Aurantiomides A−C, Quinazoline Alkaloids from the Sponge-Derived Fungus *Penicillium aurantiogriseum* SP0-19

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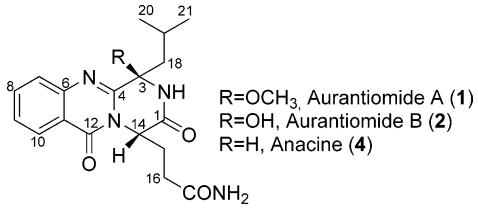
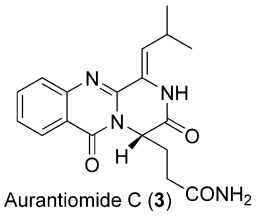
Received October 18, 2006

Three new quinazoline alkaloids, aurantiomides A (**1**), B (**2**), and C (**3**), along with the known metabolite anacine (**4**) were isolated from the sponge-derived fungus strain *Penicillium aurantiogriseum* SP0-19 by bioassay-guided fractionation. Their structures were elucidated by spectroscopic and chemical methods. Their absolute configurations were deduced by comparison of their Cotton effects with anacine (**4**) and by chemical transformations. Compounds **2** and **3** showed moderate cytotoxicities against HL-60, P388 and BEL-7402, P388 cell lines, respectively.

Sponge-derived fungi have proven to be a rich source of structurally unique and biologically active secondary metabolites.[1,2] In our search for new metabolites from sponge-derived fungi, the fungus strain SP0-19 identified as *Penicillium aurantiogriseum* (Trichomaceae) was isolated from the sponge *Mycale plumose* (Mycalidae) collected in Jiaozhou Bay, Qingdao, China. The ethyl acetate extract of the fermentation broth of *P. aurantiogriseum* SP0-19 showed a cytotoxic effect on the mouse *cdc*2 mutant cell line (tsFT210). Bioassay-guided fractionation of the fermentation extract led to the discovery of three new cytotoxic quinazoline alkaloids, aurantiomides A (**1**), B (**2**), and C (**3**), and a known quinazoline alkaloid, anacine (**4**).[3]





The bioactive ethyl acetate extract of *P. aurantiogriseum* SP0-19 was chromatographed on silica gel columns and extensive reversed-phase semipreparative HPLC to give four pure compounds (**1**−**4**).

Compound **1** was obtained as a colorless oil. High-resolution mass measurement on the pseudomolecular peak at *m*/*z* 373.1862 $[M + H]^+$ (calcd 373.1831) in HRESIMS, in combination with ^1H and ^{13}C NMR data (Table 1), supported the molecular formula $C_{19}H_{24}N_4O_4$, indicating 10 degrees of unsaturation. The UV spectrum displayed maximal absorption at 282 and 311 nm, and the IR spectrum exhibited absorption at 3217, 1632, 1606, and 773 cm^{-1}. Analysis of 1D NMR spectra data for **1** revealed the presence of one *ortho*-disubstituted benzene ring, three carbonyls, three methylenes, two methyls, and one methoxyl. From the $^1H-^1H$ COSY spectrum, the molecular fragments from C-14 to C-16, C-7 to C-10, and C-18 to C-20, C-21 could be established (Figure 1). The assignment of $C_4{=}N_5$ was based on the absence of any other sp^2-carbons bonded to C-4 and the appearance of the C-4 carbon signal at lower field (δ 147.4). Additionally, the ^{13}C resonance for the aromatic carbon C-6 was also shifted downfield (δ 146.1), implying that the C-6 was also linked to a nitrogen atom. Key correlations from H-2 to C-1, C-3, C-4 and C-14, from H-14 to C-1, C-4, and C-12, from H-18 to C-3 and C-4, and from H-22 to C-3 were observed in the HMBC experiments (Figure 1). Thus, the structure of **1** was established as 3-methoxyanacine.[3]

Compound **2** was isolated as an amorphous powder. High-resolution mass measurement on the pseudomolecular peak at *m*/*z* 397.1267 $[M + K]^+$ (calcd 397.1278) in the HRESIMS, in combination with ^1H and ^{13}C NMR data (Table 1), supported the molecular formula $C_{18}H_{22}N_4O_4$, which differed from that of **1** by loss of a CH_2 unit. Except for the absence of a methoxyl signal at δ 3.32 (s, 3H) and δ 50.5 (CH_3), the NMR data of **2** were very similar to those of **1**, indicating that the methoxyl at C-3 in **1** was substituted by the hydroxyl in **2**. Thus, the structure of **2** was elucidated as 3-hydroxyanacine.

