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Anti-hepatitis C Virus Natural Product from a Fungus, *Penicillium herquei*

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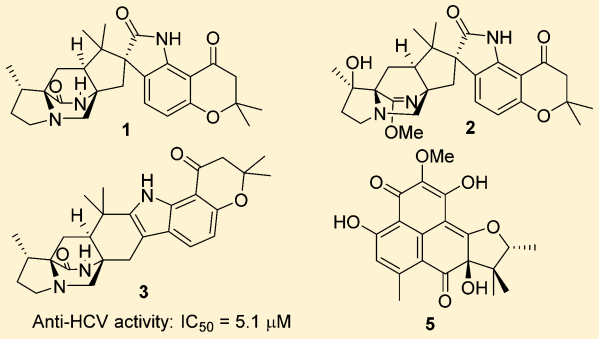
Supporting Information

ABSTRACT: New diazabicyclo[2.2.2]octane derivatives, peniciherquamides A–C (**1**–**3**), and a novel herqueinone derivative, neoherqueinone (**5**), were isolated from a fungal culture broth of *Penicillium herquei*. The structures of these novel compounds were determined by interpretation of spectroscopic data (1D/2D NMR, MS, and IR). Four known compounds, preparaherquamide (**4**), peniciherqueinone (**6**), and herqueinone/isoherqueinone (**7**/**7a**), were also obtained. The isolated compounds were tested for anti-hepatitis C virus (HCV) activity, and peniciherquamide C (**3**) was found to display an IC_{50} value of 5.1 μM. To our knowledge, this is the first report of a diazabicyclo[2.2.2]octane derivative with anti-HCV activity.



Hepatitis C virus (HCV) is a major causative agent of chronic liver disease, including liver cirrhosis and hepatocellular carcinoma.[1] Estimates predict that 170 million people, 2–3% of the world's population, are chronically infected with HCV, with 3–4 million people becoming newly infected each year.[2,3] Recently, the number of anti-HCV therapies has expanded due to the development of so-called direct-acting antivirals, which inhibit the function of viral-derived proteins, including NS3 protease, NS5A, and NS5B polymerase, in addition to PEGylated-interferon (IFN) α and ribavirin. Unfortunately, however, these therapies have significant drawbacks such as diverse efficacy among HCV genotypes, adverse effects, and especially high costs.[4] Thus, a new class of anti-HCV small molecules is desired.

In the past, we have focused on fungi isolated from seaweed, mosses, and plants to isolate novel natural products that possess biological activity.[5,6] It is thought that there are 5.1 million species of fungi on Earth, of which only about 99 000 have been reported in the literature.[7] These numbers infer that more than 98% of fungal species are still unknown to science. Because of their biodiversity, fungi are attractive organisms to screen for novel bioactive secondary metabolites in drug discovery programs. In this study, we report the isolation, structural elucidation, and anti-HCV activities of new diazabicyclo[2.2.2]octane derivatives, peniciherquamide A–C (**1**–**3**), and a novel herqueinone derivative, neoherqueinone (**5**). Additionally, the isolation and characterization of known compounds preparaherquamide (**4**),[8] peniciherqueinone (**6**),[9] and herqueinone/isoherqueinone (**7**/**7a**)[10,11] are also described.

TLC-guided fractionation of a culture extract from *Penicillium herquei* using silica gel column chromatography yielded compounds **1**–**7**. The molecular formula of $C_{27}H_{33}N_3O_4$ for compound **1** was determined by HRESIMS. The IR spectrum showed absorption bands at 3402, 3311, 1722, and 1672 cm^{-1} corresponding to amide groups, which was supported by ^1H NMR signals at δ 9.31 (1H, s) and δ 6.57 (1H, brs). As shown in Table 1, the ^{13}C NMR and DEPT spectroscopic data suggested the presence of three carbonyl and six aromatic carbon atoms, as well as five methyl groups. ^1H–^1H COSY correlations observed between two aromatic proton signals at δ 7.24 and δ 6.53 were assigned to an *ortho*-coupling (J = 8.3 Hz) between H-4 and H-5, and HMBC correlations of these protons revealed a 1,2,3,4-tetrasubstituted benzene moiety (Table 1 and Figure 2). HMBC correlations from two methyl protons to C-25 and C-26, together with correlation from H-25 to C-24 (δ 193.7), indicated the presence of a 2,2-dimethylchroman-4-one moiety. The HMBC correlations of H-22 and H-23 with C-3, C-20, and C-21 revealed the connection of C-21 to both C-3 and C-20 and established the presence of a cyclopentanoid ring. The amide

