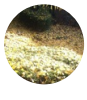
Alkaloidal Metabolites from a Marine-Derived Aspergillus sp. Fungus

ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · JANUARY 2015

Impact Factor: 3.8 · DOI: 10.1021/np500683u · Source: PubMed

6 AUTHORS, INCLUDING:



Lijuan Liao
Seoul National University
8 PUBLICATIONS **26** CITATIONS
SEE PROFILE

Juhye Shin
Korea University
183 PUBLICATIONS **2,425** CITATIONS
SEE PROFILE

JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Alkaloidal Metabolites from a Marine-Derived *Aspergillus* sp. Fungus

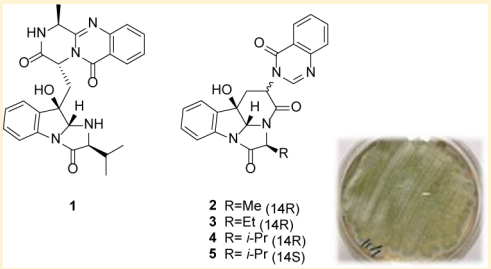
Lijuan Liao,[†] Minjung You,[†] Beom Koo Chung,[‡] Dong-Chan Oh,[†] Ki-Bong Oh,*[,‡] and Jongheon Shin*[,†]

[†]Natural Products Research Institute, College of Pharmacy, Seoul National University, San 56-1, Sillim, Gwanak, Seoul 151-742, Korea

[‡]Department of Agricultural Biotechnology, College of Agriculture and Life Science, Seoul National University, San 56-1, Sillim, Gwanak, Seoul 151-921, Korea

Supporting Information

ABSTRACT: Fumiquinazoline S (**1**), a new quinazoline-containing alkaloid, and the known fumiquinazolines F (**6**) and L (**7**) of the same structural class were isolated from the solid-substrate culture of an *Aspergillus* sp. fungus collected from marine-submerged wood. In addition, isochaetominines A–C (**2**–**4**) and 14-*epi*-isochaetominine C (**5**), new alkaloids possessing an unusual amino acid-based tetracyclic core framework related to the fumiquinazolines, were isolated from the same fungal strain. The structures of these compounds were determined by combined spectroscopic methods, and the absolute configurations were assigned by NOESY, ROESY, and advanced Marfey's analyses along with biogenetic considerations. The new compounds exhibited weak inhibition against Na^+/K^+-ATPase.



Microorganisms in marine environments have been widely recognized as emerging sources of biologically active and structurally unique secondary metabolites.[1−5] Dominated by three major phylogenetic groups, actinomycete and non-actinomycete bacteria and fungi, the novel natural products from marine microorganisms have shown a noticeable increase since 1997.[2] Marine fungi alone have produced more than 400 novel compounds, of which several have exhibited potent and diverse bioactivities.[6,7]

In our search for bioactive metabolites from the fungi of marine environments, we have recently reported novel compounds, such as acremostrictin, chrysoarticulins, herqueiazoles, herqueidiketal, and penicillipyrones, possessing unusual carbon frameworks and/or functionalities, thus contributing to the chemical library from marine-derived fungi.[8] Several of these compounds have exhibited moderate to significant inhibition of sortase A (chrysoarticulin C and herqueidiketal),[8b,c] significant induction of quinone reductase (penicillipyrone B),[8d] and moderate antioxidant activity (acremostrictin).[8a] During our search, a strain of *Aspergillus* was collected from marine-submerged decaying wood from Korea, with an organic extract that showed moderate cytotoxicity (IC_{50} 170 μg/mL) against the K562 human leukemia cell line. Furthermore, the LC-ESIMS profile of the extract revealed the presence of diverse secondary metabolites, which prompted our detailed investigation of the extract metabolites.

A solid-substrate culture of the strain followed by an extraction and chromatographic separation led to the isolation of several new alkaloids. In this study, we report the structure determinations of fumiquinazoline S (**1**), isochaetominines A–C (**2**–**4**), and 14-*epi*-isochaetominine C (**5**) and the known congeners fumiquinazolines F (**6**) and L (**7**) based on combined spectroscopic and chemical analyses. Fumiquinazoline S is a new member of the fumiquinazoline class of alkaloids, which has been reported in a number of marine-derived *Aspergillus*, *Acremonium*, and *Scopulariopsis* fungal strains. Fumiquinazolines A–P have been reported in the literature. However, fumiquinazolines K and L were doubly designated.[9−11] Accordingly, our new compound was designated fumiquinazoline S instead of either Q or R.[9−16] The isochaetominines are structurally related to the recently reported chaetominine (**8**) from the terrestrial endophytic fungus *Chaetomium* sp.[17] Our isolation of the fumiquinazolines and the isochaetominines from a common fungal strain provides insight into the biogenetic relationships of these quinazoline-containing natural products. Although these compounds were not significantly active against the K562 and A549 cell lines, several of them showed weak inhibition against Na^+/K^+-ATPase.

