JOURNAL OF NATURAL PRODUCTS

Article

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Lahorenoic Acids A–C, *ortho*-Dialkyl-Substituted Aromatic Acids from the Biocontrol Strain *Pseudomonas aurantiaca* PB-St2

Samina Mehnaz,*[,†] Rahman Shah Zaib Saleem,[‡] Basit Yameen,[‡] Isabelle Pianet,[§] Gregor Schnakenburg,[⊥] Halina Pietraszkiewicz,[∥] Fred Valeriote,[∥] Michaele Josten,[▽] Hans-Georg Sahl,[▽] Scott G. Franzblau,[○] and Harald Gross*[,#]

[†]Department of Biological Sciences, Forman Christian College University, Lahore 54600, Pakistan

[‡]Department of Chemistry, School of Sciences and Engineering, Lahore University of Management Sciences, Lahore 54792, Pakistan

[§]CESAMO-ISM, UMR 5255, CNRS, Université Bordeaux I, 351 Cours de la Libération, F-33405 Talence, France

[⊥]Institute of Inorganic Chemistry, University of Bonn, Gerhard-Domagk-Straße 1, 53121 Bonn, Germany

[∥]Henry Ford Health System, Detroit, Michigan 48202, United States

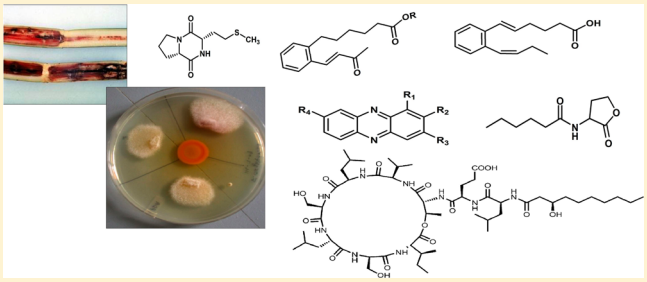
[▽]Institute for Medical Microbiology, Immunology and Parasitology (IMMIP), Pharmaceutical Microbiology Unit, University of Bonn, 53115 Bonn, Germany

[○]Institute for Tuberculosis Research, University of Illinois at Chicago, Chicago, Illinois 60612, United States

[#]Institute for Pharmaceutical Biology, University of Tübingen, Auf der Morgenstelle 8, 72076 Tübingen, Germany

Supporting Information

ABSTRACT: Three new aromatic acids, named lahorenoic acids A (**1**), B (**2**), and C (**3**), have been isolated along with the known compounds phenazine-1-carboxylic acid (**4**), 2-hydroxyphenazine-1-carboxylic acid (**5**), 2-hydroxyphenazine (**6**), 2,8-dihydroxyphenazine (**7**), cyclo-Pro-Tyr (**8**), cyclo-Pro-Val (**9**), cyclo-Pro-Met (**10**), and WLIP (**11**) and characterized from the biocontrol strain *Pseudomonas aurantiaca* PB-St2. The structures of these compounds were deduced by 1D and 2D NMR spectroscopic and mass spectral data interpretation. Compounds **2**, **4**, and **7** showed moderate antibacterial activity against mycobacteria and other Gram-positive bacteria, while **4** was also found to exhibit cytotoxic and antifungal properties.



Fungal phytopathogens such as *Fusarium*, *Rhizoctonia*, or *Colletotrichum* species cause worldwide damage to crop production. In the last decades, disease management has relied mainly on the use of synthetic fungicides. However, this strategy is challenged due to the accumulation of these compounds in the ecosystem and due to resistant fungal strains. One way of reducing the use of synthetic fungicides in agriculture is by using bacterial strains,[1] which aggressively colonize the plant rhizosphere and are able to protect their hosts from infection by soil-borne fungal pathogens.[2] The biological mechanisms underlying this phenomenon, known as biocontrol, include the secretion of both enzymes and small molecules.[2−5] One bacterial genus used for this purpose is *Pseudomonas*.[6] This group of bacteria is frequently described as being rhizosphere colonizers and are known to produce a diverse array of potent antifungal compounds, which include 2,4-diacetylphloroglucinol, phenazine-1-carboxylic acid,[7−9] pyrrolnitrin, pyoluteorin, hydrogen cyanide, lipopeptides,[10,11] and rhizoxin congeners.[12,13] On the basis of their capacity to control soil-borne phytopathogens, some *Pseudomonas* biocontrol strains have been developed into commercial products, e.g., *P. chlororaphis* MA342 (Cedomon, Cerall),[14] *P. fluorescens* A506 (BlightBan A506),[15] and *P.* sp. DSMZ 13134 (Proradix).[16,17]

In our continuing effort to explore the chemistry of pseudomonads,[10,12,13] we describe here the chemical investigation of the biocontrol strain *Pseudomonas aurantiaca* PB-St2,[18,19] which was found to inhibit the growth of *Colletotrichum falcatum*, the causative agent of the red rot disease of sugar cane (*Saccharum* sp. hybrids).[20,21] Strain PB-St2 was known to produce at least five secondary metabolites: phenazine-1-carboxylic acid (**4**), 2-hydroxyphenazine (**6**), *N*-hexanoyl homoserine lactone, hydrogen cyanide, and a siderophore of the hydroxamate type.[18,19] The antifungal properties of the strain have been attributed to the production of phenazine-1-carboxylic acid (**4**) and 2-hydroxyphenazine (**6**).[18] The high capacity for secondary metabolite production in this group of bacteria, as shown by genomic sequence data for several *Pseudomonas* spp.,[22] prompted us to continue the

