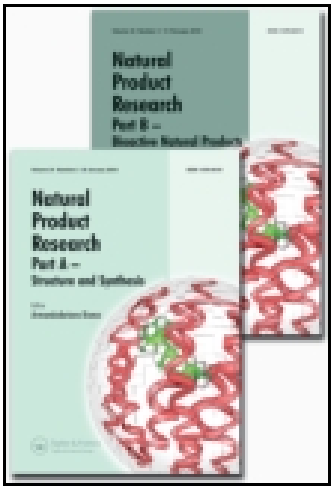

Natural Product Research: Formerly Natural Product Letters
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gnpl20

Nucleoside derivatives from the marine-derived fungus Aspergillusversicolor

Min Chen[a], Xiu-Mei Fu[a], Chui-Jian Kong[a] & Chang-Yun Wang[ab]
[a] Key Laboratory of Marine Drugs, The Ministry of Education of China, School of Medicine and Pharmacy, Ocean University of China, 5 Yushan Road, Qingdao 266003, People's Republic of China
[b] Institute of Evolution & Marine Biodiversity, Ocean University of China, Qingdao 266003, People's Republic of China
Published online: 26 Mar 2014.

To cite this article: Min Chen, Xiu-Mei Fu, Chui-Jian Kong & Chang-Yun Wang (2014) Nucleoside derivatives from the marine-derived fungus Aspergillusversicolor, Natural Product Research: Formerly Natural Product Letters, 28:12, 895-900, DOI: 10.1080/14786419.2014.891114

To link to this article: http://dx.doi.org/10.1080/14786419.2014.891114

Natural Product Research, 2014
Vol. 28, No. 12, 895–900, http://dx.doi.org/10.1080/14786419.2014.891114

Nucleoside derivatives from the marine-derived fungus *Aspergillus versicolor*

Min Chen[a], Xiu-Mei Fu[a], Chui-Jian Kong[a] and Chang-Yun Wang[ab]*

[a]*Key Laboratory of Marine Drugs, The Ministry of Education of China, School of Medicine and Pharmacy, Ocean University of China, 5 Yushan Road, Qingdao 266003, People's Republic of China;* [b]*Institute of Evolution & Marine Biodiversity, Ocean University of China, Qingdao 266003, People's Republic of China*

(*Received 27 November 2013; final version received 1 February 2014*)

Four nucleoside derivatives (**1**–**4**) were isolated from the fungus *Aspergillus versicolor* derived from the gorgonian *Dichotella gemmacea* collected in the South China Sea. Their structures were elucidated by comprehensive spectroscopic method of NMR and MS analysis. All isolated metabolites were evaluated for their cytotoxicity, antibacterial activity and lethality towards brine shrimp *Artemia salina*. Compounds **1/2** exhibited selective antibacterial activity against *Staphylococcus epidermidis* with an MIC value of 12.5 μM. It should be noted that **1** and **2**, whose structures were listed in SciFinder Scholar, had no associated reference. This is the first report about their isolation, structure elucidation and biological activities.

Keywords: marine-derived fungus; *Aspergillus versicolor*; nucleoside derivative; transesterification; antibacterial activity

1. Introduction

Marine-derived fungi, especially those belonging to the genus *Aspergillus*, are rich source of structurally novel and biologically potent natural products (Fremlin et al. 2009; Yamada et al. 2010; Blunt et al. 2013). During our search for bioactive secondary metabolites from marine-derived fungi in the South China Sea, we reported the isolation of several new compounds with antibacterial, cytotoxic and antifouling activities (Shao et al. 2011; Sun et al. 2012; Yang et al. 2012; Shao et al. 2013). Recently, the secondary metabolites were investigated for an *Aspergillus versicolor* strain isolated from the gorgonian *Dichotella gemmacea* collected from the Xisha Islands coral reef. Four nucleoside derivatives (**1**–**4**) were isolated from the fermentation extract (Figure 1). Although the nucleoside derivatives are ubiquitous, the reports on naturally occurring aroyl uridine derivatives are relatively uncommon. Especially, compounds **1** and **2**, whose structures were listed in SciFinder Scholar, had no associated reference. In this article, we report for the first time the isolation, structural elucidation and biological activities of two aroyl uridine derivatives (**1** and **2**), together with two known analogues (**3** and **4**). The mechanism of the interconversion observed between **1** and **2** are also discussed.

