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Publisher's version / Version de l'éditeur:
https://doi.org/10.1021/acs.jnatprod.5b00277
Journal of Natural Products, 78, 8, pp. 1942-1948, 2015-07-23

JOURNAL OF NATURAL PRODUCTS

Article

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Characterization of L-Digitoxosyl-phenanthroviridin from *Streptomyces venezuelae* ISP5230

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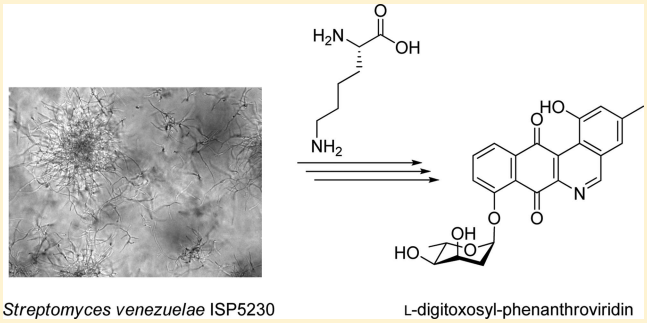
Supporting Information

ABSTRACT: The jadomycin-derived compound L-digitoxosyl-phenanthroviridin was isolated from fermentations of *Streptomyces venezuelae* ISP5230 grown in nutrient-deficient media with L-lysine as the sole nitrogen source. Structural elucidation was accomplished using a combination of high-resolution MS, LC-MS/MS, and 1D- and 2D-NMR. The compound was evaluated against the National Cancer Institute (NCI) 60 human tumor cell line screen in both the one-dose and five-dose screens, and cytotoxicity was compared to a small library of jadomycin analogues to probe the structure–activity relationship.



The angucyclines are the largest known group of natural products derived from type II polyketide synthases (PKSs).[1] The structural diversity of this large family of natural products arises from the configurations of polyaromatic angucycline backbones and the modification of these scaffolds by a wide array of post-PKS tailoring enzymes. A great deal of interest in their continued isolation and characterization is in anticipation of identifying novel bioactive natural products. The soil bacterium *Streptomyces venezuelae* ISP5230 has been studied extensively for its ability to produce the jadomycin group of natural products.[2] The jadomycins are grouped within the angucycline family and are distinguished by a characteristic modified benz[*a*]anthracene scaffold, a 2,6-dideoxysugar moiety, L-digitoxose, and an amino acid that is usually fused directly into the polyaromatic backbone as an oxazolone ring. The first isolated examples were that of the aglycone jadomycin A[3] and the glycosylated jadomycin B (Figure 1).[4,5] Interestingly, amino acid incorporation has been shown to proceed through a spontaneous process in which the enzyme JadG is responsible for a C–C bond cleavage at the B-ring (Scheme 1).[1,6,7] This proceeds via a Baeyer–Villiger-type mechanism producing a reactive aldehyde intermediate, which couples with an amino acid, forming an imine, which undergoes spontaneous cyclization and oxidation to produce a five-membered oxazolone ring (Scheme 1). This mechanism has been confirmed through both extensive biosynthetic and total synthetic studies,[7−9] and exploitation of this mechanism by precursor-directed derivatization with varying amino acids has led to the isolation of upward of 25 jadomycin analogues.[2,10−14] The glycosyltransferase JadS then appends the L-digitoxyl moiety to the aglycones, yielding the fully furnished natural products (Scheme 1).

Engineering approaches have also been successful in expanding the structural diversity of these compounds: disruption of the *jadO* gene, coding for a putative 2,3-dehydratase, resulted in the production and isolation of ILEVS1080, a differentially glycosylated analogue of jadomycin B.[17] Rohr and co-workers isolated and characterized the glycosylated analogue L-digitoxosyl-dehydrorabelomycin from a *jadG* deletion mutant (Figure 1).[18] Lacking the ability to catalyze the ring-opening step of biosynthesis, a buildup of the jadomycin precursor dehydrorabelomycin (Scheme 1) and the glycosylated analogue was observed, coupled with complete loss of jadomycin production.[18] The isolation of the phenanthroviridin aglycone has also been reported from cultures of *S. venezuelae* ISP5230 (Figure 1). It was simultaneously identified during the first isolation of jadomycin A (Figure 1).[3] However, the glycosylated analogue L-digitoxosyl-phenanthroviridin (**1**) has not been reported to date.

Recently, we reported the isolation and characterization of jadomycin Oct and jadomycin AVA, both containing eight-membered heterocyclic rings (Scheme 1).[15] Cyclization to the eight-membered ring occurs as a result of initial imine formation with the δ-amine, in preference to the α-amino group. In an effort to isolate the jadomycin incorporating L-lysine, where we anticipated formation of a nine-membered heterocyclic ring, *S. venezuelae* fermentations in the presence of L-lysine were performed. Although production of a jadomycin analogue with the appropriate m/z for L-lysine incorporation was confirmed by LC-MS/MS analysis of the crude growth

