Structure, Derivatization, and Antitumor Activity of New Griseusins from *Nocardiopsis* sp.

Jian He,† Ernst Roemer,† Corinna Lange,† Xueshi Huang,† Armin Maier,‡ Gerhard Kelter,‡ Yi Jiang,§ Li-hua Xu,§ Klaus-Dieter Menzel,† Susanne Grabley,† Heinz-Herbert Fiebig,‡ Cheng-Ling Jiang,§ and Isabel Sattler*,†

Leibniz Institute for Natural Product Research and Infection Biology, Hans-Knöll-Institute, Beutenbergstr. 11a, D-07745 Jena, Germany, Oncotest GmbH, Institut für Experimentelle Onkologie, Am Flughafen 12-14, D-79108 Freiburg, Germany, and The Key Laboratory for Microbial Resources of the Ministry of Education, Yunnan Institute of Microbiology, Yunnan University, Kunming 650091, China

Received February 14, 2007

Four new griseusins, 4′-dehydro-deacetylgriseusin A (**1**) and 2a,8a-epoxy-*epi*-deacetylgriseusin B (**2**) as new constitutional derivatives and *epi*-deacetylgriseusin A (**3**) and *epi*-deacetylgriseusin B (**4**) as new stereoisomers, were isolated from *Nocardiopsis* sp. (YIM80133, DSM16644). 4′-Dehydro-deacetylgriseusin A (**1**) showed pronounced cytotoxic potency (mean $IC_{50} = 0.430$ μM) combined with a significant selectivity for mammary cancer, renal cancer, and melanoma in a panel consisting of 37 tumor cell lines. In a clonogenic assay with tumor cells from 51 solid tumors, **1** inhibited anchorage independent growth and *in vitro* colony formation of tumor cells in a concentration-dependent and tumor type selective manner. As **1** was only a minor product, a semisynthetic preparation from the major metabolite, *epi*-deacetylgriseusin A (**3**), was achieved. Our studies also yielded 9-hydroxy-*epi*-deacetylgriseusin B methylester (**5**), 4′-dehydro-9-hydroxy-deacetylgriseusin B methylester (**6**), and 4′-dehydro-2a,8a-epoxy-deacetylgriseusin B (**7**) as new synthetic isochromanequinone derivatives, which provided a basic structure−activity relationship study.

Introduction

Cancer is still the second leading disease-related cause of death worldwide.[1] Because for many types of cancer no curative therapy is available, there is an ongoing need for novel leads for chemotherapy. Nature is the primary source for antitumor agents: about 62% of anticancer drugs commercially available in Europe and the U.S.A. are of natural origin, either derived from higher plants or from microorganisms such as actinomycetes.[2] To obtain access to new natural products, we were using remote sites for bioprospecting of microbial isolates. The extreme and unusual habitats of the Yunnan province in the southwest of China comprise vast and mostly untapped resources with regard to the biology and chemistry of their unique biodiversity. The screening program included more than 500 microbial isolates, including alkalophilic and halophilic actinomycetes, as well as epi- and endophytes from plants related to traditional Chinese medicine. Six tumor cell lines that differed in chemosensitivity toward standard chemotherapeutic agents were applied to identify cytotoxic activity in microbial extracts, fractionated extracts and finally pure compounds. Active compounds were characterized in detail for their cytotoxic activity using tumor cell lines and cell suspensions derived from tumor xenografts.

4′-Dehydro-deacetylgriseusin A (**1**) and 2a,8a-epoxy-*epi*-deacetylgriseusin B (**2**) were isolated and identified along with *epi*-deacetylgriseusin A (**3**) and *epi*-deacetylgriseusin B (**4**) from the fermentation broth of *Nocardiopsis* sp. (YIM80133,[a] DSM16644; Figure 1).[3] The structures of the new compounds were determined by MS and NMR analysis. In addition, we prepared 9-hydroxy-*epi*-deacetylgriseusin B methylester (**5**), 4′-dehydro-9-hydroxy-deacetylgriseusin B methylester (**6**), and 4′-dehydro-2a,8a-epoxy-deacetylgriseusin B (**7**) as new synthetic derivatives for a basic structure−activity relationship (SAR) study.

* To whom correspondence should be addressed. Phone: ++49 3641 656920. Fax: ++49 3641 656922. E-mail: isabel.sattler@hki-jena.de.

† Leibniz Institute for Natural Product Research and Infection Biology.

‡ Oncotest GmbH.

§ Yunnan University.

Results

The extracts of *Nocardiopsis* sp. (YIM80133, DSM1664), an actinomycetes isolated from an alkaline soil sample (pH 9−10) collected from Xinjiang province (Northwest China), showed strong cytotoxic activity against all six cell lines of the screening panel ($T/C > 70\%$; LXFL 529L, MAXF 401NL, MEXF 462NL, UXF 1138L, GXF 251L, RXF 486L). TLC analysis of the product pattern on silica gel ($CHCl_3$/MeOH = 9:1, staining with anisaldehyde/H_2SO_4) revealed intense bands (R_f: **1**, 0.6; **2**, 0.1; **3**, 0.43; **4**, 0.05) that were previously not observed.[4,5] From 180 L of culture broth from a pilot-scale fermentation of the producing strain 4′-dehydro-deacetylgriseusin A (**1**, 36 mg), 2a,8a-epoxy-*epi*-deacetylgriseusin B (**2**, 35 mg), *epi*-deacetylgriseusin A (**3**, 300 mg), and *epi*-deacetylgriseusin B (**4**, 290 mg) were isolated by column chromatography (Figure 1).

The molecular formula of compound **1**, was determined to be $C_{20}H_{16}O_9$ from HREIMS data at *m/z* 400.0821 (calcd, 400.0794), a molecular weight 2 amu less than that of de-acetylgriseusin A (**8**).[6,7] The ^{13}C NMR and DEPT data of **1**

[a] Abbreviations: YIM, Yunnan Institute of Microbiology; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen; SAR, structure−activity relationship; MS, mass spectrometry; HRESIMS, high resolution electrospray ionisation mass spectrometry; NMR, nuclear magnetic resonance; DEPT, distortionless enhancement by polarisation transfer; HMQC, heteronuclear multiple-quantum coherence; COSY, correlated spectroscopy; HMBC, heteronuclear multiple-bond correlation; NOE, nuclear Overhauser effect; NOESY, nuclear Overhauser effect spectroscopy; w, weak; vw, very weak; m, multiplet; s, singlett; d, dublett; dd, double dublett; ddd, dublett of dublett of dublett; cov, covered; CD, circular dichroism; HPLC; high performance liquid chromatography; TLC, thin layer chromatography; R_f, retention factor; *T/C*, test versus control value; LXFL 529L, tumor cell line of large cell lung cancer xenograft 529; MAXF 401NL, tumor cell line of mammary cancer xenograft 401; MEXF 462NL, tumor cell line of melanoma xenograft 462; UXF 1138L, tumor cell line of uterine cancer xenograft 1138; GXF 251L, tumor cell line of gastric cancer xenograft 251; RXF 486; tumor cell line of renal cancer xenograft 486; FCS, fetal calf serum; NMR, Naval Medical Research Institute, U.S.A.; nu/nu, mouse strain of athymic immune-deficient nude mice; RPMI, Roosevelt Park Memorial Institute; PBS, phosphate-buffered saline; BSA, bovine serum albumine; HSC-CFU, hematopoietic stem cell-colony forming unit; MRSA, methicillin resistant *Stapylococcus aureus*; PCC, pyridinium-chloro-chromat; PDC, pyridinium-di-chromat; DMSO, dimethylsulfoxide.

10.1021/jm070170n CCC: $37.00 © 2007 American Chemical Society
Published on Web 09/18/2007

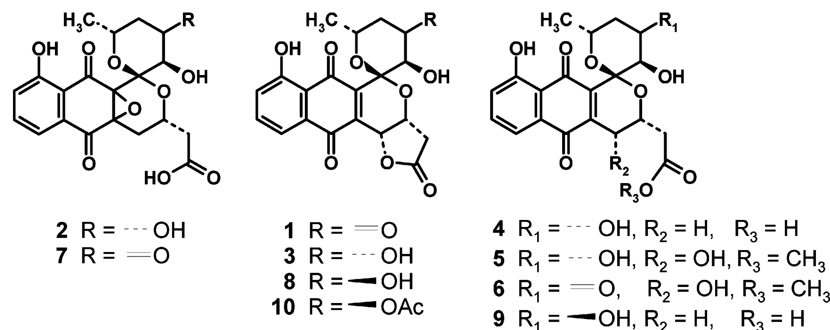

Figure 1. Chemical structures of natural products 4′-dehydro-deacetylgriseusin A (**1**), 2a,8a-epoxy-*epi*-deacetylgriseusin B (**2**), *epi*-deacetylgriseusin A (**3**), *epi*-deacetylgriseusin B (**4**), deacetylgriseusin A (**8**), and deacetylgriseusin B (**9**) as well as of synthetic derivatives 9-hydroxy-*epi*-deacetylgriseusin B methylester (**5**), 4′-dehydro-9-hydroxy-deacetylgriseusin B methylester (**6**), and 4′-dehydro-2a,8a-epoxy-deacetylgriseusin B (**7**).

