-5 position of the benz[*a*]anthracenone core.

RESULTS AND DISCUSSION

Cultivation, Isolation, and Structure Investigation. A 2.3 g portion of crude extract was obtained from a 4-day culture of *Streptomyces* sp. KY40-1. On the basis of TLC, UV, and HPLC-MS (Figure S5) of the crude extract, several angucyclines containing aquayamycin (**17**) were detected.[34] Fractionation of the extract (2.30 g), using various chromatographic techniques (Figure S1), led to the isolation of the six new saquayamycins G–K (**1**–**5**) and B1 (**6**). For comparison reasons we report here also NMR assignments and NOESY correlations of saquayamycins A and B (**7** and **8**) (Figures S3–S6), which were previously incompletely reported.[7]

Saquayamycin G (1). Compound **1** is an orange-red solid with a molecular formula of $C_{37}H_{41}O_{14}$, indicating that one sugar molecule was missing compared to saquayamycins A (**7**) and B (**8**). The ^{1}H NMR spectrum of **1** displayed the same aromatic pattern as aquayamycin (**17**), with sugar substitutions at the C-3 and C-9 positions. The aliphatic region between δ 1.37–1.26 revealed three methyl protons as doublets, indicating three 6-deoxysugar moieties. This was confirmed by the presence of the three anomeric protons at δ 5.24 (d, 3.5 Hz), 5.22 (brd, 5.5 Hz), and 4.87 (brd, 11.0 Hz), consistent with one β-D- and two α-L-glycosidically linked sugar moieties. The ^{13}C NMR/HSQC spectra of **1** established aquayamycin (**17**) as the aglycone. The last two carbonyls correspond to a quinone system, with one carbonyl chelated with a peri-hydroxy group. In the sp^3 region, two anomeric carbon signals (δ 95.5 and 92.6) were observed along with seven methine, three quaternary, five methylene, and four methyl signals. The ^{1}H and ^{13}C NMR data of **1** are closely related to saquayamycins A (**7**) and B (**8**) with $\Delta m/z$ =110, indicating the missing sugar B (L-cinerulose or L-aculose) or sugar D (L-aculose) in compounds **7** and **8**, respectively.

The HMBC and ^{1}H–^{1}H COSY correlations of **1** (Figure 1) revealed two partial structures, the aquayamycin aglycone (**17**) and a disaccharide system. The attachment of the disaccharide at the usual C-3 position was confirmed by a $^3J_{C-H}$ long-range coupling between the anomeric proton of α-L-rhodinose (δ_H 5.22) and C-3 (δ_C 82.7) of the aglycone. All three sugars A, C, and D showed the same signal patterns and connectivity as

dx.doi.org/10.1021/np300316b | J. Nat. Prod. 2012, 75, 1383–1392

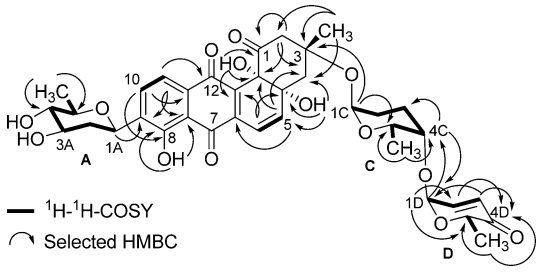


Figure 1. ^{1}H−^{1}H COSY (bold lines) and selected HMBC long-range couplings (→) of saquayamycin G (**1**).

previously found in saquayamycins A (**7**) and B (**8**). The oxygenated carbon C-4A (δ_C 78.2) of the D-olivose moiety of compound **1** appeared upfield compared to the same carbon of saquayamycin A (**7**; C-4A, δ_C = 89.3, Table 4), indicating a free OH group at C-4A of the β-D-olivose sugar moiety. The couplings and chemical shifts were in full agreement with structure **1** (Figure 1). The relative configuration of the sugar residues was further confirmed by NOESY experiments (Figure 2), determining structure **1** as 3-α-L-rhodinosyl-4−1-α-L-aculosylaquayamycin, which was subsequently named saquayamycin G.

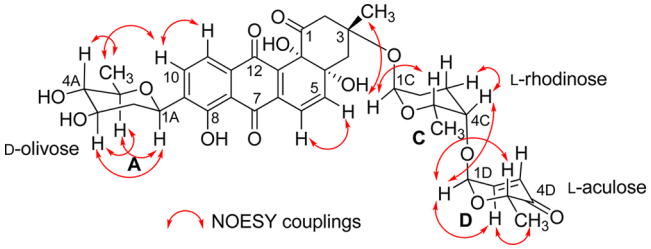


Figure 2. Selected NOESY correlations (↔) in saquayamycin G (**1**).

*Saquayamycin H (**2**).* Compound **2** was obtained from fraction FIV as an orange-red solid (Figure S1). It has similar physicochemical properties to saquayamycins G (**1**), A (**7**), and B (**8**), showing an orange fluorescence under long UV (365 nm) light and blue-violet coloration with 2 N NaOH. The molecular formula $C_{43}H_{49}NO_{16}$ of compound **2** was established by HRESIMS, indicating a nitrogen-containing compound with $\Delta m/z$ = 15 higher than saquayamycins A (**7**) and B (**8**).

The ^{1}H NMR of compound **2** was very similar to that of saquayamycin B (**8**) with the exception that one of the two doublet olefinic protons of the L-aculose moiety was missing. Instead, one singlet proton at δ 5.19 along with a broad signal with an integration of 2H at δ 4.78 was present. The ^{13}C NMR/HSQC of compound **2** displayed 43 carbon atoms as in saquayamycin B (**8**) with highly similar chemical shifts. The sole difference in the ^{13}C NMR spectrum was that the two methine carbons at C-2D (δ_C 143.3, C_q) and C-3D (δ_C 127.5, CH) of the L-aculose moiety of saquayamycin B (**8**) were shifted to δ 159.1 (C_q) and 97.4 (CH), respectively. The downfield chemical shift of the quaternary carbon at δ 159.1 in compound **2** indicated its connection to a heteroatom, which turned out to be NH_2 in this case, as shown from the broad signal in the ^{1}H NMR spectrum, in the β-position of the carbonyl.

The full NMR assignments for compound **2** were deduced from the ^{1}H−^{1}H COSY, HSQC, and HMBC experiments (Figure 3 and Tables 1 and 2), indicating the presence of the

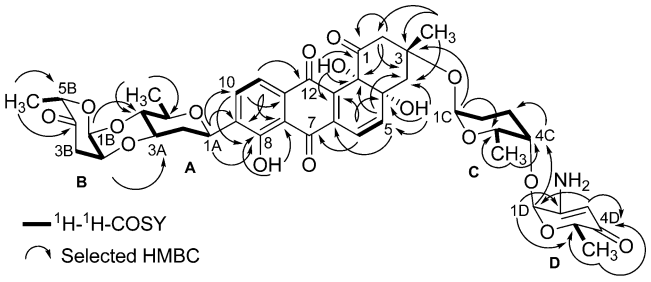


Figure 3. ^{1}H−^{1}H COSY (bold lines) and selected HMBC long-range couplings (→) of saquayamycin H (**2**).

rare aminosugar rednose, which was connected at C-4C of the α-L-rhodinose moiety instead of the α-L-aculose moiety found in the same position in saquayamycins A (**7**) and B (**8**). On the basis of NOESY experiments (Figure 4), coupling constants, and comparison with saquayamycin B (**8**), compound **2** was established as 4A-α-L-cinerulosyl-3-α-L-rhodinosyl-4C-1D-α-L-rednosylaquayamycin and consequently named saquayamycin H. The unusual trideoxy-keto-aminosugar rednose was previously reported in two anthracycline-type compounds, CG21-C[35] and rudolphomycin[36] (hence the name).

*Saquayamycin I (**3**).* Compound **3** was obtained as an orange-red solid from the same fraction as saquayamycin H (**2**), with a slightly higher polarity. Compound **3** is an isomer of saquayamycin H (**2**) with the same molecular formula of $C_{43}H_{48}NO_{16}$. The UV spectra of compound **3** showed the band at 218 nm, characteristic of the L-rednose sugar moiety (Figure S2).