2. Results and discussion

The fungal strain of *A. versicolor* was cultivated statically in a rice medium for five weeks at room temperature (RT). The culture was harvested by extraction with EtOAc to obtain a crude

*Corresponding author. Email: changyun@ouc.edu.cn

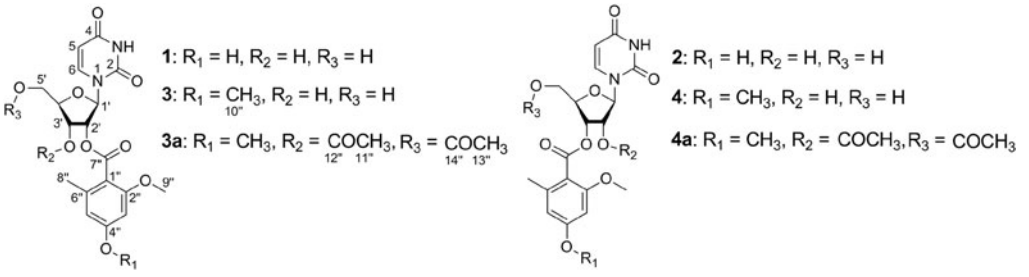

Figure 1. Structures of compounds **1**–**4**, **3a** and **4a**.

extract, which was chromatographed on silica gel (Qing Dao Hai Yang Chemical Group Co., Qingdao, China) column, Sephadex LH-20 column (Amersham Biosciences Inc., Piscataway, NJ, USA) and semi-preparative HPLC (Waters Corp., Milford, MA, USA) to obtain **1**–**4**. Interestingly, a transesterification reaction was observed in which both **1** and **2** quickly rearranged to form each other, giving a mixture of **1/2** at a ratio of 7:10 approximately in a solution of MeOH/H_2O at RT. Although **1** and **2** could be separated by HPLC using MeOH/H_2O as the mobile phase, both **1** and **2** changed into a mixture of **1/2** due to the rapid transesterification reaction (see later). The respective spectroscopic data of **1** and **2** were resolved from the spectra of **1/2**. The similar transesterification reaction was also observed for kipukasins E and D (**3** and **4**), resulting in a mixture of **3/4**. It should be noted that the constitutions of **1** and **2** were listed in SciFinder Scholar with the CAS Registry Numbers of 1136789-16-6 and 1175827-47-0, respectively; however, no reference and experimental data were found. Thus, we report the structural elucidation and give the trivial names kipukasins H and I for **1** and **2**, respectively.

Kipukasin H (**1**) was isolated as a colourless oil and has the molecular formula $C_{18}H_{20}N_2O_9$ (10 degrees of unsaturation) as determined by HR-ESI-MS. Careful comparisons of the ^{1}H and ^{13}C NMR spectra of **1** with those of kipukasin E (**3**), a nucleoside derivative from the basidioma of *Gandoderma australe* deriving *A. versicolor* fungus (Jiao et al. 2007), suggested that **1** was very similar to **3**. The most obvious difference in the ^{1}H NMR spectrum was a methoxy signal at δ_H 3.64 in **1** instead of the two methoxy signals in **3**, indicating that **1** was the demethylated derivative of **3**. The structure of **1** was independently assigned by analysing the HMQC, ^{1}H–^{1}H COSY and HMBC data (Figure S20). The selective NOE experiment was employed to assign the only one methoxy group for **1**. The irradiation of the methoxy signal of **1** resulted in obvious enhancement for the aromatic H-3″ but no obvious enhancement for the H-5″, unambiguously supporting that the methoxy group was anchored at C-2″, rather than C-4″ in **1**.