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Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Spectroscopic Data and HMBC correlations for Compounds 1−3 in $CDCl_3$

	1			2		3	
pos.	δ_C, type	δ_H (J in Hz)	HMBC	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1		9.31 (brs)	2, 3, 8, 9		9.20 (brs)		9.71 (brs)
2	183.4, C			183.5, C		140.7, C	
3	60.9, C			61.0, C		104.5, C	
4	132.3, CH	7.24 (d, 8.4)	3, 6, 8	132.4, CH	7.25 (d, 8.4)	126.9, CH	7.52 (d, 8.5)
5	109.6, CH	6.53 (d, 8.4)	6, 7, 9	109.3, CH	6.52 (d, 8.4)	109.7, CH	6.65 (d, 8.5)
6	159.2, C			159.0, C		157.5, C	
7	105.0, C			104.8, C		105.2, C	
8	142.8, C			142.9, C		134.2, C	
9	121.7, C			122.9, C		121.2, C	
10	39.6, CH_2	2.24 (d, 15.0)	2, 3, 11, 21	42.3, CH_2	2.66 (d, 14.7)	29.8, CH_2	2.84 (d, 15.1)
		1.93 (d, 15.0)	2, 9, 11, 20		1.92 (d, 14.7)		2.76 (d, 15.1)
11	61.7, C			67.3, C		56.7, C	
12	60.3, CH_2	3.64 (d, 10.8)	13, 16	57.5, CH_2	3.63 (d, 11.1)	59.8, CH_2	3.46 (d, 10.3)
		2.62 (d, 10.8)	11, 16, 20		2.13 (d, 11.1)		2.18 (m)
13	68.0, C			69.4, C		65.5, C	
14	39.9, CH	1.93 (m)	13, 15, 17, 18	77.6, C		40.4, CH	1.97 (m)
15	30.2, CH_2	2.05 (m)	14, 16	38.4, CH_2	2.22 (m)	30.9, CH_2	2.00 (m)
		1.83 (m)	14, 16, 17		1.94 (m)		1.89 (m)
16	52.8, CH_2	3.19 (ddd, 9.2, 9.0, 4.2)	13, 14, 15	52.3, CH_2	3.16 (m)	53.8, CH_2	3.21 (ddd, 9.1, 9.0, 5.5)
		2.38 (ddd, 10.0, 9.2, 4.6)	12, 15		1.94 (m)		2.29 (ddd, 10.5, 9.1, 4.6)
17	13.1, CH_3	1.40 (d, 6.9)	13, 14, 15	19.6, CH_3	1.50 (s)	13.0, CH_3	1.41 (d, 6.7)
18	173.8, C			166.8, C		173.7, C	
19	27.5, CH_2	2.08 (dd, 12.4, 10.8)	11, 13, 14, 20	22.2, CH_2	1.63 (m)	30.0, CH_2	2.19 (m)
		1.42 (m)	13, 20, 21				1.69 (dd, 12.5, 3.7)
20	53.3, CH	3.08 (dd, 10.8, 10.1)	11, 12, 19, 21, 22, 23	49.1, CH	2.52 (dd, 10.4, 10.3)	46.3, CH	2.14 (dd, 11.6, 3.7)
21	46.4, C			46.1, C		34.0, C	
22	20.8 CH_3	1.09 (s)	3, 20, 21, 23	21.4, CH_3	1.09 (s)	23.8, CH_3	1.41 (s)
23	24.0, CH_3	0.85 (s)	3, 20, 21, 22	24.4, CH_3	0.85 (s)	30.5, CH_3	1.34 (s)
24	193.7, C			193.7, C		194.1, C	
25	48.8, CH_2	2.75 (d, 16.8)	24, 26, 27, 28	48.8, CH_2	2.74 (d, 16.9)	48.7, CH_2	2.76 (s)
		2.70 (d, 16.8)	24, 26, 27, 28		2.68 (d, 16.9)		
26	79.5, C			79.3, C		79.5, C	
27	26.6, CH_3	1.47 (s)	25, 26, 28	26.7, CH_3	1.47 (s)	26.6, CH_3	1.50 (s)
28	26.8, CH_3	1.50 (s)	25, 26, 27	26.9, CH_3	1.49 (s)	26.6, CH_3	1.50 (s)
29		6.57 (brs)					5.75 (brs)
14-OH					2.77 (brs)		
18-OMe				52.2, CH_3	3.76 (s)		