Received: July 25, 2012
Published: February 12, 2013

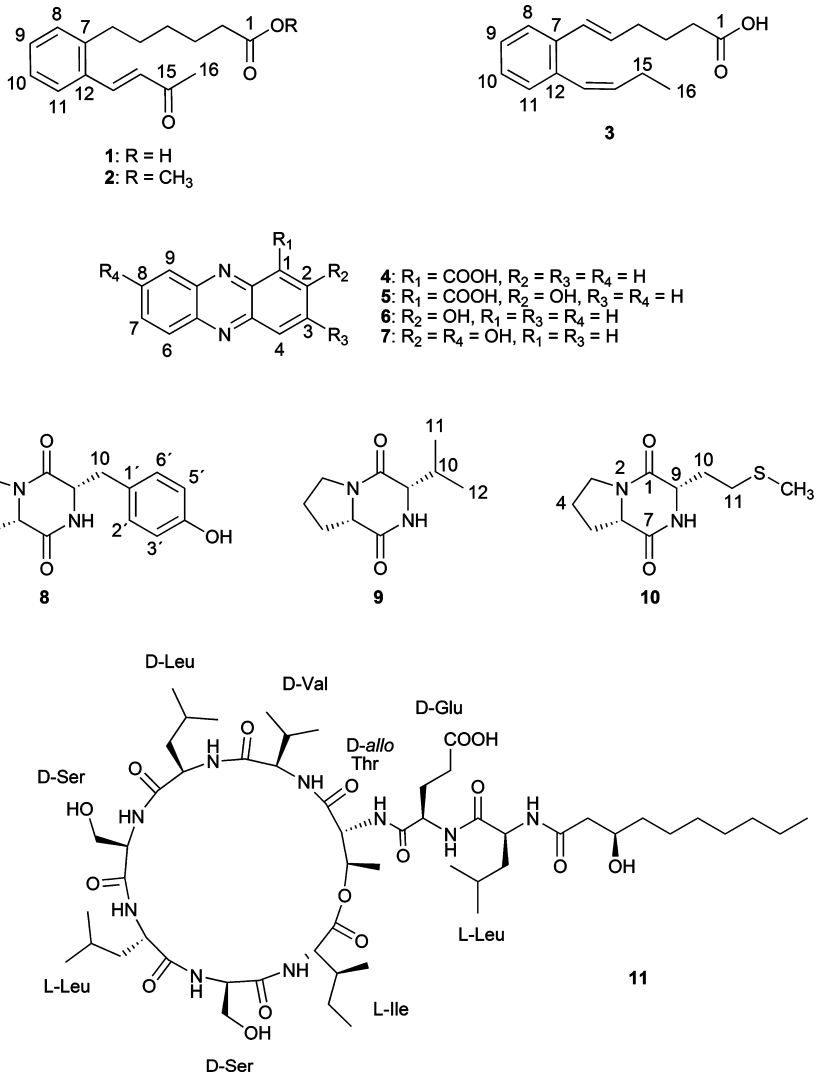


Figure 1. Compounds isolated from *Pseudomonas aurantiaca* PB-St2.

Table 1. NMR Spectroscopic Data (300 MHz, d_4-MeOH) for Lahorenoic Acids A (1), B (2), and C (3)

	lahorenoic acid A (**1**)		lahorenoic acid B (**2**)		lahorenoic acid C (**3**)	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	177.8, C[a]		175.9, C		178.1, C[a]	
2	34.5, CH_2[a]	2.33, t (7.0)	34.7, CH_2	2.36, t (7.4)	34.6, CH_2	2.37, t (7.4)
3	26.0, CH_2	1.68, m	25.8, CH_2	1.68, m	25.9, CH_2	1.82, p (7.4)
4	29.9, CH_2	1.44, m	29.8, CH_2	1.43, m	33.7, CH_2	2.29, q (7.1)
5	32.7, CH_2	1.62, m	32.6, CH_2	1.62, m	131.9, CH	6.17, dt (7.1, 15.8)
6	34.0, CH_2	2.85, t (7.7)	34.0, CH_2	2.85, t (7.6)	130.1, CH	6.60, d (15.8)
7	144.0, C		144.0, C		137.4, C	
8	131.4, CH	7.29, d (7.5)	131.4, CH	7.29, d (7.5)	126.4, CH	7.51, brd (6.4)
9	131.5, CH	7.37, dd (7.2, 7.5)	131.5, CH	7.38, dd (7.2, 7.5)	128.0, CH	7.20, m
10	127.7, CH	7.28, dd (7.2, 7.9)	127.7, CH	7.28, dd (7.2, 7.9)	127.5, CH	7.17, m
11	127.7, CH	7.70, d (7.9)	127.7, CH	7.71, d (7.9)	130.5, CH	7.13, m
12	134.0, C		134.0, C		136.7, C	
13	142.7, CH	8.00, d (16.0)	142.7, CH	8.00, d (16.0)	128.5, CH	6.49, d (11.7)
14	128.8, CH	6.78, d (16.0)	128.8, CH	6.78, d (16.0)	135.9, CH	5.78, dt (11.7, 7.5)
15	201.2, C		201.1, C		22.8, CH_2	2.11, m
16	27.7, CH_3	2.44, s	27.8, CH_3	2.44, s	14.5, CH_3	1.02, t (7.5)
17			52.0, CH_3	3.68, s		

[a]Indicated carbons showed only weak resonances in the ^{13}C NMR spectrum, but their presence and connectivity were clearly evidenced by all conducted 2D NMR experiments.

dx.doi.org/10.1021/np3005166 | *J. Nat. Prod.* 2013, 76, 135−141

chemical investigation of this *Pseudomonas* strain. Using ^{1}H NMR guided fractionation, we isolated three new *ortho*-dialkyl-substituted aromatic acids (**1**–**3**), along with a suite of previously reported metabolites belonging to the class of phenazines (**4**–**7**), diketopiperazines (**8**–**10**), and cyclic lipopetides (**11**). To date, *ortho*-dialkyl-substituted aromatic acids are rare and so far exclusively produced by Actinomycetes (e.g., serpentenes,[23,24] and demetric acid[25]) or by the marine bacterium *Pseudoalteromonas rubra* (rubrenoic acids A–C[26,27]), respectively. Indeed, lahorenoic acids A (**1**) and B (**2**) represent the first members of this family that bear a keto functionality, and this is the first report of the occurrence of this compound class in the genus *Pseudomonas*.

RESULTS AND DISCUSSION

All compounds (**1**–**11**) were isolated by the use of solvent partitioning, reversed-phase SPE (solid phase extraction), and HPLC, and their structures assigned by detailed spectroscopic analysis as detailed below.

The molecular formula of lahorenoic acid A (**1**) was determined to be $C_{16}H_{20}O_3$ by HRESIMS. The ^{13}C NMR spectrum of **1** showed 16 ^{13}C resonances (Table 1) for one methyl carbon (δ_C 27.7), five methylenes, a benzene ring, a carbon–carbon double bond (δ_C 128.8 and 142.7), a ketocarbonyl carbon (δ_C 201.2), and a carboxylic acid group (δ_C 177.8 and IR 1708 cm^{-1}). The benzene ring was suggested to be *ortho*-substituted on the basis of the coupling pattern of the aromatic protons in the ^{1}H NMR and in the ^{1}H–^{13}C-HSQC spectrum, which showed four aromatic proton resonances at δ 7.28 (1H, dd, J = 7.2, 7.9; H-10), 7.29 (1H, d, J = 7.5; H-8), 7.37 (1H, dd, J = 7.2, 7.5; H-9), and 7.70 (1H, d, J = 7.9; H-11). ^{1}H–^{1}H COSY correlations between H-8/H-9, H-9/H-10, and H-10/H-11 confirmed the connectivity deduced from coupling constant analysis. Further analysis of the ^{1}H–^{1}H-COSY NMR spectrum revealed two additional isolated ^{1}H spin systems (H_2-2 through H_2-6 and H-13/H-14), representing partial structures of the phenyl substituents in **1** (Figure 2). HMBC correlations observed from the olefinic H-13 signal to three aromatic carbon resonances for methine C-11, quaternary C-12, and quaternary C-7 (δ_C 144.0), along with correlations from olefinic H-14 to the ketone resonance at δ_C 201.2 (C-15), which was in turn correlated with H_3-16, established a 3-buten-2-one side chain located at C-12 of the benzene ring. In a similar fashion, on the basis of HMBC correlations observed between H_2-2/C-1, H_2-6/C-7, and H_2-6/CH-8, the remaining second spin system could be extended and identified as a hexanoic acid, bonded to the benzene ring via quaternary C-7. The double-bond geometry of $\Delta^{13,14}$ was *E*-configured, as determined from the coupling constant $^{3}J_{H13-H14}$ of 16.0 Hz. Therefore, compound **1** was identified as 6-(2-((*E*)-3-oxobut-1-enyl)phenyl)hexanoic acid and was given, according to its origin (Lahore, Pakistan) and chemical structure, the name lahorenoic acid A (**1**).