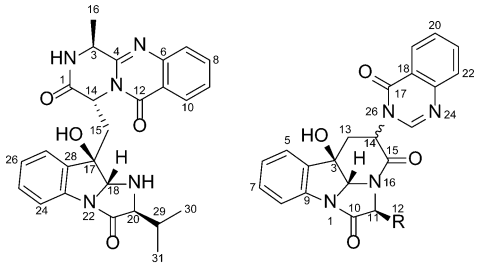
RESULTS AND DISCUSSION

The molecular formula of fumiquinazoline S (**1**) was deduced as $C_{26}H_{27}N_5O_4$ by the HRFABMS analysis. The spectroscopic data of this compound revealed the characteristic features of fumiquinazolines. That is, the presence of 12 carbon signals in the aromatic region (δ_C 150–110) in the ^{13}C NMR data, along with the corresponding proton signals (δ_H 8.2–7.0) in the ^1H NMR data and aromatic E bands in the UV spectrum, was indicative of two benzene rings in **1** (Table 1). Four carbon signals at δ_C 172.1, 168.9, 160.2, and 153.3 in the ^{13}C NMR data were thought to represent either carbonyl or imine

Special Issue: Special Issue in Honor of William Fenical

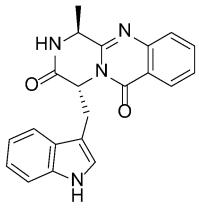
Received: September 2, 2014
Published: January 12, 2015

DOI: 10.1021/np500683u
J. Nat. Prod. 2015, 78, 349−354

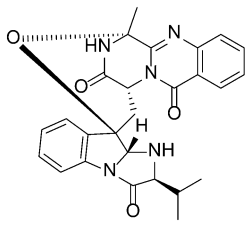


1

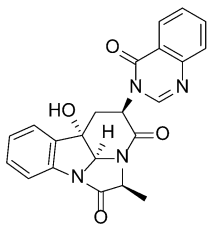
2 R=Me (14R)
3 R=Et (14R)
4 R= *i*-Pr (14R)
5 R= *i*-Pr (14S)



6



7



8 chaetominine

carbons, which was implied by the molecular formula and the strong absorption bands at 1730 and 1681 cm^{-1} in the IR spectrum.

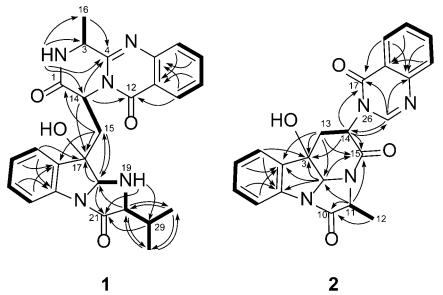
The gross structure of compound **1** was determined by a combination of 2D NMR analyses as well as comparison with the congeners **6** and **7**. First, the COSY data showed a linear spin system of four aromatic protons resonating at δ_H 8.12, 7.82, 7.66, and 7.53, thus revealing the presence of a 1,2-disubstituted benzene whose carbons were also assigned by the HSQC and HMBC data. The attachment of a carbonyl carbon at δ_C 160.2 to the ring quaternary carbon at δ_C 120.0 was secured by its HMBC correlation with a ring proton at δ_H 8.12, designating a benzamide for this portion (C-6−C-12) (Figure 1). The COSY and HSQC data also defined two spin systems consisting of a methine (δ_C 51.8, δ_H 5.58; C-14) and a methylene (δ_C 35.4, δ_H 2.58 and 1.82; C-15), and a methyl (δ_C 16.6, δ_H 1.58; C-16) and a methine group (δ_C 48.6, δ_H 4.95; C-3), respectively. The HMBC correlations of these protons and an exchangeable one at δ_H 8.61 (2-NH) with the neighboring carbons completed a 4-oxo-quinazoline-δ-lactam system found in several fumiquinazolines.[11,13]

The combined 2D NMR data also revealed the presence of another 1,2-disubstituted benzene (C-23−C-28) in **1** (Table 1). The extension of this ring to a 3-alkylindole-3-ol moiety as well as its connection to the upper portion of the molecule via a methylene (C-15) was accomplished by the HMBC correlations of an oxygenated carbon at δ_C 80.1 (C-17) and a methine carbon at δ_C 88.2 (C-18) with neighboring protons and carbons (Figure 1). The remaining portion of the molecule was also

Table 1. NMR Assignments for Compound 1 in DMSO-d_6[a]

position	δ_C, type	δ_H (J in Hz)
1	168.9, C	
3	48.6, CH	4.95, q (6.5)
4	153.3, C	
6	146.6, C	
7	126.9, CH	7.66, dd (8.3, 0.8)
8	134.5, CH	7.82, ddd (8.3, 7.5, 1.3)
9	126.7, CH	8.12, dd (8.1, 1.3)
10	126.5, CH	7.53, ddd (8.1, 7.5, 0.8)
11	120.0, C	
12	160.2, C	
14	51.8, CH	5.58, dd (9.6, 4.5)
15	35.4, CH_2	2.58, dd (14.9, 9.6)
		1.82, dd (14.9, 4.5)
16	16.6, CH_3	1.58, d (6.5)
17	80.1, C	
18	88.2, CH	5.26, dd (6.8, 1.7)
20	69.2, CH	3.54, m
21	172.1, C	
23	137.2, C	
24	115.1, CH	7.34, dd (7.9, 1.0)
25	128.8, CH	7.25, ddd (7.9, 7.5, 1.3)
26	124.6, CH	7.09, ddd (7.5, 7.5, 1.0)
27	125.5, CH	7.80, dd (7.5, 1.3)
28	138.7, C	
29	31.1, CH	1.98, m
30	18.6, CH_3	0.96, d (6.9)
31	17.6, CH_3	0.93, d (6.9)
2-NH		8.61, s
17-OH		5.70, brs
19-NH		3.51, m

[a]Data were measured at 400 and 100 MHz for ^1H and ^{13}C NMR, respectively.



1 2

Figure 1. COSY (bold lines) and selected HMBC (arrows) correlations of compounds **1** and **2**.

found to have a valine (Val)-derived residue by the COSY data, which showed a long spin system consisting of protons at δ_H 5.26 (H-18), 3.51 (19-NH), 3.54 (H-20), 1.98 (H-29), 0.96 (H-30), and 0.93 (H-31). The HMBC correlations of several of these protons with a carbonyl carbon at δ_C 172.1 (C-21) allowed the construction of an imidazolidinone moiety (Figure 1). Thus, the planar structure of fumiquinazoline S (**1**) was determined as a new derivative of the fumiquinazoline class possessing a Val-derived residue.