group was found to belong to a diazabicyclo[2.2.2]octane ring system on the basis of $^{1}H-^{1}H$ COSY and HMBC correlations (Table 1 and Figure 2). These data suggested that compound **1** was a closely related derivative of mangrovamide A (**8**), a recently reported diazabicyclo[2.2.2]octane derivative that possessed a γ-methylproline, and its ^{1}H and ^{13}C NMR data were very similar to those of **1**.[12] The consecutive $^{1}H-^{1}H$ COSY correlations from H16 to H17 (H-16/H-15/H-14/H-17) and HMBC correlations from H-14 to C-13 and C-18 (amide carbon, δ 173.8) established the presence of a β-methylproline moiety. NOESY correlations observed in **1** were used to define its relative configuration (see Figure 2). The CD spectrum of **1** showed a negative Cotton effect at 274 nm and a positive Cotton effect at 240 nm (Supporting Information) and correlates with relevant regions of that of mangrovamide A.[12] Additionally, both compounds showed a negative specific rotation [compound **1**: $[\alpha]^{26}_{D}$ −38.9 (*c* 0.502, $CHCl_3$), $[\alpha]^{23}_{D}$ −65.6 (*c* 0.0575, MeOH); mangrovamide A: $[\alpha]^{25}_{D}$ −20.3 (*c* 0.03, MeOH)].[12] Thus, the absolute configuration of compound **1** was proposed as 3*R*, 11*S*, 13*R*, 14*S*, and 20*S*, and it was named peniciherquamide A (Figure 1). Several diazabicyclo[2.2.2]octane ring derivatives bearing β-methylproline have been reported.[13] Of these derivatives, the structures of SB200437 and VM55595 were very similar to those of **1** except for the 2,2-dimethylchroman-4-one moiety.[14,15] On the other hand, diazabicyclo[2.2.2]octane ring derivatives bearing 2,2-dimethylchroman-4-one were reported to contain proline (citrinalin C) or γ-methylproline (mangrovamides) instead of β-methylproline of **1**.[12,16,17] Peniciherquamide A is the first diazabicyclo[2.2.2]octane ring derivative that possesses both β-methylproline and 2,2-dimethylchroman-4-one moieties.

The molecular formula of $C_{28}H_{35}N_3O_5$ for compound **2** was determined by HRESIMS. As shown in Table 1, the ^{1}H and ^{13}C NMR spectra suggested that the structure of compound **2** was similar to compound **1**, except for the chemical shift value of C-14 and the presence of ^{1}H (δ 3.76) and ^{13}C (δ 52.2) signals due to a methoxy group in **2**. The downfield-shifted ^{13}C signal at C-14 (δ 77.6) indicated the presence of a C-14 alcohol. On the basis of HMBC correlations from the methoxy protons to C-18, the methoxy group was found to be located at C-18 and to form an imidate. The structure of compound **2** was in agreement with the molecular formula determined by

