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Fumigatosides A–D, Four New Glucosidated Pyrazinoquinazoline Indole Alkaloids from a Jellyfish-Derived Fungus *Aspergillus fumigatus*

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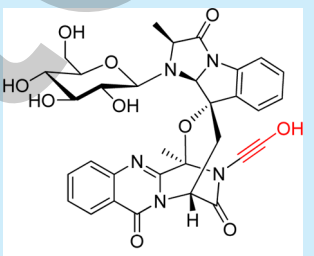
Supporting Information

ABSTRACT: Four new pyrazinoquinazoline indole glucosides (**1**–**4**) were isolated from the fungus *Aspergillus fumigatus* derived from the jellyfish *Nemopilema nomurai*. Compounds **1**–**4** represent the first examples of glycosidated fumiquinzoline-type alkaloids from nature. Compound **1** is a spirofumiquinazoline featured, with a heptacycle formed via a hemiaminal bridge, and possesses a novel *N*-ethynyl alcohol moiety hitherto undiscovered from natural sources. The structures of the compounds were elucidated by spectroscopic methods and ECD/TDDFT calculations. A possible biogenetic pathway was proposed.



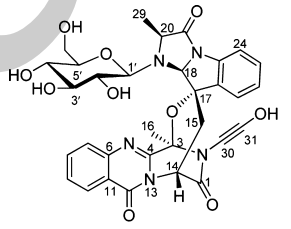
Fumigatoside A (**1**)

The sea is richly biodiverse, and numerous pharmaceutical leads have been obtained from it; the field of marine natural products has progressed over the past decades.[1] Marine microorganisms are considered an important source of bioactive secondary metabolites. These metabolites can consistently be obtained from fungi. Thus, interest in endozoic microorganisms continually increases.[2,3]

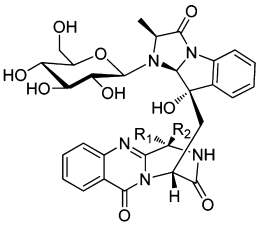
Twelve fungal strains were isolated from the jellyfish *Nemopilema nomurai* collected off the southern coast of Korea in June 2007. These strains were fermented on a small scale to obtain ethyl acetate extracts. These extracts were screened for antibacterial and antitumor activities. After screening, the bioactive strains were analyzed further for causative secondary metabolites. We previously isolated four new polyketides from the jellyfish-derived fungus *Paecilomyces variotii*, namely, paecilocins A–D. These polyketides have unique chain lengths and/or carbon skeletons.[4]

As part of our progressive program to explore the biomedical potential of endozoic fungi obtained from jellyfish, the secondary metabolites of the strain J08NF-8 were examined. The large-scale fermentation and isolation of these fungi resulted in the discovery of four new quinazoline glucosides, namely, fumigatosides A–D. These glucosides are the first examples of natural glycosidated alkaloids of the fumiquinzoline type (**1**–**4**, Figure 1).

The strain J08NF-8 was identified by analyzing the gene sequence and morphology of the internal gene spacer. The strain is closely related to the type of strain of *Aspergillus fumigates*, a common *Aspergillus* species that induces disease in individuals with immunodeficiencies.



Fumigatoside A (**1**)



Fumigatoside B (**2**) R_1 = Me, R_2 = OH
Fumigatoside C (**3**) R_1 = H, R_2 = Me
Fumigatoside D (**4**) R_1 = Me, R_2 = H

Figure 1. Structures of fumigatosides A–D.

Compound **1** was isolated as a yellow, amorphous powder. Its molecular formula was expressed as $C_{32}H_{31}N_5O_{10}$ based on high-resolution FAB-MS and NMR data. The exact mass of the $[M + H]^+$ ion at m/z 646.2096 matched well with the formula $C_{32}H_{32}N_5O_{10}$ (Δ −5.3 mmu). The IR spectrum displayed absorption bands at 3317, 1666, and 1605 cm^{-1}, which indicated the signals for an alcohol, two amides, and an aromatic ring. The ^1H and ^{13}C NMR data are listed in the Supporting Information (SI).

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The ^{1}H and ^{13}C NMR data for compound **1** revealed spectroscopic and structural features that are comparable with those of the fumiquinazoline class of fungal metabolites belonging to the spiroquinazoline family of alkaloids. These metabolites were previously obtained from a marine-derived strain of *A. fumigatus* isolated from the gastrointestinal tract of a marine fish and an *Acremonium* sp. fungus isolated from the surface of the Caribbean tunicate *Ecteinascidia turbinata*.[5,6] The NMR data for compound **1** suggested that its structure was analogous to that of the known fumiquinazoline C, except that compound **1** possessed one ethynol (δ_C 78.6 and 77.5) at N-2 instead of the amine proton, as determined by the HMBC correlation between the OH proton (δ_H 9.77 in DMSO-d_6) and the acetylene carbon C-30 (δ_C 78.6) (see SI).[5] This similarity was confirmed by high-resolution FAB-MS. The moiety of *N*-ethynyl alcohol has hitherto been undiscovered in nature. The C-16 protons of compound **1** resonated as a singlet signal, and the carbon signals for C-3 and C-15 revealed a difference in chemical shift relative to that of compound **2**. Thus, the planar structure of compound **1** linked C-3 and C-17 through ether. A correlation between H-29 (δ_H 1.37) and H_a-15 (δ_H 2.26) was observed through the NOESY spectrum, indicating that the C18−N19, N22−C21, and C17−C15 bonds had a cisoid orientation. The methyl group at C-20 was on the same side as the C17−C15 bond. The ether linkage between C-3 and C-17 confirmed that the relative configuration of the spiroquinazoline moiety was deduced as 3*R**, 14*R**, 17*S**, 18*S**, and 20*S** (Figure 2).

