Bioorganic & Medicinal Chemistry 16 (2008) 7561–7564

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Nakijiquinones G–I, new sesquiterpenoid quinones from marine sponge

Yohei Takahashi [a], Takaaki Kubota [a], Junji Ito [b], Yuzuru Mikami [b], Jane Fromont [c], Jun'ichi Kobayashi [a,*]

[a] Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan
[b] Medical Mycology Research Center, Chiba University, Chiba 260-0856, Japan
[c] Western Australian Museum, Locked Bag 49, Welshpool DC, WA 6986, Australia

ARTICLE INFO

Article history:
Received 14 June 2008
Revised 12 July 2008
Accepted 15 July 2008
Available online 18 July 2008

Keywords:
Marine sponge
Sesquiterpenoid quinones
Nakijiquinones G–I

ABSTRACT

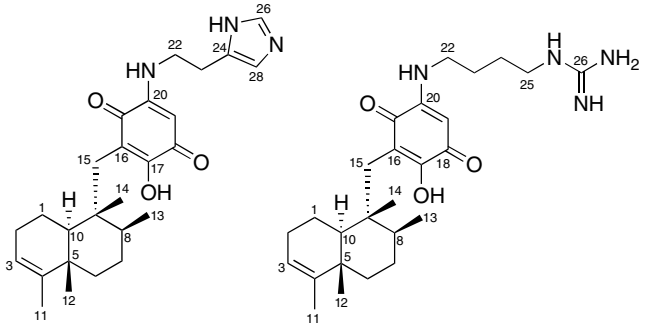
Three new sesquiterpenoid quinones, nakijiquinones G–I (**1**–**3**), containing a different amino group derived from amino acids have been isolated from Okinawan marine sponges of the family *Spongiidae*, and the structures and relative stereochemistry of **1**–**3** were elucidated on the basis of the spectral data. Nakijiquinones G–I (**1**–**3**) showed modest cytotoxicity and inhibitory activity against HER2 kinase, while nakijiquinone H (**2**) exhibited antimicrobial activity.

1. Introduction

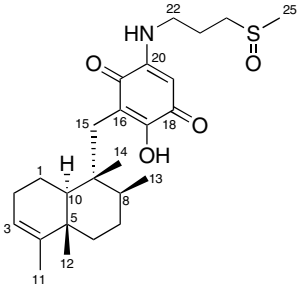
Marine sponges contain a number of unique secondary metabolites with a diversity of biological activities.[1] In our search for bioactive metabolites from marine organisms,[2] we previously isolated new sesquiterpenoid quinones, nakijiquinones A–F, from an Okinawan sponge of the family *Spongiidae*.[3–5] Further investigation of extracts of another collection of sponges of this family resulted in the isolation of three new sesquiterpenoid quinones containing a different amino group derived from amino acids, nakijiquinones G–I (**1**–**3**). Here we describe the isolation and structure elucidation of **1**–**3**.

2. Results and discussion

The sponge (SS-1074) collected off Unten Port, Okinawa, was extracted with MeOH. The MeOH extracts were partitioned between EtOAc and H_2O, and then H_2O-soluble portions were extracted with *n*-BuOH. EtOAc-soluble materials were purified by silica gel columns followed by a C_{18} column and C_{18} HPLC to afford nakijiquinone G (**1**), while nakijiquinone H (**2**) was obtained from *n*-BuOH-soluble materials by purification with silica gel columns followed by a C_{18} column and C_{18} HPLC. The MeOH extract of another collection of the sponge (SS-1047) collected off Gesashi, Okinawa, was partitioned between EtOAc and H_2O. EtOAc-soluble materials were purified by silica gel columns followed by a C_{18} column and C_{18} HPLC to give nakijiquinone I (**3**). Known related terpenoid quinines such as dictyoceratins A–C,[6,7] isospongiaquinone,[8] nakijinol,[9] nakijiquinones A–D,[3,4] and neoavarol[10] have been isolated from both sponges together with **1**–**3**.



1 **2**



3

* Corresponding author. Tel.: +81 11 706 3239; fax: +81 11 706 4989.
E-mail address: jkobay@pharm.hokudai.ac.jp (J. Kobayashi).

