Nakijiquinones J−R, Sesquiterpenoid Quinones with an Amine Residue from Okinawan Marine Sponges†

Yohei Takahashi,‡ Masahiro Ushio,‡ Takaaki Kubota,‡ Sunao Yamamoto,‡ Jane Fromont,§ and Jun'ichi Kobayashi*,‡

Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan, and Western Australian Museum, Locked Bag 49, Welshpool DC, WA 6986, Australia

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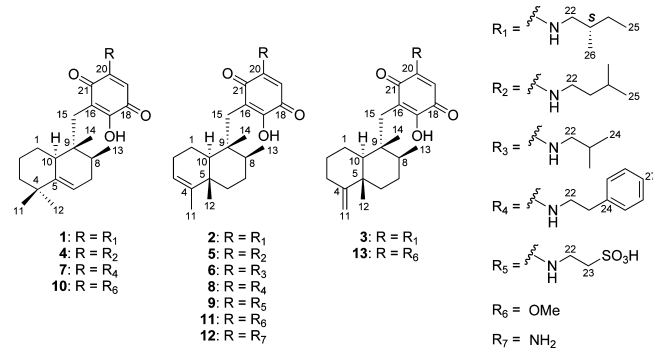
Nine new sesquiterpenoid quinones, nakijiquinones J−R (**1**−**9**), have been isolated from three collections of Okinawan marine sponges of the family Spongiidae, and the structures and configurations were elucidated from the spectroscopic data and chemical correlations. Nakijiquinones J−L (**1**−**3**), M and N (**4** and **5**, respectively), O (**6**), P and Q (**7** and **8**, respectively), and R (**9**) are new sesquiterpenoid quinones possessing (*S*)-2-methylbutylamine, isopentylamine, isobutylamine, phenethylamine, and taurine residues, respectively, attached to each quinone ring.

Sesquiterpenoid quinones and quinols are well known to be contained in several genera of marine sponges.[1] Among them, isospongiaquinone (**11**),[2] ilimaquinone (**13**),[3] and mamanuthaquinone (**10**)[4] are considered as representative compounds in this series. These structures including absolute configurations have been established by chemical degradations and chemical correlations. Isospongiaquinone (**11**) was isolated from the marine sponge *Stelospongia conulata*,[2a] and its absolute configuration was assigned by chemical correlation.[2b] The absolute configuration of ilimaquinone (**13**), isolated from the sponge *Hippospongia* sp.,[3a] was established by chemical correlation of a degradation product.[3b] Mamanuthaquinone (**10**) was first isolated from a sponge *Fasciospongia* sp., and chemical conversion revealed its absolute configuration.[4] The absolute configurations of isospongiaquinone (**11**) and ilimaquinone (**13**) were confirmed by the enantiospecific total syntheses,[5,6] while total synthesis of only the racemic form of mamanuthaquinone (**10**) has been achieved so far.[7]

In our laboratory, nakijiquinones A−I and nakijinol have been isolated from five collections of Okinawan marine sponges of the family Spongiidae.[8−12] Among them, the absolute configuration of nakijiquinone A, which possesses a glycine residue attached to a quinone ring, was elucidated from spectroscopic data and chemical degradation.[8] Additionally, we found that nakijiquinone C, possessing an L-serine residue, showed inhibitory activity against protein tyrosine kinase HER2.[9] After this report, studies on the total synthesis and structure−activity relationships of nakijiquinones were performed by Waldmann et al., and it was found that simplified analogues of nakijiquinones A−D exhibited inhibitory activities against different kinds of tyrosine kinases.[13]

Recently, we reported the structure elucidation of nakijiquinones G−I, which were isolated from two collections (SS-1047 and SS-1074) of the sponges and found to possess unique side chains derived from amino acids.[11] Further investigation of the extract of the former sponge (SS-1047) resulted in the isolation of seven new sesquiterpenoid quinones, nakijiquinones J (**1**), K (**2**), and M−Q (**4**−**8**). Moreover, two new nakijiquinones, nakijiquinones L (**3**) and R (**9**), were obtained from two collections of Okinawan marine sponges (SS-265 and SS-1208, respectively). In this paper, we describe the isolation and structure elucidation of **1**−**9**.