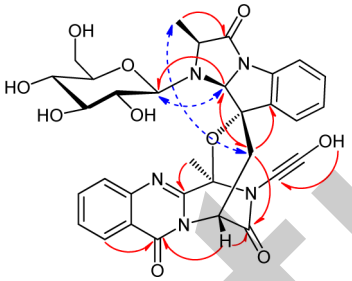

Figure 2. Key HMBC (half arrows) and NOESY (dashed arrows) correlations of compound **1**.

The absolute configuration of the aglycone moiety was determined by ECD/TDDFT calculations using the truncated structure (the sugar moiety was replaced by a methyl), which afforded the predicted gas phase and solution ECD spectra consistent with the experimental one (Figure 3). Thus, the absolute configuration was established as 3*R*, 14*R*, 17*S*, 18*S*, and 20*S*. Six additional resonances in the NMR spectrum of ^{13}C were detected, which revealed a hexose moiety in the structure. The hexose moiety was verified as a glucose by HSQC, HMBC, and COSY correlations. Further comparison with previous NMR data confirmed that this moiety is a β-D-glucoside.[7,8] The NOESY spectra showed that H-1′ was correlated with H-3′ (δ_H 3.38) and H-5′ (δ_H 3.43), thereby proving the configuration of β-D-glucoside. The HMBC correlation of the anomeric proton H-1′ (δ_H 4.33) with C-18 (δ_C 88.3) and C-20 (δ_C 57.2) established the glucosidic linkage at position N-19. This linkage was supported by the NOESY correlations between H-18 (δ_H 5.60) and H-1′ (δ_H 4.33), which were also correlated with H-3′ (δ_H 3.38) and H-5′ (δ_H 3.43). Compound **1** was defined as a fumiquinazoline-19-β-D-glucoside based on these data.

Fumigatoside B (**2**), which was also isolated as a yellow powder, was assigned the molecular formula $C_{30}H_{33}N_5O_{10}$ by the HRFABMS ion at *m/z* 646.2120 $[M + Na]^+$ and *m/z* 624.2305 $[M + H]^+$. The IR spectrum depicted absorption bands at 3371, 1647, and 1608 cm^{-1}. These bands were very similar to those of fumigatoside A (**1**).

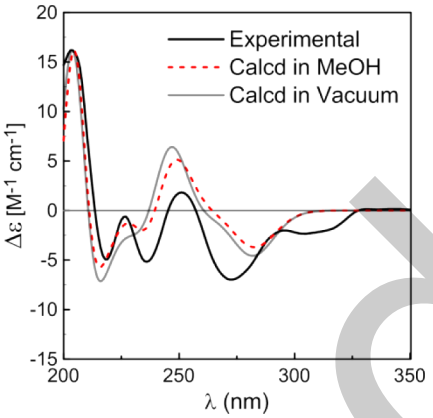

Figure 3. Comparison of the calculated and the experimental ECD spectra of compound **1**.

The ^{1}H and ^{13}C NMR data of compound **2** were directly compared with those of compound **1**. Results suggest that they share the same skeleton with the quinazoline and hexose moieties. The skeletons differ in terms of the amide proton at N-2 in compound **1** and the ether linkage detected between C-3 (δ_C 80.1) and C-17 (δ_C 80.4) instead of between two hydroxy groups (3-OH and 17-OH). The HMBC and NOESY correlations showed that the structure of the spiroquinazoline moiety in compound **2** was similar to that of the demethyl fumiquinazoline E, which was previously formed by treating fumiquinazoline C with 2% HCl in MeOH.[5] Consequently, the quinazoline moiety in compound **2** was proposed to be derived through enzyme hydrolysis of fumiquinazoline C inside the fungus *A. fumigatus*. The CD spectrum of compound **2** displayed a transition pattern similar to that of compound **1** (see SI). Meanwhile, the absolute configuration of compound **2** was determined as 3*R*, 14*R*, 17*S*, 18*S*, and 20*S* by ECD/TDDFT calculations which were carried out with the lowest-energy conformer of the truncated structure and provided simulated ECD spectra closely similar to the measured one (see SI). According to 2D NMR data, the hexose moiety in compound **2** was determined to be a β-D-glucoside. In addition, NOESY confirmed that the glucosidic linkage position of compound **2** was the same as that of compound **1**. Therefore, the structure of compound **2** was identified and given the trivial name fumigatoside B.