Table 1. ^{1}H and ^{13}C NMR Data ($CDCl_3$) of 4′-Dehydro-deacetylgriseusin A (**1**) and 2a,8a-Epoxy-*epi*-deacetylgriseusin B (**2**)

	1			**2**		
new	^{13}C[a]	^{1}H[b]	HMBC[c]	^{13}C[a]	^{1}H[b]	HMBC[c]
2	99.4			97.1		
2a	140.5			62.9		
3	187.3			193.1		
3a	115.2			114.5		
4	162.2			162.7		
4-OH		11.9 (s)			11.6 (s)	C-3a, C-4, C-5
5	125.5	7.30 (m)	C-3a, C-4, C-5, C-7a, C-8	125.1	7.22 (dd, 1.2, 8.3)	C-3a, C-4 (w), C-7
6	137.2	7.65 (m)	[d], C-5	136.9	7.58 (dd, 7.7, 7.7)	C-4, C-7a
7	119.7	7.65 (m)	[d], C-6	119.2	7.55 (dd, 1.2, 7.5)	C-3a, C-5, C-8
7a	131.2			131.1		
8	181.7			189.4		
8a	138.5			63.9		
9	68.1	5.27 (d, 3.0)	C-2a, C-8a, C-8, C-10	27.5	2.29 (dd, 11.5, 15.0)	C-8a (vw), C-10, C-11
					2.48 (dd, 2.3, 14.8)	C-2a, C-8a
10	66.7	4.68 (dd, 3.0, 4.8)	C-9, C-12	62.4	4.32 (m)	
11	36.0	2.94 (dd, 4.9, 17.8)	C-12, C-2	38.8	2.53 (1H, dd cov)	C-9, C-10
		2.67 (d, 17.8)	C-8a (w), C-9, C-10, C-12[e]		2.64 (dd, 4.3, 15.8)	C-9, C-10
12	173.1			174.1		
3′	75.9	5.47 (d, 8.6)	C-2, C-2a, C-4′	74.3	4.44 (d, 11.1)[g]	C-2a (w), C-4′, C-5′ (vw)
3′-OH		3.12 (d, 8.6)			1.9 (br)	
4′	203.0			68.9	3.88 (m)	C-3′ (vw)
4′-OH					1.9 (br	
5′	47.6	2.66 (2H, m)[f]	C-3′, C-4′, C-6′, C-7′[e]	40.1	H_{ax} 1.54 (m)	C-3′ (vw), C-4′, C-6′, C-7′ (w)
					H_{eq} 1.96 (ddd br)	C-3′, C-4′, C-7′ (w)
6′	69.5	4.21 (m)	C-2, C-4′, C-5′, C-7′	66.3	4.03 (m)	
7′	21.4	1.39 (d, 6.2)	C-2 (w), C-4′, C-5′, C-6′	21.2	1.23 (d, 6.0)	C-5′, C-6′

[a] 75 MHz. [b] 300 MHz, multiplicity, *J* in Hz. [c] 500 MHz, H → C. [d] Not distinguishable: C-3a, C-4, C-7, C-7a, C-8. [e] Cannot be unambiguously assigned. [f] H_{ax}-5′ 2.71 (ddd, 13.8, 11.0, 1.0), H_{eq}-5′ 2.58 (dd., 14.0, 3.0) in CD_3OD, 500 MHz. [g] 4.44 (d, 9.2) in CD_3OD, 500 MHz.

strongly suggested a skeleton similar to that of **8**, as indicated by eight olefinic sp^2 carbons of the naphthoquinone moiety and the respective signals of the γ-lactone and spiro-pyrane moieties. But in addition to the expected lactone and quinone carbonyls, an additional carbonyl group was identified at δ_C 203.0, whereas the hydroxylated methine at C-4′ of **8** was missing in the ^{13}C and ^{1}H NMR spectra. All other data were in good accordance with those of de-acetylgriseusin A (**8**) and were assigned on the basis of HMQC, COSY, and HMBC data (Table 1, Figure 2). HMBC correlations of the protons at C-3′, C-5′, C-6′, and C-7′ with C-4′ revealed that the new keto group was located at C-4′. The downfield shifts of the ^{13}C NMR signals by 8 ppm of the neighboring C-3′ and C-5′ and C-6′ in γ-position as compared with **8** further supported the assignment.[6] In accordance with this, H-3′ only showed a proton coupling with the geminal hydroxy group.

Concerning the relative stereochemistry of **1**, the *cis*-attachment of the γ-lactone ring was indicated by an NOE between H-9 and H-10. NOEs between H-3′ and the hydroxyl proton at C-4, as well as between H-3′ and the multiplet of the methylene protons at C-5′, were in accord with the axial position of H-3′ as in **8**.[7] Finally, a strong NOE between H-10 and H-6′ suggested the axial position of H-6′, which was also in agreement with the $^{3}J_{H-5',H-6'}$ di-axial coupling constant of 11 Hz as recorded for the ^{1}H NMR signal of H_{ax}-5′ in CD_3OD. The absolute stereochemistry of **1** was suggested to be identical to that of the known congeners on the basis of similar optical rotation values and CD spectra (**1**: $[\alpha]^{15}_{D}$ −114° (*c* 0.09, $CHCl_3$), CD (MeOH) θ_{245} −42 681, θ_{301} +16 554, θ_{346} 0, θ_{449} −4889; deacetylgriseusin A (**8**),[7−9] $[\alpha]^{15}_{D}$ −198° (*c* 0.1, $CHCl_3$), CD (MeOH) θ_{251} −32 000, θ_{289} +5500, θ_{351} 0, θ_{460} −2200). The similarity of data implied that the missing stereocenter at C-4′ does not strongly influence the chiroptical behavior of **1**. Altogether, **1** was determined to be 4′-dehydro-deacetylgriseusin A with 2*R*,9*R*,10*R*,3′*R*,6′*R* configuration.[3]

For compound **2**, the molecular formula of $C_{20}H_{20}O_{10}$, which was derived from HRESIMS (419.0948 $[M - H]^-$; calcd, 419.0978), indicated a degree of unsaturation of 11 for the chemical structure. The ^{13}C NMR spectrum ($CDCl_3$) showed a strong resemblance to that of *epi*-deacetylgriseusin B (**4**) and deacteylgriseusin B (**9**)[6−10] and revealed the presence of nine sp^2 carbons of one aromatic ring and three carbonyls (Table 1).

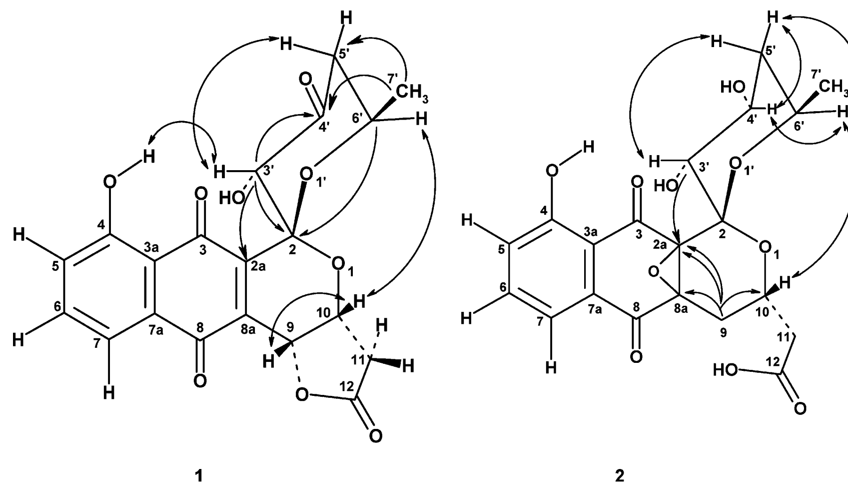

Figure 2. Crucial NMR correlations (HMBC <−>, NOE −>) of 4′-dehydro-deacetylgriseusin A (**1**) and 2a,8a-epoxy-*epi*-deacetylgriseusin B (**2**).