0968-0896/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2008.07.028

Nakijiquinone G (**1**) was obtained as an optically active red amorphous solid {$[\alpha]_D^{24}$ +109 (*c* 0.25, MeOH)}. HRFABMS analysis revealed the molecular formula to be $C_{26}H_{35}N_3O_3$ [*m*/*z* 440.2912 $(M+2H+H)^+$, Δ −0.2 mmu]. IR absorptions (3280 and 1670 cm^{-1}) implied the presence of OH and/or NH, and carbonyl functionalities. UV absorptions (λ_{max} 320 and 494 nm) suggested the existence of a quinone chromophore. Analysis of the ^1H–^1H COSY spectrum of **1** revealed connectivities of three partial structures, **a** (C-1 to C-4 and C-10, and C-4 to C-11), **b** (C-6 to C-8, and C-8 to C-13), and **c** (N-20 to C-23) as shown in Figure 1. HMBC correlations of H-3 to C-5, and H_3-12 to C-5, C-6, and C-10 indicated that C-4, C-6, C-10, and C-12 were connected to C-5. Connectivities of C-8, C-10, C-14, and C-15 to C-9 were implied by HMBC cross-peaks of H-10, H_3-13, H_3-14, and H_2-15 to C-9, and H_2-15 to C-10. UV absorptions (λ_{max} 320 and 494 nm) and ^{13}C NMR signals of C-16 (δ_C 113.9), C-17 (δ_C 158.5), C-18 (δ_C 178.3), C-19 (δ_C 92.2), C-20 (149.7), and C-21 (183.0) in **1** suggested the existence of a hydroxy quinone moiety. HMBC correlations of H-19 to C-17 and C-21 indicated that the substitution pattern of the quinone ring in **1** was the same as that of nakijiquinone A.[3] Connections of C-15 to C-16 and N-20 to C-20 were deduced from HMBC cross-peaks for H_2-15 to C-16, C-17, and C-21, and NH-20 to C-19 and C-21, and H_2-22 to C-20. Remaining three carbons (C-24, C-26, and C-28) and two nitrogen atoms were attributed to those of an imidazole ring on the basis of ^{13}C NMR chemical shifts of C-24 (δ_C 130.9), C-26 (δ_C 134.0), and C-28 (δ_C 116.2). Cross-peaks of H_2-23 to C-24 and C-28 in the HMBC spectrum revealed the connection between C-23 and C-24. Thus, the gross structure of nakijiquinone G was elucidated to be **1**.

The relative stereochemistry of a decaline moiety in nakijiquinone G (**1**) was elucidated from NOESY correlations as shown in Figure 2. The α-configuration of H-10 and β-configurations of C-12, C-13, and C-14 were deduced from NOESY correlations of H-8/H-10, H-10/H_2-15, and H_3-12/H_3-14, indicating a twist-boat conformation of ring A (C-1 to C-5 and C-10) and a chair conformation of ring B (C-5 to C-10).

Nakijiquinone H (**2**) was obtained as an optically active red amorphous solid {$[\alpha]_D^{22}$ +66 (*c* 0.25, MeOH)} and the molecular formula was established to be $C_{26}H_{40}N_4O_3$ by HRFABMS data [*m*/*z* 459.3348 [$(M+2H+H)^+$, calcd for $C_{26}H_{43}N_4O_3$, Δ +2.7 mmu]. UV absorptions (λ_{max} 321 and 495 nm) of **2** indicated the presence of a hydroxy quinone, while IR absorptions (3280 and 1670 cm^{-1}) implied the existence of OH and/or NH, and carbonyl functionalities. The ^1H and ^{13}C NMR spectra of **2** were close to those of **1** except for the side chain moiety. The ^1H–^1H COSY and HMBC spectra indicated that **2** possessed the same terpenoid quinone moiety (C-1 to C-21) as that of **1**(Fig. 3). Analysis of the ^1H–^1H COSY spectra revealed connectivities of N-20 to C-22, C-22 to C-25, and C-25 to N-25. The HMBC cross-peak of H_2-25 to imino carbon (C-26, δ_C 156.9) and the molecule formula indicated that an agmatine unit

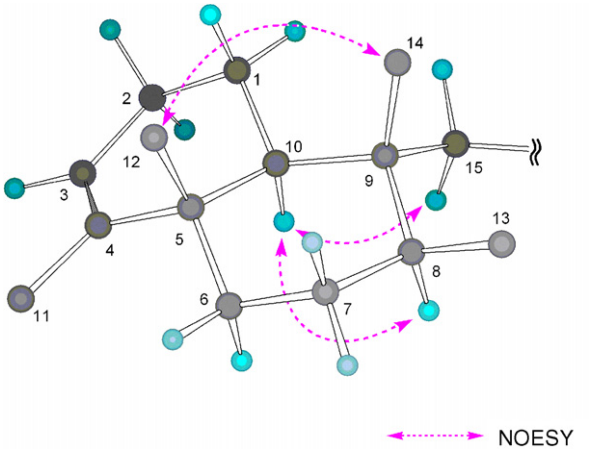


Figure 2. Selected NOESY correlations and relative stereochemistry for a decaline moiety (C-1–C-15) of nakijiquinone G (**1**) (hydrogen atoms of methyl groups were omitted).