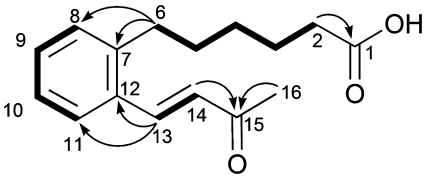


Figure 2. ^{1}H–^{1}H-COSY and ^{1}H–^{13}C long-range key correlations of **1**.

Spectroscopic analysis of compound **2** revealed that it was very closely related to lahorenoic acid A (**1**) but differed from **1** by 14 mass units. Comparison of the ^{1}H and ^{13}C chemical shifts for **1** and **2** showed a slight upfield shift for C-1 by 1.9 ppm in the ^{13}C NMR spectrum and the presence of an oxygenated methyl group (δ_H 3.68, s; δ_C 52.0) in the ^{1}H NMR and ^{13}C spectrum of **2** (Table 1). These differences could be explained by methylation of the free carboxylic acid in **2**,[28] as confirmed by an HMBC correlation from the 3H singlet at δ_H 3.68 (H_3-17) to the C-1 resonance (δ_C 175.9). Therefore, the structure of **2** was assigned as methyl 6-(2-((*E*)-3-oxobut-1-enyl)-phenyl)hexanoate and given the trivial name lahorenoic acid B. Since during the purification process methanol was applied as eluent, compound **2** could possibly represent an isolation artifact of **1**. However, LC-MS investigations using MeCN as mobile phase proved that compound **2** is already present in the crude ethyl acetate extract. Thus, we currently consider lahorenoic acid B (**2**) to be a real product of a biosynthesis pathway, and lahorenoic acid A (**1**) appears to be an intermediate in the biosynthesis of lahorenoic acid B (**2**).

High-resolution mass measurement of the sodiated molecular ion for **3** yielded a molecular formula of $C_{16}H_{20}O_2$ with 7 degrees of unsaturation. Analysis of the NMR data (Table 1) indicated that compound **3** shared many structural features with lahorenoic acid A (**1**). Differences between them, evident in their 1D spectra, resulted from the absence of one carbonyl group in **3** that was formerly observed at δ_C 201.2 in the spectrum for **1**, from the upfield shift of H_3-16, and from the appearance of signals representing a second double bond (δ_H 6.17 and 6.60) in the spectra for compound **3**. Analysis of the COSY and HMBC spectra revealed that compound **3** possessed a 1-butene side chain that is attached to a benzene ring and a 5-hexenoic acid, bonded to the benzene ring in *ortho* position. The configurations of the double bonds were found to be 5*E*, 13*Z*, as deduced from the coupling constants $^{3}J_{H5-H6}$ of 15.8 Hz and $^{3}J_{H13-H14}$ of 11.7 Hz, respectively. The deduction of the 13*Z* configuration was also supported by the typical upfield shift of δ_H 7.7 to 7.1 for the conjugated ring proton H-11, which is usually observed in this case for this compound class.[23] Therefore, compound **3** was identified as (5*E*)-6-(2-((*Z*)-but-1-enyl)phenyl)hex-5-enoic acid and given the name lahorenoic acid C (**3**).

The structures of phenazines **4**–**7**,[29–31] diketopiperazines **8**–**10**,[29,32–35] and the cyclic lipodepsipeptide "white line inducing principle" (WLIP) **11**[36] were established either by direct comparison of their spectral data with literature data or by an independent spectral analysis (see Supporting Information). Regarding the analysis of WLIP (**11**), it is noteworthy to mention that the strain PB-St2 provided false negative results in the white line in agar (WLA) test[37] (see Supporting Information). We therefore suggest that the WLA test should no longer be considered a means of unequivocal proof of WLIP production by a *Pseudomonas* strain. The application of X-ray crystallography in combination with chemical degradation studies and subsequent Marfey's analysis[38] allowed the unambiguous confirmation that compound **11** is indeed WLIP and not its epimer viscosin.[39]

Bioassay. The antifungal, antimicrobial, and cytotoxic effects of compounds **2**, **4**, **6**, **7**, and **11** were investigated. Lack of material precluded the bioassays with compounds **1**, **3**, and **5**. Diketopiperazines **8**–**10** were not included in the bioassay investigations since they were considered isolation artifacts.[40,41] While compounds **6** and **11** were inactive in all

dx.doi.org/10.1021/np3005166 | *J. Nat. Prod.* 2013, 76, 135–141

assays conducted, compounds **2**, **4**, and **7** were found to be active. Lahorenoic acid B (**2**) showed a moderate inhibition of *Mycobacterium tuberculosis* (MIC = 49.5 μg/mL) and was noncytotoxic and not antifungal. For phenazine-1-carboxylic acid (**4**) a 50% growth inhibition (IC_{50}) value of 15.6 μM was determined toward HCT-116 cells. In disc diffusion assays, **4** showed selectivity toward the solid tumor cell lines H-125, MCF-7, and LNCaP (see Supporting Information). In antimicrobial assays, compound **4** showed a moderate activity toward *M. tuberculosis* (MIC = 32.9 μg/mL) and demonstrated a 0.2 cm growth inhibition zone (reduced hyphal density) toward the fungus *Colletotrichum falcatum* at 100 μg per filter disc. 2,8-Dihydroxyphenazine (**7**) was inactive in antifungal and cytotoxicity assays but demonstrated in antibacterial tests a moderate activity toward *Arthrobacter crystallopoietes* DSM 20117 (MIC = 25 μg/mL) and *Bacillus cereus* (MIC = 25 μg/mL).

This study demonstrated, by proof of production of the new *ortho*-dialkyl-substituted aromatic acids **1**–**3** and the cyclic lipopeptide WLIP (**11**), that *P. aurantiaca* PB-St2 posssess a higher biosynthetic capacity than previously anticipated. Evaluation of the toxic and antimicrobial potential of the major metabolites confirmed at the 100 μg level phenazine-1-carboxylic acid (**4**) to be the antifungal component of the strain. However, since in parallel compound **4** exhibited also cytotoxic properties toward eukaryotic cells, the antifungal activity has to be classified as nonspecific. 2-Hydroxyphenazine (**6**), which was previously identified as an antifungal component,[18] was active only above the 200 μg level and therefore reassessed in our strict assays as not active. Furthermore, this study revealed that the secrected metabolites of PB-St2 possess moderate antibacterial properties that might additionally contribute to the biocontrol effect of the strain.