One of the three collections of the sponges of the family Spongiidae (SS-1047 collected off Gesashi, Okinawa) was extracted and partitioned as described previously,[11] and the EtOAc-soluble materials were subjected to silica gel and C_{18} columns followed by repeated C_{18} HPLC to afford nakijiquinones J (**1**), K (**2**), and M−Q (**4**−**8**) together with known sesquiterpenoids mamanuthaquinone (**10**),[4] isospongiaquinone (**11**),[2] nakijiquinones A−E and G−I,[8−11] nakijinol,[12] dictyoceratins A−C,[14,15] and an olefin regioisomer (**12**)[16] of smenospongine.[17] The sponge (SS-265) collected off Kerama Islands, Okinawa, was extracted with MeOH. The $CHCl_3$-soluble materials of the extract were separated by silica gel and C_{18} column chromatographies and C_{18} HPLC to yield nakijiquinone L (**3**) together with known related sesquiterpenoid quinones, ilimaquinone (**13**),[3] smenospongine,[17] smenospongidine,[18] smenospongorine,[18] and smenospongiarine.[18] The $CHCl_3$-soluble materials of the MeOH and MeOH/toluene (3:1) extract of the sponge (SS-1208) collected off Unten Port, Okinawa, were purified by silica gel and C_{18} column chromatographies and C_{18} HPLC to obtain nakijiquinone R (**9**).



Nakijiquinone J (**1**) was obtained as an optically active purple-red amorphous solid, and the molecular formula of **1** was revealed to be $C_{26}H_{39}NO_3$ by HREIMS data (*m*/*z* 413.2916 $[M]^+$). IR absorptions indicated the existence of OH and/or NH and carbonyl functionalities, while UV absorptions were attributed to a quinone chromophore. The ^1H NMR spectrum for **1** showed the presence of one NH proton, one olefin, and six methyl signals. The ^{13}C NMR (Table 1) spectrum gave 26 signals due to six sp^2 quaternary carbons, two sp^2 methines, two sp^3 quaternary carbons, three sp^3 methines, six sp^3 methylenes, and seven sp^3 methyls.

Detailed analyses of the $^1H-^1H$ COSY and HMQC spectra disclosed three partial structures, C-10 to C-3, C-6 to C-13, and 20-NH to C-26 (Figure 1). HMBC cross-peaks of H_3-12/C-3, H_3-12/C-4, H_3-12/C-5, and H_3-12/C-11 revealed the presence of a *gem*-dimethyl group in **1**. The ^{13}C chemical shifts for C-5 and C-6 and HMBC correlations for H-6/C-4 and H-6/C-10 indicated that the double bond was formed between C-5 and C-6 and that C-6 was

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* To whom correspondence should be addressed. Tel: +81-11-706-3239. Fax: +81-11-706-4989. E-mail: jkobay@pharm.hokudai.ac.jp.

‡ Hokkaido University.

§ Western Australian Museum.

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Table 1. ^{13}C NMR Data for Nakijiquinones J−R (**1**−**9**) (150 MHz)

position	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[a]	**6**[a]	**7**[a]	**8**[a]	**9**[b]
1	30.6	19.9	23.2	30.6	20.1	19.9	30.5	19.9	19.4
2	22.8	27.1	28.7	22.8	27.0	27.1	22.8	27.0	26.3
3	41.4	120.8	33.0	41.4	120.7	120.8	41.3	120.8	120.8
4	36.3	144.1	160.5	36.4	144.0	144.1	36.4	144.1	143.1
5	146.5	38.5	40.4	146.5	38.4	38.5	146.5	38.5	37.8
6	114.9	36.0	36.7	114.9	35.9	36.0	114.8	36.0	35.4
7	31.6	27.9	28.0	31.6	27.9	27.9	31.6	28.0	27.5
8	36.4	37.7	37.9	36.3	37.6	37.7	36.3	37.7	37.1
9	40.6	42.7	42.9	40.6	42.6	42.6	40.6	42.7	41.8
10	41.6	47.6	50.0	41.6	47.5	47.6	41.6	47.6	47.0
11	29.7	18.1	102.5	29.7	18.1	18.1	29.7	18.2	17.9
12	28.0	19.9	20.5	28.0	19.8	20.1	28.0	20.1	19.9
13	16.6	17.7	17.9[c]	16.6	17.7	17.7	16.5	17.7	17.8
14	15.9	17.3	17.2	16.0	17.2	17.3	15.9	17.3	17.2
15	32.8	32.4	32.5	32.7	32.4	32.4	32.7	32.4	32.0
16	114.5	113.8	113.5	114.5	113.8	113.9	114.7	113.9	113.6
17	156.7	157.2	157.3	156.7	157.2	157.1	156.5	156.9	158.8[d]
18	178.3	178.1	178.1	178.3	178.0	178.1	178.5	178.3	178.0
19	91.5	91.5	91.6	91.5	91.5	91.6	91.8	91.8	91.6
20	150.5	150.6	150.5	150.1	150.3	150.5	149.9	150.9	[e]
21	183.1	182.9	182.9	183.1	182.8	182.9	183.0	182.8	182.7[d]
22	48.7	48.7	48.7	41.1	41.1	50.3	44.0	44.0	39.2[d]
23	34.0	34.0	34.0	36.9	36.8	27.6	34.2	34.3	48.0
24	27.2	27.2	27.2	25.9	25.9	20.2[f]	137.4	137.4	
25	11.1	11.1	11.1	22.3[f]	22.3[f]		128.5[f]	128.6[f]	
26	17.3	17.4	17.4[c]				128.9[f]	128.9[f]	
27							127.0	127.1	