Compound **3** was also isolated as an amorphous powder. The molecular formula of **3** was determined as $C_{18}H_{20}N_4O_3$ by HRESIMS at *m*/*z* 341.1617 $[M + H]^+$ (calcd 341.1614). On the basis of the 1D NMR spectra, **3** contained the same anacine core structure as in **1** and **2**, with one double bond (a methine at δ 6.48, 1H; δ 127.8, d and a quaternary carbon at δ 127.6, s), the position of which was confirmed by the correlation of H-18 with H-19 in the $^1H-^1H$ COSY spectrum and long-range correlation of H-18 with C-4, C-20, and C-21 in the HMBC spectrum. Therefore the double bond of **3** was located at C-3 and C-18, indicating that compound **3** was a dehydrated derivative of **2**.

The CD spectra of compounds **1** and **2** were carefully compared with those of anacine (**4**) and the verrucines.[3] They showed very similar Cotton effects to anacine and verrucine A, while differing significantly from verrucine B, an epimer of verrucine A. This indicated that they had the same absolute configuration and a boat conformation of the piperazine ring with the two large substituents in a *syn*-1, 4-diaxial disposition, as in **4**.

The *Z*-configuration of the double bond $C_3{=}C_{18}$ in **3** was deduced from the downfield chemical shift of H-18 (δ 6.48), for the deshielding effect of 4-imine, which was also further confirmed by the correlation of NH (δ 8.77) with H-19 (δ 2.77) in NOESY experiments. The *S*-configuration of C-14 in **3** was assigned by

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10.1021/np060516h CCC: $37.00
Published on Web 04/25/2007

Table 1. ^{1}H and ^{13}C NMR Data for Compounds **1**−**3** ($CDCl_3$)[a]

	1		**2**[b]		**3**	
position	δ_C	δ_H (*J*/Hz)	δ_C	δ_H (*J*/Hz)	δ_C	δ_H (*J*/Hz)
1	170.2 qC		171.3 qC		166.1 qC	
2 (NH)		8.18, br s				8.77, br s
3	87.1 qC		84.1 qC		127.6 qC	
4	147.4 qC		152.0 qC		144.4 qC	
6	146.1 qC		148.2 qC		147.3 qC	
7	127.9 CH	7.76, d (7.7)	128.8 CH	7.74, d (8.3)	127.1 CH	7.69, d (8.3)
8	134.8 CH	7.80, t (7.2, 7.7)	136.1 CH	7.84, t (8.2, 8.3)	134.9 CH	7.77, t (7.7, 8.3)
9	127.8 CH	7.54, t (7.2, 7.7)	128.7 CH	7.56, t (7.8, 8.2)	127.5 CH	7.48, t (7.7, 8.3)
10	126.7 CH	8.27, d (7.7)	127.6 CH	8.23, d (7.8)	126.8 CH	8.25, d (8.3)
11	120.4 qC		121.6 qC		119.9 qC	
12	160.8 qC		162.5 qC		160.8 qC	
13						
14	55.5 CH	5.32, dd (4.9, 9.4)	56.9 CH	5.24, dd (4.9, 7.4)	54.6 CH	5.47, t (7.2)
15	28.8 CH_2	2.37, m; 2.41, m	30.7 CH_2	2.49, m; 2.35, m	28.8 CH_2	2.24, q (7.2)
16	32.3 CH_2	2.62, t (7.2)	33.1 CH_2	2.49, m	31.6 CH_2	2.46, t (7.2)
17	174.7 qC		177.4 qC		173.8 qC	
18	40.4 CH_2	2.04, dd (14.3, 6.6) 2.64, dd (14.3, 6.1)	47.7 CH_2	1.96, dd (14.2, 6.4) 2.74, dd (14.2, 6.4)	127.8 CH	6.48, d (10.4)
19	24.3 CH	1.79, m (6.6)	26.0 CH	1.81, m (6.4, 6.8)	26.2 CH	2.77, m
20	23.7 CH_3	0.91, d (6.5)	23.9 CH_3	0.82, d (6.4)	22.2 CH_3	1.18, d (5.5)
21	23.8 CH_3	1.02, d (6.6)	24.0 CH_3	0.99, d (6.8)	22.4 CH_3	1.19, d (5.5)
3-OCH_3	50.5 CH_3	3.32, s				
17-NH_2		6.60, br s; 6.25, br s				6.15, br s; 6.00, br s