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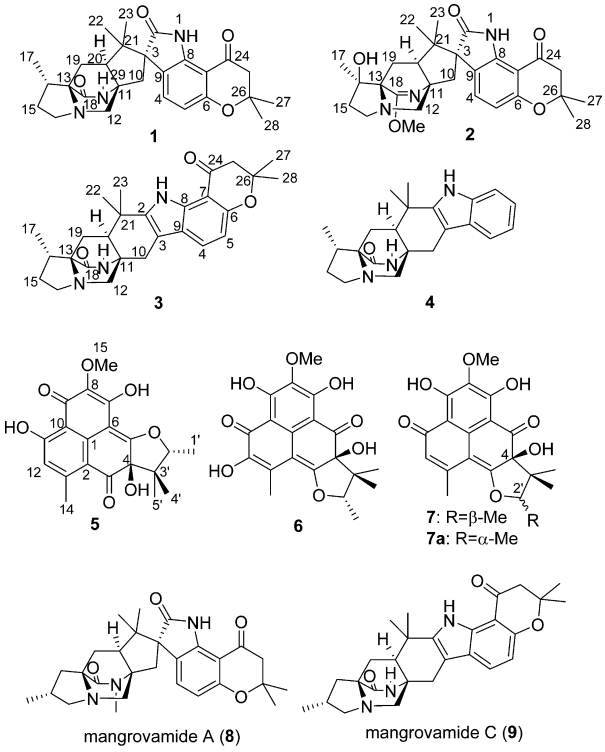

Figure 1. Structures of compounds **1**−**7**.

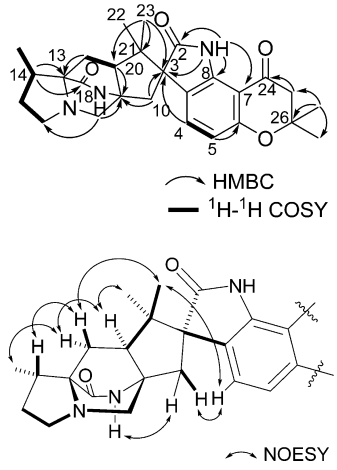

Figure 2. Key ^{1}H−^{1}H COSY, HMBC, and NOESY correlations for **1**.

HRESIMS and further confirmed by DEPT, HMQC, and HMBC experiments. The relative configuration of **2** was deduced by NOESY correlations recorded in $CDCl_3$, CD_3OD, or DMSO-d_6 (Figure 3). The relative configuration of C-14 was determined by the NOESY correlations between H-17 and MeO-18, between MeO-18 and H-16α, between H-16α and H-12α, and between H-12β and H-4. The CD spectrum of **2**, like compound **1**, showed a negative Cotton effect at 273 nm and a positive Cotton effect at 240 nm (Supporting Information), suggesting a 3*R*, 11*S*, 13*R*, 14*R*, and 20*S* configuration (Figure 1). Although several diazabicyclo[2.2.2]octane ring derivatives bearing an *N*-methyl group have been reported, to our knowledge, compound **2** (peniciherquamide B) is the first diazabicyclo[2.2.2]octane ring system to be described that possesses an *O*-methyl group to form an imidate.[13,18]

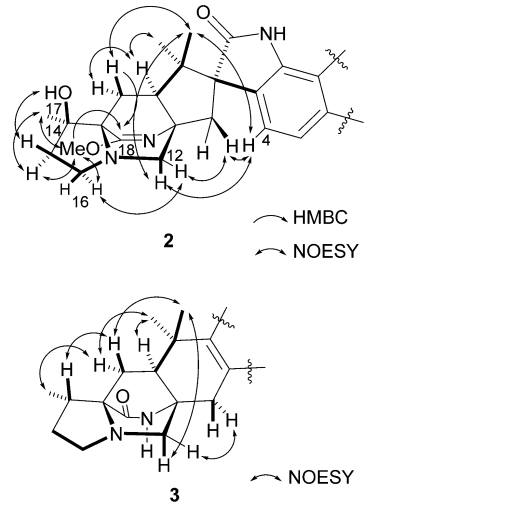

Figure 3. Key HMBC and NOESY correlations for **2** and **3**.