EXPERIMENTAL SECTION

General Experimental Procedures. High-performance liquid chromatography (HPLC) was performed using either a Merck-Hitachi system consisting of an L-6200 pump, an L-7420 UV–vis detector, a Rheodyne 7125 injector, and a Knauer interface box, controlled by Knauer EuroChrom software, or a Waters system consisting of a 600 pump, a 996 photodiode array detector, a Rheodyne 7161 injector, and a Perkin-Elmer Series 200 vacuum degasser. 1D and 2D NMR experiments were carried out on a Bruker Avance 300 DPX spectrometer equipped with a 5 mm broadband observe (BBO) probe head or a 2.5 mm selective inverse (SEI) microprobe head and on a Bruker AV-III 600 MHz spectrometer using an inverse broadband probe head, respectively. For samples, obtained in small quantities, advanced 5 mm microtubes, matched to $CDCl_3$, MeOH, or DMSO (Shigemi, Allison Park, PA, USA), were applied for ^{13}C NMR and DEPT135 NMR experiments. Spectra were referenced to residual solvent signals with resonances at $\delta_{H/C}$ 7.26/77.0 ($CDCl_3$), $\delta_{H/C}$ 3.35/49.0 (CD_3OD), and 2.50/39.5 (d_6-DMSO). UV spectra were taken on Perkin-Elmer Lambda 40 and Thermo Scientific Evolution 300 instruments, respectively. Infrared spectra were obtained employing Perkin-Elmer Spectrum BX and Bruker Vector 22 FT-IR instruments, respectively. EIMS and ESIMS spectra were recorded on a Kratos MS 50 and Bruker Daltonic's micrOTOF-Q instrument, respectively. Macherey-Nagel Polygoprep 50–60 reversed-phase silica gel was used for vacuum liquid chromatography. All solvents were purchased as HPLC grade.

Biological Material and Collection. *P. aurantiaca* PB-St2 was isolated by one of the authors (S.M.) from surface-sterilized stems of sugar cane grown in Pakistan.[18,19] The identity of the strain was proven by 16S rRNA gene sequence analysis. The 16S sequence for PB-St2 was deposited in GenBank (accession number EU761590). Reference strains *P. tolaasii* LMG 2342^T (also known as NCPPB 2192^T and ATCC 33618^T) and "*P. reactans*" LMG 5329 were obtained from the BCCM/LMG Bacteria Collection, Ghent, Belgium. *P. fluorescens* SWB 25 was kindly provided by J. M. Raaijmakers (Wageningen University, Wageningen, The Netherlands). The fungal phytopathogens *Colletotrichum falcatum* (BF166, C01148, CP77400, SPF234) and *Rhizoctonia solani* AG 2-2 and AG 4[42] were kindly provided by the Shukur Gunj Research Institute, Jhang, Pakistan, and M. Höfte, University of Ghent, Belgium, respectively. *M. tuberculosis* $H_{37}Rv$ was obtained from the American Type Culture Collection (ATCC 27294).

Fermentation and Isolation. *P. aurantiaca* PB-St-2 was grown with shaking (140 rpm) at 26 °C in four 5 L Erlenmeyer flasks, each containing 1 L of LB medium. After 72 h of incubation, cell and supernatant fractions of the cultures were separated by centrifugation at 3000 rpm for 20 min. Supernatant was acidified to pH 3 with HCl and extracted four times with chloroform (1:1, v/v), to yield 1.6 g of crude extract. The extract was fractionated by vacuum liquid chromatography (VLC) over reversed-phase silica gel using a stepwise gradient of methanol–water and finally dichloromethane to give six fractions. ^1H NMR profiling of these fractions indicated the 20% H_2O in MeOH fraction and the 100% MeOH fraction to be of further interest due to a large number of aromatic resonances in its ^1H NMR spectrum. Separation of the (80:20) VLC fraction by RP-HPLC by using a linear gradient of 55:45–100:0 MeOH–H_2O (0.05% TFA) over a period of 40 min, followed by isocratic elution at 100% MeOH for an additional 20 min (Phenomenex Luna C_{18}(2)-100A, 10 × 250, 5 μm column in combination with a Phenomenex SecurityGuard Luna-C_{18}(2) 10 × 10 mm precolumn; 1.9 mL/min flow rate; UV monitoring at 215 nm), produced 10 subfractions. ^1H NMR profiling of these HPLC subfractions showed 3, 4, and 8 to be of further interest. HPLC subfraction 3 represented pure 2,8-dihydroxyphenazine (**7**) (10.6 mg). HPLC subfractions 4 and 8 appeared to be mixtures of aromatic compounds. While the chloroform-soluble parts of HPLC subfraction 4 yielded readily pure phenazine-1-carboxylic acid (**4**) (2.0 mg), rechromatography of its methanol-soluble parts by RP-HPLC applying a linear gradient of 65:35–80:20 MeOH–H_2O (0.05% TFA) over a period of 30 min (Phenomenex Luna C_{18}(2)-100A, 10 × 250 mm, 5 μm column in combination with a Phenomenex SecurityGuard Luna-C_{18}(2) 10 × 10 mm precolumn; 2.0 mL/min flow rate; UV monitoring at 215 nm) provided pure 2-hydroxyphenazine (**6**) (3.5 mg). HPLC subfraction 8 of the (80:20) VLC fraction was also purified by C_{18} HPLC, this time using a linear gradient of 50:50–75:25 MeOH–H_2O (0.05% TFA) over a period of 30 min, followed by isocratic elution at 75:25 MeOH–H_2O (0.05% TFA) for an additional 30 min (column: Waters XTerra, 4.6 × 250 mm; 0.8 mL/min flow rate; UV monitoring at 210 nm), to yield (in order of elution) compounds **1** and **2** in a semipure form. Rechromatography of these fractions by RP-HPLC employing two different RP_{18}-HPLC columns in series (Macherey-Nagel Nucleosil 120-5, 4.6 × 250 mm column, followed by a Macherey-Nagel Nucleodur 100-5 HILIC, 4.6 × 250 mm) with a linear gradient of 50–80% ACN in H_2O (0.05% TFA) over 30 min at 0.5 mL/min, followed by isocratic elution at 80:20 ACN–H_2O (0.05% TFA) for an additional 30 min, provided compounds **1** (0.9 mg) and **2** (4.3 mg) in a pure form. RP-HPLC separation of the 100% MeOH VLC fraction (Phenomenex Luna C_{18}(2)-100A, 10 × 250 mm, 5 μm column in combination with a Phenomenex SecurityGuard Luna-C_{18}(2) 10 × 10 mm precolumn; 2.0 mL/min flow rate; UV monitoring at 210 nm) using gradient elution from 80% to 100% MeOH in H_2O (0.05% TFA) over 45 min, followed by isocratic elution at 100% MeOH for an additional 15 min, gave compounds **5** (1.2 mg) and impure WLIP (**11**) and provided additional quantities of compounds **4** and **7**. The WLIP (**11**)-containing fraction was purified twice by RP-HPLC, first by employing a Macherey-Nagel Nucleosil 120-5, 4 × 250 mm column (1.0 mL/min, 40 min gradient elution from 60% to 100% MeOH–H_2O (0.05% TFA) followed by a 20 min hold) and subsequently a Waters SymmetryShield RP_{18}, 4.6 × 250 mm column (1.0 mL/min, 40 min gradient elution from 50% to 100% ACN–H_2O (0.05% TFA) followed by a 20 min hold), to yield 6 mg of pure WLIP (**11**). Higher yields of **11** were achieved by variation of the extraction solvent (e.g., EtOAc instead of $CHCl_3$) or the fermentation medium