Received: March 31, 2015
Published: July 23, 2015

DOI: 10.1021/acs.jnatprod.5b00277
J. Nat. Prod. 2015, 78, 1942–1948

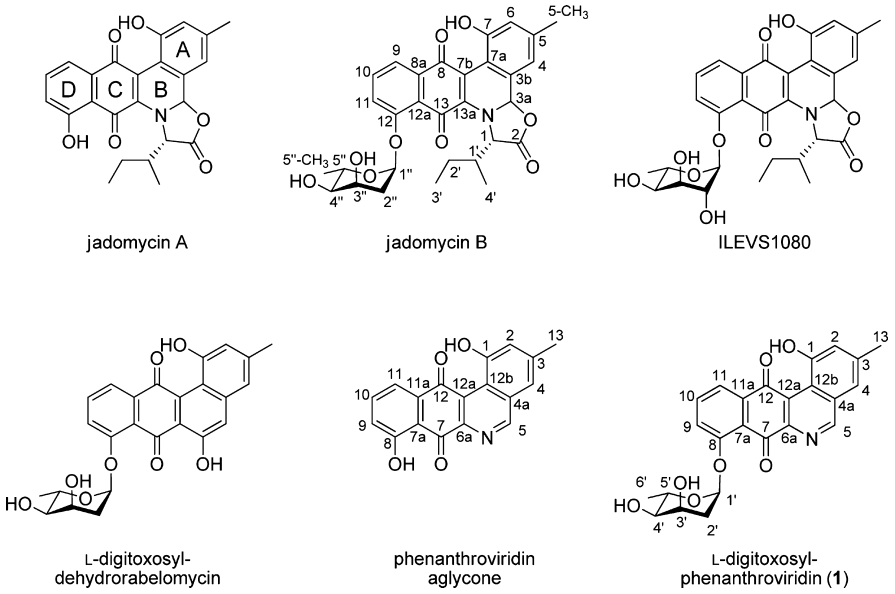
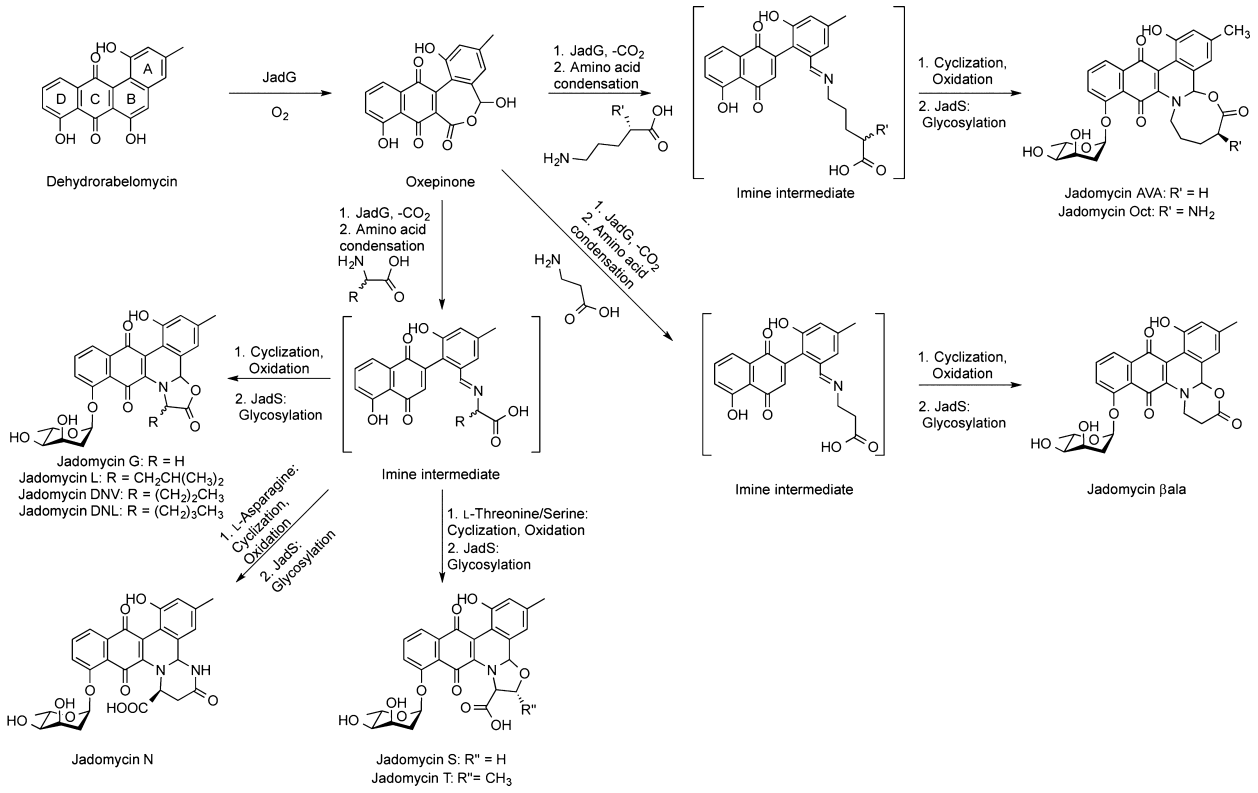


Figure 1. Structures of jadomycin A;[3] jadomycin B;[4,5] ILEVS1080;[17] L-digitoxosyl-dehydrorabelomycin;[18] phenanthroviridin aglycone;[3] and L-digitoxosyl-phenanthroviridin (**1**).

