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Antineoplastic Agents. 560. Isolation and Structure of Kitastatin 1 from an Alaskan *Kitasatospora* sp.[1]

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By utilizing a bioassay-guided separation (P388 lymphocytic leukemia and a panel of human cancer cell lines) of fermentation broths from a *Kitasatospora* sp. collected from a tundra soil sample taken at the shore of the Beaufort Sea, we have isolated three powerful (GI_{50} to 0.0006 μg/mL) cancer cell growth inhibitors (**1**−**3**) and determined their structures to be closely related cyclodepsipeptides. From 380 L fermentations of *Kitasatospora* sp. were obtained 2.6 mg of a new cyclodepsipeptide designated kitastatin 1 (**3**), accompanied by the previously known respirantin (**1**, 10.8 mg) and its valeryl homologue (**2**, 4.8 mg). The structures were determined by employment of a series of high-resolution mass and 2D NMR spectroscopic analyses. The stereochemical assignments and overall structures were confirmed by subsequent total synthesis of depsipeptide **1**, as reported in the accompanying contribution.

The actinomycete genus *Kitasatospora* has a developing history of producing biologically active metabolites, especially those with cancer cell growth inhibitory properties. An early example of the latter was the isolation of the anticancer antibiotic terpentecin from a soil *Kitasatospora* sp. (strain MF730-N6) by Umezawa and colleagues in 1985.[2] That advance was quickly followed by the isolation of anticancer carbolines from *Kitasatospora setae*[3a,b] cultured from a Spitsbergen soil sample.[4] In 1993, the stereochemically undefined cyclodepsipeptide respirantin (**1**) was isolated from a *Kitasatospora* sp. during an examination of its constituents for insecticidal activity.[5] Interestingly, in an investigation of endophytic actinomycetes on *Taxus baccata* plants, a *Kitasatospora* sp. (strain P & U 22869) was isolated and found to produce paclitaxel and related taxanes.[6] More recently, *Kitasatospora* spp. have been found to produce yeast-like pleiotropic drug-resistant pump constituents,[7] proteasome inhibitors designated tyropeptins A and B,[8] and bafilomycin-like antifungal compounds;[9] more recently, *K. cheerisanensis* was found to contain the cytotoxic bafilomycin C1-amide.[10] The bafilomycins represent a group of 16-membered macrocyclic lactones isolated from *K. setae* and several streptomyces species and are very strong cancer cell growth inhibitors.[11a−h]

In 1997, during an exploration in arctic Alaska for terrestrial and marine microorganisms that might contain anticancer constituents, a tundra specimen on the shore of the Beaufort Sea near Prudhoe Bay was collected and subsequently found to contain a Gram-positive bacterium identified as *Kitasatospora* sp. After this actinomycete was identified by 16S rRNA gene sequence similarity, it was scaled up in quarter-strength potato dextrose broth over a 7-day fermentation period. A dichloromethane extract of 4 L of broth was concentrated to a residue that inhibited growth of the P388 lymphocytic leukemia and a minipanel of human cancer cell lines. For isolation of the cancer cell growth inhibitor constituents, the fermentation was scaled up to 380 L, and separation was guided by bioassay using both the P388 and human cancer cell line systems. The resulting extract (3.4 g) was partitioned between 9:1 CH_3OH−water and hexane followed by dilution of the aqueous phase to 3:2 CH_3OH−water and extraction with CH_2Cl_2. Concentration of the latter phase provided the active fraction (0.50 g, P388 ED_{50} 0.15 μg/mL). Separation of this fraction was guided by the P388 lymphocytic leukemia cell line bioassay and was conducted using a series of gel permeation column separations on Sephadex LH-20 followed by partition chromatographic separations, again on LH-

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20, and finally by reversed-phase high-performance liquid chromatographic separations that led to cyclodepsipeptides **1** (10.8 mg), **2** (4.8 mg), and **3** (2.6 mg).