[a] In $CDCl_3$. [b] In DMSO-d_6. [c] Interchangeable. [d] Assigned from HMBC spectrum. [e] Not observed. [f] **2c**.

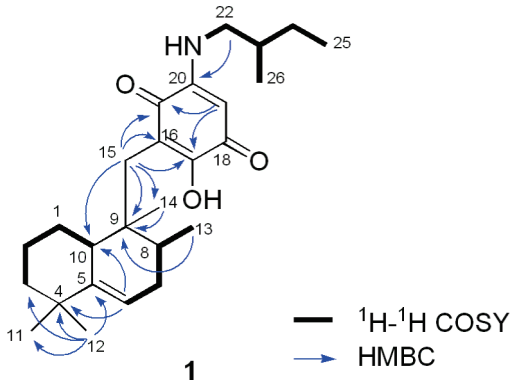

Figure 1. Selected 2D NMR correlations for nakijiquinone J (**1**).

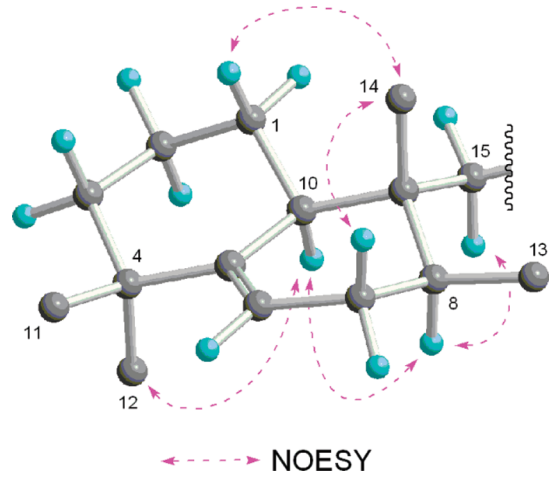

Figure 2. Selected NOESY correlations and relative configuration for the terpenoid moiety in nakijiquinone J (**1**).

connected to C-4 and C-10 via C-5. Connectivities of C-8, C-10, C-14, and C-15 through C-9 were indicated by HMBC cross-peaks of H_3-13/C-9, H_3-14/C-9, H_2-15/C-9, and H_2-15/C-10. HMBC correlations of H_2-15/C-16, H_2-15/C-17, H_2-15/C-21, H-19/C-17, and H-19/C-21 suggested that a quinone ring was attached to C-15. The presence of 17-OH was revealed by the ^{13}C chemical shift for C-17, while the connectivity of 20-NH and C-20 was indicated by the ^{13}C chemical shift for C-20 and the HMBC cross-peak of H-22/C-20. Given the molecular formula of **1**, C-19 was connected to C-18 and C-20, although HMBC correlations for H-19/C-18 and H-19/C-20 were not observed due to small $^2J_{CH}$ values. ^{1}H and ^{13}C chemical shifts of C-1−C-15 were in good agreement with those of mamanuthaquinone (**10**),[4] while those of C-16−C-21 were close to those of other aminoquinone analogues possessing the same partial structures as that of **1**.[18] Thus, the gross structure of nakijiquinone J was elucidated to be **1**.

The relative configuration of the C-1−C-15 part for **1** was deduced from NOESY correlations shown in Figure 2. NOESY cross-peaks of H-8/H-10, H-10/H_3-12, H-8/H-15b, H-1a/H_3-14, and H-7b/H_3-14 indicated that H-1b, H-8, H-10, C-12, and C-15 were α-oriented, and H-1a, H-7b, and H_3-14 were *β*-oriented. Conformations of a cyclohexane (C-1−C-5 and C-10) and a cyclohexene (C-5−C-10) ring were considered to take chair and half-chair forms, respectively. Similarity of ^{1}H and ^{13}C chemical shifts of **1** to those of mamanuthaquinone (**10**) supported that the C-1−C-15 part of **1** had the same relative configuration as that of mamanuthaquinone (**10**).[4] To elucidate the absolute configuration of **1**, chemical derivatization was carried out as follows. Treatment of mamanuthaquinone (**10**)[4] with (*S*)-(−)-2-methylbutylamine in the presence of $NaHCO_3$ for 12 h gave **1**. Physico-chemical properties of the derived **1** were coincident with those of natural **1**; thus, the absolute configuration of nakijiquinone J was assigned as **1**.