Scheme 1. Substrate Scope of Amino Acid Incorporation into the Jadomycin Backbone[8,11,13−16]



media,[19] attempts to isolate the product using standard methodologies successfully employed for other jadomycins or by chemical derivatization were unsuccessful due to compound instability.[15,20] During investigations of crude fermentation extracts, we identified an intriguing unknown amber-colored compound by TLC that proved to be sufficiently stable for isolation and characterization. Herein, we report the isolation, characterization, and cytotoxic evaluation of the new phenanthroviridin analogue L-digitoxosyl-phenanthroviridin (**1**).

RESULTS AND DISCUSSION

Fermentation, Isolation, and Purification of 1. *S. venezuelae* ISP5230 VS1099 was grown in the presence of L-lysine (60 mM) as the sole nitrogen source using literature methodology.[21] The fermentation was allowed to proceed for 48 h while monitoring by absorption spectroscopy for cell growth at 600 nm and the production of colored natural products at 526 nm (Figure S1). After 48 h, growth media was colored reddish-purple, indicating jadomycin production. The

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J. Nat. Prod. 2015, 78, 1942−1948

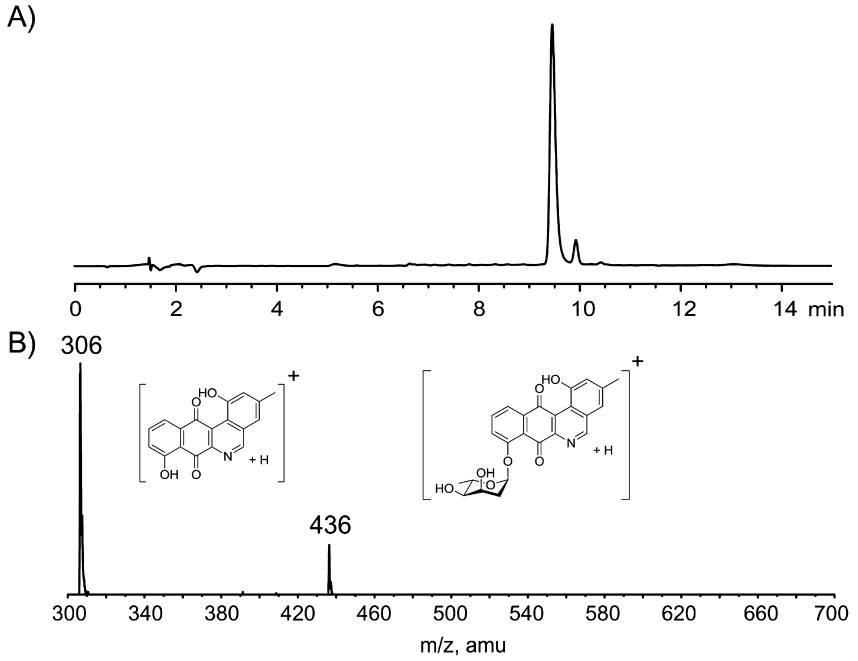

Figure 2. (A) HPLC trace of **1** (t_R = 9.47 min) monitored at 254 nm; (B) LC-MS/MS fragmentation of **1**, showing parent ion $[M + H]^+$ and fragmentation resulting from the cleavage of L-digitoxose $[M + H - \text{digitoxose}]^+$ (m/z 306).

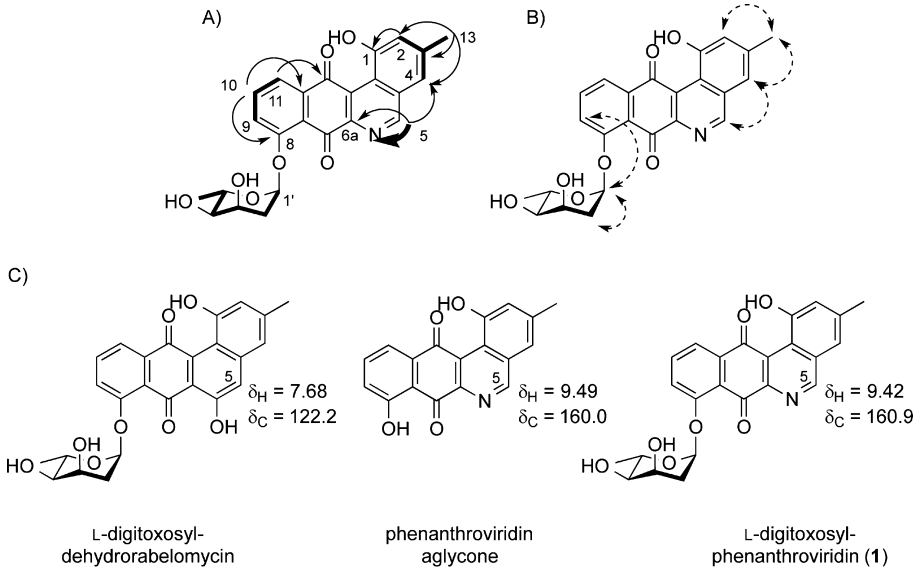

Figure 3. (A) Key $^1H-^1H$ COSY (bold), $^1H-^{13}C$ HMBC (solid arrows), and $^1H-^{15}N$ HMBC (bold arrow) correlations in **1**; (B) key NOESY (dashed arrows) correlations of **1**; (C) δ_H and δ_C values of H5 and C5 associated with the compounds L-digitoxosyl-dehydrorabelomycin,[18] phenanthroviridin aglycone,[3] and **1**.