Thus, two sp^2 carbons of the naphthoquinone skeleton of deacetylgriseusin B (**9**) were missing in **2**. In addition, **2** contained 11 sp^3 carbons, including four oxygenated methines, one ketal, three methylenes, and one methyl group next to two oxygenated quarternary carbons (δ_C 62.9, 63.9). As the latter were not seen with **9**, the C-2a/C-8a double bond was suggested to be replaced by an oxirane as in lactoquinomycin B.[11] HMBC correlations between H-9 and C-10, C-2a, and C-8a and between H-3′ and C-2 and C-2a confirmed the location of the oxirane moiety. The ^{1}H NMR spectrum of **2** ($CDCl_3$) confirmed the presence of three aromatic protons, a methyl group next to a methine, three other methines, and three methylene moieties (Table 1). The connectivities of all proton and carbon data were established by HMQC, COSY, and HMBC data. Concerning the relative stereochemistry in the upper pyrane ring, a coupling constant of 9 Hz for $^3J_{H-3',H-4'}$ strongly suggested a diaxial arrangement of H-3′ and H-4′ ($^3J_{H-3',H-4'} = 4$ Hz in griseusin A).[10,12] The axial positions of H-3′ and H-4′ were confirmed by NOEs with H_{ax}-5′ and H-6′, respectively. An NOE between H-6′ and H-10 suggested the identical relative stereochemistry for C-6′, C-2 and C-10 as in 4′-dehydro-deacetylgriseusin A (**1**). The absolute stereochemistry of compound **2** could not be determined by comparison of optical rotation and CD data with **9**. Significant differences of the chiroptical behavior indicated a strong influence by the two new stereocenters of the oxirane. By biosynthetic reasoning, an identical configuration as in **1**, **3**, and **4** was suggested. Thus, **2** was determined to be 2a,8a-epoxy-*epi*-deacetylgriseusin B with 2*S*,10*R*,3′*R*,4′*S*,6′*R* configuration, revising earlier results.[3]

The constitution of compound **3** was readily identified to be that of deacetylgriseusin A (**8**) by HREIMS, ^{13}C NMR, and ^{1}H NMR data.[7] However, detailed analysis of ^{1}H coupling data and NOE correlations (NOESY) revealed **3** to be the 4′-epimer of the known compound. As in compound **2**, the $^3J_{H-3',H-4'}$ coupling constant of 9 Hz strongly suggested a diaxial arrangement of H-3′ and H-4′. Likewise, the axial positions of H-3′ and H-4′ were confirmed by NOEs with H_{ax}-5′ and H-6′, respectively. The different relative stereochemistries of the upper pyrane moiety also resulted in significant differences for the ^{13}C NMR signals for C-3′ (δ_c 74.7 vs 67.8 in **8**[7]) and C-6′ (δ_c 67.8 vs 62.7 in **8**[7]). The identical relative stereochemistry of C-2 and the lactone moiety as in **1** and **8** was confirmed by NOEs between H-6′ and H-10 and between H-9 and H-10. The CD spectrum of **3** was very similar to that of deacetylgriseusin A (**8**), again suggesting that variations at C-4′ do not strongly influence the chiroptical data of the whole molecule. Thus, the identical absolute stereochemistry was suggested for C-10, C-2, C-3′, and C-6′ to be the same as in **1**, **8**, and **9**. Altogether, compound **3** was determined to be *epi*-deacetylgriseusin A with the 2*R*,9*R*,10*R*,3′*R*,4′*S*,6′*R* configuration. The constitution of compound **4** was determined to be that of deacetylgriseusin B (**9**) by HRESIMS, ^{1}H NMR, and ^{13}C NMR data.[7] As in compound **3**, a large $^3J_{H-3',H-4'}$ coupling constant of 9.3 Hz suggested a diaxial arrangement of H-3′ and H-4′. On the grounds of similar ^{13}C NMR and CD data as compared with **3**, compound **4** was determined to be *epi*-deacetylgriseusin B with the 2*R*,10*R*,3′*R*,4′*S*,6′*R* configuration.

As **1** was only isolated as a minor component, attempts were made to obtain larger amounts by semisynthesis starting from the major metabolite *epi*-deacetylgriseusin A (**3**). After investigating several oxidation methods, for example, with pyridinium chromates (PCC and PDC immobilized on polystyrene), tetramethylpiperidinyloxy/bisacetoxy iodine benzene (TEMPO/BAIB), Dess−Martin−Periodinane (soluble and immobilized on polystyrene), SO_3−pyridine, and chromic sulfuric acid in acetone (Jones' reagent), the only one found to be effective was Jones' reagent.[13−16] This reaction provided a nearly complete turnover of the starting material and complete selectivity for the hydroxy group at C-4′. Through careful dosage of the oxidizing reagent, it was possible to avoid unwanted excess of chromic acid in the reaction mixture. The Jones oxidation was also successfully utilized for oxidation of 2a,8a-epoxy-*epi*-deacetylgriseusin B (**2**) to give 4′-dehydro-2a,8a-epoxy-deacetylgriseusin B (**7**). Our semisynthetic studies also yielded new derivatives by opening the lactone ring using acid-catalyzed methanolysis. This reaction was initially observed as an unwanted side reaction in the chromatographic isolation procedures from the fermentation product. In this manner, 4′-dehydro-9-hydroxy-deacetylgriseusin B methyl ester (**6**) and 9-hydroxy-*epi*-deacetylgriseusin B methyl ester (**5**) were obtained from 4′-dehydro-deacetylgriseusin A (**1**) and *epi*-deacetylgriseusin A (**3**), respectively.

A basic SAR study on compounds **1**−**7**, together with griseusin A (**10**), was performed in monolayer cultures of six tumor cell lines (Figure 3). Compounds **6** and **1** proved to be most active in this series, with an overall potency of 0.155 μM and 0.392 μM, respectively (mean IC_{50} values of six tumor cell lines tested). They were followed by **5** (mean $IC_{50} = 0.688\ \mu$M), **10** (mean $IC_{50} = 2.458\ \mu$M), and **3** (mean $IC_{50} = 5.317\ \mu$M) in decreasing order of cytotoxic potency. In the tested concentration range, **2**, **4**, and **7** did not inhibit growth of tumor cell lines. Although 4′-dehydro-9-hydroxy-deacetyl-griseusin B methyl-

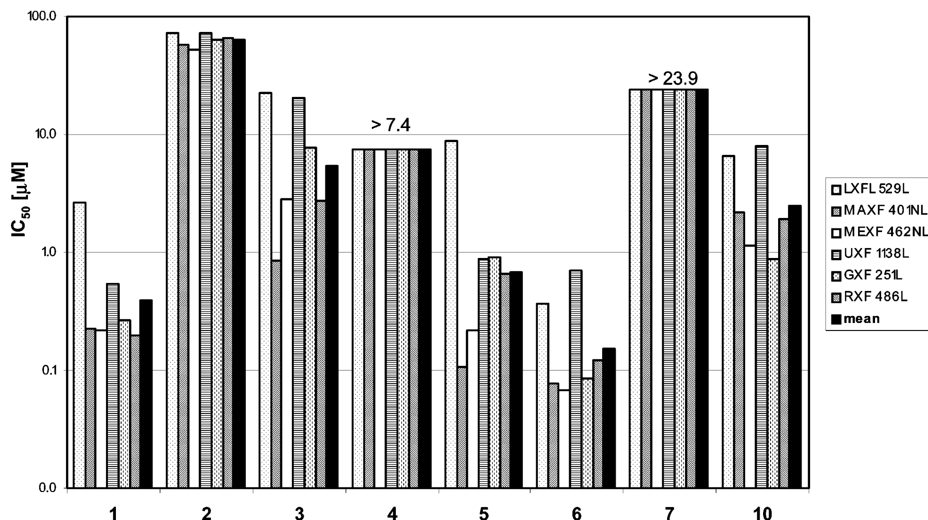

Figure 3. Basic SAR study on cytotoxicity of eight griseusin analogues tested in six tumor cell lines in monolayer culture.

ester (**6**) was more potent in the monolayer assays than 4′-dehydro-deacetylgriseusin A (**1**), it was of similar potency in the clonogenic assay (data not shown) and, thus, not further studied.