The molecular formula of nakijiquinone K (**2**) was revealed to be $C_{26}H_{39}NO_3$ from HREIMS data (*m*/*z* 413.2940 $[M]^+$), and the spectroscopic data of **2** were similar to those of **1**. Comparison of the 1D and 2D NMR data of **2** with those of other known aminoquinone derivatives implied that the sequiterpenoid quinone moiety for **2** was identical with that of isospongiaquinone (**11**)[2] and (*S*)-(−)-2-methylbutylamine was attached to the quinone moiety at C-21. The relative configuration of the C-1−C-15 part in **2** was deduced to be the same as that of isospongiaquinone (**11**)[2] from

the NOESY spectrum of **2**. The absolute configuration of **2** was elucidated from chemical conversion by the same procedure applied for **1** using isospongiaquinone (**11**)[2] as a starting material.

Nakijiquinone L (**3**) was found to have the same molecular formula as those of **1** and **2**, and the spectroscopic data of **3** were also nearly identical with those of **1** and **2**. Comparison of ^{1}H and ^{13}C NMR data of **3** with those of **2** and other known aminoquinone derivatives implied that **3** was the olefin regioisomer of **2**. The gross structure of **3** and relative configuration for the C-1−C-15 part in **3**, which were identical to those of ilimaquinone (**13**)[3] except for a side chain attached to C-20, were revealed by detailed analyses of the 2D NMR spectra of **3**. The side chain of **3** was deduced to be a 2-methylbutylamino group, which was the same as that of **1** and **2**. The absolute configuration of **3** was elucidated from chemical conversion by the same procedure applied for **1** using ilimaquinone (**13**)[3] as a starting material.

Comparison of the ^{1}H and ^{13}C NMR spectra with those of known terpenoid quinones implied that nakijiquinones M−Q (**4**−**8**) possess isopentylamine (for **4** and **5**), isobutylamine (for **6**), and phenethylamine (for **7** and **8**) moieties, respectively. Concerning the terpenoid quinone moieties, those of **4** and **7** were elucidated to be the same as that of nakijiquinone J (**1**), while those of **5**, **6**, and **8** were assigned as the same as that of nakijiquinone K (**2**), respectively, by 1D NMR spectra of **4**−**8**. On the basis of detailed analyses of spectroscopic data, the structures of nakijiquinones M−Q were elucidated to be **4**−**8**, respectively. Nakijiquinones N (**5**), O (**6**), and Q (**8**) were the olefin regioisomers of smenospongiarine,[18] smenospongorine,[18] and smenospongidine,[18] respectively, while nakijiquinones M (**4**) and P (**7**) possessed hybrid structures of the terpenoid moiety in mamanuthaquinone (**10**) and side chains in smenospongiarine or smenospongidine, respectively.

Nakijiquinone R (**9**) was obtained as a purple-red amorphous solid, and the HRESIMS data revealed the molecular fomula of **9** (*m*/*z* 450.1955 [M − H]$^-$, $C_{23}H_{32}NO_6S$). The UV and IR spectra were similar to those of **1**−**8**, except for a strong absorption at 1050 cm^{-1} in the IR spectrum, indicating the existence of the sulfonyl group. The ^{1}H and ^{13}C NMR spectra (Table1) of **9** were also analogous to those of **1**−**8** apart from two sp^3 methylene signals (CH_2-22 and CH_2-23) shifted to relatively low field. Although sp^2 quaternary carbons such as C-17, C-18, C-20, and C-21 in **9** were not observed in the ^{13}C NMR spectrum due to the limited amount of the sample, the presence of the aminoquinone moiety (C-16−C-21) was implied from the UV and IR spectra, and the presence of C-17 and C-20 was indicated from HMBC correlations for H_2-15/C-21, H-19/C-17, and H-19/C-21. Additional 2D NMR analysis revealed the structure of nakijiquinone R to be **9**.