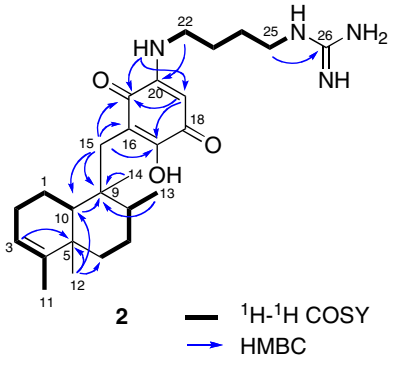


Figure 3. Selected 2D NMR correlations for nakijiquinone H (**2**).

was attached to C-20 in **2** in place of a histamine unit in **1**. Thus, the gross structure of nakijiquinone H was assigned as **2** (Fig. 3). The relative stereochemistry of a decaline ring (C-1–C-15) in **2** was elucidated to be the same as that of the corresponding moiety in **1** on the basis of the NOESY data.

Nakijiquinone I (**3**) was obtained as an optically active red amorphous solid {$[\alpha]_D^{24}$ +158 (*c* 0.25, MeOH)} and the molecular formula was established to be $C_{25}H_{37}NO_4S$ by HRFABMS data [*m*/*z* 450.2671 $(M+2H+H)^+$, Δ −1.6 mmu]. Detailed analysis of 1D and 2D NMR data suggested that **3** possessed the same terpenoid quinone moiety (C-1 to C-21) as that of **1** (Fig. 4). The molecule formula and the HMBC correlation of H_3-25 to C-24 (δ_C 50.4) implied that a 3-(methylsulfinyl)propan-1-amine unit was

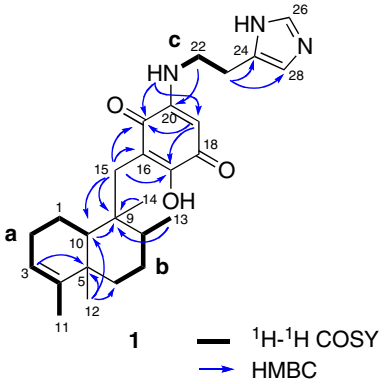


Figure 1. Selected 2D NMR correlations for nakijiquinone G (**1**).

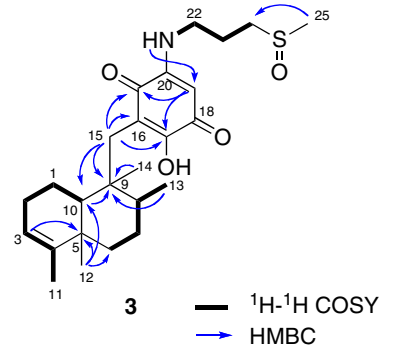


Figure 4. Selected 2D NMR correlations for nakijiquinone I (**3**).

Table 1
Antimicrobial activities of nakijiquinones G–I (**1**–**3**)

Compound	MIC (μg/mL)						
	B. subtilus	*E. coli*	*M. luteus*	*S. aureus*	*C. neoformans*	*C. albicans*	*A. niger*
1	33.3	>33.3	33.3	33.3	>33.3	>33.3	>33.3
2	33.3	>33.3	16.7	33.3	8.35	8.35	16.7
3	>33.3	>33.3	33.3	>33.3	>33.3	>33.3	>33.3

Bacteria: *Bacillus subtilis*, *Escherichia coli*, *Micrococcus luteus*, and *Stapylococcus aureus*. Fungi: *Cryptococcus neoformans*, *Candida albicans*, and *Aspergillus niger*.

attached to C-20 in **3** in place of a histamine unit in **1**, Thus, the gross structure of nakijiquinone I was assigned as **3** (Fig. 4). Analysis of the NOESY spectrum of **3** revealed that the relative stereochemistry of a decaline ring in **3** was the same as that of the C-1–C-15 moiety of **1**.