A detailed cytotoxicity analysis of 4′-dehydro-deacetylgriseusin A (**1**) included monolayer cultures of 37 different human tumor cell lines, reflecting 14 different solid tumor types. Compound **1** effected concentration-dependent inhibition of tumor cell growth with a mean IC_{50} of 0.430 μM, indicating pronounced cytotoxic potency. Cytotoxic activity was most pronounced in four out of four tumor cell lines of breast cancer (MDA-MB 231, MDA-MB 468, MCF-7, MAXF 401; IC_{50} = 0.150−0.345 μM), all five melanomas (MEXF 276L, MEXF 394NL, MEXF 462NL, MEXF 514L, MEXF 520L; IC_{50} = 0.087−0.280 μM), as well as three out of four cell lines of renal cancer (RXF 393NL, RXF 468L, RXF 944L, IC_{50} = 0.095−0.260 μM; Figure 4). Individual cell lines of bladder cancer (BXF 1218L, IC_{50} = 0.140 μM), non-small cell lung cancer (LXF 1121L, IC_{50} = 0.207 μM), and prostate cancer (PRXF 22Rv1, IC_{50} = 0.177 μM) also responded above average relative to the mean IC_{50}. Tumor types of non-small cell lung cancer (LXF 289L, LXF 529L, LXF 629L, H-460) and ovary cancer (OVXF 1619L, OVXF 899L, OVCAR-3) were generally less sensitive toward **1**.

Inhibition of clonogenicity of tumor cells was evaluated in additional tumor models using a clonogenic assay. The antiproliferative activity of **1** was evaluated in cell suspensions prepared from 51 human tumor xenografts of 17 different tumor types, which were cultured as solid tumors in serial passage on immune-deficient nude mice. The IC_{50} in the clonogenic assay ranged from 0.132 μM to 4.836 μM (mean IC_{50} = 0.972 μM), confirming the overall potency of **1**. Cytotoxic selectivity of **1** for breast cancers (MAXF 1162 and MAXF 583, IC_{50} = 0.455 μM and 0.195 μM, respectively), melanomas (MEXF 535 and MEXF 989, IC_{50} = 0.137 μM and 0.295 μM, respectively), and renal cancers (RXF 393 and RXF 944LX, IC_{50} = 0.375 μM and 0.290 μM, respectively) could also be confirmed. In addition, considerable inhibition of colony formation was observed against three out of five tumor models of colon cancer (CXF 1103, CXF 158 and CXF 264, IC_{50} = 0.257−0.432 μM), two out of two pancreatic cancers (PAXF 546 and PAXF 736, IC_{50} = 0.420 μM and 0.614 μM, respectively), as well as acute lymphoblastic leukemia (CCRF-CEM, IC_{50} = 0.132 μM). In addition, **1** was tested in a preparation of hematopoietic stem cells from cord blood as a model system for nonmalignant tissue: IC_{50} = 1.186 μM.

The observed antitumor selectivity patterns of **1** only weakly correlated with those of standard cytotoxic compounds with known mechanisms of action as deduced by COMPARE analyses. Best correlations were obtained with the alkylating agent carmustine (1,3-bis(2-chloroethyl)-l-nitrosourea, BCNU; $\rho = 0.39$) in the case of the monolayer assay data and with the alkylating agent lomustine (cyclohexylchloroethylnitrosurea, CCNU; $\rho = 0.53$) in the case of the clonogenic assay data. However, the correlation coefficients were below the significant threshold level of $\rho = 0.6$ in each case.

Discussion

Griseusins, which have been known for a long time, have been the subject of numerous biological, biosynthetic, and synthetic studies.[10,17] The known spiro-naphthoquinones of natural origin, griseusins A and B, deacetylgriseusins A and B, as well as 3′-*O*-D-forosaminyl-(+)-griseusin A, only showed structural variations in the lactone moiety and peripheral functionalities of the upper pyrane ring. Our investigation of the metabolic profile of a *Nocardiopsis* sp. (YIM80133, DSM16644) gave rise to derivatives that show novel structural variations.[18] This was also the first time stereochemical variations of the polyketide skeleton, as in 2a,8a-epoxy-*epi*-deacetylgriseusin B (**2**), *epi*-deacetylgriseusin A (**3**), and *epi*-deacetylgriseusin B (**4**), were found within this class of compounds. The configuration of the pyran hydroxyl substituent at C-4′ is obviously established by the enzymatic ketoreduction of the polyketide skeleton, in which a hydride is transferred either from the pro-*S* or from the pro-*R* side.[19] A similar occurrence of epimers by different microorganisms has been observed in related benzoisochromanequinone pathways, albeit in different types of polyketide pathways and organisms. For example, in actinorhodin and medermycin biosyntheses, C-3 ketoreductases convert a bicyclic intermediate to chiral alcohols, which are subsequently converted to (*S*)-DNPA.[20,21] A corresponding ketoreductase of the granaticin pathway exhibits the opposite stereospecificity, yielding the enantiomeric alcohol.[22,23] A corresponding enantiodivergent step most likely plays a key role in the pathways that lead to the epimeric griseusin derivatives.

The griseusins have been known as potent antimicrobial agents that are effective against Gram-positive bacteria, including MRSA.[17] The newly found 4′-keto functionality endows the

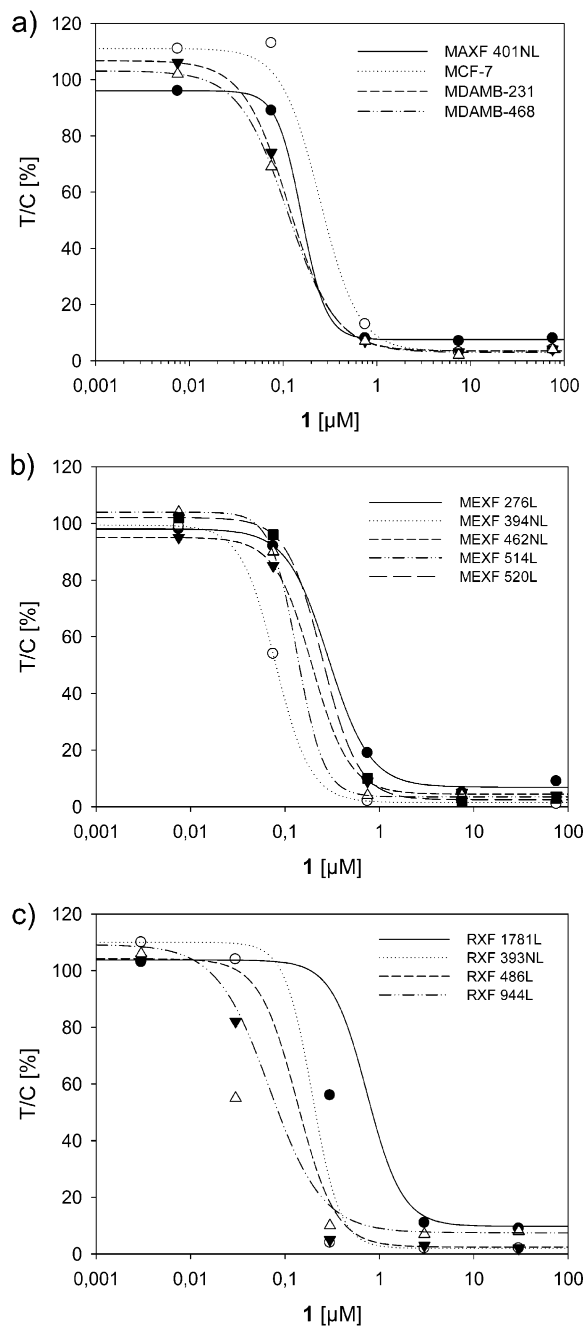

Figure 4. Concentration−response curves of 4′-dehydro-deacetylgriseusin A (**1**) tested in human tumor cell lines (monolayer culture) of (a) breast cancer, (b) melanoma, and (c) renal cancer.

griseusins with very potent cytotoxicity that has not been found in compounds of this class before. A basic SAR study confirmed that the 4′-keto group is essential for strong cytotoxic activity. Surprisingly, the oxirane of 2a,8a-epoxy-*epi*-deacetylgriseusin B (**2**) is detrimental to the cytotoxic activity of the griseusins, which cannot be reinstalled by the 4′-carbonyl functionality as seen by the inactive derivative 4′-dehydro-2a,8a-epoxy-deacetylgriseusin B (**7**). 4′-Dehydro-deacetylgriseusin A (**1**) showed significant cytotoxic potency (mean $IC_{50} = 0.430$ μM) combined with a significant selectivity for mammary cancer, renal cancer, and melanoma in a panel consisting of 37 tumor cell lines. Furthermore, **1** displayed pronounced activity and selectivity in clonogenic assays against tumor cell suspensions from 51 solid tumors. Those tumor cells, representing 17 different tumor types, were freshly prepared from solid human tumor xenografts grown in nude mice. Cells that show anchorage-independent growth in semisolid medium contain, to a certain extent, tumor stem cells that are considered to be responsible for the metastatic and infiltrative potential of a tumor.[24−27] Thus, the clonogenic assay may inter alia be used to identify candidate tumors for subsequent in vivo studies.[28−32] 4′-Dehydro-deacetylgriseusin A (**1**) selectively inhibited colony formation of tumor cells in semisolid medium and, based on the distribution of IC_{50} values, tumor types such as colon cancer, breast cancer, melanoma, pancreatic cancer, renal cancer, and leukemia could be clearly identified to be most sensitive toward **1**. Based on IC_{50} values, these sensitive tumor models were on average about 3.5-fold more sensitive than the mean IC_{50} of all models tested. Moreover, these sensitive tumor models were on average about 4-fold more sensitive than hematopoietic stem cells as representative model system for nonmalignant tissue, indicating a satisfactory therapeutic index of the compound.