Results and Discussion

In a high-resolution APCI (positive-ion mode) mass spectrum, cyclodepsipeptide **1** showed a molecular ion peak at *m*/*z* 748.3660 $[M + H]^+$ that suggested the molecular formula $C_{37}H_{53}N_3O_{13}$. The comprehensive analyses of ^1H, ^{13}C, and 2D NMR spectra allowed assignment of the following units: lactyl, 2-hydroxy-3-methylvaleryl, 4-amino-2,2,6-trimethyl-3-oxoheptanoyl, 2-hydroxy-4-methylvaleryl, 2-hydroxy-3-formylaminobenzoyl, and a threonine unit. The connection of the fragments by HMBC-NMR analyses led to assignment of depsipeptide **1** with unknown stereochemistry, as reported for the *Streptomyces* sp. constituent **1** by Urushibata[5] in 1993.

The molecular formula $C_{36}H_{51}N_3O_{13}$ was assigned to cyclodepsipeptide **2** on the basis of APCI (positive-ion mode) HRMS results, the molecular ion at *m*/*z* 734.3510 $[M + H]^+$ being 14 units (CH_2) less than **1**. The NMR proton and carbon assignments of cyclodepsipeptide **2** were deduced by comparison with those exhibited in the NMR spectra of **1**. Those comparisons revealed a 2-hydroxy-3-methylbutyryl unit in place of the 2-hydroxy-4-methylvaleryl unit of **1** and allowed assignment of cyclodepsipeptide **2**, previously described in a 1994 Japanese patent.[12]

The 380 L scale fermentation of *Kitasatospora* sp. was repeated three times in order to obtain sufficient amounts of cyclodepsipeptides **1** and **2** for attempts at obtaining crystals for X-ray crystal structure determinations. Although suitable crystals were not obtained, one of the 380 L fermentations led to isolation of a new cyclodepsipeptide designated kitastatin 1 (**3**), albeit in very low yield (2.6 mg). Kitastatin 1 (**3**) was obtained as an amorphous powder that gave a high-resolution (APCI+) mass spectral molecular ion at *m*/*z* 720.3725 $[M + 1]^+$, which corresponds to a formula 28 mass units less than that of cyclodepsipeptide **1**. The ^1H, ^{13}C, and HMQC NMR spectra of kitastatin 1 (Table 1) resembled that of respirantin (**1**). However, the high-field carbonyl signal corresponding to the aromatic formamide group of **1** was missing. That was further confirmed when ^1H, ^1H-COSY, TOCSY, and HMBC data were interpreted and pointed to a 3-amino-2-hydroxybenzoyl segment in place of the aromatic formamide as the only structural difference from cyclodepsipeptide **1**. Thus, kitastatin 1 was unequivocally assigned structure **3**.[13]

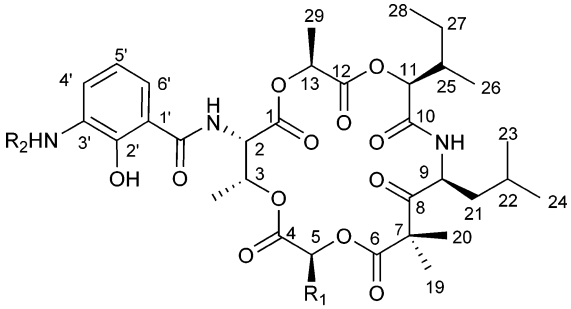
Because suitable crystallization of cyclodepsipeptides **1**−**3** was not successful and prevented investigation of the stereochemistry by X-ray crystal structure determinations, we initially focused on high-field 2D NMR approaches to define the stereochemistry. In ROESY-NMR experiments, kitastatin 1 (**3**) showed cross-peaks related to the ring system at H-2/H-3 (δ_H 5.25/6.02), H-9/H-19 (δ_H 4.84/1.10), and H-25/NH-9 (δ_H 2.11/7.53), which suggested a 2*S*, 3*R* (or 2*R*, 3*S*), 9*S* and 11*S* (or 9*R* and 11*R*) relationship. Similarly, three cross-peaks at H-2/H-3 (δ_H 5.24/6.03), H-9/H-19 (δ_H 4.83/1.09), and H-25/NH-9 (δ_H 2.09/7.50) were located in the ROESY spectrum of the cyclodepsipeptide respirantin (**1**). While these NMR experiments were in progress, we were able to unequivocally assign the stereostructure of **1** and kitastatin 1 (**3**) as 2*S*, 3*R*, 5*S*, 9*S*, 11*S*, and 13*S* by our completion of total syntheses.[13] Since we were unable to make a direct comparison of our cyclodepsipeptides **1** and **2** with authentic samples previously reported,[5,12] there still remains some minor uncertainty.