(Davis Minimal Broth without dextrose, supplemented with 20 mM glycerol (DMBgly) instead of LB medium). Adaption of the extraction procedure, using CH_2Cl_2 without prior pH adjustment by acidification of the supernatant, led to the isolation of diketopiperazines **8**–**10**. Under these conditions, 490 mg of crude extract was obtained from a 4.5 L LB-medium-based culture. The ACN-soluble parts were directly subjected to C_8 HPLC (Knauer Eurospher II C_8 100-5, 8 × 250 mm column 2.0 mL/min, 40 min gradient elution from 35% to 70% ACN–H_2O followed by a 20 min hold) to give compound **8** (2.0 mg) and a mixture of **9** and **10** and provided additional quantities of compounds **4** and **6**. Rechromatography of the diketopiperazine mixture using a C_{18} Macherey-Nagel Nucleosil 120-5, 4.0 × 250 mm column (1.0 mL/min, gradient elution from 35% to 80% ACN in 40 min, followed by a 20 min hold) gave pure **9** (2.8 mg) and **10** (0.8 mg).

Further adaption of the cultivation and extraction procedure, employing DMBgly medium on a 4.5 L scale, with only 25 h of growth and extraction with CH_2Cl_2 without prior pH adjustment yielded **3**. The ACN-soluble parts of the crude extract (110 mg) were directly subjected to HPLC (Knauer Eurospher II C_8 100-5, 8 × 250 mm column 2.0 mL/min, 30 min gradient elution from 50% to 75% ACN–H_2O followed by a 30 min hold) to afford 1.2 mg of compound **3**.

*Lahorenoic acid A (**1**):* pale yellow, amorphous solid; UV (MeOH) λ_{max} 210 nm (ε 4.23), 225sh nm (ε 4.12), 285 nm (ε 4.19); FT-IR (ATR) ν_{max} 2930, 2857, 1708, 1668, 1596, 1416, 1359, 1257, 1180, 974, 754 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z* (positive) 283.1 $[M + Na]^+$; HRESIMS *m/z* 283.1302 (calcd for $C_{16}H_{20}NaO_3$, 283.1310).

*Lahorenoic acid B (**2**):* pale yellow, amorphous solid; UV (MeOH) λ_{max} 211 nm (ε 4.23), 226sh nm (ε 4.14), 288 nm (ε 4.32); FT-IR (ATR) ν_{max} 2927, 2854, 1735, 1670, 1597, 1435, 1358, 1254, 1174, 975, 753 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z* (positive) 297.2 $[M + Na]^+$; HRESIMS *m/z* 297.1461 (calcd for $C_{17}H_{22}NaO_3$, 297.1467).

*Lahorenoic acid C (**3**):* pale yellow, amorphous solid; UV (MeOH) λ_{max} 244 nm (ε 5.31); FT-IR ν_{max} 2930, 2871, 1711, 1452, 1404, 1249, 1159, 967 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z* (positive) 267.1 $[M + Na]^+$; HRESIMS *m/z* 267.1341 (calcd for $C_{16}H_{20}NaO_2$, 267.1356).

*Phenazine-1-carboxylic acid (tubermycin B) (**4**):* yellow needles; ^1H and ^{13}C NMR data, see Table S1 of the Supporting Information; HRESIMS $[M + Na]^+$ *m/z* 247.0475 (calcd for $C_{13}H_8N_2NaO_2$, 247.0483).

*2-Hydroxyphenazine-1-carboxylic acid (**5**):* orange powder; ^1H and ^{13}C NMR data, see Table S2 of the Supporting Information; HREIMS $[M]^+$ *m/z* 240.0537 (calcd for $C_{13}H_8N_2O_3$, 240.0535).

*2-Hydroxyphenazine (**6**):* orange powder; ^1H and ^{13}C NMR data, see Table S3 of the Supporting Information; HRESIMS $[M + H]^+$ *m/z* 197.0709 (calcd for $C_{12}H_9N_2O$, 197.0715).

*2,8-Dihydroxyphenazine (**7**):* brownish-orange, amorphous solid; ^1H and ^{13}C NMR data, see Table S4 of the Supporting Information; HRESIMS $[M + H]^+$ *m/z* 213.0663 (calcd for $C_{12}H_9N_2O_2$, 213.0664).

***cyclo*-(L-Pro-L-Tyr) (maculosin-1) (8):** colorless, amorphous solid; $[\alpha]_D^{22}$ −131.3 (*c* 0.14, EtOH) (lit.,[29] −126.1 in EtOH, lit.,[32] −144.5 in MeOH); HRESIMS $[M + H]^+$ *m/z* 261.1235 (calcd for $C_{14}H_{17}N_2O_3$, 261.1239); ^1H and ^{13}C NMR data, see Supporting Information.

***cyclo*-(L-Pro-L-Val) (9):** colorless, amorphous solid; $[\alpha]_D^{22}$ −142.8 (*c* 0.20, EtOH) (lit.,[29] −139.4 in EtOH, lit.,[43] ranging from −134 to −180 in EtOH, lit.,[44] −128 in MeOH); HRESIMS $[M + Na]^+$ *m/z* 219.110 (calcd for $C_{10}H_{16}N_2NaO_2$, 219.1109); ^1H and ^{13}C NMR data, see Supporting Information.

***cyclo*-(L-Pro-L-Met) (10):** colorless, amorphous solid; $[\alpha]_D^{22}$ −85.7 (*c* 0.057, EtOH) (lit.,[29] −82.2 in EtOH); HRESIMS $[M + Na]^+$ *m/z* 251.0839 (calcd for $C_{10}H_{16}N_2NaO_2S$, 251.0830); ^1H and ^{13}C NMR data, see Supporting Information.

WLIP (11): yellowish glass; ^1H and ^{13}C NMR data, see Table S5 in the Supporting Information); HRESIMS $[M + Na]^+$ *m/z* 1148.6798 (calcd for $C_{54}H_{95}N_9O_{16}Na$, 1148.6794).