Nakijiquinones J−R (**1**−**9**) are new sesquiterpenoid quinones possessing (*S*)-(−)-2-methylbutylamine (**1**−**3**), isopentylamine (**4** and **5**), isobutylamine (**6**), phenethylamine (**7** and **8**), and taurine (**9**), respectively. These residues except for taurine are considered to be derived from L-isoleucine, L-leucine, L-valine, and L-phenylalanine, respectively. To the best of our knowledge, nakijiquinones J−L (**1**−**3**) are the first examples of the sesquiterpenoid quinones possessing (*S*)-2-methylbutylamine, while nakijiquinone R (**9**) is the fourth example of sesquiterpenoid quinones with a taurine residue.[19,20] Among nakijiquinones A−D and G−Q,[8,9,11] nakijiquinones C and D,[9] possessing a hydroxy group, are isolated as amino acid forms, while nakijiquinones A,[8] G−I,[11] J−N (**1**−**5**), P (**7**), and Q (**8**) are yielded as amine forms. Nakijiquinones B[8] and O (**6**), with an L-valine and an isobutylamine residue, respectively, are obtained as both amine and amino acid forms. Concerning the sesquiterpenoid moieties, nakijiquinones J−R (**1**−**9**) are categorized as three types, namely, the *gem*-dimethyl type (**1**, **4**, and **7**), the *endo*-olefin type (**2**, **5**, **6**, **8**, and **9**), and the *exo*-olefin type (**3**). Although a large number of sesquiterpenoids have been reported so far, sesquiterpenoids possessing the same terpenoid skeleton as those of **1**, **4**, and **7** are rare.[4,21] Comparing compounds isolated from SS-1047 with those of SS-1208, both *gem*-dimethyl types and *endo*-olefin types are not always contained in one sponge. Nakijiquinones J−R (**1**−**9**) at 1 mM were tested for inhibitory activities against EGFR and HER2 tyrosine kinases. Among them, nakijiquinones P (**7**) and R (**9**) exhibited inhibitory activities against EGFR (% inhibition, 76 and >99, respectively), while nakijiquinones N (**5**), O (**6**), and R (**9**) showed inhibitory activities against HER2 (66%, 59%, and 52%, respectively).

Experimental Section

General Methods. Optical rotations were recorded on a JASCO P-1030 polarimeter. IR and UV spectra were recorded on a Shimadzu UV-1600PC and a JASCO FT/IR-5300 spectrophotometer, respectively. ^{1}H, ^{13}C, and 2D NMR spectra were measured on a Bruker AMX-600 spectrometer using 2.5 mm microcells (Shigemi Co., Ltd.) in $CDCl_3$, DMSO-d_6, or C_5D_5N. The 7.26, 2.49, and 7.19 ppm resonances of residual $CHCl_3$, CHD_2SOCD_3, and C_5HD_4N and 77.0, 39.5, and 123.5 ppm resonances of $CDCl_3$, DMSO-d_6, and C_5D_5N were used as internal references for ^{1}H and ^{13}C chemical shifts, respectively. EIMS spectra were recorded on a JEOL FABmate spectrometer; ESIMS spectra, on a JEOL JMS-T100LP spectrometer.

Sponge Description. Sponge SS-1047 was collected at a depth of 10−20 m, off Gesashi, Okinawa, in May 2003. This specimen is dark brown and appears to be branching or may have been flattened physically at some stage after collection. The surface looks unarmoured, but this is hard to determine from the specimen. The mesohyl is very dense. The skeleton consists of primary, secondary, and tertiary fibers, which have a faint, fine pith centrally and faint laminations in the bark. The skeletal reticulations are small and dense. The primary fibers are 90 μm thick, the secondaries 40−50 μm thick, and the tertiaries 10−15 μm thick. The primary fibers are not cored, and they do not appear to bifurcate at the surface.

Sponge SS-265 was collected at a depth of 10−20 m, at Kerama Islands, in July 1984. The preserved sponge has a dark yellow-brown conulose surface and light yellow-brown interior. The mesohyl is dense; the sponge is firm and slightly compressible. Primary and secondary skeletal fibers are the same size; the tersiary skeletal fibers are finer. The primary fibers are 55 μm wide. The fibers are stratified. Primary fibers are uncored.

Sponge SS-1208 was collected at a depth of 10−20 m, off Unten-Port, Nakijin, Okinawa, in August 2007: open meshed mound, surface characters unknown, purplish-brown in EtOH, firm, slightly compressible, slightly springy sponge; reticulate fiber skeleton with primary and secondary fibers of a similar size, ~100 μm thick, mesh spaces ≤400 μm wide; fine tertiary network between with fibers, ~15 μm thick, mesh spaces ≤90 μm. All fibers are uncored and laminated with faint central pith, no surface details on specimen to determine ectosomal skeleton, and primary fibers are not cored.

All three collections of sponges were kept frozen until used. The voucher specimens were deposited at Graduate School of Pharmaceutical Sciences, Hokkaido University.