The only difference in the ^{1}H and ^{13}C NMR spectra between compounds **2** and **3** was that the ABX signals (CH-2B and CH_2-3B) of the L-cinerulose sugar moiety in **2** were missing in **3**. Instead, two CH groups of the L-aculose moiety in **3** at δ 7.07 (dd, 10.0, 3.5 Hz; δ_C 145.2) and δ 6.05 (d, 10.0 Hz; δ_C 127.3) were observed instead.

The full assignment of compound **3** was deduced from the ^{1}H−^{1}H COSY, HSQC, and HMBC experiments (Figure 5 and Tables 1 and 2), indicating the attachment of the L-rednose moiety at C-4A of the β-D-olivose moiety (sugar A). This was confirmed by long-range coupling of the proton at δ 5.50 (H-1B, δ_C 96.6) to C-4A (δ_C 88.4) and from H-4A (δ_H 3.40) to C-1B (δ_C 96.6). Thus, the rednose moiety was found in compound **3** in a different position than in saquayamycin H (**2**), and the same *O*-glycosidic linked disaccharide chain (3-α-L-rhodinosyl-4-1-α-L-aculose) as found in compounds **1**, **7**, and **8** is connected at C-3 of the aquayamycin aglycone. The relative stereochemistry of the aglycone of compound **3** was deduced from NOESY experiments (Figure 6) and coupling constants and by comparison with the related saquayamycins A (**7**), B (**8**), and H (**2**). The relative configuration of the sugar residues was established to be 4A-α-L-rednosyl-3-α-L-rhodinosyl-4C-1D-α-L-aculosylaquayamycin, confirming structure **3** as indicated in Figures 5 and 6, which was subsequently named saquayamycin I. The attachment of the L-rednose moiety in two different positions in saquayamycins H (**2**) and I (**3**) indicates acceptor substrate flexibility of the aminosugar glycosyltransferase or the involvement of two different enzymes.

*Saquayamycin J (**4**).* Compound **4** is a yellow solid with similar physicochemical properties and staining to those of the earlier isolated saquayamycins. Its molecular weight was deduced by ESIMS and (−)HRESIMS, establishing its molecular formula to be $C_{43}H_{52}O_{16}$, 4 amu higher than

dx.doi.org/10.1021/np300316b | J. Nat. Prod. 2012, 75, 1383−1392

Table 1. ^{1}H NMR (500 MHz) Assignments of Saquayamycins B (8), G (1), H (2), and I (3), δ in ppm relative to TMS (multiplicity, J/Hz)

	saquayamycin B (8)[a]	saquayamycin G (1)[a]	saquayamycin H (2)[a]	saquayamycin I (3)[a]
position	δ_H ($CDCl_3$)	δ_H ($CDCl_3$)	δ_H ($CDCl_3$)	δ_H (Acetone-d_6)
2	2.48, d (13.5, Ha), 3.15, dd (13.0, 3.0, He)	2.49, d (13.5, Ha), 3.16, dd (13.5, 3.0, He)	2.48, d (13.5, Ha), 3.16, dd (13.5, 3.0, He)	2.89, brd (13.0, Ha), 2.96, brd (13.0, He)
3-CH_3	1.37, s	1.38, s	1.37, s	1.39, s
4	1.81, d (15.5, Ha), 2.25, dd (15.5, 3.0, He)	1.81, d (15.0, Ha), 2.26, dd (15.0, 3.0, He)	1.84, d (15.0, Ha), 2.25, dd (15.5, 3.0, He)	2.22, d (15.5, Ha), 2.31, dd (15.5, 2.0, He)
4a-OH	4.31, brs	4.32, brs	4.35, brs	4.31, brs
5	6.42, d (10.0)	6.42, d (9.5)	6.42, d (10.0)	6.43, d (9.5)
6	6.87, d (9.5)	6.88, brd (10.5)	6.87, d (9.5)	6.82, d (9.5)
8-OH	12.27, s	12.28, s	12.26, s	12.32, s
10	7.85, d (7.5)	7.85, d (8.0)	7.85, d (7.5)	7.90, d (7.5)
11	7.57, d (7.5)	7.58, d (7.5)	7.57, d (7.5)	7.57, d (8.0)
12b-OH	4.56, brs	4.59, brs	4.56, brs	4.62, brs
Sugar A, β-D-olivose				
1A	4.93, brd (9.5)	4.87, brd (11.0)	4.93, brd (9.5)	4.91, brd (11.0)
2A	1.45, m (Ha), 2.41, ddd (12.5, 4.0, 2.5, He)	1.40 m (Ha), 2.44 m (He)	1.45, ddd (14.0, 11.5, 3.0, Ha), 2.41 (ddd, 12.5, 2.5, 1.5, He)	1.40, m (Ha), 2.47 brdd (12.5, 4.5)
3A	3.78, m	3.80, brm	3.78, m	3.95, m
3A-OH		3.46, brs		4.75, brs
4A	3.45, dd (9.0, 9.0)	3.16, dd (10.5, 9.0)	3.45, dd (9.0, 9.0)	3.40, dd (9.0, 9.5)
4A-OH		n.o.[b]		
5A	3.54, dq (9.0, 6.0)	3.48, m	3.54, dq (9.0, 6.0)	3.63, m
6A	1.36, d (6.5)	1.37, brd (7.5)	1.36, d (6.0)	1.43, d (6.0)
Sugar B, α-L-cinerulose or α-L-rednose				
1B	5.15, d (2.5)		5.15, d (3.0)	5.50 (s)
2B	4.31, brs		4.32, m	
2B-NH_2				6.38, brs
3B	2.60, dd (17.5, 3.0, Ha), 2.64, dd (17.5, 3.0, He)		2.60, dd (17.5, 3.0, Ha), 2.64, dd (17.5, 3.0, He)	5.10, s
5B	4.69, q (7.0)		4.69, q (6.5)	4.63, q (6.5)
6B	1.35, d (7.0)		1.34, d (6.5)	1.31, d (6.5)
Sugar C, α-L-rhodinose				
1C	5.22, brd (4.0)	5.22, brd (5.5)	5.22, d (2.5)	5.24, brs
2C	1.44, m (Ha), 2.01, m (He)	1.90, m (Ha), 2.00, m (He)	1.90, m (Ha), 2.00, m (He)	1.96−1.88, m (Ha) 1.96−1.88, m (He)
3C	1.88, m (Ha) 1.88, m (He)	1.45, m (Ha), 1.85, m (He)	1.45, m (Ha), 1.85, m (He)	1.43, m (Ha), 1.82, m (He)
4C	3.66, brs	3.67, brs	3.69, brs	3.73, brs
5C	4.21, brm	4.22, m	4.21, brq (6.5)	4.20, m
6C	1.26, d (6.5)	1.26, d (6.5)	1.30, d (7.0)	1.22, d (6.5)
Sugar D, α-L-aculose or α-L-rednose				
1D	5.24, d (3.5)	5.24, d (3.5)	5.17, s	5.38, d (3.5)
2D	6.86, dd (10.5, 3.5)	6.85, dd (10.0, 3.5)		7.07, dd (10.0, 3.5)
2D-NH_2			4.78, brs	
3D	6.01, d (10.5)	6.07, d (10.5)	5.19, s	6.05, d (10.0)
5D	4.51, q (6.5)	4.51, q (7.0)	4.32, m	4.58, q (6.5)
6D	1.34, d (6.5)	1.35, d (7.0)	1.34, d (6.5)	1.28, d (7.0)

[a]See also Figures S12−17, S20−31, and S60−65. [b]Not observed.