To investigate whether minor variations in the fermentation conditions could lead to a series of new cyclodepsipeptide antineoplastic agents, culture media were modified by addition of presumed biochemical precursors, DL-serine, 2-hydroxyvaleric acid, DL-tyrosine, or shikimic acid. Fermentation conditions as well as bioassay-guided isolation techniques were otherwise identical. As summarized in Table 2, only cyclodepsipeptides **1** and **2** were isolated following addition of the various presumed precursors to the fermentation broth.

Table 1. ^1H and ^{13}C Spectral Assignments for Kitastatin 1 (**3**, recorded in CD_2Cl_2, *J* in Hz)[a]

position	δ ^{13}C	δ ^1H	^1H,^1H-COSY	HMBC (C to H)
1-CO	168.0			H-2, H-13
2-CH	55.8	5.25 (dd, 8.5/2.5)	NH-2, H-3	H-14
3-CH	72.9	6.02 (dq, 7.0/2.5)	H-2, H-14	H-14
4-CO	172.3			H-3
5-CH	72.6	4.72 (m)	H-15a, H-15b	
6-CO	173.7		H-19, H-20	
7-C	53.7		H-19, H-20	
8-CO	208.5			H-9, H-19, H-20
9-CH	56.0	4.84 (dd, 11.0/5.5)	NH-9, H-21	H-21
10-CO	170.4			H-9, H-11
11-CH	81.4	4.74 (m)	H-25	H-26
12-CO	170.2			H-11, H-13, H-29
13-CH	71.6	5.80 (q, 6.5)	H-29	H-29
14-CH_3	16.6	1.36 (d, 6.5)	H-3	
15-CH_2	39.8	1.52 (m)	H-5, H-15b	H-16, H-17
		1.72 (m)	H-5, H-15a	
16-CH	24.9	1.74 (m)	H-18	
17-CH_3	21.6	0.89 (d, 6.5)		H-18
18-CH_3	22.9	0.94 (d, 7.0)	H-16	H-17
19-CH_3	20.0	1.10 (s)		H-20
20-CH_3	24.3	1.27 (s)		H-19
21-CH_2	43.3	1.80 (2H, t, 8.5)	H-9, H-22	H-9, H-23, H-24
22-CH	25.0	1.64 (m)	H-21, H-23, H-24	H-23, H-24
23-CH_3	21.1	0.92 (d, 7.0)	H-22	H-21
24-CH_3	23.6	0.93 (d, 7.0)	H-22	H-21
25-CH	36.9	2.11 (m)	H-11, H-26, H27	H-11, H-27
26-CH_3	14.7	0.98 (d, 7.0)	H-25	H-27a
27-CH_2	25.7	1.37 (m)	H-25, H-27b, H-28	H-27
		1.77 (m)	H-27a, H-28	
28-CH_3	10.6	0.97 (t, 7.5)	H-27a, H-27b	H-27a
29-CH_3	18.3	1.54 (d, 7.0)	H-13	H-13
1′-C	113.3			H-5′
2′-C	150.9			H-4′, H-6′
3′-C	137.0			H-5′
4′-CH	118.5	6.89 (d, 6.5)	H-5′	H-6′
5′-CH	119.3	6.78 (t, 7.5)	H-4′, H-6′	
6′-CH	114.6	7.01 (d, 8.0)	H-5′	H-4′
7′-CO	171.3		NH-2, H-6′	
NH-2		7.08 (d, 8.5)	H-2	
NH-9		7.53 (d, 9.5)		H-9
NH-3′		4.00 (s, br)		

[a] 500 MHz for ^1H NMR, 100 MHz for ^{13}C NMR.