Kipukasin I (**2**) was also isolated as a colourless oil, possessing the same molecular formula $C_{18}H_{20}N_2O_9$ as **1**. The ^{1}H and ^{13}C NMR spectra of **2** were very similar to those of **1**, with only a subtle difference in the chemical shifts and splitting patterns for partial of the ribose oxymethine proton signals in the ^{1}H NMR spectrum. The signal for H-2′ (δ_H 4.36; dd, $J = 6.6$, 5.4 Hz) was shifted upfield, while the H-3′ resonance (δ_H 5.25; dd, $J = 5.4$, 2.4 Hz) was shifted downfield in **2** compared with those of **1**, leading to the assignment of the aroyl group at C-3′ in **2**. The HMBC correlation from H-3′ to C-7″ supported this connection for **2**. Therefore, **2** is the regioisomer of **1**.

The absolute configurations of **1** and **2** were resolved based on biogenetic considerations. Because the absolute configurations of the uridine moiety contained in the known compounds kipukasins E (**3**) and D (**4**) have been previously assigned as uracil-1-β-D-ribofuranoside (Jiao et al. 2007), the uridine unite in the co-isolated compounds **1** and **2** could also be established as uracil-1-β-D-ribofuranoside, identical to their methylated derivatives **3** and **4**.

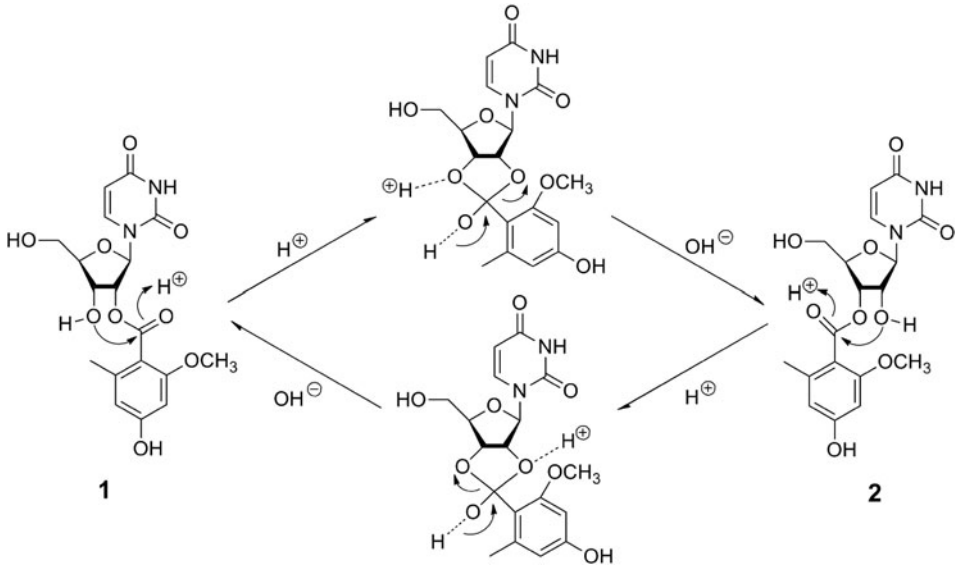

Figure 2. Mechanism of the interconversion for **1** and **2** in MeOH–H_2O.

2′/3′-Transesterification has been known to occur in some ribose derivatives (Hasegawa et al. 2001; Sauve et al. 2001; Jackson & Denu 2002). The interconversion between **1** and **2** was observed during the extraction and purification processes. The 2′-*O*-acyl ribofuranoside or 3′-*O*-acyl ribofuranoside was labile and reactive in MeOH–H_2O solution and underwent the intramolecular acyl migration to produce isomers. The aroyl in **1** was transferred from the 2′-OH position to the neighbouring 3′-OH on the ribose moiety *via* an *ortho*-acid ester intermediate (Spahn-Langguth & Benet 1992), yielding the regioisomer **2** (Figure 2). This aroyl migration was reversible, which finally achieved a dynamic equilibrium (n_1:n_2 = 7:10, approximately). The same mechanism was responsible for the interconversion between **3** and **4**. In addition, the acetylation of **3** and **4** at the hydroxyl groups on the ribose unit gave the diacetylated derivatives **3a** and **4a**, respectively, which exhibited no interconversion phenomenon. The acquisition of pure samples of **3a** and **4a** confirmed the interconversion mechanism discussed earlier.