saquayamycins A (7) and B (8), attributed to the reduction of two double bonds or ring-openings. The NMR spectrum showed the same aromatic pattern of the aquayamycin system and the characteristic ABX proton signals CH-2B and CH_2-3B along with the carbonyl of the L-cinerulose sugar moiety (sugar B, δ_C 208.0), confirming the existence of the same sugar residues A and B in compound **4** as in saquayamycin B (8). Comparing the NMR data with those of saquayamycin B (8) showed the absence of the olefinic proton signals of the L-aculose sugar moiety (2D and 3D) and the carbonyl carbon C-4D. Instead, two methylene groups and one oxygenated carbon at δ_C 29.9, 27.9, and 70.4, respectively, were observed. The 4 amu difference between saquayamycin B (8) and compound **4** was attributed to the reduction of the C-2D−C-3D double

dx.doi.org/10.1021/np300316b | J. Nat. Prod. 2012, 75, 1383−1392

Table 2. ^{13}C NMR (125 MHz) Assignments of Saquayamycins B (8), G (1), H (2), and I (3), δ in ppm relative to TMS

	saq. B (8)[a,b]	saq. G (1)[a,b]	saq. H (2)[a,b]	saq. I (3)[a,c]	saq. B1 (6)[a,b]
position	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.
1	205.0, C	205.1, C	205.2, C	205.8, C	205.1, C
2	50.4, CH_2	50.4, CH_2	50.2, CH_2	51.1, CH_2	52.9, CH_2
3	82.7, C	82.7, C	82.8, C	83.3, C	76.3, C
3-CH_3	25.6, CH_3	25.6, CH_3	25.6, CH_3	26.1, CH_3	30.5, CH_3
4	44.7, CH_2	44.7, CH_2	44.8, CH_2	43.9, CH_2	43.4, CH_2
4a	80.1, C	80.2, C	80.2, C	80.9, C	80.7, C
5	145.8, CH	145.7, CH	145.8, CH	147.2, CH	144.5, CH
6	117.6, CH	117.7, CH	117.7, CH	117.5, CH	117.7, CH
6a	138.9, C	139.0, C	140.0, C	139.4, C	139.0, C
7	188.4, C	188.4, C	188.4, C	190.1, C	188.2, C
7a	114.1, C	114.1, C	114.1, C	115.2, C	114.2, C
8	158.1, C	158.2, C	158.1, C	158.4, C	158.2, C
9	138.0, C	138.6, C	138.1, C	138.8, C	138.2, C
10	133.8, CH	133.9, CH	133.8, CH	134.1, CH	134.0, CH
11	119.9, CH	119.9, CH	119.8, CH	119.7, CH	120.0, CH
11a	130.7, C	130.6, C	130.7, C	132.0, C	130.6, C
12	182.4, C	182.4, C	182.4, C	183.2, C	182.2, C
12a	139.0, C	139.0, C	140.0, C	140.4, C	138.3, C
12b	77.6, C	77.6, C	77.6, C	78.2, C	76.3, C
Sugar A, β-D-olivose					
1A	71.6, CH	71.4, CH	71.6, CH	71.9, CH	71.6, CH
2A	36.9, CH_2	39.5, CH_2	36.9, CH_2	40.5, CH_2	36.9, CH_2
3A	76.8, CH	73.1, CH	76.8, CH	71.9, CH	76.9, CH
4A	74.6, CH	78.2, CH	74.6, CH	88.4, CH	74.7, CH
5A	74.7, CH	76.1, CH	74.7, CH	75.5, CH	74.8, CH
6A	17.6, CH_3	18.3, CH_3	17.6, CH_3	19.1, CH_3	17.7, CH_3
Sugar B, α-L-cinerulose or α-L-rednose					
1B	91.5, CH		91.5, CH	96.6, CH	91.6, CH
2B	71.3, CH		71.3, CH	161.1, C	71.3, CH
3B	40.1, CH_2		40.2, CH_2	95.6, CH	40.2, CH_2
4B	208.0, C		208.1, C	193.2, C	208.0, C
5B	77.9, CH		77.9, CH	71.0, CH	78.0, CH
6B	16.3, CH_3		16.4, CH_3	16.6, CH_3	16.4, CH_3
Sugar C, α-L-rhodinose					
1C	92.6, CH	92.6, CH	92.6, CH	92.9, CH	
2C	24.9, CH_2	24.9, CH_2	24.9, CH_2	25.2, CH_2	
3C	24.7, CH_2	24.7, CH_2	24.8, CH_2	25.6, CH_2	
4C	76.3, CH	76.4, CH	76.7, CH	77.2, CH	
5C	67.1, CH	67.1, CH	67.2, CH	67.4, CH	
6C	17.3, CH_3	17.4, CH_3	17.7, CH_3	17.6, CH_3	
Sugar D, α-L-aculose or α-L-rednose					
1D	95.5, CH	95.5, CH	96.7, CH	96.1, CH	
2D	143.3, CH	143.3, CH	159.1, C	145.2, CH	
3D	127.5, CH	127.5, CH	97.4, CH	127.3, CH	
4D	197.0, C	197.1, C	195.2, C	197.3, C	
5D	70.8, CH	70.8, CH	70.5, CH	71.1, CH	
6D	15.4, CH_3	15.4, CH_3	16.0, CH_3	15.5, CH_3	

[a]See also Figures S12−17, S20−31, S48−53, and S60−65. [b]$CDCl_3$. [c]Acetone-d_6.

bond and the C-4D carbonyl group. The OH group at C-4D could be axial or equatorial. The structure of **4** was further confirmed by 2D NMR experiments (Figures 7 and 8), exhibiting the same sugar residues A (D-olivose), B (L-cinerulose), and C (L-rhodinose) and connectivity as found in **8** (Figures 7 and 8). However, on the basis of coupling constants and NOESY correlations the fourth sugar, residue D, was deduced to be L-rhodinose instead of L-aculose, which was typically found in the same position, e.g., in compounds **1**, **3**, **7**, and **8**. Hence the new compound **4** was determine to be 4A-α-L-cinerulosyl-3-α-L-rhodinosyl-4C-1D-α-L-rhodinosylaquayamycin and named saquayamycin J. This is the first angucycline with a disaccharide side chain consisting of the same sugar building blocks (α-L-rhodinose) attached to C-3.

Saquayamycin K (5). Compound **5** was isolated as a yellow-orange solid. The two isomers **4** and **5** were separated by HPLC followed by PTLC (see Figure S8). The (−)-HRESIMS suggested the molecular formula $C_{43}H_{52}O_{16}$ for **5** with a $\Delta m/z$ 4 amu higher than saquayamycin A (**7**), corresponding to two fewer double-bond equivalents. Compound **5** has the same

dx.doi.org/10.1021/np300316b | J. Nat. Prod. 2012, 75, 1383−1392

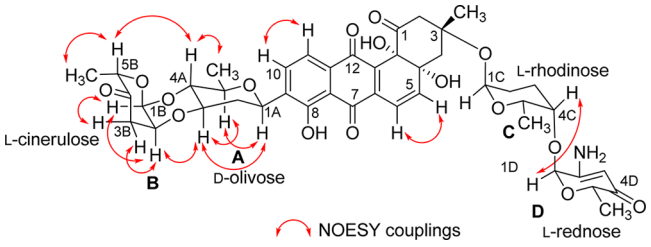


Figure 4. Selected NOESY correlations (↔) in saquayamycin H (**2**).

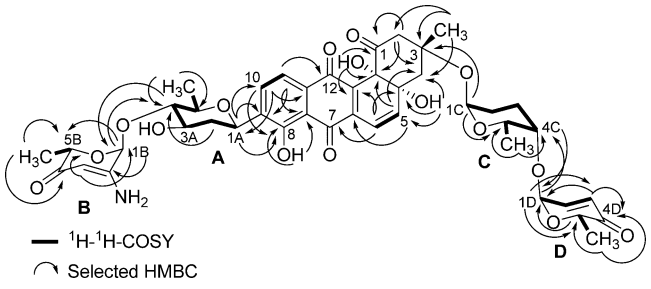


Figure 5. ^{1}H−^{1}H COSY (bold lines) and selected HMBC long-range couplings (→) of saquayamycin I (**3**).