1 $R_1 = CH_2CH(CH_3)_2$, R_2 = CHO, respirantin
2 $R_1 = CH(CH_3)_2$, R_2 = CHO
3 $R_1 = CH_2CH(CH_3)_2$, R_2 = H, kitastatin 1

Figure 1. Cyclodepsipeptides **1**−**3**.

The bioassay-directed separation clearly indicated that cyclodepsipeptides **1**−**3** are the most important cancer cell growth inhibitor constituents of *Kitasatospora* sp. When evaluated against the murine P388 lymphocytic leukemia and six human cancer cell lines, they exhibited extraordinary cancer cell growth inhibitory properties (Table 3). Since the minor structural differences between compounds **1**, **2**, and **3** did not greatly affect the cancer cell growth inhibitory activities, it appears that the overall stereochemistry of the macrocyclic lactone and side chain are of greater importance.

Table 2. Results of Fermentation Experiments (runs A−D)[a]

cyclodepsipeptide and CH_2Cl_2 fraction	A (mg)	B (mg)	C (mg)	D (mg)
CH_2Cl_2 fraction[b]	60.0	92.0	185.0	140.0
cyclodepsipeptide 1	7.0	5.3	3.7	9.0
cyclodepsipeptide 2	1.7	2.0	0.9	2.3

[a] Run A: culture media with addition of DL-serine. Run B: culture media with addition of 2-hydroxyvaleric acid. Run C: culture media with addition of DL-tyrosine. Run D: culture media with addition of shikimic acid. [b] CH_2Cl_2 extract fraction (from each 380 L fermentation) obtained from the $CH_2Cl_2/CH_3CHOH-H_2O$ (3:2) solvent partition isolation step.

Structural modifications of kitastatin 1[13] are in progress as well as preclinical development. Interestingly, no previous cancer cell growth inhibitory activity has been reported for cyclodepsipeptides **1** and **2**.[5,12]

In addition to the human cancer cell line activity, cyclodepsipeptide **1** had activity against the pathogenic fungus *Cryptococcus neoformans* (minimum inhibitory concentration [MIC] = 2 μg/mL), and cyclodepsipeptide **2** had marginal activity against the opportunistic bacterium *Micrococcus luteus* (MIC = 64 μg/mL). Kitastatin 1 (**3**) had marginal activity against *C. neoformans* and *Enterococcus faecalis* (MIC = 64 μg/mL).

Experimental Section

General Experimental Procedures. Solvents used for the chromatographic procedure were redistilled. Sephadex LH-20 employed for gel permeation and partition chromatography was obtained from Pharmacia Fine Chemicals AB, Upsala, Sweden. The silica gel GHLF Uniplates for thin-layer chromatography were supplied by Analtech, Inc. The TLC results were viewed under UV light and developed with ceric sulfate−sulfuric acid (heating for 3 min). Analytical HPLC was conducted using a Hewlett-Packard model 1100 HPLC coupled with a diode-array detector and an evaporative light scattering detector. Reversed-phase HPLC was performed on a Zorbax SB C18 column attached to a Waters 600E instrument with a 2487 dual-λ absorbance detector.

The melting points were recorded with a Kofler melting point instrument. Optical rotation data were determined with a Perkin-Elmer 241 polarimeter. UV spectra were acquired using a Perkin-Elmer Lambda 3β UV/vis spectrophotometer equipped with a Hewlett-Packard laser jet 2000 plotter. IR spectra were recorded with an Avatar 360 FT-IR instrument with the sample prepared in $CHCl_3$ film. High-resolution mass spectra were obtained with a JEOL LCmate magnetic sector instrument by APCI in positive-ion mode with a polyethylene glycol reference. The NMR experiments were conducted using a Varian Unity INOVA-500 spectrometer operating at 500 or 400 MHz for ^{1}H NMR as well as 2D NMR referenced to tetramethylsilane and at 100 MHz for ^{13}C NMR referenced to tetramethylsilane.