Extraction and Isolation. The sponge SS-1047 (0.30 kg, wet weight) was extracted and partitioned as described previously.[11] The EtOAc-soluble materials (1.2 g) were subjected to a silica gel column (*n*-hexane/EtOAc) to give less polar fractions α and β, a mixture of isospongiaquinone and mamanuthaquinone, and a polar fraction containing nakijiquinone I. Fraction α was purified by C_{18} column (MeOH/H_2O) and C_{18} HPLC (Wakosil-II 5C18AR, Wako Pure Chemical Industries, Ltd., 250 × 10 mm; eluent, MeCN/H_2O/CF_3CO_2H, 90:10:0.05; flow rate, 2.0 mL/min; UV detection at 300 nm) to yield nakijiquinones P (**7**, 2.8 mg, 0.00093% wet weight) and Q (**8**, 24.7 mg, 0.0082%). Fraction β was separated by C_{18} column (MeOH/H_2O/CF_3CO_2H) and C_{18} HPLC (Luna 5u Phenyl-Hexyl, Phenomenex, 250 × 10 mm; eluent, MeCN/H_2O/CF_3CO_2H, 80:20:0.05; flow rate, 2.0 mL/min; UV detection at 300 nm) to obtain nakijiquinone O (**6**, 0.9 mg, 0.00030%) and two crude fractions, γ and δ. Fraction γ was purified by C_{18} HPLC (Luna 5u C18(2), Phenomenex, 250 × 10 mm; MeOH/H_2O/Et_2NH, 70:30:0.1; flow rate, 2.0 mL/min; UV detection at 300 nm) to afford nakijiquinones K (**2**, 1.4 mg, 0.00047%) and N (**5**, 4.0 mg, 0.0013%). Fraction δ was purified by a C_{18} HPLC (Luna 5u Phenyl-Hexyl, 250 × 10 mm; MeOH/H_2O/Et_2NH, 65:35:0.1; flow rate, 2.0 mL/min; UV detection at 300 nm) to give nakijiquinones J (**1**, 0.7 mg, 0.00023%) and M (**4**, 1.6 mg, 0.00053%). Mamanuthaquinone (**10**) and isospongiaquinone (**11**) were isolated by C_{18} column (MeOH/H_2O) and C_{18}

HPLC (Luna 5u Phenyl-Hexyl, 250 × 10 mm; eluent, MeOH/H_2O/CF_3CO_2H, 85:15:0.05; flow rate, 2.5 mL/min; UV detection at 320 nm). A part of the sponge SS-265 (1.4 kg, wet weight) was extracted with MeOH (4.3 and 3.2 L). The MeOH extract (68.4 g) was partitioned between $CHCl_3$ and H_2O. Part of the $CHCl_3$-soluble materials (2.3 g) was subjected to silica gel column (*n*-hexane/EtOAc), C_{18} column (MeOH/H_2O), silica gel column (*n*-hexane/acetone), and repeated C_{18} HPLC (Wakosil-II 5C18AR, 250 × 10 mm; eluent, MeCN/H_2O/CF_3CO_2H, 90:10:0.1; flow rate, 2.0 mL/min; UV detection at 300 nm and Luna 5u C18(2), 250 × 10 mm; MeOH/H_2O/Et_2NH, 70:30:0.1; flow rate, 2.0 mL/min; UV detection at 300 nm) to afford nakijiquinone L (**3**, 1.8 mg, 0.00013%), ilimaquinone (**13**), smenospongine, smenospongidine, smenospongorine, and smenospongiarine. The sponge SS-1208 (0.4 kg, wet weight) was extracted with MeOH (3 × 0.8 L) and MeOH/toluene (3:1) (1 × 0.8 L). The combined extract (15.9 g) was partitioned between $CHCl_3$ and H_2O (3 × 500 mL). $CHCl_3$-soluble portions (2.7 g) were purified by silica gel column (*n*-hexane/EtOAc and $CHCl_3$/MeOH), C_{18} column (MeOH/H_2O/CF_3CO_2H), and repeated C_{18} HPLC (Luna 5u Phenyl-Hexyl, 250 × 10 mm; eluent, MeCN/H_2O/CF_3CO_2H, 70:30:0.1; flow rate, 2.0 mL/min; UV detection at 300 nm and Wakosil-II 5C18AR, 250 × 10 mm; eluent, MeCN/H_2O/CF_3CO_2H, 75:25:0.1; flow rate, 2.0 mL/min; UV detection at 300 nm) to yield nakijiquinone R (**9**, 0.8 mg, 0.00020%) and nakijiquinones A−E and G.