A structural comparison of the congeners revealed a biogenetic relationship between **1** and **7**, whose absolute configurations were assigned by X-ray crystallographic analysis.[11] Although several biosynthetic postulations would be plausible for the structural conversion between these

DOI: 10.1021/np500683u
J. Nat. Prod. 2015, 78, 349−354

compounds, the configurations at C-14, C-17, C-18, and C-20 are likely to be the same in these two compounds, whereas the configuration at the reaction center C-3 in **1** may be either intact or reversed, depending on the reaction pathway. This hypothesis was supported by the ROESY data for **1**, in which a conspicuous cross-peak was found at H-18/H-29 (Figure 2).

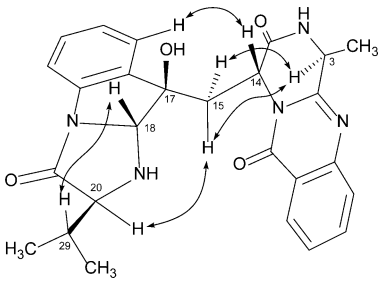


Figure 2. Selected ROESY correlations of compound **1**.

Although the desired cross-peak at 17-OH/H-18 was not found, it was compensated by another cross-peak at H-15 (δ_H 2.58)/H-20 because a DFT model calculation (Supporting Information) revealed a spatial proximity between these protons only by the β-orientation of the 17-OH proton at the tetracyclic frame. Similarly, H-14 was placed at the β-orientation based on the cross-peaks at H-3/H-15 (δ_H 2.58), H-3/H-15 (δ_H 1.82), and H-14/H-27.

The significant ROESY correlations of H-3 with both of the H-15 methylene protons coupled with the lack of a ROESY correlation of H-16 with the latter protons assigned an α-orientation for the H-3 to the quinazoline plane, which was supported by the ^{13}C NMR data. A literature study showed that the C-3 asymmetric center significantly influences the chemical shifts of the C-16 methyl carbon (*cis* δ_C ~24.9, *trans* δ_C ~16.8), possibly due to the spatial crowding of the substituents at C-14 (*cis* δ_C ~52.7 and *trans* δ_C ~49.2).[12−14] The chemical shifts of C-16 and C-14 at δ_C 16.9 and 49.1 in $CDCl_3$, respectively, in the ^{13}C NMR data fit well with the α-orientation (*trans* orientation of C-15 with C-16) of H-3 (Supporting Information). Thus, the relative configurations of the stereogenic carbon centers of **1** were unambiguously assigned throughout the molecule. Compound **1** has a Val-derived unit at C-21. The absolute configuration of this unit was determined to be L by advanced Marfey's analysis (Experimental Section).[18] Therefore, the overall absolute configuration of **1** was assigned as 3*S*, 14*R*, 17*R*, 18*R*, and 20*S*.

The molecular formula of isochaetominine A (**2**) was deduced as $C_{22}H_{18}N_4O_4$ by the HRFABMS analysis. The gross NMR features of this compound were highly reminiscent of **1**, indicating the presence of a quinazoline and a 3-alkylindolin-3-ol as substructures. The replacement of the Val-derived residue with an alanine (Ala)-derived one was also found by the NMR data (δ_C 174.4, 59.7, and 13.8, δ_H 4.58 and 1.57). However, a detailed examination of the ^{13}C and ^{1}H NMR data revealed that the signals of the several carbons and protons, particularly those near the δ-lactam C-14, noticeably differ from those of **1** in the chemical shifts and the multiplicities, which prompted an extensive interpretation of the full spectroscopic data (Tables 2 and 3).

First, a combination of the COSY, HSQC, and HMBC data secured the presence of a 1,2-disubstituted benzene in **2** and assigned all of the carbons and protons in this moiety. A three-bond HMBC correlation with an aromatic proton at δ_H 8.14

Table 2. ^{13}C NMR (ppm, type) Assignments for Compounds 2−5 in DMSO-d_6

position	2[a]	3[b]	4[b]	5[b]
2	82.9, CH	83.3, CH	83.7, CH	84.5, CH
3	74.1, C	74.0, C	73.9, C	76.7, C
4	135.8, C	135.4, C	134.9, C	137.5, C
5	124.6, CH	124.6, CH	124.6, CH	124.6, CH
6	125.2, CH	125.2, CH	125.2, CH	125.5, CH
7	130.1, CH	130.0, CH	130.1, CH	129.7, CH
8	114.1, CH	114.1, CH	114.2, CH	114.7, CH
9	139.7, C	139.9, C	140.3, C	137.6, C
10	174.4, C	175.2, C	173.9, C	169.7, C
11	59.7, CH	65.3, CH	69.4, CH	69.6, CH
12	13.8, CH_3	21.7, CH_2	28.2, CH	30.3, CH
13	34.7, CH_2	34.4, CH_2	34.1, CH_2	38.2, CH_2
14	55.6, CH	55.7, CH	55.7, CH	49.1, CH
15	164.2, C	164.6, C	164.9, C	167.2, C
17	159.9, C	159.8, C	159.8, C	159.9, C
18	121.4, C	121.5, C	121.5, C	121.0, C
19	126.2, CH	126.2, CH	126.2, CH	126.4, CH
20	127.4, CH	127.4, CH	127.4, CH	127.2, CH
21	134.7, CH	134.7, CH	134.7, CH	134.7, CH
22	127.2, CH	127.2, CH	127.2, CH	127.1, CH
23	147.5, C	147.5, C	147.5, C	147.2, C
25	146.7, CH	146.7, CH	146.9, CH	146.7, CH
27		10.9, CH_3	20.2, CH_3	19.1, CH_3
28			18.7, CH_3	19.0, CH_3

[a]Data were measured at 150 MHz. [b]Data were measured at 125 MHz.