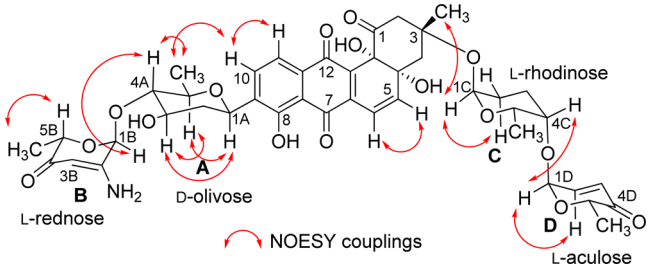


Figure 6. Selected NOESY correlations (↔) in saquayamycin I (**3**).

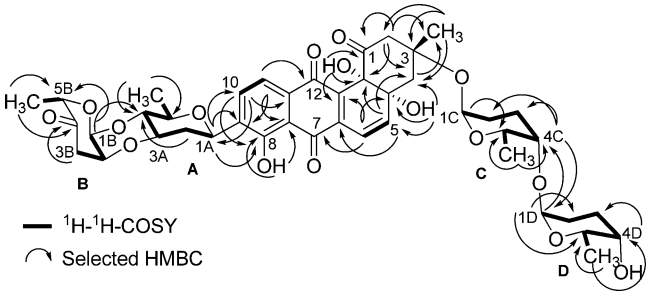


Figure 7. ^{1}H−^{1}H COSY (bold lines) and selected HMBC (→) correlations of saquayamycin J (**4**).

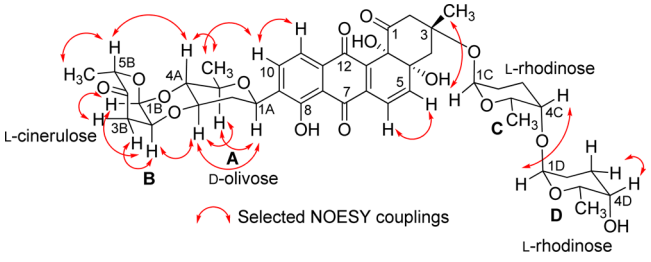


Figure 8. Selected NOESY correlations (↔) in saquayamycin J (**4**).

mass as saquayamycin J (**4**). The ^{1}H and ^{13}C NMR spectrum of compound **5** was similar to those of saquayamycins A (**7**) and J (**4**), except in the olefinic region, where one set of double-bond protons of an L-aculose moiety was observed, not two as in **7**. This indicates that one of the L-aculose moieties of saquayamycin A (**7**) was reduced to an L-rhodinose moiety to give compound **5**. The overall structure was investigated by 2D NMR studies (Figures 9 and 10; Tables 3 and 4) to determine

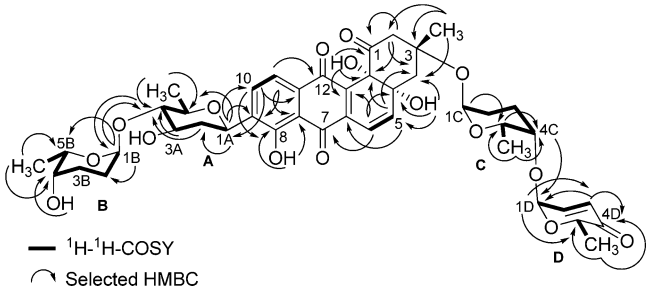


Figure 9. ^{1}H−^{1}H COSY (bold lines) and selected HMBC long-range couplings (→) of saquayamycin K (**5**).

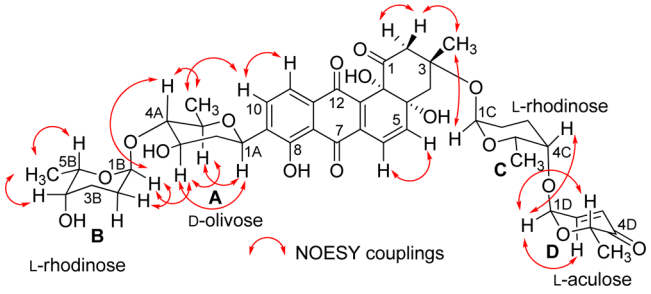


Figure 10. Selected NOESY correlations (↔) in saquayamycin K (**5**).

at which positions the L-aculose and L-rhodinose moieties were located. In the HMBC spectrum, a ^3J coupling was observed from the L-rhodinose anomeric proton at δ 4.87 (δ_C 98.9) to C-4A (δ_C 89.2) and from H-4A (δ 3.01) to C-1B (δ_C 98.9), fixing the linkage of the L-rhodinose sugar moiety at C-4A of the D-olivose (sugar A). The structure of **5** and its configuration was finally determined to be 4A-α-L-rhodinosyl-3-α-L-rhodinosyl-4C-1D-α-L-aculosylaquayamycin (Figures 9 and 10) and subsequently named saquayamycin K. An α-L-rhodinosyl sugar moiety (B) was reported in the same position in A-7884 (**16**), where it is inserted between a D-olivose and an L-aculose residue. The fact that a second α-L-rhodinose moiety was found in two different positions in the isomers **4** and **5** indicates again either a high flexibility of the corresponding glycosyltransferase or two different glycosyltransferases, similar to the glycosyltransferase responsible for the L-rednose transfer in saquamycins H (**2**) and I (**3**).

*Saquayamycin B1 (**6**).* Compound **6** was isolated as a yellow solid, after several chromatographic purifications of the main fraction FI. The (−)HRESIMS-derived molecular formula $C_{31}H_{32}O_{12}$, 224 amu less than saquayamycins A (**7**) and B (**8**), indicates two missing sugar residues. The UV data and the proton NMR spectrum of compound **6** revealed its similarity to saquayamycin B (**8**), except the protons and carbon atoms of the sugar residues C (L-rhodinose) and D (L-aculose) were absent (Tables 1 and 3). The chemical shift of C-3 was upfield (δ 76.3; in saquayamycin B, δ_C 82.7), due to the attachment of a free hydroxyl group. Thus, compound **6** was identified as saquayamycin B1, which had been previously reported together with saquayamycins A1 (**11**) and C1 (**12**), obtained through acid hydrolysis of saquayamycins B (**8**), A (**7**), and C (**9**),

dx.doi.org/10.1021/np300316b | J. Nat. Prod. 2012, 75, 1383−1392

Table 3. ^{1}H NMR Assignments (500 MHz, $CDCl_3$) of Saquayamycins A (7), J (4), K (5), and B1 (6), δ in ppm relative to TMS (multiplicity, J/Hz)