Specimen Collection and Fermentation. Soil samples were collected in clean plastic bags by one of us (G.R.P.) on the shore tundra near Prudhoe Bay (Beaufort Sea), Alaska, and shipped by air to our laboratory. Soils were aseptically diluted and spread on quarter-strength potato dextrose agar (Difco) containing soil extract (1 kg/L commercial potting soil was heated, filtered, sterilized, and used at a final concentration of 133 mL/L in all preparations). *Kitasatospora* sp. was identified by 16S rRNA gene sequence similarity (Accugenix, Newark, DE). Results from the MicroSeq database based on the first 500 base pairs of the 16S rRNA gene placed the bacterium in the genus *Kitasatospora* sp. (% difference = 1.91, confidence level to genus). Isolated colonies were subcultured and fermented in potato dextrose broth/soil extract, and extracts were screened against the murine P388 lymphocytic leukemia cell line and a minipanel of human cancer cell lines. Prior to large-scale fermentation (6 L flasks containing 4 L of media), the P388 and human cancer cell line activity of the actinomycete was determined to be optimum in quarter-strength potato dextrose broth for 7 days. All activity peak experiments and large-scale fermentations were performed at room temperature with shaking. The voucher specimen is maintained at Arizona State University.

Extraction and Solvent Partition of *Kitasatospora* sp. The microbial broth (380 L; a series over the period Feb 2003−May 2003) was extracted (3×) with CH_2Cl_2 (1/2 volume). The CH_2Cl_2 extract was dried (3.4 g) and then redissolved in 2 L of 9:1 CH_3OH-H_2O and partitioned (4×) with CH_2Cl_2 (2 L per pass). The CH_2Cl_2 was quickly removed *in vacuo*, and the residue was redissolved in 9:1 CH_3OH-H_2O and partitioned (4×) with hexane. After dilution to 3:2 CH_3OH-H_2O, the aqueous phase was partitioned (4×) with CH_2Cl_2 to give 0.5 g of a CH_2Cl_2-soluble fraction (P388 ED_{50}, 0.15 μg/mL).

Isolation of Cyclodepsipeptides 1, 2, and 3. The cancer cell inhibitory CH_2Cl_2 fraction (0.5 g), obtained as described in the preceding experiment, was passed in CH_3OH through a column of Sephadex LH-20. Two resulting bioactive (cancer cell line bioassay) fractions were combined and chromatographed on a Sephadex LH-20 column using hexane−toluene−CH_3OH (3:1:1) as eluent, which led to the concentration into one fraction of the inhibitory activity (57 mg, P388 ED_{50} 0.02 μg/mL). Further separation of the active fraction was performed using reversed-phase HPLC. Initially, analytical HPLC of the fraction was conducted on a HP1100 series instrument with both ELSD and UV detectors to locate the target peaks. The fraction was then separated by semipreparative HPLC on a Waters instrument with a Zorbax SB C18 column (9.6 × 250 mm) in acetonitrile−H_2O (40% to 90% in 45 min) at 4 mL/min flow rate. Cyclodepsipeptides **1**, **2**, and **3** were obtained by concentration of the eluting fractions with peaks at retention times of 42.5, 40, and 45 min, respectively.

The experimental results summarized in Table 2 were obtained by employment of the preceding procedure.