The molecular formula of $C_{27}H_{33}N_3O_3$ for compound **3** was determined by HRESIMS. The ^{1}H and ^{13}C NMR spectra of compound **3** were similar to those of an indole derivative, preparaherquamide (**4**), except for the presence of a carbonyl carbon, an sp^3 quaternary carbon, a methylene carbon, and two methyl carbons in **3** (Table 1). The chemical shift values and 2D NMR analyses revealed that compound **3** contained a 2,2-dimethylchroman-4-one moiety like compounds **1** and **2**. Thus, the structure of compound **3** (peniciherquamide C) was determined (Figure 1), and the relative configurations were confirmed by NOESY experiments (Figure 3). In this case, compound **3** was a regioisomer of mangrovamide C (**9**), in which the position of the methyl group on pyrrolidine ring was shifted, originating from the biosynthetic intermediates (β-methyl proline versus γ-methyl proline).[12,13]

Compound **4** was identified as preparaherquamide by comparison of its ^{1}H and ^{13}C NMR and MS with data previously reported in the literature.[8,19,20]

The molecular formula of $C_{20}H_{20}O_7$ for compound **5** was determined by HRESIMS and found to be the same as that of herqueinone (**7**). The ^{1}H and ^{13}C NMR spectra of **5** were very similar to those of **7** (Table 2). Further analyses by DEPT, ^{1}H−^{1}H COSY, HMQC, and HMBC experiments indicated that **5** is an isomer of **7**. Weak four-bond HMBC correlations from H-12 and H-14 to a carbonyl carbon signal (δ 193.4) were observed, which allowed us to assign the ketone functionality at C-3 (Figure 4). All carbon signals were assigned on the basis of the HMBC spectrum. Thus, we proposed the structure of **5** as shown in Figure 1, which suggested that **5** was a closely related derivative of herqueichrysin and desmethylherqueichrysin.[21,22] Compound **5** is an oxidative form of herqueichrysin and has a hydroxyl group at C-4. The *cis* relationships of H-2′/OH-4 were deduced from the NOE correlation between these protons (Figure 4). Thus, the structure of compound **5** was determined (Figure 1) and named neoherqueinone. The absolute configuration of **5** remains to be determined due to its unstable nature and lack of sufficient material for analysis.

Compound **6** was identified as peniciherqueinone by comparison of its ^{1}H and ^{13}C NMR and MS data with those recently reported in the literature.[9] An inseparable mixture of compounds **7** and **7a** was also obtained (**7**:**7a** = 2:1). ^{1}H and ^{13}C NMR as well as MS data of compound **7** were in complete agreement with those of herqueinone.[23,24] In previous reports,

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Table 2. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Spectroscopic Data for Compound 5 in DMSO-d_6

pos.	δ_C, type	δ_H (J in Hz)
1	138.4, C	
2	115.5, C	
3	193.4, C	
4	79.0, C	
5	176.3, C	
6	98.1, C	
7	157.7, C	
8	133.1, C	
9	181.1, C	
10	111.9, C	
11	165.8, C	
12	116.5, CH	6.62 (s)
13	148.1, C	
14	23.6, CH_3	2.57 (s)
15	60.0, CH_3	3.72 (s)
1′	13.0, CH_3	1.34 (d, 6.6)
2′	89.4, CH	4.83 (q, 6.6)
3′	45.6, C	
4′	16.6, CH_3	1.33 (s)
5′	16.3, CH_3	0.77 (s)
4-OH		7.13 (s)
11-OH		15.31 (brs)

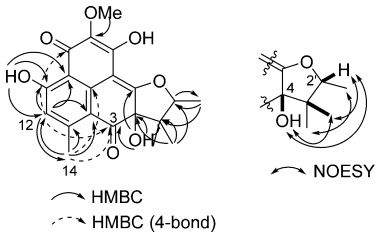

Figure 4. Key HMBC and NOESY correlations for **5**.

herqueinone extracted from the mycelium of *P. herquei* was invariably contaminated with isoherqueinone.[25,26] Compound **7a** was found to be isoherqueinone by NMR and MS analysis, in which the *cis* relationships of H-2′/OH-4 were deduced from the NOE correlation between these protons.