cells were removed, and the clarified growth media was applied to a reversed-phase phenyl column enabling retention of aromatic organic compounds. The aromatic organic material was eluted with 100% methanol and extracted with water and ethyl acetate, yielding ~45 mg L^{-1} of crude solid material. TLC analysis of the ethyl acetate extract identified a yellow compound as the predominant natural product in the mixture. Preparative TLC was performed on the material, yielding 8 mg of the amber yellow solid, compound **1** (4 mg L^{-1}), of sufficient purity for characterization.

Structural Elucidation of 1. High-resolution mass spectrometry (HRMS) identified a molecular formula of $C_{24}H_{21}NO_7$. LC-MS/MS analysis scanning for $[M + H]^+$ identified an m/z of 436, with fragmentation to m/z of 306. This fragmentation pattern is typical of jadomycin-like molecules and represents the loss of L-digitoxose, identifying a glycosylation of the unknown compound (Figure 2).[11]

Structural characterization was accomplished using a combination of ^1H, ^{13}C, COSY, edited-HSQC, HMBC, and NOESY NMR spectroscopy experiments. The ^1H NMR and COSY experiments confirmed the presence of the L-digitoxose spin system (C1′ through C6′). In addition, the characteristic jadomycin A-ring (C2, C4, and C13) and D-ring (C9 through C11) spin systems were observed. An exchangeable singlet (not observable in MeOD-d_4) at δ_H = 11.84 ppm was also present, corresponding to the 1-OH. HMBC data provided core

DOI: 10.1021/acs.jnatprod.5b00277
J. Nat. Prod. 2015, 78, 1942−1948

connectivity typical of a jadomycin (Figure 3). No signals arising from the incorporation of L-lysine (or any other amino acid) were present. The key signal in the ^{1}H NMR was a nonexchangeable CH proton at δ_H = 9.42 ppm with an HSQC correlation to a carbon at δ_C = 160.9 ppm. This relatively downfield chemical shift suggested a distinct heteroaromatic chemical environment resembling that of the phenanthroviridin aglycone at C5, as opposed to L-digitoxosyl-dehydrorabelomycin, which lacks the heteroaromatic ring (Figure 3). HMBC and 2D-NOESY correlations from H5 to C4 and H4, respectively, confirmed the proximity of the proton to the A-ring; HMBC analysis also identified a correlation to the C6a position of the B-ring. ^{1}H−^{15}N HMBC analysis established a correlation between H5 and N6, confirming the presence of a heteroaromatic nitrogen. Glycosylation at the 8-position was confirmed by NOESY correlations between H9 of the D-ring and H1′ of the L-digitoxose moiety and an HMBC correlation between H1′ and C8 of the D-ring (Figure 3). Stereochemistry of the sugar moiety was inferred as L-digitoxose based on past X-ray crystallographic studies of jadomycin B.[22] The biosynthetic production of the dideoxysugar is well established for this family of molecules, and the NMR data associated with the sugar moiety match those of previously published jadomycin and jadomycin-like compounds.[18] These data are consistent with our proposed structure of **1** as a functionalized benzo[*b*]phenanthridine framework glycosylated with L-digitoxose. A full tabulated list of ^{1}H and ^{13}C chemical shift and 2D-correlations for compound **1** can be found in Table 1.

Our difficulties in isolation of the jadomycin L-lysine derivative paralleled work by Yang and co-workers, who attempted purification of the compound but were hampered by product instability and low yields that led to incomplete structural characterization.[10] We propose **1** is likely a stable degradation product of the L-lysine analogue. Isolation of **1** presented an opportunity to test the bioactivity of a unique jadomycin-like analogue and to probe the structure−activity relationship associated with the oxazolone ring system.

Cytotoxic Activity. Compound **1** was selected for evaluation against the National Cancer Institute's (NCI) 60 DTP human tumor cell line screen. All screening was carried out according to the NCI protocol with the exception of solvent, where ethanol was substituted for dimethyl sulfoxide due to compound stability. Initial single dose screening (10 μM) identified sufficient cytotoxicity (Table S1) for further testing in a five-dose assay (10 nM to 100 μM) using $\log_{10}$ concentration intervals. Percent growth was plotted as a function of the concentration of **1** giving dose−response curves for each tumor cell line (Figures S3 and S4). From these curves the GI_{50} (growth inhibition of 50%), TGI (drug concentration resulting in total growth inhibition), and LC_{50} (lethal concentration resulting in 50% tumor death) were calculated. With the exception of leukemia, compound **1** showed respectable cytotoxicity, in many cases resulting in complete or near-complete tumor death at higher concentrations (100 μM).

Having access to cytotoxicity data for a series of jadomycin derivatives previously reported by our lab, direct comparison of the GI_{50}, TGI, and LC_{50} values of **1** to this group of compounds was explored. It was discovered that despite lacking an amino acid side chain and an oxazolone ring, **1** had comparable bioactivity when compared to this small library of natural and semisynthetic jadomycins. These data are summarized in Table 2.