Fumigatoside C (**3**) was isolated as a white, amorphous powder. Its spectroscopic data were similar to those of compound **2**. This result suggests that the two compounds have the same molecular skeleton. Its molecular formula was expressed as $C_{30}H_{33}N_5O_9$ based on HRFABMS and NMR data. The exact mass of the $[M + Na]^+$ ion at *m/z* 630.2151 matched well with the formula $C_{30}H_{33}N_5O_9Na$ (Δ −2.5 mmu), thereby indicating the loss of one oxygen equivalent as compared with the formula of compound **2**. The ^{1}H and ^{13}C NMR spectra of the spiroquinazoline moiety in compound **3** were similar to those of compound **2**, except that the C-3 hydroxyl group in compound **2** was replaced in compound **3** by a proton signal resonating at δ_H 5.02 (δ_C 48.9) ppm (3-H). The HMBC correlation of the anomeric proton H-1′ (δ_H 4.30) to C-18 (δ_C 88.9) and C-20 (δ_C 57.9) also established the glucosidic linkage at position N-19. The hexose moiety was verified as a β-D-glucoside by HSQC,

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HMBC, and COSY correlations. With respect to corresponding CD transitions, the pattern exhibited by compound **3** was similar to those of compounds **1** and **2** (see SI). The absolute configuration of **3** was further determined as 3*R*, 14*R*, 17*S*, 18*S*, 20*S* based on ECD/TDDFT calculations (see SI).

The molecular formula of fumigatoside D (**4**) was similar to that of compound **3**. The general features of the spectra of compound **4** closely resembled those of compound **3** except for the signals of C-1, C-3, C-15, and C-16 in the ^{13}C NMR spectrum. This result implies that compound **4** is a stereoisomer of compound **3** at C-3, C-14, or both. In the NOE experiments, compound **3** exhibited NOEs between H-3 and H_b-15, whereas compound **4** displayed NOEs between 16-H and 15-H_b. The transition pattern of the CD spectrum of compound **4** was similar to those of the other compounds. However, its CE was positive at 230 nm, unlike that of compound **3**. The CD Cotton effects induced by compound **4** were also quite similar to those of 3-hydroxyglyantrypine at 230 ($\Delta\varepsilon$ +4.64) and 217 nm ($\Delta\varepsilon$ −13.8) (see SI). This result implies that the *R*-configuration at C-14 of compound **4** is identical to that of compound **3**. Thus, compounds **4** and **3** are stereoisomers at C-3.[9] The ECD/TDDFT calculations also confirmed that the absolute configuration of the spiroquinazoline moiety in compound **4** was 3*S*, 14*R*, 17*S*, 18*S*, 20*S* (see SI). The results of 1D and 2D NMR proved that the hexose moiety was a β-D-glucoside and that the glucosidic linkage position in compound **4** was the same as that in the other compounds.

To determine the inhibitors of surfactant protein binding to the human NK-1 receptor, spiroquinazoline, which is the first member of the spiroquinazoline family of alkaloids, was isolated from the fungus *Aspergillus flavipes*, whose carbon skeleton was new at the time.[10] Although more than 20 natural products closely resembling spiroquinazoline have been developed, analogs were isolated from a variety of fungi, particularly *A. fumigatus*. The fumiquinazoline glucosides fumigatosides A−D were isolated from a natural source for the first time.

Fumigatosides A−D are supposed precursors of fumiquinazolines prior to enzymolysis. However, the hexose moiety may have been biotransformed from rice solid media; thus, a possible biogenetic pathway is proposed (see SI). The spiroquinazoline family of alkaloids contains a pyrazino[2,1-*b*]quinazoline-3,6-dione moiety and an indole ring. In all members, anthranilic acid and tryptophan substructures were detected. Therefore, fumigatosides A−D may have also been derived from anthranilic acid, tryptophan, and alanine.[11,12] Upon the introduction of L-alanine, fungi produce fumigatosides A−C but not fumigatoside D. Fumigatoside A (**1**) alone has a spiral structure, which may be helpful in resolving some contradictory biosyntheses of this class of compounds.[13] To address the indistinct biosynthesis of this class of compounds, however, further study is necessary because the *N*-ethynyl alcohol moiety has not been discovered in nature thus far.

The various biological activities of fumiquinazolines have attracted the attention of researchers interested in analgesics or insecticides. Fumiquinazolines are moderately cytotoxic and reportedly exhibit antitumor activity against several cancer cell lines.[14] Thus, the antibacterial activities of compounds **1**−**4** were evaluated against a panel of strains, including *Staphyloccocus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 13883, *Acinetobacter baumannii* ATCC 19606, *Aeromonas hydrophila* ATCC 7966, and *Enterococcus faecalis* ATCC 29212. The cytotoxicities of the compounds were also assessed against 10 cell lines of solid human tumors (K562, A549, Huh-7, H1975, MCF-7, U937, BGC823, HL60, Hela, and MOLT-4). However, neither antibacterial activity nor cytotoxicity was observed.

■ ASSOCIATED CONTENT

Supporting Information

Plausible biogenetic pathway, Experimental Section, full spectroscopic and computational data for compounds **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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