Nakijiquinones G–I (**1**–**3**) are new sesquiterpenoid quinones having an amine residue such as histamine, agmatine, and 3-(methylsulfinyl)propan-1-amino group, respectively, although some sesquiterpenoid quinones containing an amino acid residue have been isolated from a marine sponge.[3,4] Nakijiquinones G–I (**1**–**3**) showed modest cytotoxicity against P388 murine leukemia (IC_{50}, 3.2, 2.4, and 2.9 μg/mL, respectively), L1210 murine leukemia (IC_{50}, 2.9, 8.5, and 2.4 μg/mL, respectively), and KB human epidermoid carcinoma cells (IC_{50}, 4.8, >10, and 5.6 μg/mL, respectively) in vitro. Nakijiquinones G–I (**1**–**3**) exhibited inhibitory activity against HER2 kinase.[11] Furthermore, antimicrobial activities of **1**–**3** are shown in Table 1. In particular, nakijiquinone H (**2**) showed antibacterial activity against *Micrococcus luteus* (MIC, 16.7 μg/mL), and antifungal activities against *Cryptococcus neoformans* (MIC, 8.35 μg/mL), *Candida albicans* (MIC, 8.35 μg/mL), and *Aspergillus niger* (MIC, 16.7 μg/mL).

3. Experimental

3.1. General

Optical rotations were recorded on a JASCO P-1030 polarimeter. IR and UV spectra were recorded on JASCO FT/IR-230 and Shimadzu UV-1600PC spectrophotometer, respectively. ^1H and ^{13}C NMR spectra were recorded on a Bruker AMX-600 spectrometer using 2.5 mm micro cells (Shigemi Co., Ltd) for DMSO-$_{d6}$. The 2.49 and 49.8 ppm resonances of residual DMSO-d_6 were used as internal references for ^1H and ^{13}C NMR spectra, respectively. FAB mass spectra were obtained on a JEOL JMS-700TZ spectrometer.

3.2. Sponge description

The sponge (SS-1047) is dark brown and appears to be branching or may have been flattened physically at some stage after collection. The surface looks unarmoured. The mesohyl is very dense. The skeleton consists of primary, secondary and tertiary fibres, which have a faint, fine pith centrally and faint laminations in the bark. The skeletal reticulations are small and dense. The primary fibres are 90 μm thick, the secondaries 40–50 μm thick, and the tertiaries 10–15 μm thick. The other sponge (SS-1074) could be the same as SS-1047. The voucher specimens were deposited at Graduate School of Pharmaceutical Sciences, Hokkaido University.

3.3. Collection, extraction and isolation

The sponge (SS-1074) was collected off the Unten Port, Okinawa. The sponge (0.6 kg, wet weight) was extracted with MeOH. The MeOH extract (20.2 g) was partitioned between EtOAc and H_2O, and H_2O-soluble portions were extracted with *n*-BuOH. EtOAc-soluble materials (2.2 g) were purified by silica gel columns (*n*-hexane/acetone, and then $CHCl_3$/MeOH/H_2O) followed by a C_{18} column (MeOH/H_2O/TFA) and C_{18} HPLC (Wakosil-II 5C18 AR, Wako Pure Chemical Ind., Ltd, 10 × 250 mm; eluent MeOH/H_2O/TFA, 80:20:0.05; flow rate, 3.0 mL/min; UV detection at 300 nm) to afford nakijiquinone G (**1**, 1.8 mg, t_R 8.0 min). A part (1.3 g) of *n*-BuOH-soluble materials (1.9 g) was purified by silica gel columns ($CHCl_3$/MeOH/H_2O) followed by a C_{18} column (MeOH/H_2O/TFA) and C_{18} HPLC (Wakosil-II 5C18 AR, 10 × 250 mm; eluent MeOH/H_2O/TFA, 80:20:0.05; flow rate, 3.0 mL/min; UV detection at 300 nm) to give nakijiquinone H (**2**, 3.4 mg, t_R 9.6 min). Another sponge (SS-1047, 0.3 kg, wet weight) collected off Gesashi, Okinawa, was extracted with MeOH. The extract (10.8 g) was partitioned between EtOAc and H_2O. The EtOAc-soluble materials (1.2 g) was purified by silica gel columns (*n*-hexane/EtOAc, then $CHCl_3$/MeOH/H_2O) followed by a C_{18} column (MeOH/H_2O/TFA) and C_{18} HPLC (Luna 5u Phenyl-Hexyl, Phenomenex, 250 × 10 mm; eluent, MeCN/H_2O/TFA, 70:30:0.05; flow rate, 1.5 mL/min; UV detection at 300 nm) to yield nakijiquinone I (**3**, 3.5 mg, t_R 20.4 min).