Table 3. ^{1}H NMR (δ, mult (J in Hz)) Assignments for Compounds 2−5 in DMSO-d_6

position	2[a]	3[b]	4[b]	5[b]
2	5.88, s	5.85, s	5.81, s	5.78, s
5	7.40, dd (8.0, 1.0)	7.52, dd (7.4, 1.0)	7.54, dd (7.5, 1.3)	7.47, dd (7.4, 0.9)
6	7.20, ddd (8.0, 8.0, 1.3)	7.24, ddd (7.5, 7.4, 1.4)	7.26, ddd (7.8, 7.5, 1.3)	7.24, ddd (7.4, 7.4, 1.2)
7	7.38, ddd (8.0, 8.0, 1.0)	7.42, ddd (7.6, 7.5, 1.0)	7.43, ddd (7.8, 7.5, 1.3)	7.41, ddd (7.8, 7.4, 0.9)
8	7.47, dd (8.0, 1.3)	7.43, dd (7.6, 1.4)	7.46, dd (7.5, 1.3)	7.49, dd (7.8, 1.2)
11	4.58, q (7.3)	4.45, dd (9.4, 5.9)	4.21, d (9.3)	4.37, d (6.7)
12	1.57, d (7.3)	2.06, m	2.44, m	2.26, m
		2.00, m		
13	2.92, dd (14.3, 7.0)	2.97, dd (14.2, 7.2)	3.01, dd (14.0, 7.9)	2.93, dd (13.0, 13.0)
	2.65, dd (14.3, 5.1)	2.72, dd (14.2, 5.2)	2.77, dd (14.0, 5.3)	2.49, dd (13.0, 3.5)
14	4.88, dd (7.0, 5.1)	4.89, dd (7.2, 5.2)	4.86, dd (7.9, 5.3)	5.98, dd (13.0, 3.5)
19	8.14, dd (8.0, 1.3)	8.17, dd (7.9, 1.2)	8.18, dd (7.9, 1.3)	8.20, dd (7.9, 1.3)
20	7.56, ddd (8.0, 8.0, 1.0)	7.59, ddd (7.9, 7.9, 1.0)	7.59, ddd (7.9, 7.5, 1.0)	7.58, ddd (7.9, 7.6, 0.9)
21	7.84, ddd (8.0, 8.0, 1.3)	7.87, ddd (7.9, 7.9, 1.2)	7.87, ddd (7.7, 7.5, 1.3)	7.86, ddd (7.8, 7.6, 1.3)
22	7.67, dd (8.0, 1.0)	7.71, dd (7.9, 1.0)	7.71, dd (7.7, 1.0)	7.70, dd (7.8, 0.9)
25	8.25, s	8.29, s	8.30, s	8.23, s
27		1.11, t (7.3)	1.12, d (6.7)	1.09, d (6.7)
28			1.15, d (6.7)	1.05, d (6.7)
3-OH	6.25, brs	6.28, brs	6.30, brs	6.77, brs

[a]Data were measured at 600 MHz. [b]Data were measured at 500 MHz.

DOI: 10.1021/np500683u
J. Nat. Prod. 2015, 78, 349−354

(H-19) placed a carbonyl carbon at δ_C 159.9 as a substituent (C-17) (Figure 1). Interestingly, this carbon showed an additional correlation with an isolated singlet proton at δ_H 8.25, which correlated with the aromatic carbon at δ_C 127.2 (C-22). The downfield chemical shift of this proton-bearing carbon at δ_C 146.7 defined it as an amidine methine carbon (C-25), corresponding to the C-4 carbon in **1**. Therefore, unlike the additional six-membered lactam system present in **1** and other fumiquinazoline metabolites, the 4-oxo-quinazoline moiety of **2** was directly attached to the remaining portion of the compound through a single bond at N-26, which is discussed later.

A combination of 2D NMR data revealed the presence of another 1,2-disubstituted benzene moiety. Despite the noticeable shifts of the protons and the carbons in the NMR data, the extension to a 3-alkylindolin-3-ol, identical to **1**, was accomplished by the HMBC analysis (H-2/C-3, H-2/C-4, H-2/C-9, and H-5/C-3, Figure 1). The connection of the Ala-derived residue to the indolinol moiety through the construction of an imidazolidinone ring was also accomplished by long-range correlations at H-11/C-2, H-11/C-10, and H-11/C-15. Thus, **2** was found to possess the same indolinol-imidazolidinone tricyclic substructure as found in **1**.

Compound **1** possessed a three-carbon moiety (C-13, C-14, and C-15) connecting the indolinol and quinazoline moieties. The ^{13}C NMR data for **2** also showed signals of three carbons at δ_C 164.2 (C), 55.6 (CH), and 34.7 (CH_2) having corresponding multiplicities to those of **1**. Although the chemical shifts of these carbons and their attached protons at δ_H 4.88, 2.92, and 2.65 in the ^{1}H NMR data were significantly shifted compared with those of **1**, the COSY and HMBC data showed the same linear assembly of these groups in **2**. In addition, the HMBC data showed the key correlations at H-2/C-15, H-13/C-2, H-13/C-3, H-13/C-4, and H-14/C-3, constructing a δ-lactam moiety consisting of indolinol-imidazolidinone and three carbons at C-13, C-14, and C-15 (Figure 1). As previously mentioned, the connection between this lactam and 4-oxo-quinazoline via a C−N single bond was also accomplished by the long-range correlations at H-14/C-17, H-14/C-25, and H-25/C-14 in the HMBC data. Thus, the planar structure of isochaetominine A (**2**) was defined as a 4-oxo-quinazoline-containing alkaloid, identical to chaetominine (**8**).[17]

A comparison of the main framework of this compound with that of fumiquinazolines **1**, **6**, and **7** indicated that **2** and the fumiquinazolines are biogenetically related, but that the exact relationship is unclear at this time. A literature study revealed that the hexacyclic framework of **2** is unusual; the only previous example is the recently found chaetominine from the terrestrial endophytic *Chaetomium* sp. fungus, whose planar structure is identical to **2**. However, the NMR chemical shifts of several carbons and protons of these compounds significantly differ from each other, suggesting a diastereomeric relationship between them.