Table 1. NMR Spectroscopic Data (^{1}H: 700 MHz and ^{13}C: 176 MHz, CD_2Cl_2) for L-Digitoxosyl-phenanthroviridin (1)

position	δ_C, type	δ_H (*J* in Hz)[a]	COSY	HMBC	NOESY
1	155.3, C				
2	123.6, CH	7.32, d (1.4)	4, 13	1, 4, 13	13
3	144.2, C				
4	121.5, CH	7.46, brs	2, 13	2, 4a, 5, 13	5, 13
4a	132.8, C				
5	160.9, CH	9.42, s		4a, 4, 6a, 12b	4
6a	147.4, C				
7	181.5, C				
7a	120.1, C				
8	156.2, C				
9	121.9, CH	7.63, d (8.5)	10	7a, 11	1′
10	136.2, CH	7.76, t (8.1)	9, 11	8, 11a	
11	122.7, CH	8.00, d (7.6)	10	7a, 9, 12	
11a	136.3, C				
12	191.1, C				
12a	126.9, C				
12b	120.6, C				
13	21.7, CH_3	2.55, s	2, 4	2, 3, 4	2, 4
1′	95.2, CH	5.96, d (3.2)	2′	8, 3′	9, 2a′, 2b′
2a′	35.6, CH_2	ax: 2.24, ddd (15.1, 3.2, 3.2)[b]	1′, 2b′, 3′	none	1′, 2b′, 3′, 4′
2b′	35.6, CH_2	eq: 2.52, ddd (15.1, 3.2, 0.9)[c]	1′, 2a′, 3′	3′, 4′	1′, 2a′, 3′
3′	66.5, CH	4.11, dq (10.1, 3.2)	2a′, 2b′, 3′-OH, 4′	none	2a′, 2b′ 4′
4′	72.9, CH	3.24, brm	3′, 4′-OH, 5′	none	2a′, 3′
5′	66.6, CH	3.79, dq (10.1, 6.2)	4′, 6′	none	6′
6′	18.1, CH_3	1.22, d (6.2)	5′	4′, 5′	5′
1-OH		11.84, brs		1, 2, 3	
3′-OH		5.20, brd (10.6)	3′		
4′-OH		2.89, brd (8.7)	4′		

[a]All coupling constants reported were determined using the TopSpin3.2 multiplet tool software; any minor inconsistences are to be attributed to signal broadness and error associated with the measurements. [b]Ax = axial proton at the 2′-position. [c]Eq = equatorial proton at the 2′-position.

In an effort to probe the mechanism of action of **1**, a standard COMPARE[23,24] analysis screening against the NCI standard agents database using GI_{50}, TGI, and LC_{50} values was performed. The screen identified mediocre correlation (Pearson correlation coefficient (PCC) < 0.58) between **1** and the standard agents database, a library of 171 known cytotoxic compounds, when compared to GI_{50}, TGI, and LC_{50} values. Results showed correlations to a wide variety of compounds including known antimitotic agents, alkylating agents, topoisomerase II inhibitors, and a series of compounds with listed unknown function (Figure 4). The highest correlation obtained was associated with trimethyltrimethylolmelamine (PCC = 0.573) while comparing LC_{50} data (Figure 4). This correlation, together with a GI_{50} correlation to pancratiastatin (PCC = 0.467), has been previously reported for structurally similar naphthoquinone moiety containing compounds.[25]

DOI: 10.1021/acs.jnatprod.5b00277
J. Nat. Prod. 2015, 78, 1942−1948

Table 2. Summary of NCI 60 Cancer Cell Line Screen GI_{50}, TGI, and LC_{50} Values of 1 Compared to a Small Library of Jadomycin Analogues[a]

compound[a]	concentration range (nM–μM)[b]	GI_{50} (μM)		TGI (μM)		LC_{50} (μM)	
		median (range)	n	median (range)	n	median (range)	n
1	10–100	2.14 (0.27–16.6)	57	5.75 (1.4–48)	56	18.2 (4.7–96)	41
jadomycin G (Scheme 1)	10–100	1.68 (0.20–21.4)	54	9.6 (0.40–85)	54	38.5 (0.78–96)	42
jadomycin L (Scheme 1)	10–100	1.82 (0.28–11.2)	58	4.1 (0.76–85)	51	9.3 (4.0–79)	43
jadomycin DNV (Scheme 1)	3.2–32	4.79 (0.59–9.1)	59	10 (1.23–31)	57	19 (2.4–32)	42
jadomycin DNL (Scheme 1)	3.2–32	3.89 (0.47–9.3)	59	8.7 (0.91–26)	56	18 (1.8–32)	44
jadomycin T (Scheme 1)	10–100	1.35 (0.17–2.6)	59	3.02 (0.48–8.9)	58	6.5 (1.4–41)	48
S1	1.6–16	3.5 (0.45–16.6)	54	7.2 (1.3–14.9)	40	11.8 (7.7–16.3)	17
S3	2.5–25	6.1 (0.7–23.6)	51	11.9 (2.6–4.6)	28	14.9 (13–21.4)	10
S4	1.6–16	3.1 (0.6–10)	55	6.8 (2.3–15.1)	46	11.6 (8.2–16.3)	25
S5	1.6–16	3.7 (0.5–9.3)	54	6.6 (1.6–15.6)	42	10.9 (8.3–15)	19
S6	1.6–16	3.1 (0.5–15.6)	57	6.3 (2.1–16)	51	9.8 (1.0–16.5)	26