Nakijiquinone J (1): purple-red, amorphous solid; $[\alpha]^{23}_D$ −42 (*c* 0.25, $CHCl_3$); IR (film) ν_{max} 3290, 1680, 1650, 1590, 1520, 1460, 1390, 1200 cm^{-1}; UV (MeOH) λ_{max} 338 (log ϵ 4.06), 511 nm (2.63); ^1H NMR ($CDCl_3$), see Supporting Information; ^{13}C NMR ($CDCl_3$), see Table 1 and Supporting Information; EIMS *m/z* (%) 413 (M^+, 15), 223 (100), 191 (10), 168 (15), 166 (14), 152 (16), 119 (18); HREIMS *m/z* 413.2916 $[M]^+$ (calcd for $C_{26}H_{39}NO_3$, 413.2930).

Nakijiquinone K (2): purple-red, amorphous solid; $[\alpha]^{21}_D$ +136 (*c* 0.25, $CHCl_3$); IR (film) ν_{max} 3270, 1680, 1650, 1590, 1510, 1450, 1380, 1210 cm^{-1}; UV (MeOH) λ_{max} 336 (log ϵ 4.09), 505 nm (2.67); ^1H NMR ($CDCl_3$), see Supporting Information; ^{13}C NMR ($CDCl_3$), see Table 1 and Supporting Information; HREIMS *m/z* 413.2940 $[M]^+$ (calcd for $C_{26}H_{39}NO_3$, 413.2930).

Nakijiquinone L (3): purple-red, amorphous solid; $[\alpha]^{22}_D$ +33 (*c* 0.2, $CHCl_3$); IR (film) ν_{max} 3280, 1640, 1590, 1510, 1380, 1200 cm^{-1}; UV (MeOH) λ_{max} 501 (log ϵ 2.88), 327 (4.17), 243 (3.86), 208 nm (4.25); ^1H NMR ($CDCl_3$), see Supporting Information; ^{13}C NMR ($CDCl_3$), see Table 1 and Supporting Information; EIMS *m/z* (%) 413 (M^+, 15), 223 (100), 191 (3), 166 (10), 152 (10), 95 (10); HREIMS *m/z* 413.2934 $[M]^+$ (calcd for $C_{26}H_{39}NO_3$, 413.2930).

Nakijiquinone M (4): purple-red, amorphous solid; $[\alpha]^{21}_D$ −38 (*c* 0.2, $CHCl_3$); IR (film) ν_{max} 3270, 1680, 1650, 1590, 1510, 1460, 1380, 1200 cm^{-1}; UV (MeOH) λ_{max} 338 (log ϵ 4.21), 515 nm (2.63); ^1H NMR ($CDCl_3$), see Supporting Information; ^{13}C NMR ($CDCl_3$), see Table 1 and Supporting Information; EIMS *m/z* (%) 413 (M^+, 24), 223 (100), 191 (13), 166 (20), 152 (17), 119 (20); HREIMS *m/z* 413.2947 $[M]^+$ (calcd for $C_{29}H_{37}NO_3$, 413.2930).

Nakijiquinone N (5): purple-red, amorphous solid; $[\alpha]^{21}_D$ +124 (*c* 0.25, $CHCl_3$); IR (film) ν_{max} 3270, 1680, 1640, 1590, 1510, 1380, 1210 cm^{-1}; UV (MeOH) λ_{max} 336 (log ϵ 4.17), 515 nm (2.55); ^1H NMR ($CDCl_3$), see Supporting Information; ^{13}C NMR ($CDCl_3$), see Table 1 and Supporting Information; EIMS *m/z* (%) 413 (M^+, 7), 223 (100), 191 (3), 166 (8), 152 (9), 107 (8), 95 (18); HREIMS *m/z* 413.2947 $[M]^+$ (calcd for $C_{26}H_{39}NO_3$, 413.2930).

Nakijiquinone O (6): purple-red, amorphous solid; $[\alpha]^{23}_D$ +160 (*c* 0.1, $CHCl_3$); IR (film) ν_{max} 3270, 1730, 1640, 1590, 1510, 1380, and 1210 cm^{-1}; UV (MeOH) λ_{max} 334 (log ϵ 4.29), 509 nm (2.86); ^1H NMR ($CDCl_3$), see Supporting Information; ^{13}C NMR ($CDCl_3$), see Table 1 and Supporting Information; EIMS *m/z* (%) 399 (M^+, 8), 209 (100), 191 (3), 166 (11), 152 (9), 107 (9), 95 (22); HREIMS *m/z* 399.2790 $[M]^+$ (calcd for $C_{25}H_{37}NO_3$, 399.2773).