[a] ^{1}H, ^{13}C NMR, HMBC, and ^{1}H−^{1}H COSY spectra were obtained at 600, 150, and 600 MHz, respectively. [b] Recorded in CD_3OD.

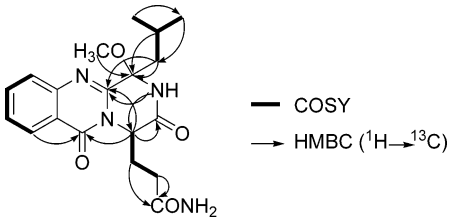

Figure 1. Key COSY and HMBC correlations of compound **1**.

the chemical transformation from **2** to **3**. When **2** was dehydrated with *p*-toluenesulfonic acid in benzene,[4] compound **3** was yielded and identified by the same HPLC properties and optical rotation ([α] = +29.5) as the isolated sample (Figure 2).

The cytotoxicities of compounds **1**−**3** were evaluated against the P388, BEL-7402, A-549, and HL-60 cell lines by the MTT method.[5] Compound **2** exhibited moderate cytotoxic activities against HL-60 and P388 cell lines with IC_{50} values of 52 and 54 μg/mL, respectively, while compound **3** selectively inhibited BEL-7402 and P388 cell lines with IC_{50} values of 62 and 48 μg/mL, respectively. Compound **1** was inactive.

Fungi are capable of incorporating anthranilic acid and amino acids into a variety of fungal quinazoline metabolites. One class of the metabolites is anacine, which is composed of anthranilic acid, leucine, and glutamine. Anacine was first isolated from *P. aurantiogriseum* and originally identified as a benzodiazepine structure.[6] Later Larsen[3] showed that anacine had a quinazoline structure that was further confirmed by the total synthesis.[7] Recently Larsen reported the isolation of verrucines A and B, both of which are composed of anthranilic acid, phenylalanine, and glutamine, from *P. verrucosum*.[3] To date, six anacine quinazoline compounds have been isolated from fungi in all. We reported the cytotoxic activities for the three aurantiomides (**1**−**3**) in this paper. To our best knowledge, this is the first report on the cytotoxic activities of this kind of compound.

Experimental Section

General Experimental Procedures. Optical rotations were obtained on a JASCO P-1020 digital polarimeter. UV spectra were recorded on a Beckman DU 640 spectrophotometer. IR spectra were taken on a Nicolet NEXUS 470 spectrophotometer in KBr discs. ^{1}H and ^{13}C NMR, DEPT, and 2D-NMR spectra were recorded on a JEOL JNM-ECP 600 spectrometer using TMS as internal standard, and chemical shifts were recorded as δ values. ESIMS were measured on a Q-TOF Ultima Global GAA076 LC mass spectrometer. Semipreparative HPLC was performed using an ODS column [Shin-pak ODS (H), 20 × 250 mm, 5 μm, 4 mL/min].

Fungal Material and Fermentation. The fungus *P. aurantiogriseum* SP0-19 was isolated from the sponge *Mycale plumose* collected in Jiaozhou Bay, Qingdao, China. It was identified according to its morphological characteristics and preserved in the China Center for Type Culture Collection, No. CCTCC M205048. Working stocks were prepared on potato dextrose agar slants stored at 4 °C. The producing fungal strain *P. aurantiogriseum* SP0-19 was inoculated into a 500 mL cylindrical flask containing 100 mL of the liquid medium consisting of sorbitol 2%, maltose 2%, glutamine 1%, KH_2PO_4 0.05%, $MgSO_4 \cdot 7H_2O$ 0.03%, tryptophan 0.05%, yeast extract 0.3%, and seawater