The COMPARE algorithm provides preliminary information on a possible mechanism of action of a compound based on in vitro data obtained in a panel of tumor cell lines tested in monolayer culture or tumor cells from xenografts tested in a clonogenic assay.[33] The cytotoxic efficacy of a compound reveals a selectivity pattern reflecting a characteristic distribution of sensitive and resistant tumors. IC_{50} values of a test compound obtained in tumor cells within this pattern are ranked and correlated to those of standard agents obtained in the same tumor cells. Standard agents in the reference database represent the main mechanisms of action known for anticancer drugs, for example, alkylating agents, antimetabolites, cyclin-dependent kinase (Cdk) inhibitors, DNA binders, histone deactylase inhibitors, heat shock protein 90 (HSP-90) inhibitors, protein kinase C (PKC) modulators, proteasome inhibitors, serin/threonin kinase inhibitors, topoisomerase inhibitors, tubulin binders, and tyrosin kinase inhibitors. 4′-Dehydro-deacetylgriseusin A (**1**) was identified to be COMPARE-negative and, thus, could act by a mechanism of action that was not represented by the standard agents or possibly one that is not even yet known.

In summary, four new griseusins, 4′-dehydro-deacetylgriseusin A (**1**) and 2a,8a-epoxy-*epi*-deacetylgriseusin B (**2**) as new constitutional derivatives and *epi*-deacetylgriseusin A (**3**) and *epi*-deacetylgriseusin B (**4**) as new stereoisomers, were isolated from *Nocardiopsis* sp. (YIM80133, DSM16644). 4′-Dehydro-deacetylgriseusin A (**1**) showed pronounced cytotoxic potency (mean $IC_{50} = 0.430$ μM) combined with a significant selectivity for mammary cancer, renal cancer, and melanoma in a panel consisting of 37 tumor cell lines. A basic SAR study confirmed that the novel 4′-keto group is essential for strong cytotoxic activity.

Experimental Section

General Experimental Procedures. NMR spectra were recorded on a Bruker DPX-300 and a Bruker DRX-500, as indicated. IR spectra were recorded on a Bruker FT-IR (IFS 55) spectrometer. Electrospray MS were recorded by use of a VG Quattro Micromass spectrometer. High-resolution EI mass spectra (with perfluorokerosine as a standard) were measured with the AMD-402 instrument of *Be* geometry equipped with direct inlet system (AMD Intectra Harpstedt, Germany). UV spectra were recorded on a Cary 1 Bio UV−visible spectrophotometer (Varian). TLC: silica gel plates (silica gel $60F_{254}$ on aluminum foil or glass, Merck). LC: silica gel (0.040∼0.063 mm, Merck). Sephadex-LH 20 (Pharmacia).

Strain Isolation, Characterization, and Cultivation. *Nocardiopsis* sp. (YIM80133, DSM16644) was isolated from an alkaline soil sample (pH 9−10) collected from Xinjiang in the Northwest of China by using standard dilution plating procedures with an alkaline isolation medium.[37] The strain was characterized by a well-

developed white to beige aerial mycelium, a reddish brown to dark brown substrate mycelium, and the formation of a brown soluble pigment on yeast extract−malt extract agar. In old cultures, hyphae completely fragmented into spores. In liquid culture, characteristic zigzag hyphae were observed. The cell wall contains meso-2,6-diaminopimelic acid (meso-DAP). Phenotypic identification as *Nocardiopsis* sp. was confirmed by 16S rDNA analysis (AMODIA Bioservice GmbH, Braunschweig) and sequence comparison with GenBank data (NCBI, National Institute of Health) by 99.6% similarity.

Laboratory cultivation of the organism was performed on yeast extrac−malt extract agar that consisted of glucose (0.4%), malt extract (1.0%), yeast extract (0.4%), and agar (2.0%) or in liquid culture broth of the same composition without agar. The pH value of the medium was adjusted to pH 9.5−10.0 with sterilized NaOH (32%) after sterilization. The incubation temperature was 28 °C. Initial inoculation of liquid cultures was achieved by adding slants (1 cm^2) of well-grown agar cultures. Stock cultures were maintained either in the vapor phase of liquid nitrogen by adding 5% DMSO to the culture medium or at −80 °C by adding glycerol (1:1).

For screening scale compound production, *Nocardiopsis* sp. (YIM80133, DSM16644) was cultivated under shaking conditions for 48 h at 28 °C in 500 mL Erlenmeyer flasks containing 100 mL of the yeast extract−malt extract medium. These cultures (1 mL) were used as a seed culture for the inoculation of 100 mL production medium (500 mL Erlenmeyer flasks) consisting of soybean flour (2%) and mannitol (2%) at pH 9.5−10.0. Incubation at 28 °C lasted for 6 days. Large-scale cultivation was performed in a 300 L fermentor vessel filled with 200 L of soybean−mannitol production medium at pH 9.5−10.0. As seed culture, 3200 mL of a 48 h old shaking flask culture in the production medium (8 × 400 mL in 1 L flasks) was used. The fermentation was carried out for 4 days with aeration at 50 L/min and stirring at 200 rpm.

Natural Product Isolation and Purification. After harvesting, the culture broth was filtered and the filtrate was absorbed on Amberchrom (6 L), from which it was eluted with a H_2O/MeOH gradient. The cytotoxic 100% MeOH fractions 4−7 (2.5 L each) were combined and concentrated to an aqueous residue. After liquid/liquid extraction with EtOAc (3 × 1000 mL), the combined EtOAc extracts were evaporated under reduced pressure and lyophilized to afford 12 g of semisolid red material. This material was dissolved in methanol (15 mL) and subjected to column chromatography on Sephadex LH-20 (6 × 90 cm, MeOH) to furnish fractions 1−4 that by TLC analysis contained the material of interest. Column chromatography of fraction 1 on silica gel (2.5 × 80 cm) and elution with $CHCl_3$/MeOH = 30:1 afforded compounds **1** (40 mg) and **3** (300 mg). Chromatography of fraction 2 on Sephadex LH-20 (2.5 × 80 cm, MeOH) and preparative HPLC [GILSON, column: SP 250/21 Nucleosil 100-7 C18, Macherey−Nagel (2.2 × 21 cm), MeOH/H_2O gradient, flow rate 10 mL/min] afforded compound **2** (25 mg). Compound **4** (290 mg) was purified from fraction 3 by repeated chromatography on silica gel (1 × 40 cm, $CHCl_3$/MeOH from 20:1 to 3:1).

4′-Dehydro-deacetylgriseusin A (1). Orange solid; $[\alpha]^{15}_D$ −114° (*c* 0.09, $CHCl_3$); CD (MeOH) θ_{217} −8609, θ_{222} −7462, θ_{245} −42 681, θ_{301} +16 554, θ_{346} 0, θ_{449} −4889; IR ν_{max} (KBr) 1779.8, 1735.2, 1651.3, 1618.7, 1454.9, 1204.9 cm^{-1}; UV λ_{max} ($CHCl_3$; log ϵ) 256.0 (4.18), 445.0 (3.76) nm; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 423.1 [M + Na]$^+$, 822.9 [2M + Na]$^+$; HRESIMS 423.0645 [M + Na]$^+$ ($C_{20}H_{16}O_9Na$; calcd, 423.0692); HREIMS 400.0821 [M]$^+$ ($C_{20}H_{16}O_9$; calcd, 400.0794).

2a,8a-Epoxy-*epi*-deacetylgriseusin B (2). Yellow oil; $[\alpha]^{15}_D$ +9.2° (*c* 0.8, MeOH); CD (MeOH) θ_{214} −13 978, θ_{21} −12 670, θ_{226} −13 149, θ_{242} −1678, θ_{260} −8993, θ_{297} +203, θ_{309} −579, θ_{324} −531, θ_{367} −8295; IR ν_{max} (KBr) 3319.5, 1788.3, 1697.7, 1650.7, 1455.1, 1273.3, 765.3 cm^{-1}; UV λ_{max} ($CHCl_3$; log ϵ) 242.00 (3.85), 288.4 (sh), 368.0 (3.39) nm; ^1H and ^{13}C data, see Table 1; ESIMS *m/z* 443.1 [M + Na]$^+$, 862.8 [2M + Na]$^+$; HREIMS [M − H]$^-$ 419.0948 ($C_{20}H_{20}O_{10}$; calcd, 419.0978).