X-ray Crystallographic Analysis of 2-Hydroxyphenazine (6) and WLIP (11). The data collection was performed on a NONIUS KappaCCD diffractometer (area detector) for **6** and a Bruker X8-KappaApexII diffractometer for **11** using graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). Both diffractometers were equipped with a low-temperature device (Cryostream, Oxford Cryosystems, 123(2) K, Bruker Kryoflex, 100(2) K). Intensities were measured by fine-slicing psi and omega scans and corrected for background, polarization, and Lorentz effects. A semiempirical absorption correction from equivalent reflections was applied for the data sets following Blessing's method.[45] The structures were solved by direct methods and refined anisotropically by the least-squares procedure implemented in the SHELX program system.[46] The hydrogen atoms were included isotropically using the riding model on the bound carbon atoms. The illustrations of the molecular structures were prepared with Diamond 2.1c.[47] Crystals of 2-hydroxyphenazine (**6**) were obtained by slow evaporation from an ethyl acetate solution stored at 4 °C. Monoclinic space group $C2/c$ with a = 18.9536(13) Å, b = 4.3422(2) Å, c = 22.3807(17) Å, α = 90.00°, β = 96.752(3)°, γ = 90.00°, V = 1829.2(2) Å^3 at 123(2) K with Z = 8; 5879 reflections measured, 2154 independent reflections (R_{int} = 0.0734). The final R_1 values were 0.0485 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1089 ($I > 2\sigma(I)$). The final R_1 values were 0.0890 (all data). The final $wR(F^2)$ values were 0.1234 (all data). Crystals of WLIP (**11**) were generated in a two-staged process: 6.3 mg of WLIP (**11**) was dissolved in a vial in 0.5 mL of DMSO, followed by addition of 1.5 mL of methanol. Seed crystals were obtained by slow evaporation of the solvent in a 100 mL round-bottom flask at room temperature after 24 h. Upon freeze-drying of the sample, suitable seed crystals were selected and inserted into a DMSO–methanol solution of 3.1 mg of compound **11**. Crystals suitable for X-ray analysis were obtained by slow evaporation at room temperature after several weeks. Orthorhombic space group $P2_12_12_1$ with a = 14.1536(5) Å, b = 18.7258(6) Å, c = 24.3604(8) Å, V = 6456.4(4) Å^3 at 100(2) K with Z = 4; 25 141 reflections measured, 8473 independent reflections (R_{int} = 0.0207). The final R_1 values were 0.0370 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0933 ($I > 2\sigma(I)$). The final R_1 values were 0.0443 (all data). The final $wR(F^2)$ values were 0.0994 (all data). CCDC 874817 (**6**) and CCDC 919229 (**11**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. The absolute configuration fixed by the side-chain stereochemistry of threonine and glutamic acid was supported by a Marfey analysis.[38]

Absolute Configuration of the Glu2 and Thr3 Residues of WLIP (11). WLIP (1.0 mg) was dissolved in 400 μL of 6 N HCl and was hydrolyzed in a sealed vial at 110 °C for 15 h, using an oil bath. The solvent was removed by using a stream of dry N_2. A 1 M $NaHCO_3$ solution (100 μL) was added to the dried hydrolysate of **11**, as well as to standards of L-Glu, D-Glu, L-Thr, L-*allo*-Thr, D-Thr, and D-*allo*-Thr. A solution of 1% L-FDAA in acetone (50 μL) was added to each reaction batch. Each vial was sealed and incubated at 80 °C for 30 min. To quench the reactions, 50 μL of 2 N HCl was added to each sample and evaporated to dryness. The Marfey's derivatives of the hydrolysate and standards were resuspended in a mixture of DMSO (150 μL) and ACN (150 μL), and analyzed by C_{18} HPLC (1.0 mL/min, 40 min gradient elution from 25% to 100% ACN–H_2O acidified with 0.05% TFA, UV detection at 340 nm). Retention times for the amino acid standards were L-Thr 10.5 min, L-*allo*-Thr 10.6 min, D-Thr 11.5 min, D-*allo*-Thr 10.9 min, L-Glu 12.8 min, and D-Glu 12.1 min, while the hydrolysate gave peaks at 10.9 and 12.1 min. Co-injection of the hydrolysate with the corresponding standards unequivocally established the analyzed residues as D-Glu and D-*allo*-Thr.

White-Line-in-Agar Test. The white line test was performed according to the method of Wong and Preece.[37] *P. aurantiaca* was streaked on King's B medium at 1 cm distance next to *Pseudomonas tolaasii* LMG 2342^T, and the formation of a white precipitate in the agar was evaluated after 24 to 72 h growth at 26 °C. *Pseudomonas reactans* LMG 5329 (WLIP producer) and *Pseudomonas fluorescens* SWB 25 (viscosin producer)[48] were included as positive and negative controls, respectively. Assays were carried out in duplicates and repeated twice.

Antibacterial Assay. The antibacterial activity was determined as previously published[49] by agar diffusion assays. In brief, culture plates

(5% sheep blood Columbia agar, BD) were overlaid with 3 mL of tryptic soy soft agar, inoculated with tryptic soy broth (TSB, Oxoid) growth suspension of the bacteria to be tested. Compounds were diluted with DMSO to a concentration of 1 mg/mL, and 3 μL of this dilution was spotted on the surface of the agar; the diameter of the inhibition zone was measured after 24 h incubation at 37 °C. The indicator strains represent clinical isolates from distinct patients.[49] These strains were maintained on Mueller–Hinton agar or on blood agar. MIC determinations were carried out in microtiter plates. *Arthrobacter crystallopoietes* DSM 20117 was grown in tryptic soy broth (Oxoid); all other strains were grown in half-concentrated Mueller–Hinton broth (Oxoid). MICs with 2-fold serial dilution steps were performed (1:2). Bacteria were added to give a final concentration of 10^5 CFU in a volume of 0.2 mL. After incubation for 24 h at 37 °C, the MIC was read as the lowest compound concentration causing inhibition of visible growth. Results are mean values of three independent determinations. Pure compounds demonstrating an MIC higher than 50 μg/mL were regarded as inactive samples.

Antituberculosis Bioassay. The virulent H_{37}Rv strain of *M. tuberculosis* (ATCC27294, American Type Culture Collection, Rockville, MD, USA) was used for the antituberculosis bioassay.[50,51] The test materials were dissolved in DMSO at 10 mg/mL and tested in a series of 2-fold dilutions with the highest concentration of 100 μg/mL (and 1% v/v DMSO). Samples were incubated at 37 °C for 7 days with *M. tuberculosis* in a 96-well plate, and then cell growth was determined using the Alamar Blue dye with fluorometric detection. The MIC was defined as the lowest concentration resulting in 90% or greater inhibition of fluorescence compared to controls (*M. tuberculosis* without sample). Rifampicin and INH were used as positive controls, exhibiting MICs of 0.02–0.07 and 0.02–0.24 μg/mL, respectively, while DMSO at a final concentration of 1% v/v served as a negative control. Pure compounds demonstrating an MIC higher than 50 μg/mL were regarded as inactive samples.