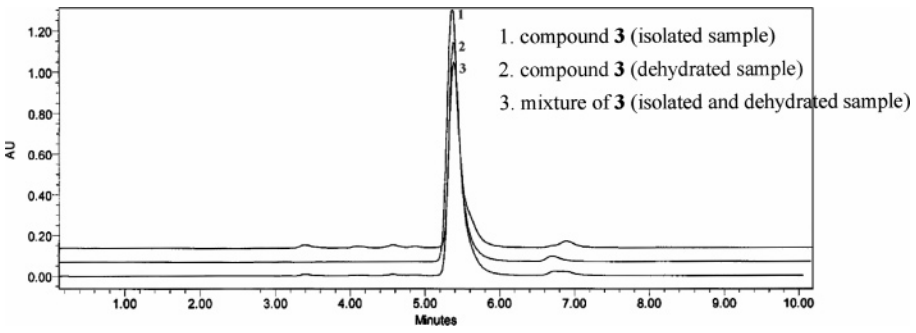

Figure 2. HPLC profiles of compound **3**.

(adjusted to pH 6.5 prior to sterilization) and cultured at 28 °C for 48 h on a rotary shaker at 120 rpm. The seed culture was transferred into two hundred 500 mL conical flasks (150 mL/flask), and the fermentation was performed at 28 °C for 10 days with an agitation rate of 120 rpm.

Extraction and Separation. The fermented whole broth (30 L) was filtered through cheese cloth to separate supernatant and mycelia. The former was concentrated under reduced pressure to about one-quarter of the original volume and then extracted three times with EtOAc to give an EtOAc solution, while the latter was extracted three times with acetone. The acetone solution was concentrated under reduced pressure to afford an aqueous solution. The aqueous solution was extracted three times with EtOAc to give another EtOAc solution. Both EtOAc solutions were combined and concentrated under reduced pressure to give a crude extract (20 g).

Isolation of Secondary Metabolites. The EtOAc extract (20 g) was subjected to Si gel CC and eluted with gradient elution of *n*-hexane−EtOAc and acetone−MeOH, respectively. The fraction eluted with *n*-hexane−EtOAc (4:1) was further purified by HPLC on ODS with 60% MeOH to give **1** (20 mg) and **2** (18 mg). The fraction eluted with acetone−MeOH (99:1) was separated by HPLC on ODS using 50% MeOH as eluting solvent to afford **3** (15 mg) and **4** (12 mg).

Chemical Transformation of 2 to 3. Aurantiomide B (**2**, 5.4 mg) and *p*-TsOH (2 mg) in benzene (4 mL) were reacted for 1 h at refluxing temperature. The mixture was cooled to RT, and then 2 mL of EtOAc and 4 mL of 5% $NaHCO_3$ were added. The organic phase was washed three times with H_2O (15 mL) and then dried, filtered, and evaporated to give 4.6 mg of **3** (90% yield). Synthesized **3** showed the same physical properties as the isolated sample of **3** by HPLC and optical rotation, $[\alpha]_D^{24}$ +29.5 (*c* 0.09, $CHCl_3$).

Biological Assays. Active fractions were assayed using the MTT method with the mouse temperature-sensitive *cdc2* mutant cell line, tsFT210. The cytotoxic activities of compounds **1**−**3** were evaluated by the MTT method using P388, A-549, and HL-60 cell lines. The cell lines were grown in RPMI-1640 supplemented with 10% FBS under a humidified atmosphere of 5% CO_2 and 95% air at 37 °C (tsFT210 cell line at 32 °C). Cell suspensions (200 μL) at a density of 5×10^4 cell mL^{-1} were plated in 96-well microtiter plates and incubated for 24 h at the above conditions. Next, 2 μL of the test compounds in DMSO at different concentrations was added to each well and further incubated for 72 h in the same conditions. MTT solution (20 μL, 5 mg/mL in IPMI-1640 medium) was added to each well and incubated for 4 h. Old medium (150 μL) containing MTT was then gently replaced by DMSO and pipetted to dissolve any formazan crystals formed. Absorbance was then determined on a Spectra Max Plus plate reader at 540 nm.