Compound **2** possessed four asymmetric carbon centers at C-2, C-3, C-11, and C-14 with configurations assigned by NOESY (Figure 3) and the chemical analyses. The H-2 methine proton showed strong cross-peaks with the 3-OH and H-12 in the NOESY data, placing all of these protons on the same face of the tetracyclic plane. In contrast, the H-14 methine proton did not exhibit cross-peaks with any of these protons but showed strong cross-peaks with both of the neighboring H-13 methylenes, matching the *gauche* conformation based on the

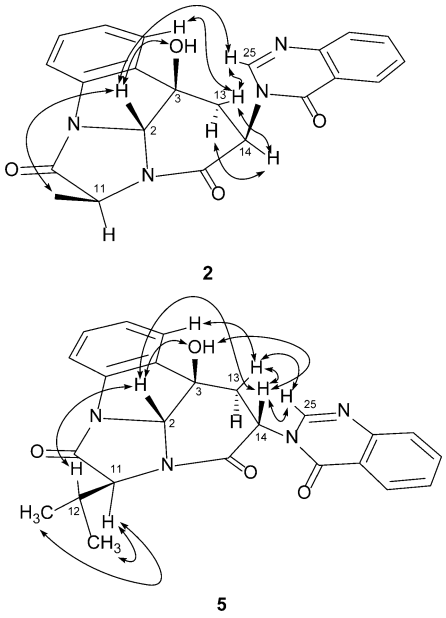


Figure 3. Selected NOESY and ROESY correlations of compounds **2** and **5**.

proton coupling constants ($J_{11,12}$ = 7.0 and 5.1 Hz) on the pseudo-boat form of the lactam ring deduced by the DFT model study (Supporting Information). Therefore, H-14 must be oriented on the opposite face of the tetracyclic plane, which coincided well with the cross-peak at H-2/H-25. For compound **2**, the absolute configuration of the Ala-derived residue at C-10 was determined as L by advanced Marfey's analysis (Supporting Information). These results assigned the 2*R*, 3*R*, 11*S*, and 14*R* absolute configuration, which was distinguished from the 2*S*, 3*S*, 11*S*, and 14*R* configuration of chaetominine based on X-ray crystallographic analysis.[17] Therefore, compound **2** is the a bis-epimer of chaetominine at C-2 and C-3.

Isochaetominine B (**3**) was isolated as a pale yellow solid, which was determined as $C_{23}H_{20}N_4O_4$ by HRFABMS analysis. The NMR data for this compound were similar to those of **2**, and all of the key structural features were intact in **3**. In the ^{13}C NMR data, the only significant difference was the addition of a methylene carbon at δ_C 21.7 (Table 2). A corresponding difference was found in the ^{1}H NMR data, in which the signals of the new methylene protons were observed at δ_H 2.06 and 2.00. In addition, the multiplicity of a doublet methyl proton was changed to a triplet (J = 7.3 Hz) (Table 3). These spectroscopic differences were readily accommodated by the replacement of the C-12 methyl with an ethyl group, which was confirmed by combined 2D NMR methods. Therefore, the Ala-derived residue must be replaced with a 2-amino-butanoic acid-derived residue. The ROESY data showed the same cross-peaks as **2**, which along with the L configuration for the newly appearing 2-amino-butanoic acid residue by advanced Marfey's analysis assigned the same absolute configuration as **2**. Thus, the structure of isochaetominine B (**3**) was defined as a new alkaloid possessing an unusual L-2-amino-butanoic acid-derived lactam moiety.

The molecular formula of isochaetominine C (**4**) was deduced as $C_{24}H_{22}N_4O_4$ by HRFABMS analysis. The NMR data for this compound were similar to those of **3**, with the replacement of signals of the C-12 group with an isopropyl group (δ_C 28.2, 20.2, and 18.7, δ_H 2.44, 1.15, and 1.12) (Tables

DOI: 10.1021/np500683u
J. Nat. Prod. 2015, 78, 349−354

2 and 3). This replacement of an Ala-derived residue in **2** with a valine (Val)-derived one was confirmed by the combined 2D NMR data. The NOESY cross-peaks at H-2/3-OH, H-2/H-12, and H-13/H-14 along with the L configuration of the newly appearing Val-derived residue by advanced Marfey's analysis assigned the same 2*R*, 3*R*, 11*S*, and 14*R* configuration as **2** and **3**. Thus, the structure of isochaetominine C (**4**) was determined as a new alkaloid, possessing an L-Val-derived lactam moiety.

Finally, the molecular formula of 14-*epi*-isochaetominine C (**5**) was deduced as $C_{24}H_{22}N_4O_4$, which is identical to **4**, by HRFABMS analysis. Despite the noticeable shifts of several carbons, the ^{13}C NMR data for this compound showed the same signal distribution and multiplicities as **4** (Table 2). The same trend was also found in the ^1H NMR data, in which most of the signals showed similar chemical shifts and coupling constants to **4** (Table 3). Accordingly, **4** and **5** must be diastereomeric to each other, which was supported by the combined COSY, HSQC, and HMBC analyses, in which the identical proton−proton and carbon−proton correlations were found between these compounds. The ROESY data showed cross-peaks at H-2/3-OH and H-2/H-12, confirming the same *syn* orientations for these protons as for the other isochaetominines (Figure 3). However, additional cross-peaks were found at H-2/H-14 and 3-OH/H-14, adding the H-14 methine proton in a *syn* orientation to the other key protons. Furthermore, the 4-oxo-quinazoline moiety was spatially close to the main framework by the cross-peaks at H-13/H-25 and H-14/H-25. Because the L configuration was found for the Val-derived moiety (C-10, C-11, C-12, C-27, C-28) by advanced Marfey's analysis, the overall ROESY data confidently assigned the 2*R*, 3*R*, 11*S*, and 14*S* configuration for **5**, reversed at C-14 from **2**−**4**. This interpretation coincides well with the ^{13}C and ^1H NMR data, in which the conspicuous differences were concentrated at the signals of the carbons and the protons near C-14 (Tables 2 and 3). This conclusion was further supported by the opposite sign of the specific rotation of **5** compared with the other isochaetominines. Thus, the structure of **5** was determined as the 14-*epi*-derivative of isochaetominine C (**4**).