***epi*-Deacetylgriseusin A (3).** Yellow solid; $[\alpha]^{15}_D$ −100.9° (*c* 0.2, MeOH); CD (MeOH) θ_{249} −44 360, θ_{285} +9850, θ_{463} −3488; UV λ_{max} (MeOH; log ϵ) 212.0 (4.48), 251.0 (3.94), 431.0 (3.52) nm; IR ν_{max} (KBr) 3403.9, 1781.23, 1646.6, 1455.6, 1254.74, 1155.7, 1044.6, cm^{-1}; ^1H NMR (δ, $CDCl_3$) 1.20 (d, 6.2 Hz, H-7′), 1.58 (m, H-5′ax), 1.98 (ddd, J = 12.9, 4.7, 2.1 Hz, H-5′eq), 2.76 (d, J = 17.8 Hz, H-11b), 2.99 (dd, J = 4.8, 17.8 Hz, H-11a), 3.92 (m cov, H-4′), 3.96 (ddd cov, H-6′), 4.42 (d, J = 9.0 Hz, H-3′), 4.69 (dd, J = 4.7, 2.9 Hz, H-10), 5.25 (d, J = 2.9 Hz, H-9), 7.24 (m, H-5), 7.61 (m, H-7), 7.61 (m, H-6); ^{13}C NMR (δ, $CDCl_3$) 20.9 (q, C-7′), 36.2 (t, C-11), 39.7 (q, C-5′), 66−0 (d, C-10), 67.8 (d, C-6′), 68.6 (d, C-4′), 68.9 (t, C-9), 74.7 (d, C-3′), 97.4 (s, C-2), 115.3 (s, C-3a), 119.3 (d, C-7), 125.3 (d, C-5), 131.2 (s, C-7a), 136.7 (d, C-6), 138.3 (s, C-8a), 142.8 (s, C-2a), 162.0 (s, C-4), 174.5 (s, C-12), 181.9 (s, C-8), 187.2 (s, C-3); ^{13}C NMR (δ, CD_3OD) 21.4 (C-7′), 36.9 (C-11), 42.0 (C-5′), 67.4 (C-10), 69.0 (C-6′), 70.5 (C-4′), 75.4 (C-3′), 68.4 (C-9), 98.8 (C-2), 116.3 (C-3a), 126.0 (C-5), 119.9 (C-7), 132.3 (C-7a), 138.0 (C-6), 140.1 (C-8a), 144.3 (C-2a), 162.6 (C-4), 177.2 (C-12), 183.4 (C-8), 188.7 (C-3); ESIMS *m/z* 357.1 [M − CO_2]$^-$, 400.9 [M − H]$^-$, 825.0 [2M + Na]$^-$; HREIMS [M]$^+$ 402.0961 ($C_{20}H_{18}O_9$; calcd, 402.0951).

***epi*-Deacetylgriseusin B (4).** Yellow solid; $[\alpha]^{15}_D$ −131.7.9° (*c* 0.05, MeOH); CD (MeOH) θ_{252} −21 436, θ_{291} +9896, θ_{345} −2554; UV λ_{max} (MeOH; log ϵ) 212.0 (4.48), 251.0 (3.94), 431.0 (3.52) nm; ^1H NMR (δ, CD_3OD) 1.20 (d, 6.3 Hz, H-7′), 1.42 (m, H-5′), 1.97 (m, H-5′), 2.17 (dd, J = 11.8, 14.8 Hz, H-9a), 2.50 (dd, J = 2.8, 14.8 Hz, H-9b), 2.72 (m, 2H, H-11), 3.93 (m, H-4′), 4.17 (m, H-6′), 4.30 (m, H-10), 4.36 (d, J = 9.3 Hz, H-3′), 7.28 (dd, J = 1.2, 8.4 Hz, H-5), 7.52 (dd, J = 1.2, 7.5 Hz, H-7), 7.68 (dd, J = 7.5, 8.4 Hz, H-6); ^{13}C NMR (δ, CD_3OD) 21.2 (C-7′), 40.4 (C-11), 42.3 (C-5′), 67.0 (C-10), 63.9 (C-6′), 69.5 (C-4′), 75.3 (C-3′), 29.1 (C-9), 98.7 (C-2), 115.6 (C-3a), 125.8 (C-5), 120.0 (C-7), 132.6 (C-7a), 137.2 (C-6), 138.2 (C-8a), 148.9 (C-2a), 163.7 (C-4), 174.5 (C-12), 184.4 (C-8), 190.6 (C-3); HRESIMS [M − H]$^-$ 403.0999 ($C_{20}H_{19}O_9$; calcd, 403.1029).

Oxidation of *epi*-Deacetylgriseusin A (3). To a solution of 200 mg (498 μmol) of compound **3** in 15 mL of acetone, 600 μL of freshly prepared Jones reagent were added in three portions every 60 min at room temperature. The mixture was stirred for an additional 2 h and was worked up by filtration through a short silica column. Then compound **1** was purified by repetitive column chromatography on silica gel with a stepwise gradient from dichloromethane to dichloromethane/ethylacetate = 3/1 to obtain 160 mg (80%) of **1**. The data for structural assignment were identical to those obtained for compound **1**.

9-Hydroxy-*epi*-deacetylgriseusin B Methylester (5). A solution of 50 mg (124 μmol) of compound **3** in 4 mL of methanol was acidified with 40 μL of concd hydrochloric acid and was allowed to stand at room temperature overnight. After evaporation of the solvent, the crude product was obtained. The purification was conducted using preparative plate chromatography on silica gel, first developing with dichloromethane/ethylacetate = 1/1, then with dichloromethane/ethylacetate = 1/2 to obtain 30 mg (56%) of compound **5** and some recovered starting material **3** (5 mg). ^{13}C NMR (δ, $CDCl_3$) 20.9 (q, C-7′), 34.9 (t, C-11), 39.8 (t, C-5′), 51.9 (q, OCH_3), 60.0 (d, C-9)*, 67.01 (d, C-6′)*, 67.04 (d, C-10)*, 69.0 (d, C-4′)*, 74.9 (d, C-3′), 97.9 (s, C-2), 115.3 (s, C-3a), 119.0 (d, C-7), 125.3 (d, C-5), 131.2 (s, C-7a), 136.3 (d, C-6), 139.3 (s, C-2a), 144.7 (s, C-8a), 161.9 (s, C-4),171.4 (s, C-12), 183.4 (s, C-8), 187.9 (s, C-3); * = exchangeable; HRESIMS *m/z* 457.1113 [M + Na]$^+$ ($C_{21}H_{22}O_{10}Na$; calcd, 457.1111).

4′-Dehydro-9-hydroxy-deacetylgriseusin B Methylester (6). A solution of 50 mg (125 μmol) of compound **1** in 20 mL of methanol was acidified with 60 μL of concd hydrochloric acid and was allowed to stand at room temperature overnight. After evaporation of the solvent, 38 mg (70%) of the crude product was obtained. Purification was effected using preparative TLC on silica gel, and the compound was eluted with dichloromethane/ethylacetate = 3/1 using two developments. ^{13}C NMR (δ, $CDCl_3$) 21.5 (q, C-7′), 34.7 (t, C-11), 47.5 (t, C-5′), 51.9 (q, OCH_3), 59.8 (d, C-9), 67.8 (d, C-10)*, 68.8 (d, C-6′)*, 76.0 (d, 3′), 100.0 (s, C-2), 115.3 (s, C-3a), 119.4 (d, C-7), 125.4 (d, C-5), 131.4 (s, 7a), 136.7 (d, C-6), 137.0 (s, C-2a), 145.1 (s, C-8a), 162.1 (s, C-4), 171.1 (s,

C-12), 182.7 (s, C-8), 188.1 (s, C-3), 203.7 (s, C-4′); * = exchangeable; HRESIMS *m/z* 455.0910 [M + Na]$^+$ ($C_{21}H_{20}O_{10}Na$; calcd, 455.0887).