	saquayamycin A (7)[a]	saquayamycin J (4)[a]	saquayamycin K (5)[a]	saquayamycin B1 (6)[a]
position	δ_H	δ_H	δ_H	δ_H
2	2.47, d (13.5, Ha),	2.47, d (13.0, Ha),	2.49, d (13.5, Ha),	2.60, d (13.5, Ha),
	3.13, dd (13.5, 2.5, He)	3.16, dd (13.0, 3.0, He)	3.16, dd (13.5, 3.0, He)	2.93, dd (12.5, 2.5, He)
3-CH_3	1.36, s	1.38, s	1.38, s	1.27, s
3-OH				3.90, brs[b]
4	1.81, d (15.0, Ha), 2.24, dd (15.5, 3.0, He)	1.81, d (15.5, Ha), 2.24, dd (15.0, 2.5, He)	1.82, d (15.5, Ha), 2.26, dd (15.5, 3.0, He)	1.82, d (15.0, H_a), 2.25, dd (14.0, 2.0, He)
4a-OH	4.30, brs	4.38, brs	4.31, brs	3.54, brs[b]
5	6.40, d (10.0)	6.42, d (10.0)	6.42, d (10.0)	6.38, d (10.0)
6	6.86, d (9.5)	6.87, d (9.5)	6.89, d (10.0)	6.87, d (9.5)
8-OH	12.25, s	12.27, s	12.26, s	12.26, s
10	7.83, d (8.0)	7.85, d (8.0)	7.85, d (8.0)	7.87, d (7.5)
11	7.56, d (7.5)	7.57, d (8.0)	7.58, d (7.5)	7.59, d (8.0)
12b-OH	4.56, brs	4.56, brs	4.57, brs	4.98, brs[b]
Sugar A, β-D-olivose				
1A	4.84, brd (11.0)	4.93, brd (9.5)	4.84, brd (10.0)	4.94, brd (10.0)
2A	1.45, m (Ha), 2.48, m (He)	1.41, m (Ha), 2.41, ddd, 12.5, 4.5, 2.0, He)	1.33 m (Ha), 2.49 m (He)	1.40, m (Ha), 2.42, ddd (12.5, 4.5, 2.0, He)
3A	3.86, m	3.78, m	3.79, m	3.79, m
3A-OH	4.23, brs		3.46, brs	
4A	3.17, dd (9.0, 8.5)	3.45, dd (9.0, 9.0)	3.01, dd (8.5, 8.5)	3.46, dd (9.0, 9.0)
5A	3.53, m	3.54, m	3.53, m	3.55, m
6A	1.35, d (6.0)	1.37, d (6.0)	1.33, d (6.5)	1.38, d (6.0)
Sugar B, α-L-aculose or α-L-cinerulose or α-L-rhodinose				
1B	5.34, d (3.5)	5.15, d (3.0)	4.87, brs	5.16, d (3.0)
2B	6.81, dd (10.0, 3.5)	4.31, brm	1.90−1.80, m (Ha, He)	4.32, brq (3.0)
3B	6.10, d (10.5)	2.60, dd (17.5, 3.0, Ha), 2.65, dd (17.5, 3.5, He)	1.20, m (Ha), 1.90, m (He)	2.60, dd (16.5, 3.0, H_a), 2.66, dd (17.5, 3.5, He)
4B			3.32, brm	
4B-OH			4.93, brs	
5B	4.72, q (6.5)	4.69, q (7.0)	3.84, m	4.70, q (7.0)
6B	1.39, d (7.0)	1.34, d (7.0)	1.29, d (6.0)	1.35, d (6.5)
Sugar C, α-L-rhodinose				
1C	5.21, brs	5.23, brd (2.5)	5.23, brs	
2C	1.45, m (Ha), 1.88, m (He)	1.42, m (Ha), 1.80, m (He)	1.45, m (Ha), 1.90, m (He)	
3C	1.99−1.88, m (Ha, He)	1.76, m (Ha, He)	2.01−1.90, m (Ha, He)	
4C	3.65, brs	3.54, brm	3.67, brs	
5C	4.18, m	4.15, m	4.22, m	
6C	1.24, d (6.0)	1.20, d (6.0)	1.26, d (7.0)	
Sugar D, α-L-aculose or α-L-rhodinose				
1D	5.22, d (3.5)	4.76, brd (2.5)	5.24, d (3.5)	
2D	6.85, dd (9.5, 3.5)	1.70, m (Ha), 1.95, m (He)	6.86, dd (10.0, 3.5)	
3D	6.04, d (10.0)	1.80, m (Ha, He)	6.07, d (10.5)	
4D		3.26, brm		
4D-OH		5.27, brs		
5D	4.50, q (7.0)	3.61, m	4.52, q (6.5)	
6D	1.32, d (7.0)	1.21, d (6.0)	1.34, d (7.0)	

[a]See also Figures S32−37, S40−45, and S48−59. [b]Assignments may be interchanged among the signals.

respectively,[4,5] although not as a natural product. The reported NMR data of saquayamycin B1 were identical to those from our isolated compound **6**. We further confirmed the structure of **6** as saquayamycin B1 by full NMR assignments using 2D NMR experiments (Figure 11). On the basis of NOESY experiments (Figure 12), coupling constants, and comparison with the reported data of saquayamycin B1,[4] compound **6** was shown to have the same configuration as saquayamycin B1.

Cytotoxicity Assays. The cytotoxic activity of saquayamycins G−K (**1**−**5**) and B1 (**6**) and saquayamycins A and B (**7** and **8**) in comparsion with landomycin A[22,37−39] was determined using PC3 (prostate cancer) and H460 (non-small -cell lung cancer) cell lines (Figures S66 and S67 and Table 5). The results indicate that the cytotoxic activity of the molecules is altered, corresponding to their substitution and connectivity patterns (Table 5). The compounds with an ether linkage between C-3A and C-2B of the sugar D-olivose (A) and L-cinerulose (B) sugar moieties showed slightly better activity than the opened one, indicating that the rigidity of these two doubly linked sugar moieties may play an important role in improving the activity. Notably, compounds with fewer sugar residues are less active. The α,β-conjugated double bond (Michael acceptor) in the L-

dx.doi.org/10.1021/np300316b | J. Nat. Prod. 2012, 75, 1383−1392

Table 4. ^{13}C NMR (125 MHz) Assignments of Saquayamycins A (7), J (4), and K (5) in $CDCl_3$, δ in ppm relative to TMS

	saq. A (7)	saq. J (4)	saq. K (5)		saq. A (7)	saq. J (4)	saq. K (5)
position	δ_C, mult.[a]	δ_C, mult.[a]	δ_C, mult.[a]	position	δ_C, mult.[a]	δ_C, mult.[a]	δ_C, mult.[a]
1	205.1, C	205.0, C	205.1, C	5A	74.5, CH	74.7, CH	74.7, CH
2	50.3, CH_2	50.4, CH_2	50.4, CH_2	6A	18.5, CH_3	17.6, CH_3	18.7, CH_3
3	82.7, C	82.6, C	82.8, C	**Sugar B, α-L-aculose or α-L-cinerulose or α-L-rhodinose**			
3-CH_3	25.6, CH_3	25.6, CH_3	25.6, CH_3	1B	95.2, CH	91.5, CH	98.9, CH
4	44.6, CH_2	44.7, CH_2	44.7, CH_2	2B	142.4, CH	71.3, CH	27.3, CH_2
4a	80.1, C	80.1, C	80.2, C	3B	127.4, CH	40.1, CH_2	30.1, CH_2
5	145.7, CH	145.8, CH	145.7, CH	4B	195.4, C	208.0, C	71.7, CH
6	117.6, CH	117.6, CH	117.7, CH	5B	71.6, CH	77.9, CH	71.5, CH
6a	138.9, C	138.9, C	139.0, C	6B	15.3, CH_3	16.3, CH_3	18.0, CH_3
7	188.3, C	188.5, C	188.4, C	**Sugar C, α-L-rhodinose**			
7a	114.1, C	114.2, C	114.1, C	1C	92.5, CH	92.8, CH	92.6, CH
8	158.1, C	158.1, C	158.3, C	2C	24.8, CH_2	25.0, CH_2	24.9, CH_2
9	138.3, C	138.0, C	138.8, C	3C	24.7, CH_2	24.7, CH_2	24.7, CH_2
10	133.7, CH	133.8, CH	133.8, CH	4C	76.3, CH	74.4, CH	76.4, CH
11	119.8, CH	119.8, CH	119.9, CH	5C	67.1, CH	67.6, CH	67.1, CH
11a	130.6, C	130.7, C	130.6, C	6C	17.3, CH_3	17.4, CH_3	17.4, CH_3
12	182.4, C	182.4, C	182.5, C	**Sugar D, α-L-aculose or α-L-rhodinose**			
12a	138.9, C	139.1, C	139.0, C	1D	95.4, CH	98.8, CH	95.5, CH
12b	77.6, C	77.6, C	77.6, C	2D	143.3, CH	29.9, CH_2	143.3, CH
Sugar A, β-D-olivose				3D	127.4, CH	27.9, CH_2	127.5, CH
1A	71.2, CH	71.6, CH	71.2, CH	4D	197.0, C	72.3, CH	197.1, CH
2A	39.0, CH_2	36.9, CH_2	38.9, CH_2	5D	70.7, CH	70.4, CH	70.8, CH
3A	71.4, CH	76.9, CH	71.6, CH	6D	15.3, CH_3	18.0, CH_3	15.4, CH_3
4A	89.3, CH	74.6, CH	89.2, CH				

[a]See also Figures S32−37, S40−45, and S54−59.