Antifungal Bioassay. The antifungal activity of PB-St2 was examined using four strains of *Colletotrichum falcatum,* isolated from local sugar cane varieties (BF166, C01148, CP77400, and SPF234). Potato dextrose agar (PDA; Difco, MI, USA) was used for these assays. A small plug cut from a growing edge of the mycelium of the target fungus was seeded at the center of the medium in a Petri plate. Aliquots (25, 50, 75, 100, 150, and 200 μg) of purified compounds dissolved in suitable solvents (methanol, chloroform, or DMSO) were applied on sterilized filter paper discs, placed 2 cm away from the fungal plug, and air-dried in a laminar flow cabinet before incubation. Plates were incubated for 10–15 days at 25 °C. Antifungal activity was observed by formation of a zone of inhibition of mycelial growth around the disc. Discs with methanol, chloroform, and DMSO were used as negative controls. These assays were repeated three times in duplicate. Pure substances demonstrating no effect up to 100 μg per disc were regarded as inactive.

Disc Diffusion Soft-Agar Colony Formation Assay. An *in vitro* cell-based assay was employed to identify the cytotoxicity and the solid tumor selectivity for pure compounds. Cell lines used in this study included cell lines of murine (L1210 leukemia, C38 colon carcinoma, CFU-GM normal) and human origin (H116 colon carcinoma, H125 lung adenocarcinoma, MCF-7 and MDA-MB-231 breast adenocarcinoma, LNCaP prostate adenocarcinoma, OVCAR-3 ovarian adenocarcinoma, U251N glioma, Panc-1 pancreatic carcinoma, CCRF-CEM leukemia, CFU-GM normal). Samples were dissolved in 250 μL of DMSO, and a 15 μL aliquot was applied to a cellulose disc in an agar plate containing cells. After a period of incubation, a zone of cell colony inhibition (z) was measured from the edge of the disc to the edge of colony growth and expressed as zone units (zu), where 200 zu was equal to 6 mm. General cytotoxic activity for a given sample was defined as an antiproliferation zone of 300 zu or greater. The differential cytotoxicity[52] of a pure compound was expressed by observing a zone differential of 250 units or greater between any solid-tumor cell (murine or human) and their corresponding leukemia cells or normal cells.

IC_{50} Determination. Human colon carcinoma cells (HCT-116)[53] were grown in 5 mL of culture medium (RPMI 1640 plus 15% fetal bovine serum containing 1% penicillin–streptomycin and 1% glutamine)[54] at 37 °C and 5% CO_2 from a starting cell density of 5 × 10^4 cells/T25 flask. On day 3, the cells were exposed to different concentrations of the corresponding pure compound. The flasks were incubated for 120 h (5 days) at 5% CO_2 and 37 °C, and the cells were harvested with trypsin, washed once with Hanks' balanced salt solution, resuspended in Hanks' balanced salt solution, and counted using a hemocytometer. The results were normalized to an untreated control. The 50% inhibitory concentration (IC_{50}) was determined by nonlinear regression analysis using Prism 4.0 software (GraphPad, San Diego, CA, USA). Pure compounds demonstrating an IC_{50} value higher than 20 μg/mL were regarded as inactive samples.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR and MS spectral data of compounds **1**–**11**, X-ray structures of **6** and **11**, and detailed results of biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(S.M.) Tel: +92 42 99231581, ext. 544. Fax: 92 42 99230703. E-mail: saminamehnaz@fccollege.edu.pk. (H.G.) Tel: +49 7071 2976970. E-mail: harald.gross@uni-tuebingen.de.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We gratefully acknowledge M. Höfte, Ghent University, Belgium, and J. M. Raaijmakers, Wageningen University, The Netherlands, for providing the strains *Rhizoctonia solani* AG2-2/AG4 and *Pseudomonas fluorescens* SWB 25, respectively. We thank S. Kehraus, University of Bonn, for invaluable help regarding structure elucidation. S.M. thanks Q. Zaheer-ul-Haq, University of Karachi, for technical support. H.G. and S.M. would like to gratefully acknowledge the generous contribution of the Alexander von Humboldt Foundation, which provided financial support for conducting this research (Georg Forster Fellowship awarded to S.M.). G.S. thanks A. C. Filippou, University of Bonn, for support. This research was also supported by the Deutsche Forschungsgemeinschaft (DFG) grants GR 2673/2-1 (H.G.) and Sa292/13-1 (H.G.S.) within the Research Unit FOR854.

■ REFERENCES

(1) Gerhardson, B. *Trends Biotechnol.* **2002**, *20*, 338–343.
(2) Compant, S.; Duffy, B.; Nowak, J.; Clément, C.; Barka, E. A. *Appl. Environ. Microbiol.* **2005**, *71*, 4951–4959.
(3) Ligon, J. M.; Hill, D. S.; Hammer, P. E.; Torkewitz, N. R.; Hofmann, D.; Kempf, H.-J.; van Pée, K.-H. *Pest Manage. Sci.* **2000**, *56*, 688–695.
(4) Naik, P. R.; Sahoo, N.; Goswami, D.; Ayyadurai, N.; Sakthivel, N. *Microb. Ecol.* **2008**, *56*, 492–504.
(5) Feklistova, I. N.; Meksimova, N. P. *Microbiology* **2008**, *77*, 207–212.
(6) Walsh, U. F.; Morrissey, J. P.; O'Gara, F. *Curr. Opin. Biotechnol.* **2001**, *12*, 289–295.
(7) Thomashow, L. S.; Weller, D. M. *J. Bacteriol.* **1988**, *170*, 3499–3508.
(8) D'aes, J.; Hua, G. K.; De Maeyer, K.; Pannecoucque, J.; Forrez, I.; Ongena, M.; Dietrich, L. E.; Thomashow, L. S.; Mavrodi, D. V.; Höfte, M. *Phytopathology* **2011**, *101*, 996–1004.
(9) Chin-A-Woeng, T. F. C.; Bloemberg, G. V.; van der Bij, A. J.; van der Drift, K. M. G. F.; Schripsema, J.; Kroon, B.; Scheffer, R. J.; Keel,