4′-Dehydro-2a,8a-epoxy-*epi*-deacetylgriseusin B (7). To a solution of 10 mg (23.8 μmol) of compound **2** in 2 mL of acetone, 29 μL of freshly prepared Jones reagent was added in three portions (every 30 min) at room temperature. After stirring for three additional hours, the mixture was filtered through a short silica column. Purification was achieved by column chromatography on silica gel, and the compound was eluted with a stepwise gradient of dichloromethane to dichloromethane/ethylacetate = 3/1 to obtain 3 mg of compound **7**. ^{1}H NMR (δ, $CDCl_3$) 1.42 (d, 6.2 Hz, H-7′), 2.58 (m, 2H, H_2-5′), 2.17 (dd, J = 11.5, 14.8 Hz, H-9a), 2.58 (dd, J = 2.5, 14.9 Hz, H-9b), 2.56 (m, 2H, H_2-11), 4.38 (m, H-6′), 4.36 (m, H-10), 5.40 (s br, H-3′), 7.28 (dd, J = 1.1, 8.1 Hz, H-5), 7.57 (dd, J = 1.1, 8.1 Hz, H-7), 7.64 (dd, J = 8.1, 8.1 Hz, H-6); HRESIMS *m/z* 441.0787 [M + Na]$^+$ ($C_{20}H_{18}O_{10}Na$; calcd, 441.0798).

Monolayer Assay. A modified propidium iodide assay was used to determine the cytotoxic activity of the compounds against human tumor cell lines. The test procedure has been described elsewhere.[34] Cell lines tested were derived from patient tumors engrafted as a subcutaneously growing tumor in NMRI nu/nu mice, or obtained from the American Type Culture Collection, Rockville, MD, National Cancer Institute, Bethesda, MD, or Deutsche Sammlung von Mikroorganismen and Zellkulturen, Braunschweig, Germany. Briefly, human tumor cells lines were grown at 37 °C in a humidified atmosphere (95% air, 5% CO_2) in monolayer cultures in RPMI 1640 medium supplemented with 10% FCS and phenol red (PAA, Cölbe, Gemany). Cells were trypsinized and maintained weekly. Cells were harvested from exponentially growing cultures by trypsination, counted, and plated in 96-well flat-bottomed microplates (140 μL of cell suspension, 5×10^3 to 10×10^3 cells/well). After a 24 h recovery to allow cells to resume exponential growth, 10 μL of culture medium (six control wells per plate) or medium containing the test drug were added to the wells. Each drug concentration was plated in triplicate. After 4 days of incubation the culture medium was replaced by fresh medium containing 6 μg/mL of propidium iodide. Microplates were then kept at −18 °C for 24 h to give a total cell kill. After thawing of the plates, fluorescence was measured using the Cytofluor 4000 microplate reader (Perseptive Biosystems; excitation 530 nm, emission 620 nm). The amount of viable cells was proportional to the fluorescence intensity.

Clonogenic Assays with Human Tumor Xenografts and Hematopoietic Stem Cells. Effects of the test compounds on clonogenicity of tumor cells were investigated in a clonogenic assay. Tumor xenografts were derived from patient tumors engrafted as a subcutaneously growing tumor in NMRI nu/nu mice obtained from Oncotest's breeding facility.[35,36] Details of the test procedure have been described earlier.[28] Briefly, solid human tumor xenografts were removed from mice under sterile conditions, mechanically disaggregated and subsequently incubated with an enzyme cocktail consisting of collagenase type IV (41 U/mL), DNase I (125 U/mL), hyaluronidase type III (100 U/mL), and dispase II (1.0 U/mL) in RPMI 1640 medium at 37 °C for 45 min. Cells were passed through sieves of 200 μm and 50 μm mesh size and washed twice with sterile PBS buffer. The percentage of viable cells was determined in a Neubauer-hemocytometer using trypan blue exclusion. The bottom layer consisted of 0.2 mL/well Iscove's Modified Dulbecco's medium (IMDM, Life Technologies), supplemented with 20% (v/v) FCS (Sigma), 0.01% (w/v) gentamicin (Life Technologies), and 0.75% (w/v) agar (BD Biosciences). A total of 1.5×10^4 to 4×10^4 cells were added to 0.2 mL of the same culture medium supplemented with 0.4% (w/v) agar and plated in 24-multiwell dishes onto the bottom layer. The test compounds were applied by continuous exposure (drug overlay) in 0.2 mL of culture medium. Every dish included six untreated control wells and drug-treated groups in triplicate at six concentrations. Cultures were incubated at 37 °C and 7.5% CO_2 in a humidified atmosphere for 7−20 days and monitored closely for colony growth using an inverted microscope. Within this period, in vitro tumor growth led to the formation of colonies with a diameter of >50 μm. At the time of maximum colony formation, counts were performed with an automatic image analysis system (OMNICON 3600, Biosys GmbH). A total of 24 h prior to evaluation, vital colonies were stained with a sterile aqueous solution of 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (1 mg/mL, 100 μL/well).

For testing hematopoietic stem cells, samples of human umbilical cord blood were diluted 2- to 3-fold with PBS containing 0.1% (w/v) BSA. Peripheral blood mononuclear cells were enriched from the respective samples by Ficoll Paque density gradient centrifugation and washed twice with PBS containing 0.1% (w/v) BSA. The resulting cell suspension was stored in aliquots in freezing medium at −80 °C. Aliquots were thawed for testing as appropriate. The colony forming test was performed using 24-well plates and HSC−CFU (Miltenyi Biotec) as culture medium. The 25 000 cells/mL of the above-mentioned preparation were seeded in a final volume of 500 μL per well. Solutions of the test compounds were added directly to the medium. Every dish included six untreated control wells and drug-treated groups in triplicate at five concentrations. Three wells of the test plate were filled with 1 mL of sterile water to ensure that maximum humidity was attained during the subsequent incubation period. Cultures were incubated at 37 °C and 7.5% CO_2 in a humidified atmosphere for 12 days. Colony growth was evaluated using an inverted microscope.

Data Evaluation, Mean Graph Analysis, and COMPARE Analysis. Antiproliferative efficacies of test compounds in both assays were described by inhibitory concentrations (IC_{50} values), reflecting concentration-dependent cytotoxicity. Extrapolated IC_{50} values were given if the exact value could not be determined within the test range and if linear regression of existing T/C values resulted in IC_{50} values within a range of 3-fold the highest test concentration. In the case of resistant cell lines, exhibiting no activities, IC_{50} values were expressed to be greater than the highest test concentration. Antitumor selectivity patterns were obtained by mean graph analyses, where the distribution of IC_{50} values obtained for a test compound in the individual tumor types was given in relation to the mean IC_{50} value, obtained for all tumors tested. The individual IC_{50} values were expressed as bars on a logarithmically scaled axis. The mean graph analysis therefore represents an antiproliferative fingerprint of a compound.

For the COMPARE analysis antitumor selectivity patterns were compared by ranking and correlating individual IC values of a test compound with those IC values of standard agents in the same tumor models.[33] Similarities in antitumor selectivity of the test compound to those of the standard drugs were expressed quantitatively by the Spearman rank correlation coefficient rho (ρ). Compounds that have a high correlation coefficient have generally been found to have similar mechanisms of action. Spearman correlations with $\rho \geq 0.5$ were considered as relevant. A high correlation ($\rho \geq 0.6$) to a specific standard agent indicated a similar mechanism of action (COMPARE-positive). Low correlations to all standard agents indicated a mechanism of action that was not represented by the standard agent database or could even represent an unknown mechanism of action (COMPARE-negative).

Acknowledgment. Excellent collaboration with HKI's NMR and MS units and acquisition of CD data by Prof. A. Zeeck (University of Göttingen) is gratefully acknowledged. We are particularly grateful for fruitful discussions with Prof. C. Hertweck. Generous funding was provided by BMBF (0312848 and CHN01/328).

Supporting Information Available: Physicochemical characterization data for all compounds and mean-graph analyses of 4′-dehydro-deacetylgriseusin A (**1**) in monolayer and clonogenic cell line panels. This information is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Reddy, L.; Odhav, B.; Bhoola, K. D. Natural products for cancer prevention: A global perspective. *Pharmacol. Ther.* **2003**, *99*, 1−13.

(2) Newman, D. J.; Cragg, G. M.; Snader, K. M. Natural products as sources of new drugs over the period 1981−2002. *J. Nat. Prod*. **2003**, *66*, 1022−1037.

(3) Grabley, S.; Sattler, I.; Roemer, E.; Huang, X. S.; He, J.; Fiebig, H. H.; Maier, A.; Kelter, G. Griseusin derivatives and their use as antitumor agents. EP 04024779.3 (application 18.10.2004).

(4) Grabley, S.; Thiericke, R.; Sattler, I. Tools for drug discovery: Natural product-based libraries. *Ernst. Schering Res. Found. Workshop* **2000**, 217−252.