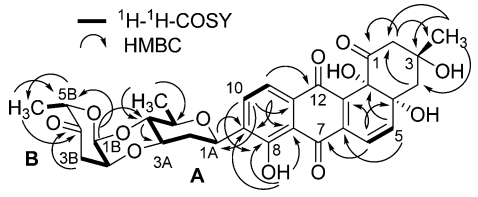


Figure 11. ^{1}H−^{1}H COSY (bold lines) and selected HMBC (→) correlations of saquayamycin B1 (6).

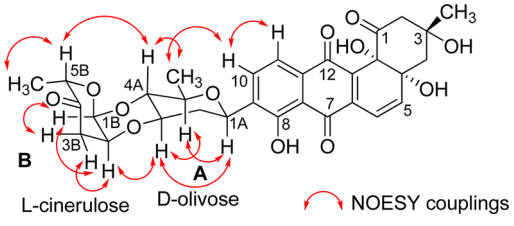


Figure 12. Selected NOESY (↔) correlations in saquayamycin B1 (6).

aculose moiety of saquayamycin B (8) is advantageous compared to the saturated L-rhodinose sugar moiety in the same position of saquayamycin J (4). In the H460 cell line, saquayamycin H (2), with the rare L-rednose sugar moiety, was slightly more active than saquayamycin B (8), which has the L-aculose moiety in the same position, suggesting a potential improvement of activity through the amino group located at the β-position of the L-aculose moiety. Currently, we are investigating the molecular mechanism by which saquayamycins exert their cytotoxic effects on both PC3 and H460 cells.

EXPERIMENTAL SECTION

General Experimental Procedures. UV spectra were recorded on a Shimadzu UV-1800 (model TCC-240A) UV spectrometer. NMR spectra were measured on a Varian Vnmr 500 (^{1}H, 500 MHz; ^{13}C, 125 MHz) spectrometer. ESIMS was recorded on a Finnigan LCQ ion trap mass spectrometer. HRMS was recorded by ESIMS on an Agilent LC/MSD TOF (resolution: 10 000; 3 ppm mass accuracy; inlet systems: Agilent Technologies 1200 Series LC pumps) mass spectrometer (manufacturer: Agilent, Palo Alto, CA, USA). HPLC purifications were carried out using a Symmetry Prep C_{18} 7 μm column (7.8 × 300 mm) on a binary LC system (solvent A: 0.2% aq. formic acid, solvent B: acetonitrile; flow rate: 2.0 mL min^{-1}; 0−15 min, 75−0% A (linear gradient), 15−20 min 0% A and 100% B, 20−22 min 0−75% A (linear gradient), 22−27 min 75% A). HPLC-MS analyses were carried out using a Symmetry analysis C_{18} 5 μm column (4.6 × 250 mm) on a binary LC system. Flash chromatography was carried out on silica gel MN 60 (140−270 mesh ASTM). R_f values were measured on Polygram SIL G/UV$_{254}$ (Macherey-Nagel & Co.). Size exclusion chromatography was performed using Sephadex LH-20 (GE Healthcare).

Cell Viability Assay. Prostate cancer cell line PC3 and non-small-cell lung cancer cell line H460 were used to determine the cytotoxicity of saquayamycins G−K (1−5), B1 (6), A (7), and B (8) and landomycin A. Cells were plated in 96-well plates at a density of 5 × 10^3 cells per well in 100 μL of full growth media and allowed to adhere overnight. The following day, media was replaced with 100 μL of fresh media containing DMSO control or serial concentrations of the test compounds, and plates were incubated for 48 h at 37 °C. At the end of the incubation period, 10 μL of Resazurin (Sigma-Aldrich, St. Louis, MO, USA) was added to each well and further incubated for 3 h at 37 °C. Cell viability was determined by measuring the fluorescence at 560 nm excitation wavelength and 590 nm emission wavelength using a Molecular Devices Spectramax M5 plate reader. Fluorescence was measured at the time of treatment (time zero) and subtracted from the fluorescence values obtained after 48 h of treatment. Fifty percent growth inhibition (GI_{50}) was measured using nonlinear regression

dx.doi.org/10.1021/np300316b | J. Nat. Prod. 2012, 75, 1383−1392

Table 5. Cytotoxic Activity of the New Angucyclines 1−6, Compared with the Previously Known Saquayamycins A (7) and B (8) and with the Angucyclin Lead Compound Landomycin A[37−39] (GI_{50} values, μM)

		PC3 cells, 48 h		H460 cells, 48 h	
no.	name and structure	GI_{50} (μM)	95% confidence intervals	GI_{50} (μM)	95% confidence intervals
1	saquayamycin G (R^1 = I, R^2 = X)	0.5535	0.4879−0.6280	6.718	4.771−9.459
2	saquayamycin H (R^1 = II, R^2 = XI)	0.7898	0.7354−0.8482	3.302	2.102−5.188
3	saquayamycin I (R^1 = III, R^2 = X)	0.9149	0.8087−1.035	7.943	5.557−11.35
4	saquayamycin J (R^1 = II, R^2 = XII)	0.1791	0.1425−0.2251	5.694	4.284−7.567
5	saquayamycin K (R^1 = V, R^2 = X)	0.1478	0.09491−0.2303	7.281	5.519−9.605
6	saquayamycin B1 (R^1 = II, R^2 = H)	1.759	0.2774−11.16	13.20	7.965−21.87
7	saquayamycin A (R^1 = IV, R^2 = X)	0.1057	0.09697−0.1153	6.195	4.617−8.312
8	saquayamycin B (R^1 = II, R^2 = X)	0.07454	0.06720−0.08267	3.929	2.895−5.333
	landomycin A	0.5505	0.4982−0.6081	4.109	2.548−6.626

analysis and by fitting a sigmoidal dose−response curve to the data using GraphPad Prism (GraphPad Software Inc., Version 5.0). The well-studied cytostatic angucycline lead compound landomycin A was used as a positive control standard.[37−39] Experiments were performed in four replicates.

SG-Medium. Glucose (20.0 g), yeast extract (5.0 g), Soytone (10.0 g), $CoCl_2 \cdot 6H_2O$ (1.0 mg), and calcium carbonate (2.0 g) were dissolved in 1 L of demineralized water. The suspension (pH 7.2) was sterilized by autoclaving for 33 min at 121 °C.

M_2-Agar. Glucose (4.0 g), yeast extract (4.0 g), malt extract (10.0 g), and agar (15.0 g) were dissolved in 1 L of demineralized water, then sterilized for 33 min at 121 °C. The medium was adjusted to pH 7.2 with 2 N NaOH before sterilization.