[a]Compound list includes jadomycin G,[13] jadomycin L,[14] jadomycin DNV,[11] jadomycin DNL,[11] and jadomycin T (Scheme 1).[13] Compounds **S1** and **S3–S6** are a series of semisynthetic jadomycin triazoles (Figure S6).[12] [b]All values are expressed as median (range), where *n* = the number of cancer cell lines in which GI_{50}, TGI, or LC_{50} were quantifiable below the maximal concentrations used in each experiment.

Correlations, although poor, between **1** and the anthracycline antibiotics daunorubicin (PCC = 0.495), deoxydoxorubicin (PCC = 0.492), and cyanomorpholino-ADR (PCC = 0.522) were observed when comparing LC_{50} values. These clinically used anthracyclines exhibit potent antitumor properties. They impart cytotoxicity via intercalation into DNA, allowing the formation of a stable complex with topoisomerase II, inhibiting the enzymes ability to effectively cut DNA disrupting tumor proliferation.[26,27] The jadomycin family of natural products shares structural similarities to these compounds, and jadomycin inhibition of topoisomerase II has been speculated in the past.[28] This may suggest a similar mode of action for **1** and other jadomycins.

When compared to the synthetic compound database (>40 000 compounds, including synthetic compounds and natural products of known structure), improved correlations between **1** and a number of jadomycins previously isolated in our lab were observed (correlations ~0.7–0.8). Thus, it can be concluded that **1** has a similar mode of action to jadomycins incorporating amino acids through precursor-directed biosynthesis and that the cytotoxic effect may not be strongly dependent on the incorporated amino acid. Rather, the amino acid functionality may tune other physiochemical properties of the natural product. This discovery illustrates the opportunity to direct efforts toward modifying the sugar moiety or derivatizing the polyaromatic backbone in order to identify new analogues with improved or altered bioactivity, while concurrently varying the amino acid incorporated into the jadomycin scaffold to adjust physicochemical properties.

EXPERIMENTAL SECTION

General Experimental Procedures. All reagents were purchased from commercial sources and used without further purification unless otherwise stated. All solvents used for compound purification were HPLC grade. Preparative TLC was carried out using 20 × 20 cm glass-backed plates (SiliCycle) layered with 1000 μm silica. Compound **1** was characterized by liquid chromatography tandem-mass spectrometry (LC-MS/MS), HRMS, and 1D- and 2D-NMR spectroscopy. Low-resolution LC-MS/MS spectra were obtained on an Applied Biosystems hybrid triple quadrupole linear ion trap (2000 Qtrap) mass spectrometer using an electrospray ionization (ESI+) source. This was coupled with an Agilent 1100 high-performance liquid chromatography (HPLC) instrument with a Phenomenex Kinetex 2.6 μm Hilic column (150 mm × 2.10 mm). Samples were prepared in methanol, and 5 μL aliquots were analyzed. Elution of compounds was accomplished using an isocratic gradient of 7:3 CH_3CN/2 mM ammonium acetate in water (pH 5.5) with a flow rate of 120 μL min^{-1} for 10 min. HRMS data were recorded on a Bruker Daltonics MicroTOF Focus mass spectrometer using an ESI+ source. Enhanced product ionization (EPI) was performed with a capillary voltage of +4500 kV, declustering potential of +80 V, and curtain gas of 10 arbitrary units. EPI scans were conducted over a range of 300–700 *m*/*z* scanning for $[M + H]^+$ and the appropriate jadomycin fragmentation. Scans were conducted using two steps, 300.0 to 320 amu (0.005 s) and 300.0 to 700.0 amu (0.100 s). Spectra were analyzed using Analyst software version 1.4.1 (Applied Biosystems). NMR analysis of **1** was performed on either a Bruker AV 500 MHz spectrometer (^1H: 500 MHz, ^{13}C: 125 MHz) equipped with an autotune and match (ATMA) broadband observe SmartProbe located at the Nuclear Magnetic Resonance Research Resource (NMR-3) facility (Dalhousie University) or a Bruker AV-III 700 MHz spectrometer (^1H: 700 MHz, ^{13}C: 176 MHz) equipped with an ATMA 5 mm TCI cryoprobe located at the Canadian National Research Council Institute for Marine Biosciences (NRC-IMB) in Halifax, Nova Scotia. All spectra were recorded in CD_2Cl_2. Chemical shifts (δ) are given in ppm and calibrated to residual solvent peaks [^1H ($CHDCl_2$): 5.32 ppm; ^{13}C ($^{13}CD_2Cl_2$): 54.0 ppm]. Structural characterization and signal assignments were accomplished using ^1H NMR chemical shifts, multiplicities, and ^{13}C NMR chemical shifts. In addition, ^1H–^1H correlated spectroscopy (COSY), ^1H–^{13}C heteronuclear single quantum coherence (HQSC) NMR, ^1H–^{13}C heteronuclear multiple bond correlation (HMBC) NMR, ^1H–^{15}N HMBC, and 2D ^1H–^1H nuclear Overhauser effect spectroscopy (NOESY) experiments were used in NMR analysis.