Certain fumiquinazoline metabolites have been reported to exhibit weak activities against cancer cell lines,[12,13] proliferation of cancer cell lines,[16] and/or fungal strains,[14] whereas chaetominine exhibited cytotoxicity against K562 leukemia and SW1116 colon cancer cell lines comparable to 5-fluorouracil.[17] In our measurement of cytotoxicity, however, **1**−**7** were inactive (**1**−**5**: IC_{50} > 13−50 μM, **6** and **7**: IC_{50} > 100 μM) against the K562 and A549 cell lines (Supporting Information).[19] In the antimicrobial activity tests against selected strains of Gram-positive and Gram-negative bacteria and pathogenic fungi, only compound **6** displayed weak inhibition (MIC 50 μM) against *Bacillus subtilis*.[20] These alkaloids also exhibited a weak inhibition (IC_{50} values were 34, 78, 20, 38, 57, 17, and 20 μM for **1**−**7**, respectively) against Na^+/K^+-ATPase.[21] None of these compounds were active against the enzymes sortase A or isocitrate lyase.

In conclusion, five new alkaloidal metabolites, fumiquinazoline S (**1**), isochaetominines A−C (**2**−**4**), and 14-*epi*-isochaetominine C (**5**), along with the known fumiquinazolines F (**6**) and L (**7**), were isolated from a marine-derived *Aspergillus* sp. fungus. Compound **1** is a new member of the fumiquinazoline alkaloids, whereas **2**−**5** had a structural similarity only with chaetominine, which has been recently isolated from a terrestrial endophytic fungus. Furthermore, the differences in the relative and absolute configurations among these compounds provide additional structural novelty to the isochaetomines. These compounds exhibited weak inhibition against Na^+/K^+-ATPase.

EXPERIMENTAL SECTION

General Experimental Procedures. The optical rotations were measured on a JASCO P-1020 polarimeter using a 1 cm cell. The UV spectra were acquired with a Hitachi U-3010 spectrophotometer. The CD spectra were obtained on a Chirascan-Plus CD spectrometer. The IR spectra were recorded on a JASCO 4200 FT-IR spectrometer, using a ZnSe cell. The NMR spectra were recorded on Bruker Avance 400, 500, and 600 spectrometers. The proton and carbon NMR spectra were measured in a DMSO-d_6 solution at 400 and 100 MHz (**1**), 600 and 150 MHz (**2**), and 500 and 125 MHz (**3**−**5**), respectively. HRFABMS data were acquired using a Jeol JMS 700 mass spectrometer with *meta*-nitrobenzyl alcohol (NBA) as the matrix at the Korea Basic Science Institute (Daegu, Korea). The HPLC analyses were performed on a Spectrasystem p2000 equipped with a refractive index detector (Spectrasystem RI-150). All of the solvents used were spectroscopic grade or distilled from glass prior to use.

Isolation and Identification of the Fungal Strain. The fungal strain *Aspergillus* sp. (strain number F452) was isolated from submerged decaying wood off the shore of Jeju Island, Korea, in November 2011. The strain was identified using standard molecular biological protocols by DNA amplification and sequencing of the ITS region. The genomic DNA extraction was performed using Intron's i-Genomic BYF DNA extraction mini kit according to the manufacturer's protocol. The nucleotide sequence of F452 has been deposited in the GenBank database under the accession number KF384188. The 18S rDNA sequence of this strain showed a 99% identity with *Aspergillus versicolor* Ppf48 (GenBank accession number GU586852).

Fermentation and Isolation. The isolated strain was cultivated on a YPG agar plate (5 g of yeast extract, 5 g of peptone, 10 g of glucose, 16.0 g of agar, 24.8 g of artificial sea salt; Instant Ocean in 1 L of distilled H_2O) for 7 days. The agar plugs (1 cm × 1 cm, 5 pieces each) were inoculated into 100 mL of the YPG media in a 250 mL Erlenmeyer flask for 7 days, then separately transferred to 2.8 L glass Fernbach flasks with rice media (1 g of peptone, 1 g of yeast extract, 200 g of rice, 5 g of artificial sea salt; Instant Ocean in 200 mL of distilled H_2O in each flask, boiled in an autoclave for 20 min at 120 °C; 8 flasks total). The fermentation in the rice media was conducted under static conditions for 6 weeks and then was extracted by EtOAc (1 L × 3). The solvent was evaporated to obtain a brown organic extract (5.8 g). The extract was separated by C_{18} reversed-phase vacuum flash chromatography using a sequential mixture of MeOH and H_2O as the eluents (seven fractions in the gradient, H_2O−MeOH, from 60:40 to 0:100), acetone, and, finally, EtOAc. On the basis of the results of the ^1H NMR analysis, the fractions that eluted with H_2O−MeOH (40:60, 1010 mg; 30:70, 1019 mg) were chosen for the separation. The fraction that eluted with H_2O−MeOH (40:60) was separated by semipreparative reversed-phase HPLC (H_2O−MeOH, 50:50, 2.0 mL/min) to afford compounds **1** and **5**. The fraction that eluted with H_2O−MeOH (30:70) was separated by semipreparative reversed-phase HPLC (H_2O−MeOH, 40:60, 2.0 mL/min) to yield, in order of elution, compounds **2**, **3**, **4**, **6**, and **7**. The purified metabolites were isolated in the following amounts: 6.4, 10.0, 9.4, 4.0, 8.9, 3.2, and 5.5 mg for **1**−**7**, respectively.