C.; Bakker, P. A. H. M.; Tichy, H.-V.; de Bruijn, F. J.; Thoms-Oates, J. E.; Lugtenberg, B. J. J. *Mol. Plant-Microbe Interact.* **1998**, *11*, 1069−1077.
(10) Gross, H.; Stockwell, V. O.; Henkels, M. D.; Nowak-Thompson, B.; Loper, J. E.; Gerwick, W. H. *Chem. Biol.* **2007**, *14*, 53−63.
(11) Berry, C.; Fernando, W. G. D.; Loewen, P. C.; de Kievit, T. R. *Biol. Control* **2010**, *55*, 211−218.
(12) Loper, J. E.; Gross, H. *Eur. J. Plant Pathol.* **2007**, *119*, 265−278.
(13) Loper, J. E.; Henkels, M. D.; Shaffer, B. T.; Valeriote, V. A.; Gross, H. *Appl. Environ. Microbiol.* **2008**, *74*, 3085−3093.
(14) Johnsson, L.; Hökeberg, M.; Gerhardson, B. *Eur. J. Plant Pathol.* **1998**, *104*, 701−711.
(15) Stockwell, V. O.; Johnson, K. B.; Sugar, D.; Loper, J. E. *Phytopathogy* **2010**, *100*, 1330−1339.
(16) Buddrus-Schiemann, K.; Schmid, M.; Schreiner, K.; Welzl, G.; Hartmann, A. *Microb. Ecol.* **2010**, *60*, 381−393.
(17) van Rad, U.; Mueller, M. J.; Durner, J. *New Phytol.* **2005**, *165*, 191−202.
(18) Mehnaz, S.; Baig, D. N.; Jamil, F.; Weselowski, B.; Lazarovits, G. *J. Microbiol. Biotechnol.* **2009**, *19*, 1688−1694.
(19) Mehnaz, S.; Baig, D. N.; Lazarovits, G. *J. Microbiol. Biotechnol.* **2010**, *20*, 1614−1623.
(20) Singh, K.; Singh, R. H. In *Sugarcane−Major Diseases*; Ricaud, C.; Egan, B. T.; Gillaspie, A. G., Jr.; Hughes, C. G., Eds.; Elsevier: New York, 1988; pp 169−188.
(21) Singh, K.; Singh, R. P. In *Sugarcane Diseases of the World: Major Diseases*; Ricaud, C. G.; Egan, B. T.; Gillaspie, A. G.; Hughes, C. G., Eds.; Elsevier: Amsterdam, The Netherlands, 1989; pp 169−188.
(22) Gross, H.; Loper, E. L. *Nat. Prod. Rep.* **2009**, *26*, 1408−1446.
(23) Wenzel, S. C.; Bode, H. B. *J. Nat. Prod.* **2004**, *67*, 1631−1633.
(24) Ritzau, M.; Drautz, H.; Zähner, H.; Zeeck, A. *Liebigs Ann. Chem.* **1993**, 433−435.
(25) DeVault, R. L.; Schmitz, H.; Hooper, I. R. *Antimicrob. Agents Chemother.* **1965**, *5*, 796−800.
(26) Holland, G. S.; Jamieson, D. D.; Reichelt, J. L.; Viset, G.; Wells, R. J. *Chem. Ind.* **1984**, *23*, 850−851.
(27) Sánchez, L. G.; Castillo, E. N.; Maldonado, H.; Chávez, D.; Somanathan, R.; Aguirre, G. *Synth. Commun.* **2008**, *38*, 54−71.
(28) Williamson, K. L.; Ul Hasan, M.; Clutter, D. R. *J. Magn. Reson.* **1978**, *30*, 367−383.
(29) Jayatilake, G. S.; Thornton, M. P.; Leonard, A. C.; Grimwade, J. E.; Baker, B. J. *J. Nat. Prod.* **1996**, *59*, 293−296.
(30) Römer, A.; Budzikiewicz, H.; Korth, H.; Pulverer, G. *Tetrahedron Lett.* **1979**, *20*, 509−512.
(31) Geiger, A.; Keller-Schierlein, W.; Brandl, M.; Zähner, H. *J. Antibiot.* **1988**, *41*, 1542−1551.
(32) Akkermann, D. Molekularbiologische und naturstoffchemische Untersuchungen ausgewählter Bakterienstämme. Ph.D. Thesis, University of Bonn, Germany, 2004.
(33) Böhlendorf, B.; Forche, E.; Bedorf, N.; Gerth, K.; Irschik, H.; Jansen, R.; Kunze, B.; Trowitzsch-Kienast, W.; Reichenbach, H.; Höfle, G. *Liebigs Ann.* **1996**, 49−53.
(34) Degrassi, G.; Aguilar, C.; Bosco, M.; Zahariev, S.; Pongor, S.; Venturi, V. *Curr. Microbiol.* **2002**, *45*, 250−254.
(35) Tezuka, Y.; Huang, Q.; Kikuchi, T.; Nishi, A.; Tubaki, K. *Chem. Pharm. Bull.* **1994**, *42*, 2612−2617.
(36) Mortishire-Smith, R. J.; Nutkins, J. C.; Packman, L. C.; Brodey, C. L.; Rainey, P. B.; Johnstone, K.; Williams, D. H. *Tetrahedron* **1991**, *47*, 3645−3654.
(37) Wong, W. C.; Preece, T. F. *Appl. Bacteriol.* **1979**, *47*, 401−407.
(38) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591−596.
(39) Laycock, M. V.; Hildebrand, P. D.; Thibault, P.; Walter, J. A.; Wright, J. L. C. *J. Agric. Food Chem.* **1991**, *39*, 483−489.
(40) Tamura, S.; Suzuki, A.; Aoki, Y.; Otake, N. *Agric. Biol. Chem.* **1964**, *28*, 650−652.
(41) Mitscher, L. A.; Kunstmann, M. P.; Martin, J. H.; Andres, W. W.; Evans, R. H., Jr.; Sax, K. J.; Patterson, E. L. *Experientia* **1967**, *23*, 796.
(42) Nerey, Y.; Pannecoucque, J.; Hernandez, H. P.; Diaz, M.; Espinosa, R.; de Vos, S.; van Beneden, S.; Herrera, L.; Höfte, M. *J. Phytopathol.* **2010**, *158*, 236−243.
(43) Adamczeski, M.; Reed, A. R.; Crews, P. *J. Nat. Prod.* **1995**, *58*, 201−208.
(44) Takeda, Y.; Fujita, T.; Shingu, T.; Ogimi, C. *Chem. Pharm. Bull.* **1987**, *35*, 2569−2573.
(45) Blessing, R. H. *Acta Crystallogr. A* **1995**, *51*, 33.
(46) Sheldrick, G. M. *SHELXS97* and *SHELXL97*; University of Göttingen: Germany, 1997.
(47) Brandenburg, K. *DIAMOND* 2.1c; Crystal Impact GbR: Bonn, Deutschland, 1999.
(48) de Bruijn, I.; de Kock, M. J. D.; Yang, M.; de Waard, P.; van Beek, T. A.; Raaijmakers, J. M. *Mol. Microbiol.* **2007**, *63*, 417−428.
(49) Reder-Christ, K.; Schmidt, Y.; Dörr, M.; Sahl, H.-G.; Josten, M.; Raaijmakers, J. M.; Gross, H.; Bendas, G. *BBA Biomembr.* **2012**, *1818*, 566−573.
(50) Collins, L. A.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004−1009.
(51) Falzari, K.; Zhu, Z.; Pan, D.; Liu, H.; Hongmanee, P.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **2005**, *49*, 1447−1454.
(52) Valeriote, F.; Grieshaber, C. K.; Media, J.; Pietraszkewicz, H.; Hoffmann, J.; Pan, M.; McLaughlin, S. *J. Exp. Ther. Oncol.* **2002**, *2*, 228−236.
(53) Brattain, M. G.; Fine, W.; Khaled, F.; Thompson, J.; Brattain, D. E. *Cancer Res.* **1981**, *41*, 1751−1756.
(54) Moore, G. E.; Woods, L. K. *Methods Cell Sci.* **1977**, *3*, 503−509.