3.4. Nakijiquinone G (1)

Red amorphous solid; $[\alpha]_D^{24}$ +109 (*c* 0.25, MeOH); IR (film) ν_{max} 3280, 1670, 1590, 1510, 1460, 1380, 1210, and 1190 cm^{-1}; UV (MeOH) λ_{max} 320 (ε 11,600), and 494 nm (1000); ^1H NMR (DMSO-d_6) δ 8.85 (1H, br s, H-26), 7.82 (1H, br s, 20-NH), 7.39 (1H, br s, H-28), 5.43 (1H, s, H-19), 5.04 (1H, br s, H-3), 3.41 (2H, br d, J = 5.6 Hz, H_2-22), 2.90 (2H, br s, H_2-23), 2.42 (1H, d, J = 13.6 Hz, H-15a), 2.30 (1H, d, J = 13.6 Hz, H-15b), 1.96 (1H, m, H-1a), 1.85 (2H, br s, H_2-2), 1.53 (1H, m, H-6a), 1.46 (3H, br s, H_3-11), 1.32 (1H, m, H-1b), 1.26 (2H, m, H_2-7), 1.21 (1H, m, H-8), 0.95 (2H, m, H-6b and H-10), 0.92 (3H, s, H-12), 0.89 (3H, d, J = 5.9 Hz, H_3-13), and 0.74 (3H, s, H_3-14); ^{13}C NMR (DMSO-d_6) δ 183.0 (s, C-21), 178.3 (s, C-18), 158.5 (s, C-17), 149.7 (s, C-20), 143.2 (s, C-4), 134.0 (d, C-26), 130.9 (s, C-24), 120.7 (d, C-3), 116.2 (d, C-28), 113.9 (s, C-16), 92.2 (d, C-19), 47.2 (d, C-10), 42.0 (s, C-9), 41.0 (t, C-22), 37.9 (s, C-5), 37.3 (d, C-8), 35.6 (t, C-6), 32.0 (t, C-15), 27.6 (t, C-7), 26.5 (t, C-2), 22.9 (t, C-23), 19.9 (q, C-12), 19.5 (t, C-1), 18.0 (q, C-11), 17.8 (q, C-13), and 17.2 (q, C-14); FABMS (positive, glycerol matrix) *m*/*z* 440 $(M+2H+H)^+$; HRFABMS *m*/*z* 440.2912 [$(M+2H+H)^+$, calcd for $C_{26}H_{38}N_3O_3$, 440.2914].

3.5. Nakijiquinone H (2)

Red amorphous solid; $[\alpha]_D^{22}$ +66 (*c* 0.25, MeOH); IR (film) ν_{max} 3280, 1670, 1600, 1540, 1460, 1380, and 1200 cm^{-1}; UV (MeOH) λ_{max} 321 (ε 9200), and 495 nm (650); ^1H NMR (DMSO-d_6) δ 7.81 (1H, br t, J = 5.9 Hz, 20-NH), 7.63 (1H, br t, J = 5.6 Hz, 25-NH), 7.6–6.8 (3H, br, guanidine-NH), 5.33 (1H, s, H-19), 5.04 (1H, br s, H-3), 3.13 (2H, td, J = 6.5 and 6.2 Hz, H_2-22), 3.08 (2H, dt, J = 6.3 and 6.3 Hz, H_2-25), 2.42 (1H, d, J = 13.6 Hz, H-15a), 2.31 (1H, d, J = 13.6 Hz, H-15b), 1.99 (1H, m, H-1a), 1.87 (2H, m, H_2-2), 1.53 (3H, m, H-6a and H_2-23), 1.46 (3H, br s, H_3-11), 1.46 (2H, overlapped, H_2-24), 1.33 (1H, m, H-1b), 1.26 (2H, m, H_2-7), 1.21 (1H, m, H-8), 0.95 (2H, m, H-6b and H-10), 0.92 (3H, s, H-12), 0.89 (3H, d, J = 5.8 Hz, H_3-13), and 0.74 (3H, s, H_3-14); ^{13}C NMR (DMSO-d_6) δ 183.2 (s, C-21), 177.9 (s, C-18), 158.7 (s, C-17), 156.9 (s, C-26), 150.0 (s, C-20), 143.3 (s, C-4), 120.8 (d, C-3), 113.8 (s, C-16), 91.7 (d, C-19), 47.2 (d, C-10), 42.0 (s, C-9), 41.7 (t, C-22), 40.5 (t, C-25), 37.9 (s, C-5), 37.4 (d, C-8), 35.6 (t, C-6), 32.0 (t, C-15), 27.6 (t, C-7), 26.5 (t, C-2), 26.1 (t, C-24), 24.6 (t, C-23), 19.9 (q, C-12), 19.5 (t, C-1), 18.0

(q, C-11), 17.8 (q, C-13), and 17.2 (q, C-14); FABMS (positive, glycerol matrix) m/z 459 $(M+2H+H)^+$; HRFABMS m/z 459.3348 $[(M+2H+H)^+$, calcd for $C_{26}H_{43}N_4O_3$, 459.3321].