Nakijiquinone P (7): purple-red, amorphous solid; $[\alpha]^{23}_D$ −14 (*c* 0.2, $CHCl_3$); IR (film) ν_{max} 3290, 1730, 1650, 1590, 1510, 1460, 1380, 1360, 1220 cm^{-1}; UV (MeOH) λ_{max} 336 (log ϵ 4.28), 507 nm (2.84); ^1H NMR ($CDCl_3$), see Supporting Information; ^{13}C NMR ($CDCl_3$), see Table 1 and Supporting Information; EIMS *m/z* (%) 447 (M^+, 9), 257 (100), 191 (2), 166 (20), 152 (5), 105 (10), 95 (15); HREIMS *m/z* 447.2790 $[M]^+$ (calcd for $C_{29}H_{37}NO_3$, 447.2773).

Nakijiquinone Q (8): purple-red. amorphous solid; $[\alpha]^{25}_D$ +180 (*c* 0.1, $CHCl_3$); IR (film) ν_{max} 3270, 1730, 1640, 1590, 1510, 1460, 1380, 1210 cm^{-1}; UV (MeOH) λ_{max} 335 (log ϵ 4.20), 502 nm (2.74); ^1H NMR ($CDCl_3$), see Supporting Information; ^{13}C NMR ($CDCl_3$), see Table 1 and Supporting Information; EIMS *m/z* (%) 447 (M^+, 25), 257 (100), 209 (17), 191 (18), 168 (45), 166 (48), 152 (17), 119 (42), 105 (40); HREIMS *m/z* 447.2783 $[M]^+$ (calcd for $C_{29}H_{37}NO_3$, 447.2773).

Nakijiquinone R (9): purple-red, amorphous solid; $[\alpha]^{22}_D$ +38 (*c* 0.2, MeOH); IR (KBr) ν_{max} 3450, 1640, 1600, 1530, 1380, 1210 cm^{-1}; UV (MeOH) λ_{max} 237 (log ϵ 2.8), 345 (4.00), 513 nm (2.47); ^1H NMR (DMSO-d_6), see Supporting Information; ^{13}C NMR (DMSO-d_6), see Table 1 and Supporting Information; ESIMS (neg) *m/z* 450 $[M - H]^-$; HRESIMS (neg) *m/z* 450.1955 $[M - H]^-$ (calcd for $C_{23}H_{32}NO_6S$, 450.1950).

Conversion of Mamanuthaquinone (10) to Nakijiquinone J (1). A mixture of mamanuthaquinone (**10**, 2.1 mg, 5.9 μmol) and (*S*)-(−)-2-methylbutylamine (1.0 μL, 0.74 mg, 8.5 μmol) in the presence of $NaHCO_3$ (22.9 mg) in EtOH (1 mL) was stirred at room temperature for 12 h. After filtration, the filtrate was evaporated *in vacuo*, and the residue was purified by C_{18} HPLC (Luna 5u Phenyl-Hexyl, 250 × 10 mm; eluent, MeOH/H_2O/CF_3CO_2H, 85:15:0.1; flow rate, 2.0 mL/min; UV detection at 300 nm) to yield nakijiquinone J (**1**, 0.9 mg, 2.2 μmol, 37%): ^1H and ^{13}C NMR (C_5D_5N), see Supporting Information.

Conversion of Isospongiaquinone (11) to Nakijiquinone K (2). Nakijiquinone K (**2**) was obtained from isospongiaquinone (**11**, 5.0 mg, 14.0 μmol), (*S*)-2-methylbutylamine (2.5 μL, 1.8 mg, 20.7 μmol), $NaHCO_3$ (11.7 mg), and EtOH (1 mL) in 40% yield (2.3 mg, 5.6 μmol) by the same procedure as described above: ^1H and ^{13}C NMR ($CDCl_3$), see Supporting Information.

Conversion of Ilimaquinone (13) to Nakijiquinone L (3). Nakijiquinone L (**3**) was obtained from ilimaquinone (**13**, 6.3 mg, 17.6 μmol), (*S*)-2-methylbutylamine (2.5 μL, 1.8 mg, 20.7 μmol), $NaHCO_3$ (31.2 mg), and EtOH (1 mL) in 43% yield (3.1 mg, 7.5 μmol) by the same procedure as described above: ^1H and ^{13}C NMR ($CDCl_3$), see Supporting Information.

Kinase Inhibition Assays. The inhibitory activities of tested compounds against EGFR and HER2 were evaluated by using Z′-LYTE kinase assay kit (tyrosine 4 peptide for EGFR and tyrosine 6 peptide for HER2, respectively) and EGFR and HER2 kinases provided by Invitrogen. The assay was carried out essentially according to the kit instructions.

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Supporting Information Available: A list of new and known sesquiterpenoid quinones contained in each of five sponges of the family Spongiidae studied in our laboratory and ^1H and ^{13}C NMR data for **1**−**9** and derived **1**−**3**. These materials are available free of charge via the Internet at http://pubs.acs.org.

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