Cyclodepsipeptide 1: colorless, amorphous powder (10.8 mg); mp 118−120 °C; ^{1}H NMR (CD_2Cl_2, 500 MHz) δ 12.61 (1H, s, 2′-OH), 8.54 (1H, d, J = 6.0 Hz, H-4′), 8.47 (1H, s, H-8′), 7.92 (1H, s, 3′-NH), 7.50 (1H, d, J = 7.5 Hz, 9-NH), 7.40 (1H, d, J = 6.0 Hz, H-6′), 7.16 (1H, d, J = 7.0 Hz, 2-NH), 6.97 (1H, t, J = 6.0 Hz, H-5′), 6.03 (1H, m, H-3), 5.80 (1H, q, J = 6.0 Hz, H-13), 5.24 (1H, dd, J = 1.6,7.0 Hz, H-2), 4.83 (1H, m, H-9), 4.73 (1H, m, H-11), 4.71 (1H, m, H-5), 2.09 (1H, m, H-25), 1.80 (2H, m, H-21), 1.76 (1H, m, H-27b), 1.70 (1H, m, H-16), 1.68 (1H, m, H-15b), 1.62 (1H, m, H-22), 1.54 (1H, m, H-15a), 1.54 (3H, d, J = 6.0 Hz, H-29), 1.36 (3H, d, J = 6.0 Hz, H-14), 1.33 (1H, m, H-27a), 1.27 (3H, s, H-20), 1.09 (3H, s, H-19), 0.96 (3H, d, J = 6.0 Hz, H-26), 0.95 (3H, t, J = 6.0 Hz, H-28), 0.93 (3H, d, J = 6.0 Hz, H-18), 0.92 (3H, d, J = 6.0 Hz, H-23), 0.91 (3H, d, J = 6.0 Hz, H-24), 0.89 (3H, d, J = 6.0 Hz, H-17); ^{13}C NMR (CD_2Cl_2, 100 MHz) δ 208.0(C-8), 173.7(C-6), 172.3(C-4), 170.7(C-7′), 170.4(C-10), 170.1(C-12), 167.8(C-1), 159.3(C-8′), 150.9(C-2′), 127.9(C-3′), 125.0(C-4′), 120.7(C-6′), 119.2(C-5′), 113.3(C-1′), 81.4(C-11), 72.7(C-3), 72.6(C-5), 71.7(C-13), 56.8(C-9), 56.0(C-2), 54.2(C-7), 43.3(C-21), 39.7(C-15), 36.8(C-25), 25.7(C-27), 24.9(C-16), 24.8(C-22), 24.3(C-20), 23.6(C-23), 23.0(C-18), 21.5(C-17), 21.0(C-24), 20.0(C-19), 18.3(C-29), 16.6(C-14), 14.5(C-26), 10.6(C-28); selected HBMC (C to H) C-1 to H-2, H-13; C-2 to H-14; C-3 to H-14; C-4 to H-3, H-5; C-6 to H-19, H-20; C-7 to H-19, H-20; C-8 to H-9, H-19, H-20; C-9 to H-21; C-10 to H-9, 10-NH, H-11; C-11 to H-26; C-12 to H-11, H-13, H-29; C-13 to H-29; HRMS (APCI positive-ion mode) m/z 748.3660 $[M + H]^+$ (calcd for $C_{37}H_{54}N_3O_{13}$, 748.3657).

Cyclodepsipeptide 2: colorless, amorphous powder (4.8 mg); mp 117−120 °C; ^{1}H NMR (CD_2Cl_2, 400 MHz) δ 12.62 (1H, s, 4′-OH), 8.53 (1H, d, J = 8.0 Hz, H-4′), 8.50 (1H, s, H-8′), 8.02 (1H, s, 3′-NH), 7.52 (1H, d, J = 10.0 Hz, 9-NH), 7.43 (1H, d, J = 8.0 Hz, H-6′), 7.18 (1H, d, J = 8.0 Hz, 2-NH), 6.99 (1H, t, J = 7.6 Hz, H-5′), 6.02 (1H, m, H-3), 5.82 (1H, q, J = 7.2 Hz, H-13), 5.26 (1H, d, J = 8.8 Hz, H-2), 4.85 (1H, m, H-9), 4.77 (1H, m, H-11), 4.54 (1H, m, H-5), 2.12 (1H, m, H-15), 2.10 (1H, m, H-24), 1.83 (2H, t, J = 6.0 Hz, H-20), 1.75 (1H, m, H-26b), 1.62 (1H, m, H-21), 1.56 (3H, d, J = 6.4 Hz, H-28), 1.39 (3H, d, J = 6.4 Hz, H-14), 1.37 (1H, m, H-26a), 1.29 (3H, s, H-19), 1.11 (3H, s, H-18), 0.98 (3H, d, J = 6.4 Hz, H-17), 0.98 (3H, d, J = 6.4 Hz, H-25), 0.97 (3H, t, J = 6.4 Hz, H-27), 0.95 (3H, d, J = 6.4 Hz, H-16), 0.92 (3H, d, J = 6.4 Hz, H-23), 0.92 (3H, d, J = 6.4 Hz, H-24); ^{13}C NMR (CD_2Cl_2, 100 MHz) δ 207.5(C-8), 173.6-(C-6), 170.9(C-4), 170.9(C-7′), 170.6(C-10), 170.0(C-12), 167.7(C-1), 159.5(C-8′), 150.8(C-2′), 127.9(C-3′), 125.0(C-4′), 120.7(C-6′), 119.1(C-5′), 113.5(C-1′), 81.0(C-11), 78.3(C-5), 72.7(C-3), 71.6(C-13), 56.7(C-9), 55.9(C-2), 53.8(C-7), 42.8(C-20), 36.8(C-24), 30.3 (C-15), 25.4(C-26), 24.8(C-19), 24.1(C-21), 23.6(C-23), 20.0(C-22), 19.8(C-18), 18.4(C-17), 18.1(C-28), 17.7(C-16), 16.6(C-14), 14.4(C-25), 10.4(C-27); HRMS (APCI positive-ion mode) m/z 734.351 $[M + H]^+$ (calcd for $C_{36}H_{52}N_3O_{13}$, 734.3500).