Compounds **1**−**7** were evaluated for their anti-HCV activity, according to a procedure described previously.[27] Of the obtained compounds, compound **3** exhibited significant anti-HCV activity with an IC_{50} value of 5.1 μM. Interestingly, the other diazabicyclo[2.2.2]octane derivatives **1**, **2**, and **4** showed no detectable anti-HCV activity (IC_{50} > 40 μM). Herqueinone derivatives **5**−**7** also failed to display any measurable anti-HCV activity. To our knowledge, peniciherquamide C (**3**) is the first diazabicyclo[2.2.2]octane derivative reported to show anti-HCV action.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were recorded on a JASCO P-1010 digital polarimeter (Jasco Corp., Tokyo, Japan) at room temperature. UV spectra were obtained on a GE Healthcare Ultraspec 2100 Pro spectrophotometer (GE Healthcare, Piscataway, NJ, USA). CD spectra were recorded using a JASCO J-720 CD spectrometer at a concentration of 1.0×10^{-4} M in MeOH at 25 °C using 10 mm path-length quartz cuvettes. Infrared spectra (IR) were recorded on a Horiba FREEXACT-II FT-720 spectrophotometer (Horiba Ltd., Kyoto, Japan) and reported as wavenumbers (cm^{-1}). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz spectrometer (Avance DRX-400; Bruker, Billerica, MA, USA), using a $CDCl_3$ or DMSO-d_6 solution (with TMS for ^{1}H NMR and $CDCl_3$ or DMSO-d_6 for ^{13}C NMR as an internal reference). Chemical shifts are expressed in δ (ppm) relative to TMS or residual solvent resonance, and coupling constants (J) are expressed in Hz. Mass spectra (MS) were obtained on an Applied Biosystems mass spectrometer (APIQSTAR Pulsar I; Applied Biosystems, Darmstadt, Germany) under conditions of high resolution, using poly(ethylene glycol) as the internal standard. Analytical TLC was carried out on precoated silica gel 60 F254 plates (Merck, Darmstadt, Germany).

Isolation and Cultivation of Fungi. Seaweeds were collected in Toba, Mie, Japan, and suspended in sterilized H_2O. The suspension was spread onto potato dextrose agar (PDA) plates (Difco & BBL, Franklin Lakes, NJ, USA), and the plates were incubated for 1−2 weeks at 37 °C. Fungi growing on this plate were transferred onto individual PDA plates and cultured under the same conditions. Cultures were repeated two to five times to obtain pure mycelium strains. The fungus producing the new compounds reported here was identified as *Penicillium herquei* Bainier & Sartory by Techno Suruga Laboratory Co., Ltd. (Shizuoka, Japan). The ITS-5.8S rDNA of this strain showed 100% sequence identity with *P. herquei* P14190 (GenBank accession number JQ863240).