(5) Grabley, S.; Thiericke, R.; Zeeck, A. The chemical screening approach. In *Drug Discovery from Nature*; Springer-Verlag: Heidelberg, 1999; pp 124−148.

(6) Tsuji, N.; Kamigauchi, T.; Nakai, H.; Shiro, M. X-ray analysis of dibromogriseusin A, revised absolute configuration of griseusins. *Tetrahedron Lett.* **1983**, *24*, 389−390.

(7) Igarashi, M.; Chen, W.; Tsuchida, T.; Umekita, M.; Sawa, T.; Naganawa, H.; Hamada, M.; Takeuchi, T. 4′-Deacetyl-(−)-griseusins A and B, new naphthoquinone antibiotics from an actinomycete. *J. Antibiot*. **1995**, *48*, 1502−1505.

(8) Kometani, T.; Takeuchi, Y.; Yoshii, E. Pyranonaphthoquinone antibiotics. 3. Synthesis of (+)-9-deoxygriseusin B and absolute configuration revision of griseusins A and B. *J. Org. Chem*. **1983**, *47*, 4725−4730.

(9) Kometani, T.; Takeuchi, Y.; Yoshii, E. Pyranonaphthoquinone antibiotics. 4. Total synthesis of (+)-griseusin A, an enantiomer of the naturally occurring griseusin A. *J. Org. Chem*. **1983**, *48*, 2314−2318.

(10) Tsuji, N.; Kobayashi, M.; Wakisaka, Y.; Kawamura, Y.; Mayama, M. New antibiotics, griseusins A and B. Isolation and characterization. *J. Antibiot*. **1976**, *29*, 7−9.

(11) Okabe, T.; Nomoto, K.; Tanaka, N. Lactoquinomycin B, a novel antibiotic. *J. Antibiot*. **1986**, *39*, 1−5.

(12) Maruyama, M.; Nishida, C.; Takahashi, Y.; Naganawa, H.; Hamada, M.; Takeuchi, T. 3′-*O*-alpha-D-forosaminyl-(+)-griseusin A from *Streptomyces griseus*. *J. Antibiot*. **1994**, *47*, 952−954.

(13) Drewry, D. H.; Coe, D. M.; Poon, S. Solid-supported reagents in organic synthesis. *Med. Res. Rev*. **1999**, *19*, 97−148.

(14) Kang, G. D.; Howard, P. W.; Thurston, D. E. Synthesis of a novel C2-aryl substituted 1,2-unsaturated pyrrolobenzodiazepine. *Chem. Commun*. *(Cambridge)* **2003**, 1688−1689.

(15) Stephan, E.; Zen, R.; Authier, L.; Jaouen, G. Improved synthesis of a protected 11-oxoestrone. *Steroids* **1995**, *60*, 809−811.

(16) Hu, T.; Schaus, J. V.; Lam, K.; Palfreyman, M. G.; Wuonola, M.; Gustafson, G.; Panek, J. S. Total synthesis and preliminary antibacterial evaluation of the RNA polymerase inhibitors (±)-myxopyronin A and B. *J. Org. Chem*. **1998**, 2401−2406.

(17) Brimble, M. A. Synthetic studies toward pyranonaphthoquinone antibiotics. *Pure Appl. Chem.* **2000**, *72*, 1635−1639.

(18) Li, X.; Zheng, Y.; Sattler, I.; Lin, W. Griseusin C. A novel quinone derivative from a marine-derived fungus *Penicillium* sp. *Arch. Pharm. Res*. **2006**, *29*, 942−945.

(19) Hertweck, C. L. A.; Rebets, Y.; Bechthold, A. Type II polyketide synthases: Gaining a deeper insight into enzymatic teamwork. *Nat. Prod. Rep.* **2007**, *24*, 162−190.

(20) Ichinose, K.; Surti, C.; Taguchi, T.; Malpartida, F.; Booker-Milburn, K. I.; Hopwood, D. A. Proof that the ACTVI genetic region of *Streptomyces coelicolor* A3(2) is involved in stereospecific pyran ring formation in the biosynthesis of actinorhodin. *Bioorg. Med. Chem. Lett*. **1999**, *9*, 395−400.

(21) Ichinose, K.; Ozawa, M.; Itou, K.; Kunieda, K.; Ebizuka, Y. Cloning, sequencing and heterologous expression of the medermycin biosynthetic gene cluster of *Streptomyces* sp. AM-7161: Towards comparative analysis of the benzoisochromanequinone gene clusters. *Microbiology* **2003**, *149*, 1633−1645.

(22) Ichinose, K.; Taguchi, T.; Bedford, D. J.; Ebizuka, Y.; Hopwood, D. A. Functional complementation of pyran ring formation in actinorhodin biosynthesis in *Streptomyces coelicolor* A3(2) by ketoreductase genes for granaticin biosynthesis. *J. Bacteriol*. **2001**, *183*, 3247−3250.

(23) Taguchi, T.; Ebizuka, Y.; Hopwood, D. A.; Ichinose, K. A new mode of stereochemical control revealed by analysis of the biosynthesis of dihydrogranaticin in *Streptomyces violaceoruber* Tu22. *J. Am. Chem. Soc*. **2001**, *123*, 11376−11380.

(24) Park, C. H.; Bergsagel, D. E.; McCulloch, E. A. Mouse myeloma tumor stem cells: A primary cell culture assay. *J. Natl. Cancer Inst*. **1971**, *46*, 411−422.

(25) Reya, T.; Morrison, S. J.; Clarke, M. F.; Weissman, I. L. Stem cells, cancer, and cancer stem cells. *Nature* **2001**, *414*, 105−111.

(26) Al-Hajj, M.; Wicha, M. S.; Benito-Hernandez, A.; Morrison, S. J.; Clarke, M. F. Prospective identification of tumorigenic breast cancer cells. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 3983−3988.

(27) Beachy, P. A.; Karhadkar, S. S.; Berman, D. M. Tissue repair and stem cell renewal in carcinogenesis. *Nature* **2004**, *432*, 324−331.

(28) Fiebig, H. H.; Maier, A.; Burger, A. M. Clonogenic assay with established human tumour xenografts: Correlation of in vitro to in vivo activity as a basis for anticancer drug discovery. *Eur. J. Cancer* **2004**, *40*, 802−820.

(29) Berger, D. P.; Henss, H.; Winterhalter, B. R.; Fiebig, H. H. The clonogenic assay with human tumor xenografts: Evaluation, predictive value and application for drug screening. *Ann. Oncol*. **1990**, *1*, 333−341.

(30) von Hoff, D. D. Human tumor cloning assays: Applications in clinical oncology and new antineoplastic agent development. *Cancer Metastasis Rev*. **1988**, *7*, 357−371.

(31) Scholz, C. C.; Berger, D. P.; Winterhalter, B. R.; Henss, H.; Fiebig, H. H. Correlation of drug response in patients and in the clonogenic assay with solid human tumour xenografts. *Eur. J. Cancer* **1990**, *26*, 901−905.

(32) Fiebig, H. H.; Burger, A. M. Human tumor xenografts and explants. In *Animal models in cancer research*; Teicher B. A., Ed.; Humana Press, Inc: Totowa, NJ, 2001; pp 113−137.

(33) Paull, K. D.; Shoemaker, R. H.; Hodes, L.; Monks, A.; Scudiero, D. A.; et al. Display and analysis of patterns of differential activity of drugs against human tumor cell lines: development of mean graph and COMPARE algorithm. *J. Natl. Cancer Inst*. **1989**, *81*, 1088−1092.

(34) Dengler, W. A.; Schulte, J.; Berger, D. P.; Mertelsmann, R.; Fiebig, H. H. Development of a propidium iodide fluorescence assay for proliferation and cytotoxicity assays. *Anticancer Drugs* **1995**, *6*, 522−532.

(35) Fiebig, H. H.; Dengler, W. A.; Roth, T. Human tumor xenografts: Predictivity, characterization, and discovery of new anticancer agents. In *Relevance of tumor models for anticancer drug development. Contrib. Oncol.*; Fiebig H. H., Burger A. M., Eds.; Karger: Basel, 1999; pp 29−50.

(36) Fiebig, H. H.; Berger, D. P.; Dengler, W. A.; Wallbrecher, E.; Winterhalter, B. R. Combined in vitro/in vivo test procedure with human tumor xenografts. In *Immunodeficient mice in oncology. Contrib. Oncol.;* Fiebig H. H., Berger D. P., Eds.; Karger: Basel, 1992; pp 321−351.

(37) Fiedler, H. J.; Mai, H. Methoden der Bodenanalyse, Bd. 2 Mikrobiologische Methoden; Verlag Theodor Steinkopff: Dresden, Germany, 1973; pp 12−15.

JM070170N