Supporting Information

Perinadine A, A Novel Tetracyclic Alkaloid from Marine-Derived Fungus *Penicillium* *citrinum*

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Experimental Section

General Experimental Procedures. ^1H and ^{13}C NMR spectra were obtained on a Varian Unity INOVA 600 spectrometer using TMS as an internal standard. HSQC experiments were optimized for $^1J_{\text{CH}}=140$ Hz and HMBC experiments for $^nJ_{\text{CH}}=8\text{Hz}$. Negative-mode ESI mass spectra were obtained on a JEOL 700TZ spectrometer.

Fungal Material and Fermentation. The fungus *Penicillium citrinum* (strain number N055) was isolated from the gastrointestinal tract of a parrot fish *Scarus ovifrons*, which was collected at Hedo Cape, Okinawa Island. Subcultures of the fungus are deposited at Graduate School of Pharmaceutical Sciences, Hokkaido University. The fungus was grown in PYG seawater medium (peptone, 1 %; D-glucose, 2 %; yeast extract, 0.5 %; pH 7.5) rotary-shaking at 500 rpm for 10 days at 25 °C. The culture broth (12 L) was filtered.

Extraction and Separation. The supernatant (12 L) of the culture broth was extracted with EtOAc (1 L x 2). Parts (676 mg) of the EtOAc-soluble portions (1.54 g, wet weight) were subjected to SiO_2 gel ($\text{CHCl}_3/\text{MeOH}$, 95:5) and then amino- SiO_2 gel

(*n*-hexane/EtOAc, 1:1 → 0:1) column chromatographies followed by C₁₈ HPLC (YMC Pack Pro C18, YMC Co., Ltd., 10 x 250 mm; eluent, CH₃CN/H₂O/CF₃CO₂H, 75:25:0.05; flow rate, 3 mL/min; UV detection at 220 nm) to afford perinadine A (**1**, 5.1 mg, wet weight, *t_R* 19.6 min). Scalusamides A³ (**2**, 10.6 mg), B (1.0 mg), and C (0.9 mg) and known pyrrolo[2,1-*b*]oxazine compound **3**³ and its dihydro congener⁴ were obtained from different fractions of the EtOAc soluble materials.

Perinadine A (1): colorless amorphous solid; $[\alpha]_D^{22}$ -33° (*c* 1.0, CHCl₃); IR (KBr) $\nu_{\max}$ 3300 ~ 2700 (br), 3211, 1724, and 1650 cm⁻¹; UV (MeOH) $\lambda_{\max}$ 315 (ε 1700), 252 (3600), and 215 nm (16000); CD (MeOH) λ_{ext} 313 (Δε -0.5), 257 (+1.3), 224 (-0.6), and 214 nm (+0.6); ¹H and ¹³C NMR, see Table 1; FABMS *m/z* 498 (M-H)⁻; HRFABMS (*m/z* 498.2510 [(M-H)⁻, calcd for C₂₈H₃₆NO₇, 498.2517].

LC-NMR Analysis of Perinadine A (1). A Varian Unity INOVA 600 MHz NMR instrument equipped with a ¹H{¹³C/¹⁵N} 4 mm/120 μL pulse field gradient triple-resonance interchangeable flow cell microflow LC/NMR probe (IFC Probe) was used. Reverse phase HPLC was carried out on a Varian (Palo Alto, CA) modular HPLC system, comprising a Varian Prostar 230 pump, Valco injection valve, and a Varian

Prostar 330 UV detector. The separation was achieved by using C₁₈ HPLC (YMC Pack ODS-AM, YMC Co., Ltd., 250 × 10 mm; eluent, CD₃CN/D₂O/CF₃CO₂H 75:25:0.05; flow rate, 3mL/min; UV detection at 220 nm). perinadine A (**1**, 40 µg) was injected, and peaks **A** (*t_R* 20.3 min) and **B** (*t_R* 22.4 min) were afforded. The ¹H NMR data of peaks **A** and **B** were recorded using the stopped-flow mode of the peak, which afforded all the typical resonance with 10000 scan. Solvent suppression was achieved with the water suppression enhanced through T1 effects (WET) technique, and a residual methyl signal of CH₃CN was set to δ_H 1.96.

Figure S1. ¹H NMR spectrum of perinadine A (1) in CDCl₃.

Figure S2. ^{13}C NMR spectrum of perinadine A (1) in CDCl_3 .

Figure S4. TOCSY spectrum of perinadine A (1) in CDCl_3 .

Figure S5. HMQC spectrum of perinadine A (1) in CDCl_3 .

Figure S6. ROESY spectrum (part) of perinadine A (1) in CDCl₃ at 0 °C.

Figure S7. HMBC spectrum of perinadin A (1) in CDCl₃.

Figure S8. NOESY spectrum of perinadin A (1) in CDCl_3 .