Fermentation, Extraction, and Isolation. *Streptomyces* sp. KY40-1 (originally isolated, purified, and taxonomically identified by M. K. Kharel from a soil sample collected from the foothills of the Appalachian Mountains near Cave Run Lake, Kentucky, USA, maintained as glycerol spore suspension at −80 °C and named KY002)[7] was cultivated on M_2-agar plates at 28 °C for 3 days. With pieces of well-grown agar cultures of *Streptomyces* sp. KY40-1, a 250 mL Erlenmeyer flask preculture containing 100 mL of SG-medium was inoculated and cultivated at 28 °C (250 rpm) for 3 days. The obtained 100 mL of preculture was used to inoculate 60 250 mL Erlenmeyer flasks (each with 1 mL of preculture), each containing 100 mL of SG-medium, and incubated at 28 °C and 250 rpm. After 4 days the culture broth was harvested. The obtained reddish-brown culture broth was mixed with Celite and filtered, affording the mycelium and water phases. The mycelium was extracted with EtOAc (4 × 300 mL), sonicated, and filtered, while the water phase was extracted with EtOAc (3 × 2 L). Extracts were combined and evaporated to dryness under vacuum at 35 °C, giving 2.30 g of a dark red solid crude extract, whose chromatographic purification yielded known metabolites and six new congeners (**1**−**6**). The crude extract (2.30 g) was chromatographed on silica gel (column 2 × 50 cm) using a stepwise MeOH−CH_2Cl_2 gradient (0−100% MeOH) and monitoring by TLC to yield fractions FI (1.0 g, yellow-orange solid), FII (0.1 g, orange solid), FIII (0.2 g, orange-red solid), FIV (0.15 g, orange-red solid), FV (0.1 g, orange solid), FVI (0.95 g, orange solid), and FVII (0.1 g, yellow-orange solid) (see also Supporting Information Figure S1). Purification of fraction FI using HPLC (SymmetryPrep C_{18} 7 μm, 7.8 × 300 mm column; MeCN−H_2O, 0.2% formic acid; flow rate 2.0 mL min^{-1}) afforded saquayamycin A (**7**; orange-red solid, 26.3 mg) and saquayamycin B (**8**; yellow-orange powder, 95.3 mg) along with the subfraction FIC. Further fractionation and purification of the subfraction FIC using PTLC (see Figure S1, for the PTLC eluting system conditions) and Sephadex LH-20 (2 × 50 cm, 50% MeOH−CH_2Cl_2) yielded saquayamycins B1 (**6**; yellow solid, 2.3 mg), J (**4**; yellow-orange solid, 10.7 mg), and K (**5**; yellow-orange solid, 8.3 mg), and further separation and purification of fractions FIII and FIV following Figure S1 gave saquayamycins G (**1**; orange-red solid, 10.5 mg), H (**2**; orange-red solid, 8.3 mg), and I (**3**; orange-red solid, 9.2 mg) along with 4′,7-dihydroxyisoflavanone (white solid, 8.7 mg). Fractions FII and FV−FVII were excluded on the basis of the TLC and HPLC-MS analysis, since no major products were isolated in sufficient amounts for NMR characterization (Figure S1).

*Saquayamycin G (**1**):* orange-red solid; R_f 0.56 (silica gel, MeOH−CH_2Cl_2, 7:93 v/v), blue-violet coloration with 2 N NaOH; UV/vis (MeOH) λ_{max} (log ε) 218 (4.50), 330 sh (4.05), 433 (4.14), 468 sh (4.11) nm; ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; (−)-APCI MS m/z 709 $[M - H]^-$; (−)-ESIMS m/z 709 $[M - H]^-$; (+)-ESIMS m/z 711 $[M + H]^+$; (−)-HRESIMS m/z 709.2501 $[M - H]^-$ (calcd for $C_{37}H_{41}O_{14}$, 709.2502) and m/z 691.2393 $[M - H_2O - H]^-$ (calcd for $C_{37}H_{39}O_{13}$, 691.2396).

*Saquayamycin H (**2**):* orange-red solid; orange fluorescence under long UV (365 nm); R_f 0.47 (silica gel, MeOH−CH_2Cl_2, 7:93 v/v); blue-violet coloration with 2 N NaOH; UV/vis (MeOH) λ_{max} (log ε) 218 (4.66), 281 (4.59), 330 sh (4.14), 433 (4.27), 468 sh (4.22) nm; ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; (−)-APCI MS m/z 834 $[M - H]^-$; (−)-ESIMS m/z 834 $[M - H]^-$; (+)-ESIMS m/z 836 $[M + H]^+$; (−)-HRESIMS m/z 834.2968 $[M - H]^-$ (calcd for $C_{43}H_{48}NO_{16}$, 834.2978) and m/z 816.2862 $[M - H_2O - H]^-$ (calcd for $C_{43}H_{46}NO_{15}$, 816.2872).

*Saquayamycin I (**3**):* orange-red solid; orange fluorescence under long UV (365 nm); R_f 0.29 (silica gel, MeOH−CH_2Cl_2, 7:93 v/v); blue-violet coloration with 2 N NaOH; UV/vis (MeOH) λ_{max} (log ε) 218 (4.65), 281 (4.50), 329 sh (4.15), 435 (4.25), 468 sh (4.20) nm; ^{1}H NMR (acetone-d_6, 500 MHz), see Table 1; ^{13}C NMR (acetone-d_6, 125 MHz), see Table 2; (−)-APCI MS m/z 834 $[M - H]^-$; (−)-ESIMS m/z 834 $[M - H]^-$; (+)-ESIMS m/z 836 $[M + H]^+$; (−)-HRESIMS m/z 834.2968 $[M - H]^-$ (calcd for $C_{43}H_{48}NO_{16}$, 834.2978) and m/z 816.2862 $[M - H_2O - H]^-$ (calcd for $C_{43}H_{46}NO_{15}$, 816.2872).

*Saquayamycin J (**4**):* yellow-orange solid; UV absorbing (254 nm), orange-red fluorescence under long UV (365 nm); R_f 0.41 (silica gel, MeOH−CH_2Cl_2, 7:93 v/v), 0.17 (silica gel, 70% EtOAc−*n*-hexane); blue-violet coloration with 2 N NaOH; UV/vis (MeOH) λ_{max} (log ε) 218 (4.61), 328 sh (4.15), 431 (4.25), 468 sh (4.20) nm; ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 3; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 4; (−)-APCI MS m/z 823 $[M - H]^-$; (−)-ESIMS m/z 823 $[M - H]^-$; (+)-ESIMS m/z 825 $[M + H]^+$; (−)-HRESIMS m/z 823.3189 $[M - H]^-$ (calcd for $C_{43}H_{51}O_{16}$, 823.3182), and m/z 805.3085 $[M - H_2O - H]^-$ (calcd for $C_{43}H_{49}O_{15}$, 805.3077).

*Saquayamycin K (**5**):* yellow-orange solid; UV absorbing (254 nm), orange-red fluorescence under long UV (365 nm); R_f 0.42 (silica gel, MeOH−CH_2Cl_2, 7:93 v/v), 0.21 (silica gel, 70% EtOAc/*n*-hexane); blue-violet coloration with 2 N NaOH; UV/vis (MeOH) λ_{max} (log ε) 218 (4.66), 328 sh (4.15), 434 (4.26), 468 sh (4.21) nm; ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 3; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 4; (−)-APCI MS m/z 823 $[M - H]^-$; (−)-ESIMS m/z 823 $[M - H]^-$; (+)-ESIMS m/z 825 $[M + H]^+$; (−)-HRESIMS m/z 823.3189 $[M - H]^-$ (calcd for $C_{43}H_{51}O_{16}$, 823.3182), and m/z 805.3085 $[M - H_2O - H]^-$ (calcd for $C_{43}H_{49}O_{15}$, 805.3077).

*Saquayamycin B1 (**6**):* yellow solid; UV absorbing (254 nm); R_f 0.50 (silica gel, MeOH−CH_2Cl_2, 7:93 v/v); blue-violet coloration with 2 N NaOH; UV/vis (MeOH) λ_{max} (log ε) 218 (4.23), 328 sh (3.88),

dx.doi.org/10.1021/np300316b | J. Nat. Prod. 2012, 75, 1383−1392

429 (3.98), 468 sh (3.94) nm; ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 3; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; (−)-APCI MS m/z 595 $[M - H]^-$; (−)-ESIMS m/z 595 $[[M - H]^-$; (+)-ESIMS m/z 597 $[M + H]^+$; (−)-HRESIMS m/z 595.1821 $[M - H]^-$ (calcd for $C_{31}H_{31}O_{12}$, 595.1821), m/z 577.1724 $[M - H_2O - H]^-$ (calcd for $C_{31}H_{29}O_{11}$, 577.1715), m/z 559.1604 $[M - 2H_2O - H]^-$ (calcd for $C_{31}H_{27}O_{10}$, 559.1609), and m/z 1191.3703 $[2M - H]^-$ (calcd for $C_{62}H_{63}O_{24}$, 1191.3714).

*Saquayamycin A (**7**):* orange-red solid; UV absorbing (254 nm); orange-red fluorescence under long UV (365 nm); R_f 0.69 (silica gel, MeOH−CH_2Cl_2, 7:93 v/v); blue-violet coloration with 2 N NaOH; ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 3; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 4; (−)-APCI MS m/z 819 $[M - H]^-$.