Compounds **1**–**4** were evaluated for antibacterial activity against seven strains of pathogenic bacteria. Compounds **1/2** exhibited selective antibacterial activity against *Staphylococcus epidermidis* with an MIC value of 12.5 μM, while their methylated derivatives **3/4** were found to be inactive against the tested pathogenic bacteria at a concentration of 50 μM. It indicated that the hydroxyl at C-4″ may have a positive contribution to the antibacterial activity. In addition, compounds **1**–**4** were also tested for their lethal activity towards brine shrimp *Artemia salina*. Only compounds **1/2** were active with an LC_{50} value of 8.4 μg/mL. Compounds **1/2** and **3/4** exhibited no cytotoxic activity against human erythroleukaemia K562 and human promyelocytic leukaemia HL-60 cell lines.

3. Experimental

3.1. *General experimental procedures*

UV spectra were obtained on a Beckman DU 640 spectrophotometer (Beckman Coulter, Inc., Brea, CA, USA). IR spectra were recorded on a Nicolet-Nexus-470 spectrometer (Nicolet Corp., Madison, WI, USA) using KBr pellets. NMR spectra were acquired using a JEOL JEM-ECP NMR spectrometer (JEOL Ltd., Tokyo, Japan) (600 MHz for ^1H and 150 MHz for ^{13}C), using

TMS as internal standard. ESI-MS spectra were obtained from a Micromass Q-TOF spectrometer (Waters Corp., Milford, MA, USA). HPLC was performed on a Waters 1525 system coupled with a Waters 2996 photodiode array detector, using a C_{18} column (Kromasil, 5 μm, 250 mm × 10 mm) for semi-preparation HPLC. Silica gel (Qing Dao Hai Yang Chemical Group Co., Qingdao, China 200–300 mesh) and Sephadex LH-20 (Amersham Biosciences Inc.) were used for column chromatography (CC). Precoated silica gel plates (Yan Tai Zi Fu Chemical Group Co., Yantai, China G60, F-254) were used for thin layer chromatography.

3.2. *Fungal material*

The marine-derived fungus *A. versicolor* was isolated from the inner part of a fresh gorgonian *D. gemmacea*, which was collected from the Xisha Islands coral reef of the South China Sea in December 2009. The strain was deposited at the Key Laboratory of Marine Drugs, The Ministry of Education of China, School of Medicine and Pharmacy, Ocean University of China, Qingdao, People's Republic of China. This fungus strain was identified as *A. versicolor* according to its morphological traits and a molecular biological protocol by amplification and sequencing of the DNA sequences of the ITS region of the rRNA gene. The 565 base-pair ITS sequence had 99% sequence identity to that of the *A. versicolor* strain ATCC 9577 (NCBI GenBank accession number AY373880).

3.3. *Fermentation, extraction and isolation*

The fungal strain of *A. versicolor* was cultivated statically in a rice medium (100 mL seawater, 100 g rice) in 1-L Erlenmeyer flasks for five weeks at RT. The fermented rice substrate (20 flasks) was extracted with EtOAc (3 × 300 mL for each flask), and the solvent was combined and concentrated *in vacuo* to afford a residue (17.5 g). The residue was separated into five fractions (Fr.1–Fr.5) by silica gel CC using a step-gradient elution with MeOH–CH_2Cl_2 (0–100%). Fr.3 was isolated by silica gel CC eluting with CH_2Cl_2–MeOH (10:1, v/v), and was further purified by using semi-preparative HPLC eluting with 35% MeOH–H_2O to yield **3** (80 mg) and **4** (96 mg). Fr.4 was subjected to Sephadex LH-20 CC eluting with a mixture of CH_2Cl_2–MeOH (1:1, v/v), and was further purified by semi-preparative HPLC (20% MeOH–H_2O) to obtain **1** (12 mg) and **2** (15 mg). All the isolated compounds turned to be a mixture rapidly.