Aurantiomide A (1): colorless oil; $[\alpha]_D^{24}$ +16 (*c* 0.05, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 282 (3.90), 311 (3.71) nm; IR (KBr) ν_{max} 3217, 1632, 1606, 773 cm^{-1}; CD (MeOH, *c* 0.50), λ_{max} ($\Delta\epsilon$) 197 (+6.25), 210 (−10.11), 235 (+19.16), 310 (+2.01), 320 (+2.15) nm; ^1H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 373.1862 $[M + H]^+$ (calcd for $C_{19}H_{25}N_4O_4$, 373.1831).

Aurantiomide B (2): amorphous powder; $[\alpha]_D^{24}$ +96.5 (*c* 0.09, $CHC1_3$), $[\alpha]_D^{24}$ +172 (*c* 1.8, CH_3OH); UV (MeOH) λ_{max} (log ϵ) 280 (4.60), 311 (4.28) nm; IR (KBr) ν_{max} 3217, 1632, 1606, 773 cm^{-1}; CD (MeOH, *c* 0.50), λ_{max} ($\Delta\epsilon$) 210 (−39.21), 227 (+68.36), 255 (+6.35), 310 (+11.25), 320 (+10.25) nm; ^1H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 397.1267 $[M + K]^+$ (calcd for $C_{18}H_{22}N_4O_4K$, 397.1278).

Aurantiomide C (3): amorphous powder; $[\alpha]_D^{24}$ +25.8 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 314 (4.48) nm; IR (KBr) ν_{max} 3197, 1634, 1606, 1582, 1561, 764 cm^{-1}; CD (MeOH, *c* 0.60), λ_{max} ($\Delta\epsilon$) 230 (+4.95), 235 (+5.11), 245 (−2.76), 275 (−2.11), 320 (+1.85) nm; ^1H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 341.1617 $[M + H]^+$ (calcd for $C_{18}H_{21}N_4O_3$, 341.1614).

Anacine (4): amorphous powder; $[\alpha]_D^{24}$ +233.3 (*c* 0.21, MeOH); UV (MeOH) λ_{max} (log ϵ) 226 (4.23), 270 (3.60) nm; IR (KBr) ν_{max} 3191, 1632, 1601, 771 cm^{-1}; CD (MeOH, *c* 0.50), λ_{max} ($\Delta\epsilon$) 307 (+5.25), 232 (+27.32), 207 (−18.63), 197 (+14.47) nm; ^1H NMR (600 MHz, $CDCl_3$) δ 8.21 (1H, d, J = 7.7 Hz, H-10), 8.05 (1H, s, H-2), 7.73 (1H, 't'-like, J = 7.2, 8.3 Hz, H-8), 7.61 (1H, d, J = 8.3 Hz, H-7), 7.45 (1H, 't'-like, J = 7.2, 7.7 Hz, H-9), 5.19 (1H, s, H-14), 4.57 (1H, s, H-3), 2.67 (2H, br s, H-16), 2.38 (1H, m, H-15a), 2.20 (1H, m, H-15b), 1.92 (1H, m, H-19), 1.90 (2H, m, H-18), 1.04 (3H, d, J = 6.4 Hz, H-20), 1.03 (3H, d, J = 6.4 Hz, H-21); ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.5 (C, C-17), 168.3 (C, C-1), 160.8 (C, C-12), 150.9 (C, C-4), 147.2 (C, C-6), 134.8 (CH, C-8), 127.0 (CH, C-9), 126.9 (CH, C-7), 126.7 (CH, C-10), 119.8 (C, C-11), 54.7 (CH, C-3), 54.7 (CH, C-14), 47.1 (CH_2, C-18), 32.3 (CH_2, C-16), 29.3 (CH_2, C-15), 24.6 (CH, C-19), 23.2 (CH_3, C-20), 21.1 (CH_3, C-21); ESIMS *m*/*z* 343 $[M + H]^+$.

Acknowledgment. This work was financially supported by the Shandong Province Natural Science Fund (No. Z2006C01), project of Chinese National Programs for High Technology Research and Development (No. 2003AA624020), and the project of international cooperation of Australian and Chinese (2005DFA30030-4). The sponge *Mycale plumose* was identified by J. H. Li, Institute of Oceanology, Chinese Academy of Sciences, and the fungus strain *Penicillium aurantiogriseum* SP0-19 was identified by Y. G. Zhou, Institute of Microbiology, Chinese Academy of Sciences.

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NP060516H