*Saquayamycin B (**8**):* yellow-orange powder; UV absorbing (254 nm); orange-red fluorescence under long UV (365 nm); R_f 0.70 (silica gel, MeOH−CH_2Cl_2, 7:93 v/v); blue-violet coloration with 2 N NaOH; UV/vis (MeOH) λ_{max} (log ε) 218 (4.50), 330 sh (4.12), 428 (4.24), 468 sh (4.20) nm; ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; (−)-APCI MS m/z 819 $[M - H]^-$.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

HPLC analysis chromatogram of the crude extract obtained from *Streptomyces* sp. KY40-1; workup procedure scheme; HRMS, NMR, and UV spectra of angucyclines (**1**−**8**); TLC of strain extract, cultivation figures, and PTLC isolation of saquayamycins J (**4**) and K (**5**). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: jrohr2@email.uky.edu. Fax: 859-257-7564.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the mass spectrometry facility of the Biotechnology Center of the University of Wisconsin for the HRESI mass spectra. This work was supported by grants CA 102102 and CA 091901of the National Institutes of Health to J.R.

■ REFERENCES

(1) Krohn, K.; Rohr, J. *Top. Curr. Chem.* **1997**, *188*, 127−195.
(2) Rohr, J.; Thiericke, R. *Nat. Prod. Rep.* **1992**, *9*, 103−137.
(3) Kharel, M. K.; Pahari, P.; Shepherd, M. D.; Tibrewal, N.; Nybo, S. E.; Shaaban, K. A.; Rohr, J. *Nat. Prod. Rep.* **2012**, *29*, 264−325.
(4) Uchida, T.; Imoto, M.; Watanabe, Y.; Miura, K.; Dobashi, T.; Matsuda, N.; Sawa, T.; Naganawa, H.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **1985**, *38*, 1171−1181.
(5) Sekizawa, R.; Iinuma, H.; Naganawa, H.; Hamada, M.; Takeuchi, T.; Yamaizumi, J.; Umezawa, K. *J. Antibiot.* **1996**, *49*, 487−490.
(6) Ströch, K.; Zeeck, A.; Antal, N.; Fiedler, H. P. *J. Antibiot.* **2005**, *58*, 103−110.
(7) Abdelfattah, M. S.; Kharel, M. K.; Hitron, J. A.; Baig, I.; Rohr, J. *J. Nat. Prod.* **2008**, *71*, 1569−1573.
(8) Henkel, T.; Zeeck, A. *J. Antibiot.* **1990**, *43*, 830−837.
(9) Rohr, J.; Zeeck, A. *J. Antibiot.* **1987**, *40*, 459−467.
(10) Rohr, J.; Zeeck, A.; Floss, H. G. *J. Antibiot.* **1988**, *41*, 126−129.
(11) Rohr, J.; Beale, J. M.; Floss, H. G. *J. Antibiot.* **1989**, *42*, 1151−1157.
(12) Rohr, J. *J. Chem. Soc., Chem. Commun.* **1990**, 113−114.
(13) Rohr, J. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1051−1053.
(14) Rohr, J. *J. Antibiot.* **1989**, *42*, 1482−1488.
(15) Rix, U.; Remsing, L. L.; Hoffmeister, D.; Bechthold, A.; Rohr, J. *ChemBioChem* **2003**, *4*, 109−111.
(16) Rohr, J. *J. Chem. Soc., Chem. Commun.* **1989**, 492−493.
(17) Fedoryshyn, M.; Nur-e-Alam, M.; Zhu, L.; Luzhetskyy, A.; Rohr, J.; Bechthold, A. *J. Biotechnol.* **2007**, *130*, 32−38.
(18) Henkel, T.; Rohr, J.; Beale, J. M.; Schwenen, L. *J. Antibiot.* **1990**, *43*, 492−503.
(19) Weber, S.; Zolke, C.; Rohr, J.; Beale, J. M. *J. Org. Chem.* **1994**, *59*, 4211−4214.
(20) Zhu, L.; Luzhetskyy, A.; Luzhetska, M.; Mattingly, C.; Adams, V.; Bechthold, A.; Rohr, J. *ChemBioChem* **2007**, *8*, 83−88.
(21) Zhou, M.; O'Doherty, G. A. *Org. Lett.* **2008**, *10*, 2283−2286.
(22) Ostash, B.; Korynevska, A.; Stoika, R.; Fedorenko, V. *Mini Rev. Med. Chem* **2009**, *9*, 1040−1051.
(23) Shaaban, K. A.; Srinivasan, S.; Kumar, R.; Damodaran, C.; Rohr, J. *J. Nat. Prod.* **2011**, *74*, 2−11.
(24) Shaaban, K. A.; Stamatkin, C.; Damodaran, C.; Rohr, J. *J. Antibiot.* **2011**, *64*, 141−150.
(25) Sezaki, M.; Kondo, S.; Maeda, K.; Umezawa, H.; Ono, M. *Tetrahedron* **1970**, *26*, 5171−90.
(26) Ohta, K.; Kamiya, K. *J. Chem. Soc., Chem. Commun.* **1981**, 154−155.
(27) Ohta, K.; Mizuta, E.; Okazaki, H.; Kishi, T. *Chem. Pharm. Bull.* **1984**, *32*, 4350−4359.
(28) Alvi, K. A.; Baker, D. D.; Stienecker, V.; Hosken, M.; Nair, B. G. *J. Antibiot.* **2000**, *53*, 496−501.
(29) Erb, A.; Luzhetskyy, A.; Hardter, U.; Bechthold, A. *ChemBioChem* **2009**, *10*, 1392−1401.
(30) Ren, X.; Lu, X.; Ke, A.; Zheng, Z.; Lin, J.; Hao, W.; Zhu, J.; Fan, Y.; Ding, Y.; Jiang, Q.; Zhang, H. *J. Antibiot.* **2011**, *64*, 339−343.
(31) Ding, C.; Tu, S.; Li, F.; Wang, Y.; Yao, Q.; Hu, W.; Xie, H.; Meng, L.; Zhang, A. *J. Org. Chem.* **2009**, *74*, 6111−6119.
(32) Martin, G. D. A.; Tan, L. T.; Jensen, P. R.; Dimayuga, R. E.; Fairchild, C. R.; Raventos-Suarez, C.; Fenical, W. *J. Nat. Prod.* **2007**, *70*, 1406−1409.
(33) Schneemann, I.; Kajahn, I.; Ohlendorf, B.; Zinecker, H.; Erhard, A.; Nagel, K.; Wiese, J.; Imhoff, J. F. *J. Nat. Prod.* **2010**, *73*, 1309−1312.
(34) Sezaki, M.; Hara, T.; Ayukawa, S.; Takeuchi, T.; Okami, Y. *J. Antibiot.* **1968**, *21*, 91−97.
(35) Johdo, O.; Yoshioka, T.; Naganawa, H.; Takeuchi, T.; Yoshimoto, A. *J. Antibiot.* **1996**, *49*, 669−675.
(36) Nettleton, D. E., Jr.; Balitz, D. M.; Doyle, T. W.; Bradner, W. T.; Johnson, D. L.; O'Herron, F. A.; Schreiber, R. H.; Coon, A. B.; Moseley, J. E.; Myllymaki, R. W. *J. Nat. Prod.* **1980**, *43*, 242−258.
(37) Depenbrock, H.; Bornschlegl, S.; Peter, R.; Rohr, J.; Schmid, P.; Schweighart, P.; Block, T.; Rastetter, J.; Hanauske, A. R. *Ann. Hematol.* **1996**, *73* (Supl. II), A80/316.
(38) Korynevska, A.; Heffeter, P.; Matselyukh, B.; Elbling, L.; Micksche, M.; Stoika, R.; Berger, W. *Biochem. Pharmacol.* **2007**, *74*, 1713−1726.
(39) Crow, R. T.; Rosenbaum, B.; Smith, R., 3rd; Guo, Y.; Ramos, K. S.; Sulikowski, G. A. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1663−1666.