Cyclodepsipeptide 3: colorless, amorphous powder; mp 126−129 °C; $[\alpha]^{24}_D$ −10 (c 0.07, CH_3OH); UV (CH_3OH) λ_{max} 231, 328 nm; IR

Table 3. Inhibition of the Murine P388 Lymphocytic Leukemia (ED_{50} μg/mL) and Human Cancer Cell Line (GI_{50} μg/mL) by Cyclodepsipeptides **1**, **2**, and **3**

cyclodepsipeptides	P388 murine leukemia	BXPC-3 pancreas	MCF-7 breast	SF268 CNS	NCI-H460 lung	KM20L2 colon	DU-145 prostate
1	0.0037	0.47	0.0006	0.0016	0.0006	0.0006	0.00018
2	0.033	1.2	0.00064	0.016	0.00063	0.00058	<0.0001
3	0.045	0.0066	0.004	0.0035	<0.001	0.0024	0.0026

($CHCl_3$), ν_{max} 1745, 1712, 1665, 1644, 1502, and 1525 cm^{-1}; HRMS (APCI positive-ion mode) *m/z* 720.3725 $[M + H]^+$ (calcd for $C_{36}H_{54}N_3O_{12}$, 720.3708); ^1H and ^{13}C NMR data see Table 1.

Cancer Cell Line Bioassay Procedures. The inhibition of cancer cell growth was evaluated using the National Cancer Institute's standard sulforhodamine B assay as previously described.[14] Cells in a 5% fetal bovine serum/RPMI1640 mdium solution were inoculated in 96-well plates and incubated for 24 h. Serial dilutions of the compounds were next added. After 48 h, the plates were fixed with trichloroacetic acid, stained with rhodamine B, and read with an automatic microplate reader. Growth inhibition of 50% (ED_{50} or the drug concentration causing a 50% reduction in the net protein increase) was calculated from optical density data with Immunosoft software. Murine leukemia P388 cells were incubated in a 10% horse serum/Fisher medium solution for 24 h followed by a 48 h incubation with serial dilutions of the compounds. Cell growth inhibition (ED_{50}) was then calculated using a Z1 Beckman/Coulter particle counter.

Antimicrobial Susceptibility Testing. Cyclodepsipeptides **1** and **2** were screened against the bacteria *Stenotrophomonas maltophilia* ATCC 13637, *Micrococcus luteus* Presque Isle 456, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Enterobacter cloacae* ATCC 13047, *Enterococcus faecalis* ATCC 29212, *Streptococcus pneumoniae* ATCC 6303, *Neisseria gonorrhoeae* ATCC 49226, and the fungi *Candida albicans* ATCC 90028 and *Cryptococcus neoformans* ATCC 90112, according to established broth microdilution susceptibility assays.[15,16] Owing to a paucity of material, compound **2** was tested against *S. maltophilia*, *M. luteus*, *S. aureus*, *E. coli*, and *C. albicans* only. Compounds were reconstituted in a small volume of sterile DMSO and diluted in the appropriate media immediately prior to susceptibility experiments. The minimum inhibitory concentration was defined as the lowest concentration of compound that inhibited all visible growth of the test organism (optically clear). Assays were repeated a minimum of two times on separate days.

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