3.3.1. *Kipukasins H and I (**1/2**)*

Colourless oil. UV (MeOH) λ_{max} (log ε): 205 (2.56), 260 (1.84) nm. IR (KBr) ν_{max} 3774, 3445, 1679, 1649, 1558, 1539, 1513, 1397, 1085 cm^{-1}. ESI-MS *m/z* 409.2 $[M + H]^+$, 431.2 $[M + Na]^+$. HR-ESI-MS *m/z* 409.1237 $[M + H]^+$ (calcd for $C_{18}H_{21}N_2O_9$, 409.1242). Kipukasin H (**1**): ^{1}H NMR (dimethyl sulfoxide (DMSO)-d_6, 600 MHz, δ, ppm): δ 11.39 (1H, brs, 3-N*H*), 9.83 (1H, brs, 4″-O*H*), 7.94 (1H, d, *J* = 7.8 Hz, H-6), 6.27 (1H, d, *J* = 2.4 Hz, H-3″), 6.22 (1H, d, *J* = 2.4 Hz, H-5″), 6.08 (1H, d, *J* = 6.6 Hz, H-1′), 5.70 (1H, d, *J* = 7.8 Hz, H-5), 5.55 (1H, d, *J* = 5.4 Hz, 3′-O*H*), 5.31 (1H, dd, *J* = 6.6, 5.4 Hz, H-2′), 5.23 (1H, t, *J* = 5.4 Hz, 5′-O*H*), 4.29 (1H, dt, *J* = 5.4, 3.0 Hz, H-3′), 3.91 (1H, q, *J* = 3.0 Hz, H-4′), 3.64 (3H, s, H-9″), 3.57–3.67 (2H, m, H_2-5′), 2.15 (3H, s, H-8″). ^{13}C NMR (DMSO-d_6, 150 MHz, δ, ppm): 166.8 (C, C-7″), 163.6 (C, C-4), 160.3 (C, C-4″), 159.0 (C, C-2″), 151.0 (C, C-2), 141.2 (CH, C-6), 138.7 (C, C-6″), 113.8 (C, C-1″), 109.5 (CH, C-5″), 102.8 (CH, C-5), 97.4 (CH, C-3″), 86.4 (CH, C-1′), 85.7 (CH, C-4′), 75.4 (CH, C-2′), 69.4 (CH, C-3′), 61.4 (CH_2, C-5′), 56.1 (CH_3, C-9″), 20.0 (CH_3, C-8″). Kipukasin I (**2**): ^{1}H NMR (DMSO-d_6, 600 MHz, δ, ppm): 11.36 (1H, brs, 3-N*H*), 9.85 (1H, brs, 4″-O*H*), 7.88 (1H, d, *J* = 7.8 Hz, H-6), 6.29

(1H, d, $J = 2.4$ Hz, H-3″), 6.24 (1H, d, $J = 2.4$ Hz, H-5″), 5.83 (1H, d, $J = 6.6$ Hz, H-1′), 5.73 (1H, brs, 5′-O*H*), 5.71 (1H, d, $J = 7.8$ Hz, H-5), 5.55 (1H, brs, 2′-O*H*), 5.25 (1H, dd, $J = 5.4$, 2.4 Hz, H-3′), 4.36 (1H, dd, $J = 6.6$, 5.4 Hz, H-2′), 4.07 (1H, q, $J = 2.4$ Hz, H-4′), 3.70 (3H, s, H-9″), 3.64 (2H, m, H-5′), 2.19 (3H, s, H-8″). ^{13}C NMR (DMSO-d_6, 150 MHz, δ, ppm): 166.4 (C, C-7″), 162.8 (C, C-4), 159.5 (C, C-2″), 159.5 (C, C-4″), 150.6 (C, C-2), 140.2 (CH, C-6), 137.9 (C, C-6″), 113.4 (C, C-1″), 108.8 (CH, C-5″), 102.1 (CH, C-5), 96.7 (CH, C-3″), 85.7 (CH, C-1′), 82.7 (CH, C-4′), 73.2 (CH, C-3′), 71.4 (CH, C-2′), 60.9 (CH_2, C-5′), 55.4 (CH_3, C-9″), 19.4 (CH_3, C-8″).