Fumiquinazoline S (1): pale yellow, amorphous solid; $[\alpha]_D^{25}$ −105 (*c* 0.5, MeOH), −151 (*c* 0.25, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 205 (4.37), 226 (4.14), 257 (3.80), 266 (3.62), 279 (3.36), 304 (3.05), 317 (2.93) nm; CD (MeOH) λ ($\Delta\varepsilon$) 210 (+13.64), 233 (−12.88), 287 (−1.33) nm; IR (ZnSe) ν_{max} 3395, 1730, 1681, 1605 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; HRFABMS *m*/*z* 474.2143 $[M + H]^+$ (calcd for $C_{26}H_{28}N_5O_4$, 474.2141).

Isochaetominine A (2): pale yellow, amorphous solid; $[\alpha]_D^{25}$ −63 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.60), 225 (4.44), 275 (4.07), 305 (3.35) nm; CD (MeOH) λ ($\Delta\varepsilon$) 204 (−15.10), 219

DOI: 10.1021/np500683u
J. Nat. Prod. 2015, 78, 349−354

(+5.78), 247 (−3.24), 285 (+0.52), 304 (−1.57) nm; IR (ZnSe) ν_{max} 3364 (br), 1725, 1673, 1607 cm^{-1}; ^1H and ^{13}C NMR data, Tables 2 and 3, respectively; HRFABMS *m/z* 403.1404 $[M + H]^+$ (calcd for $C_{22}H_{19}N_4O_4$, 403.1406).

Isochaetominine B (3): pale yellow, amorphous solid; $[\alpha]_D^{25}$ −73 (*c* 0.6, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.60), 226 (4.45), 275 (4.09), 305 (3.34) nm; CD (MeOH) λ ($\Delta\varepsilon$) 203 (−18.11), 218 (+8.17), 243 (−13.14), 286 (+0.75), 303 (−1.30) nm; IR (ZnSe) ν_{max} 3358 (br), 1726, 1678, 1608 cm^{-1}; ^1H and ^{13}C NMR data, Tables 2 and 3, respectively; HRFABMS *m/z* 417.1566 $[M + H]^+$ (calcd for $C_{23}H_{21}N_4O_4$, 417.1563).

Isochaetominine C (4): pale yellow, amorphous solid; $[\alpha]_D^{25}$ −90 (*c* 0.6, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.58), 224 (4.42), 274 (4.03), 303 (3.33) nm; CD (MeOH) λ ($\Delta\varepsilon$) 201 (−19.25), 217 (+9.06), 244 (−6.53), 274 (+0.29), 303 (−2.28) nm; IR (ZnSe) ν_{max} 3362 (br), 1723, 1676, 1610 cm^{-1}; ^1H and ^{13}C NMR data, Tables 2 and 3, respectively; HRFABMS *m/z* 431.1721 $[M + H]^+$ (calcd for $C_{24}H_{23}N_4O_4$, 431.1719).

14-*epi*-Isochaetominine C (5): pale yellow, amorphous solid; $[\alpha]_D^{25}$ +33 (*c* 0.7, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.58), 224 (4.42), 274 (4.03), 302 (3.30) nm; CD (MeOH) λ ($\Delta\varepsilon$) 204 (−15.10), 219 (+5.78), 247 (−3.24), 285 (+0.52), 305 (+0.85) nm; IR (ZnSe) ν_{max} 3370 (br), 1726, 1677, 1609 cm^{-1}; ^1H and ^{13}C NMR data, Tables 2 and 3, respectively; HRFABMS *m/z* 431.1720 $[M + H]^+$ (calcd for $C_{24}H_{23}N_4O_4$, 431.1719).

Advanced Marfey's Analysis of Compounds 1−5.[18] Compound **1** (0.4 mg) was dissolved in 0.5 mL of 6 N HCl and heated to 110 °C for 1 h. The solution was evaporated with distilled H_2O three times to remove the trace HCl under vacuum. The divided hydrolysate (0.2 mg each) was treated with 100 μL of 1 N $NaHCO_3$ followed by 50 μL of 1% L- or D-FDAA in acetone. The mixture was stirred at 80 °C for 15 min. After quenching by the addition of 50 μL of 2 N HCl, the mixture was analyzed by ESI-LC/MS to assign the chirality of the amino acids. The retention times of the L- and D-FDAA-derivatized hydrolysates were 19.0 and 22.9 min, respectively. Compounds **2**−**5** were prepared and analyzed using the same procedure. The results demonstrated that all of the amino acids in compounds **1**−**5** were in the L-form (Supporting Information).

Biological Assays. The cytotoxicity assays were performed in accordance with protocols reported in the literature.[19] Antimicrobial[20] and Na^+/K^+-ATPase[21] assays were performed according to previously reported methods.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The ^1H NMR, ^{13}C NMR, and 2D NMR spectra of compounds **1**−**5**, advanced Marfey's analyses for compounds **1**−**5**, and results of the bioactivity test are available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*(K.-B. Oh) Tel: 82 2 880 4646. Fax: 82 2 873 3112. E-mail: ohkibong@snu.ac.kr.