Fermentation, Extraction, and Isolation. All media was prepared with distilled water unless otherwise stated. Media utilized for this study included MYM broth [maltose 4 g/L, yeast extract 4 g/L, malt extract 10 g/L]; MYM agar [maltose (4 g/L), yeast extract (4 g/L), malt extract (10 g/L), agar 15 (g/L), pH 7.0]; MSM media [$MgSO_4$ (0.4 g/L), MOPS (3.77 g/L), salt solution (9 mL 1% w/v NaCl, 1% w/v $CaCl_2$), $FeSO_4{\cdot}7H_2O$ (4.5 mL 0.2% w/v), trace mineral solution (4.5 mL), pH 7.5]. Trace mineral solution [$ZnSO_4{\cdot}7H_2O$ (880 mg/L), $CuSO_4{\cdot}5H_2O$ (39 mg/L), $MnSO_4{\cdot}4H_2O$ (6.1 mg/L), H_3BO_3 (5.7 mg/L), $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (3.7 mg/L). *S. venezuelae* ISP5230 VS1099 was grown on MYM agar plates supplemented with apramycin (50 μg mL^{-1}) for 1–3 weeks.[29,30] Fermentations were carried out using modified conditions for jadomycin production with L-lysine (60 mM) as the sole nitrogen source.[21] A 1 × 1 cm lawn of *S. venezuelae* was used to inoculate 250 mL of MYM media (250 mL in 4 × 1 L flasks, total volume = 1 L). Fermentations were incubated at 30 °C with agitation (250 rpm) for 16–24 h. The cell suspension was centrifuged at 3750 rpm (4 °C) for 30 min. The supernatant was