Extraction and Purification of Compounds. The isolated fungal strain was cultured by transferring a small piece of agar from the culture plate into 12 2-L Erlenmeyer flasks each containing potato dextrose broth (19 g) (Difco and BBL) in H_2O (0.8 L). The culture (9.6 L) was grown under static conditions at room temperature and in the dark for 20 days. The culture broth was filtered through sterile cheesecloth to remove fungal mycelia, and the filtrate then extracted using CH_2Cl_2. The organic layer was evaporated *in vacuo* to obtain a crude extract (1.7 g). This crude extract was separated by silica gel column chromatography with toluene−EtOAc (100:0−0:100) to give fractions 1−13. Fraction 7 was separated by silica gel column chromatography with toluene−EtOAc (15:1) to give herqueinone/isoherqueinone (**7**/**7a**) (21.5 mg). Fraction 9 was subjected to silica gel column chromatography with hexane−EtOAc (1:0−1:4) to give compound **5** (1.8 mg) as an orange solid and compound **6** (10.4 mg) as a yellow solid. Fraction 10 was separated by silica gel column chromatography with toluene−EtOAc (20:1−10:1) to give fractions 10-1−10-7. Fraction 10-5 was subjected to HPLC using a reverse-phase column (Shiseido Capcell Pak C_{18}, 5 μm, 20 × 250 mm, gradient elution with 50−100% MeCN−H_2O, flow rate 5 mL/min) to furnish preparaherquamide (**4**) (1.1 mg). Fraction 10-6 was subjected to HPLC using a reverse-phase column (Shiseido Capcell Pak C_{18}, 5 μm, 20 × 250 mm, gradient elution with 50−100% MeCN−H_2O, flow rate 5 mL/min) to give compound **3** (0.9 mg) as a yellow oil. Fraction 12 was subjected to silica gel column chromatography with $CHCl_3$−MeOH (300:1) to give compounds **1** (24.8 mg) and **2** (1.2 mg), which were both colorless oils.

*Peniciherquamide A (**1**):* colorless oil; $[\alpha]^{26}_D$ −38.9 (*c* 0.502, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 358 (3.51), 278 (3.75), 259 (3.96) nm; CD (*c* 1.0×10^{-4}, MeOH) $\Delta\varepsilon$ (nm) −4.7 (274), +18.4 (240); IR (film) ν_{max} 3402, 3311, 2933, 2879, 1722, 1672, 1618, 1464, 1377, 1313, 1261, 1178, 1122 cm^{-1}; HRESIMS *m*/*z* 486.2374 $[M + Na]^+$ (calcd for $C_{27}H_{33}N_3O_4Na$, 486.2363); ^{13}C and ^{1}H data, see Table 2.

*Peniciherquamide B (**2**):* colorless oil; $[\alpha]^{21}_D$ −62.0 (*c* 0.062, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 357 (3.64), 259 (4.05) nm; CD (*c* 1.0×10^{-4}, MeOH) $\Delta\varepsilon$ (nm) −4.5 (273), +8.4 (240); IR (film) ν_{max} 3402, 2970, 2939, 2854, 1728, 1674, 1612, 1466, 1373, 1319, 1288, 1257, 1180, 1126 cm^{-1}; HRESIMS *m*/*z* 494.2652 $[M + H]^+$ (calcd for $C_{28}H_{36}N_3O_5$, 494.2649); ^{13}C and ^{1}H data, see Table 2.

*Peniciherquamide C (**3**):* yellow oil; $[\alpha]^{21}_D$ −21.4 (*c* 0.0515, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 374 (3.74), 331 (3.90), 243 (4.40) nm; IR (film) ν_{max} 3440, 2962, 2900, 1666, 1620, 1581, 1466, 1365, 1311, 1234, 1203, 1165, 1119 cm^{-1}; HRESIMS *m*/*z* 448.2598 $[M + H]^+$ (calcd for $C_{27}H_{34}N_3O_3$, 448.2594); ^{13}C and ^{1}H data, see Table 3.

*Preparaherquamide (**4**):* $[\alpha]^{23}_D$ +31.2 (*c* 0.026, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (1H, brs), 7.43 (d, *J* = 7.7 Hz, 1H), 7.32 (d, *J* = 8.0 Hz, 1H), 7.17 (dd, *J* = 8.0, 7.0 Hz, 1H), 7.10 (dd, *J* = 7.7, 7.0

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Table 3. Anti-HCV Activities of Compounds 1−7

compound	IC_{50} (μM)[a]
1	>40
2	>40
3	5.1 ± 1.0
4	>40
5	>40
6	>40
7	>40

[a]Data, mean ± SD (n = 3).