*(J. Shin) Tel: 82 2 880 2484. Fax: 82 2 762 8322. E-mail: shinj@snu.ac.kr.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to the National Instrumentation Center for Environmental Management (Seoul, Korea) for providing the mass data and the National Center for Inter-University Research Facilities, Seoul National University, for providing the NMR data. This work was supported by the BK21 Plus Program in 2014 and the National Research Foundation of Korea (NRF) grants funded by the Korean government (Ministry of Science, ICT and Future Planning) (2010-0020429).

■ DEDICATION

Dedicated to Dr. William Fenical of Scripps Institution of Oceanography, University of California−San Diego, for his pioneering work on bioactive natural products.

■ REFERENCES

(1) Fenical, W. *Chem. Rev.* **1993**, *93*, 1673−1683.
(2) Bugni, T. S.; Ireland, C. M. *Nat. Prod. Rep.* **2004**, *21*, 143−163.
(3) Fenical, W.; Jensen, P. R. *Nat. Chem. Biol.* **2006**, *2*, 666−673.
(4) Cragg, G. M.; Grothaus, P. G.; Newman, D. J. *Chem. Rev.* **2009**, *109*, 3012−3043.
(5) Bhatnagar, I.; Kim, S. K. *Mar. Drugs* **2010**, *8*, 2673−2701.
(6) Hu, G.-P.; Yuan, J.; Sun, L.; She, Z.-G.; Wu, J.-H.; Lan, X.-J.; Zhu, X.; Lin, Y.-C.; Chen, S.-P. *Mar. Drugs* **2011**, *9*, 514−525.
(7) Blunt, J. W.; Copp, B. R.; Keyzers, R. A.; Munro, M. H. G.; Prinsep, M. R. *Nat. Prod. Rep.* **2014**, *31*, 160−258 and earlier reports in the series.
(8) (a) Elin, J.; Oh, H.; Jang, K. H.; Lee, S. K.; Oh, D.-C.; Oh, K.-B.; Shin, J. *J. Nat. Prod.* **2011**, *74*, 2592−2594. (b) Jeon, J.-e.; Elin, J.; Oh, H.; Park, W.; Oh, D.-C.; Oh, K.-B.; Shin, J. *Tetrahedron Lett.* **2013**, *54*, 3111−3115. (c) Elin, J.; Lee, J.-H.; Liao, L.; Park, W.; Park, S.; Oh, D.-C.; Oh, K.-B.; Shin, J. *Org. Lett.* **2013**, *15*, 1286−1289. (d) Liao, L.; Lee, J.-H.; You, M.; Choi, T. J.; Park, W.; Lee, S. K.; Oh, D.-C.; Oh, K.-B.; Shin, J. *J. Nat. Prod.* **2014**, *77*, 406−410.
(9) Afiyatullov, S. S.; Zhuravleva, O. I.; Antonov, A. S.; Kalinovsky, A. I.; Pivkin, M. V.; Menchinskaya, E. S.; Aminin, D. L. *Nat. Prod. Commun.* **2012**, *7*, 497−500.
(10) Zhou, Y.; Debbab, A.; Mándi, A.; Wray, V.; Schulz, B.; Müller, W. E. G.; Kassack, M.; Lin, W. H.; Kurtán, T.; Proksch, P.; Aly, A. H. *Eur. J. Org. Chem.* **2013**, 894−906.
(11) Shao, C.-L.; Xu, R.-F.; Wei, M.-Y.; She, Z.-G.; Wang, C.-Y. *J. Nat. Prod.* **2013**, *76*, 779−782.
(12) Numata, A.; Takahashi, C.; Matsushita, T.; Miyamoto, T.; Kawai, K.; Usami, Y.; Matsumura, E.; Inoue, M.; Ohishi, H.; Shingu, T. *Tetrahedron Lett.* **1992**, *33*, 1621−1624.
(13) Takahashi, C.; Matsushita, T.; Doi, M.; Minoura, K.; Shingu, T.; Kumeda, Y.; Numata, A. *J. Chem. Soc., Perkin Trans. 1* **1995**, 2345−2353.
(14) Belofsky, G.; Anguera, M.; Jensen, P.; Fenical, W.; Köck, M. *Chem.—Eur. J.* **2000**, *6*, 1355−1360.
(15) Heredia, M.; Cuesta, E.; Avendaño, C. *Tetrahedron* **2002**, *58*, 6163−6170.
(16) Han, X.; Xu, X.; Cui, C.; Gu, Q. *Chin. J. Med. Chem.* **2007**, *17*, 232−237.
(17) Jiao, R.; Xu, S.; Liu, J.; Ge, H.; Ding, H.; Xu, C.; Zhu, H.; Tan, R. *Org. Lett.* **2006**, *8*, 5709−5712.
(18) (a) Fujii, K.; Ikai, Y.; Mayumi, T.; Oka, H.; Suzuki, M.; Harada, K.-i. *Anal. Chem.* **1997**, *69*, 3346−3352. (b) Fujii, K.; Ikai, Y.; Oka, H.; Suzuki, M.; Harada, K.-i. *Anal. Chem.* **1997**, *69*, 5146−5151. (c) Woo, J.-K.; Jeon, J.-e.; Kim, C.-K.; Sim, C. J.; Oh, D.-C.; Oh, K.-B.; Shin, J. *J. Nat. Prod.* **2013**, *76*, 1380−1383.
(19) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55−63.
(20) Oh, K.-B.; Lee, J. H.; Chung, S.-C.; Shin, J.; Shin, H. J.; Kim, H.-K.; Lee, H.-S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 104−108.
(21) (a) Johansson, M.; Karlsson, L.; Wennergren, M.; Jansson, T.; Powell, T. L. *J. Clin. Endocrinol. Metab.* **2003**, *88*, 2831−2837. (b) Chung, S.-C.; Jang, K. H.; Park, J.; Ahn, C.-H.; Shin, J.; Oh, K.-B. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1958−1961.

DOI: 10.1021/np500683u
J. Nat. Prod. 2015, 78, 349−354