DOI: 10.1021/acs.jnatprod.5b00277
J. Nat. Prod. 2015, 78, 1942–1948

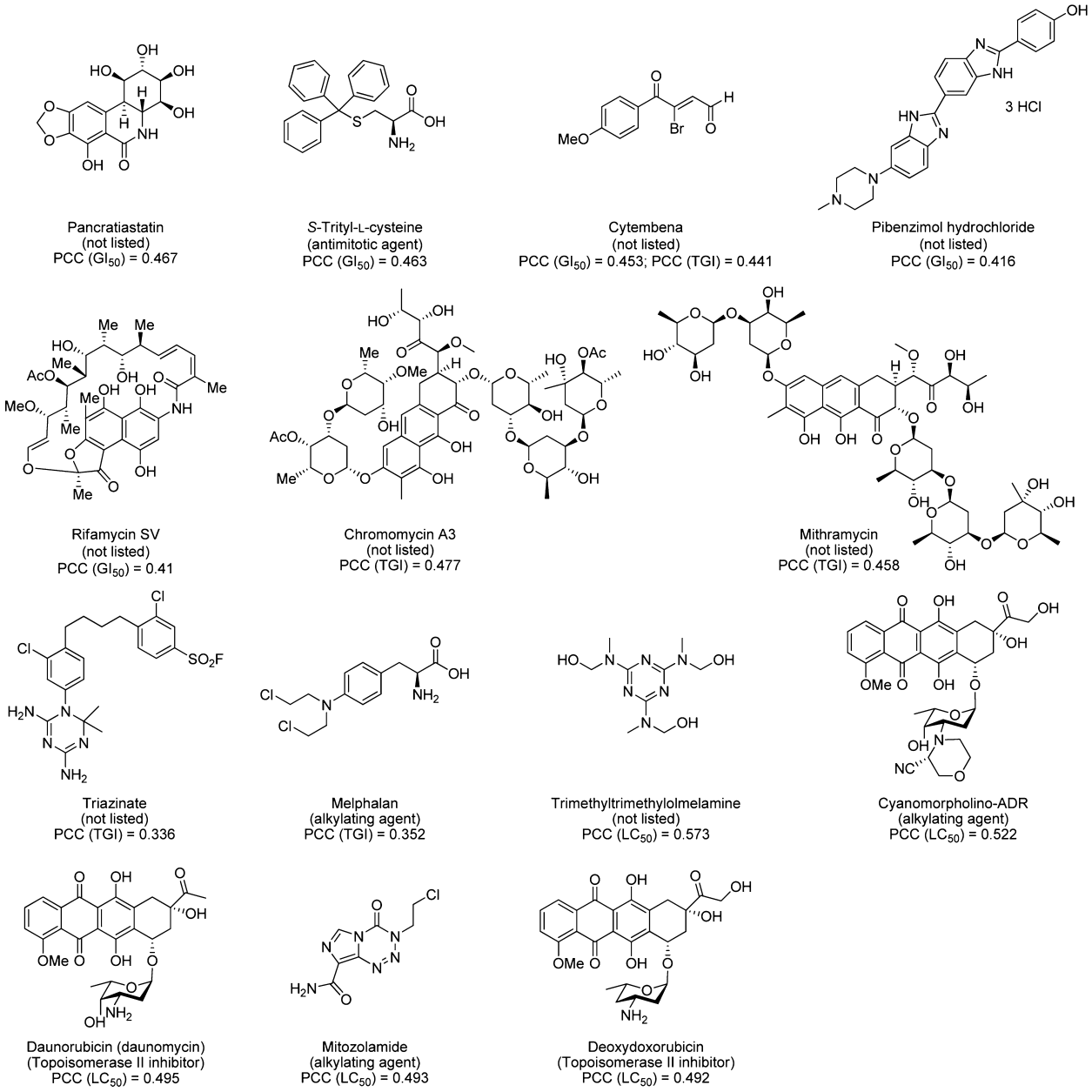

Figure 4. Top identified compounds by COMPARE analysis for each of GI_{50}, TGI, and LC_{50} correlations with **1** comparing against the NCI standard agents database. Proposed functions according to the NCI Web site are given underneath each compound in parentheses. Pearson correlation coefficient (PCC) values are listed with the appropriate parameter (GI_{50}, TGI, or LC_{50}) in parentheses (http://dtp.nci.nih.gov/docs/cancer/searches/standard_agent_table.html).

removed, and the cell pellet washed with 100 mL of MSM containing no amino acid. After centrifugation and removal of the supernatant, the wash was repeated. The pellet was resuspended in 100 mL of MSM without amino acid. MSM media (250 mL in 8 × 1 L flasks, total volume = 2 L, pH 7.5) containing L-lysine (final concentration 60 mM) was supplemented with glucose (final concentration 33 mM) and phosphate (final concentration 50 μM) before inoculation with the *S. venezuelae* ISP5230 VS1099.[21] The cell suspension was added to the MSM−L-lysine media to an initial OD_{600} of 0.6. The growth was immediately ethanol shocked with 100% ethanol (30 mL L^{-1}) and incubated at 30 °C with agitation (250 rpm) for 48 h. At 24 h, the pH of the media was adjusted back to pH 7.5 with 1 M NaOH. Bacterial cultures were monitored by absorbance at 600 nm (OD_{600}), and colored natural product production was monitored by absorbance of clear growth media at 526 nm (Figure S1). After 48 h bacterial cells were removed via suction filtration through Whatman No. 5 filter paper, followed by 0.45 μm then 0.22 μm Millipore Durapore membrane filters. The media was passed through a reversed-phase SiliCycle phenyl column (70 g) and washed with distilled water to remove water-soluble material. The remaining material was eluted from the column with 100% methanol and dried *in vacuo*. The crude mixture was dissolved in 250 mL of H_2O and extracted with EtOAc (3 × 250 mL). The EtOAc fractions were dried down *in vacuo*. The crude ethyl acetate extract was dissolved in minimal CH_2Cl_2 and loaded onto preparative TLC plates. The plates were developed three times with 5% methanol in CH_2Cl_2, and the band of interest was scraped from the glass backing and eluted with 100% methanol, yielding 16 mg of material. Further purification was accomplished by preparative TLC using a 1:1 EtOAc/CH_3CN solvent system and developing the plate twice for optimal separation. The band of interest was scraped from the glass backing, eluted with 100% methanol, and dried *in vacuo*. Dry material was brought up in CH_2Cl_2, filtered to remove residual silica, and dried, yielding 8 mg of **1** (4 mg L^{-1}) as a single diastereomer, as determined by ^1H NMR.

DOI: 10.1021/acs.jnatprod.5b00277
J. Nat. Prod. 2015, 78, 1942−1948

L-Digitoxosyl-phenanthroviridin (1): amber yellow solid; TLC R_f 0.46 (silica gel, 9:1 CH_2Cl_2/MeOH); HPLC t_R 9.47 min; ^1H NMR (CD_2Cl_2, 700 MHz) and ^{13}C NMR (CD_2Cl_2, 176 MHz) see Table 1; UV−vis (2.25×10^{-4} M, MeOH) λ_{max} (ε) = 303 nm (3911), 369 nm (1444); LRMS (ESI^+) MS/MS (436) found 436 $[M + H]^+$, 306 $[M + H -$ digitoxose$]^+$; HRMS (ESI^+) 458.1192 found, 458.1210 calculated for $C_{24}H_{21}NNaO_7$.

■ ASSOCIATED CONTENT

Supporting Information

Supporting Information includes ^1H NMR, $^1H-^1H$ COSY, ^{13}C NMR, $^1H-^{13}C$ edited-HSQC, $^1H-^{13}C$ HMBC, $^1H-^{15}N$ HMBC, and 2D NOESY spectra of **1**. One- and five-dose NCI 60 DTP human tumor cell line screen data of **1** are presented. Full tabulated COMPARE results and structures of compounds showing correlations to **1** are illustrated. Growth curves of *S. venezuelae* and media compositions are also included. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00277.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank NSERC, CIHR, and CHRP for financial support. We would like to thank Dr. N. Merkley and I. Burton at the NRC-IMB for their NMR support on the 700 MHz instrument and X. Feng for acquisition of HRMS data. We would also like to thank S. Forget for her help editing the manuscript.

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DOI: 10.1021/acs.jnatprod.5b00277
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