Hz, 1H), 5.87 (brs, 1H), 3.47 (d, J = 10.5 Hz, 1H), 3.21 (m, 1H), 2.91 (d, J = 15.1 Hz, 1H), 2.80 (d, J = 15.1 Hz, 1H), 2.30 (ddd, 9.4, 9.4, 4.5, 1H), 2.21 (d, 10.5, 1H), 2.20 (m, 2H), 2.02 (m, 2H), 1.97 (m, 1H), 1.69 (m, 1H), 1.41 (d, J = 7.7 Hz, 3H), 1.40 (s, 3H), 1.33 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.7, 140.8, 136.3, 126.9, 121.9, 119.5, 117.9, 110.6, 104.6, 65.5, 59.8, 56.7, 53.8, 46.3, 40.4, 34.0, 30.6, 30.5, 30.2, 29.9, 24.0, 13.0; ESIMS m/z 372 $[M + Na]^+$.

Neoherqueinone (**5**): orange solid; $[\alpha]^{21}_D$ −408.6 (c 0.046, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 425 (3.69), 350 (3.86), 312 (4.33), 298 (4.46), 259 (4.33) nm; IR (film) ν_{max} 3269, 3006, 2970, 2933, 2877, 1668, 1628, 1570, 1458, 1389, 1342, 1300, 1034 cm^{-1}; HRESIMS m/z 395.1106 $[M + Na]^+$ (calcd for $C_{20}H_{20}O_7Na$, 395.1101); ^{13}C and ^{1}H data, see Table 1.

Peniciherqueinone (**6**): $[\alpha]^{22}_D$ +374.6 (c 0.063, $CHCl_3$); [lit. $[\alpha]^{28}_D$ +92.62 (c 1.68, $CHCl_3$)].[9]

Herqueinone (**7**) *and isoherqueinone* (**7a**): ^{1}H NMR (400 MHz, DMSO-d_6) **7**, δ 15.72 (s, 1H), 13.16 (s, 1H), 7.52 (s, 1H), 6.34 (s, 1H), 4.85 (q, J = 6.8 Hz, 1H), 3.77 (s, 3H), 2.50 (s, 3H), 1.55 (d, J = 6.8 Hz, 3H), 1.37 (s, 3H), 0.98 (s, 3H); **7a**, δ 15.70 (s, 1H), 13.24 (s, 1H), 7.47 (s, 1H), 6.34 (s, 1H), 4.99 (q, J = 6.6 Hz, 1H), 3.77 (s, 3H), 2.48 (s, 3H), 1.38 (d, J = 6.6 Hz, 3H), 1.32 (s, 3H), 0.78 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) **7**, δ 197.1, 186.4, 178.9, 163.0, 162.0, 151.0, 139.0, 131.3, 122.8, 109.2, 103.2, 102.7, 96.1, 79.0, 60.0, 43.1, 23.9, 23.8, 18.7, 16.1; **7a**, δ 197.8, 186.5, 178.1, 163.0, 162.0, 150.9, 139.0, 131.3, 123.0, 109.3, 103.1, 102.7, 90.6, 78.6, 60.0, 46.1, 23.9, 16.2, 16.0, 13.0; ESIMS m/z 395 $[M + Na]^+$.

Anti-HCV Assay. The anti-HCV assay was performed as described previously.[27] In brief, Huh-7.5.1 cells were treated with HCV J6/JFH1 at a multiplicity of infection of 0.15 for 4 h. The cells were then washed and cultured with growth medium in the presence of various concentrations of each compound for 72 h. The infectivity of HCV in the medium was quantified by a focus-forming assay with Huh-7.5.1 cells. Cell viability at 72 h post-treatment was simultaneously measured by MTT assay. Normalized infectivity was calculated as HCV infectivity divided by cell viability.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00555.

^{1}H and ^{13}C NMR, COSY, HMQC, HMBC, and NOESY spectra for **1**−**3** and **5** and CD spectra for compounds **1**−**3** (PDF)

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Notes

The authors declare no competing financial interest.

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