3.4. *Preparation of acetylated derivatives of 3 and 4*

To a solution of **3/4** (20 mg) in dry acetone (2.0 mL), Ac_2O (100 μL) and K_2CO_3 (10 mg) were added at RT, and the reaction mixture was stirred for 6 h. The solvent was evaporated *in vacuo* and the residue was purified by semi-preparative HPLC eluting with 80% MeOH–H_2O to give **3a** (6.9 mg) and **4a** (7.8 mg).

3.4.1. *Diacetylkipukasin E (**3a**)*

Colourless oil. ^{1}H NMR ($CDCl_3$, 600 MHz, δ, ppm): 8.82 (1H, brs, 3-N*H*), 7.41 (1H, d, $J = 8.4$ Hz, H-6), 6.32 (2H, d, $J = 2.4$ Hz, H-3″, H-5″), 6.10 (1H, d, $J = 6.0$ Hz, H-1′), 5.79 (1H, dd, $J = 8.4$, 1.8 Hz, H-5), 5.52 (1H, dd, $J = 6.0$, 3.6 Hz, H-3′), 5.42 (1H, t, $J = 6.0$ Hz, H-2′), 4.43–4.46 (2H, m, H-4′, Ha-5′), 4.37 (1H, dd, $J = 13.8$, 3.6 Hz, Hb-5′), 3.81 (6H, s, H-9″, H-10″), 2.31 (3H, s, H-8″), 2.16 (3H, s, H-11″), 2.06 (3H, s, H-13″). ESI-MS *m/z* 529.1 $[M + Na]^+$.

3.4.2. *Diacetylkipukasin D (**4a**)*

Colourless oil. ^{1}H NMR ($CDCl_3$, 600 MHz, δ, ppm): 8.53 (1H, brs, 3-N*H*), 7.42 (1H, d, $J = 8.4$ Hz, H-6), 6.31 (1H, d, $J = 1.8$ Hz, H-3″ or H-5″), 6.29 (1H, d, $J = 1.8$ Hz, H-5″ or H-3″) 6.07 (1H, d, $J = 5.4$ Hz, H-1′), 5.79 (1H, dd, $J = 8.4$, 1.8 Hz, H-5), 5.56 (1H, t, $J = 5.4$ Hz, H-3′ or H-2′), 5.43 (1H, t, $J = 5.4$ Hz, H-2′ or H-3′), 4.34–4.39 (3H, m, H-4′, H-5′), 3.80 (3H, s, H-9″ or H-10″), 3.76 (3H, s, H-10″ or H-9″), 2.29 (3H, s, H-8″), 2.16 (3H, s, H-11″ or H-13″), 2.08 (3H, s, H-13″ or H-11″). ESI-MS *m/z* 529.1 $[M + Na]^+$.

3.5. *Biological assays*

The antibacterial assay was carried out as described previously (Pierce et al. 2008). Seven bacterial strains were used, including *Bacillus cereus* (ACCC 11077), *Micrococcus luteus* (ATCC 49732), *S. epidermidis* (ATCC 12228), *Staphylococcus aureus* (ATCC 27154), *Escherichia coli* (ATCC 25922), *Vibrio parahemolyticus* (ATCC 17802) and *Pseudomonas putida* (ATCC 49128), and ciprofloxacin was used as a positive control. The brine shrimp lethality assay was performed on *A. salina* according to the published protocols (Meyer et al. 1982; Solis et al. 1993). DMSO was used as a positive control. The cytotoxic activities against human erythroleukaemia K562 and human promyelocytic leukaemia HL-60 cell lines were determined by the MTT method (Mosmann 1983). Adriamycin was used as a positive control.

Supplementary material

The 1D and 2D NMR, and MS spectra of **1/2**, the ^{1}H NMR and MS spectra of **3a** and **4a** and selected ^{1}H–^{1}H COSY and HMBC correlations for **1** are available online.

Acknowledgements

We thank Dr Chang-Lun Shao for collection of the gorgonian samples. This work was supported by the National Natural Science Foundation of China (Nos 41130858; 81172977), the National High Technology Research and Development Program of China (863 Program) (No. 2013AA093001) and the Fundamental Research Funds for the Central Universities of China (No. 201122005).

References

Blunt JW, Copp BR, Keyzers RA, Munroa MHG, Prinsep MR. 2013. Marine natural products. Nat Prod Rep. 30:237–323.

Fremlin LJ, Piggott AM, Lacey E, Capon RJ. 2009. Cottoquinazoline A and cotteslosins A and B, metabolites from an Australian marine-derived strain of *Aspergillus versicolor*. J Nat Prod. 72:666–670.

Hasegawa H, Akira K, Shinohara Y, Kasuya Y, Hashimoto T. 2001. Kinetics of intramolecular acyl migration of 1β-*O*-acyl glucuronides of (*R*)- and (*S*)-2-phenylpropionic acids. Biol Pharm Bull. 24:852–855.

Jackson MD, Denu JM. 2002. Structural identification of 2′- and 3′-*O*-acetyl-ADP-ribose as novel metabolites derived from the sir2 family of β-NAD^+-dependent histone/protein deacetylases. J Biol Chem. 277:18535–18544.

Jiao P, Mudur SV, Gloer JB, Wicklow DT. 2007. Kipukasins, nucleoside derivatives from *Aspergillus versicolor*. J Nat Prod. 70:1308–1311.

Meyer BN, Ferrigni NR, Putnam JE, Jacobson LB, Nicols DE, Mclaughlin JL. 1982. Brine shrimp: a convenient bioassay for active plant constituents. Planta Med. 45:31–34.

Mosmann T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 65:55–63.

Pierce CG, Uppuluri P, Teistan AR, Wormley JFL, Mowat E, Ramage G, Lopez-Ribot JL. 2008. A simple and reproducible 96-well plate-based method for the formation of fungal biofilms and its application to antifungal susceptibility testing. Nat Protoc. 3:1494–1500.

Sauve AA, Celic I, Avalos J, Deng H, Boeke JD, Schramm VL. 2001. Chemistry of gene silencing: the mechanism of NAD^+-dependent deacetylation reactions. Biochemistry. 40:15456–15463.

Shao CL, Wu HX, Wang CY, Liu QA, Xu Y, Wei MY, Qian PY, Gu YC, Zheng CJ, She ZG, Lin YC. 2011. Potent antifouling resorcylic acid lactones from the gorgonian-derived fungus *Cochliobolus lunatus*. J Nat Prod. 74:629–633.

Shao CL, Xu RF, Wei MY, She ZG, Wang CY. 2013. Structure and absolute configuration of fumiquinazoline L, an alkaloid from a gorgonian-derived *Scopulariopsis* sp. fungus. J Nat Prod. 76:779–782.

Solis PN, Wright CW, Anderson MM, Gupta MP, Phillipson JD. 1993. A microwell cytotoxicity assay using *Artemia salina* (brine shrimp). Planta Med. 59:250–252.

Spahn-Langguth H, Benet LZ. 1992. Acyl glucuronides revisited: Is the glucuronidation process a toxification as well as a detoxification mechanism? Drug Metab Rev. 24:5–48.

Sun LL, Shao CL, Chen JF, Guo ZY, Fu XM, Chen M, Chen YY, Li R, Nicole J, She ZG, et al. 2012. New bisabolane sesquiterpenoids from a marine-derived fungus *Aspergillus* sp. isolated from the sponge *Xestospongia testudinaria*. Bioorg Med Chem Lett. 22:1326–1329.

Yamada T, Kitada H, Kajimoto T, Numata A, Tanaka R. 2010. The relationship between the CD Cotton effect and the absolute configuration of FD-838 and its seven stereoisomers. J Org Chem. 75:4146–4153.

Yang KL, Wei MY, Shao CL, Fu XM, Guo ZY, Xu RF, Zheng CJ, She ZG, Lin YC, Wang CY. 2012. Antibacterial anthraquinone derivatives from a sea anemone-derived fungus *Nigrospora* sp. J Nat Prod. 75:935–941.