3.6. Nakijiquinone I (3)

Red amorphous solid; $[\alpha]_D^{24}$ +158 (c 0.25, MeOH); IR (film) ν_{max} 3270, 1650, 1590, 1510, 1460, 1380, 1210, and 1020 cm^{-1}; UV (MeOH) λ_{max} 323 (ε 16,100) and 496 nm (1100); ^1H NMR (DMSO-d_6) δ 7.88 (1H, br s, 20-NH), 5.37 (1H, s, H-19), 5.04 (1H, br s, H-3), 3.25 (2H, br d, J = 6.0 Hz, H_2-22), 2.77 (1H, m, H-24a), 2.66 (1H, m, H-24b), 2.50 (3H, s, H_3-25), 2.42 (1H, d, J = 13.6 Hz, H-15a), 2.31 (1H, d, J = 13.6 Hz, H-15b), 1.99 (1H, m, H-1a), 1.89 (4H, m, H_2-2 and H_2-23), 1.53 (1H, m, H-6a), 1.46 (3H, br s, H_3-11), 1.33 (1H, m, H-1b), 1.26 (2H, m, H_2-7), 1.22 (1H, m, H-8), 0.97 (2H, m, H-6b and H-10), 0.92 (3H, s, H-12), 0.90 (3H, d, J = 5.6 Hz, H_3-13), and 0.74 (3H, s, H_3-14); ^{13}C NMR (DMSO-d_6) δ 183.1 (s, C-21), 178.0 (s, C-18), 158.5 (s, C-17), 149.9 (s, C-20), 143.2 (s, C-4), 120.7 (d, C-3), 113.8 (s, C-16), 91.9 (d, C-19), 50.4 (t, C-24), 47.1 (d, C-10), 41.9 (s, C-9), 41.2 (t, C-22), 38.0 (q, C-25), 37.8 (s, C-5), 37.2 (d, C-8), 35.5 (t, C-6), 32.0 (t, C-15), 27.6 (t, C-7), 26.5 (t, C-2), 20.69/20.68 (t, C-23), 19.9 (q, C-12), 19.5 (t, C-1), 18.0 (q, C-11), 17.8 (q, C-13), and 17.2 (q, C-14); FABMS (positive, glycerol matrix) m/z 450 $(M+2H+H)^+$; HRFABMS m/z 450.2671 $[(M+2H+H)^+$, calcd for $C_{25}H_{40}NO_4S$, 450.2687].

Acknowledgments

We thank Ms. S. Oka and A. Miyao, Center for Instrumental Analysis, Hokkaido University, for measurements of FAMBS, and Z. Nagahama, K. Uehara, and S. Furugen for their help with the sponge collection. This work was partly supported by a Grant-in-Aid from the Uehara Memorial Foundation and a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References and notes

1. Blunt, J. W.; Copp, B. R.; Hu, W.-P.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2008**, *25*, 35.
2. Araki, A.; Kubota, T.; Tsuda, M.; Mikami, Y.; Fromont, J.; Kobayashi, J. *Org. Lett.* **2008**, *10*, 2099.
3. Shigemori, H.; Madono, T.; Sasaki, T.; Mikami, Y.; Kobayashi, J. *Tetrahedron* **1994**, *50*, 8347.
4. Kobayashi, J.; Madono, T.; Shigemori, H. *Tetrahedron* **1995**, *51*, 10867.
5. Takahashi, Y.; Kubota, T.; Kobayashi, J. *Bioorg. Med. Chem.* **2008**, in press.
6. Nakamura, H.; Deng, S.; Kobayashi, J.; Ohizumi, Y.; Hirata, Y. *Tetrahedron* **1986**, *42*, 4197.
7. Kushlan, D. M.; Faulkner, D. J.; Parkanyi, L.; Clardy, J. *Tetrahedron* **1989**, *45*, 3307.
8. Kazlauskas, R.; Murphy, P. T.; Warren, R. G.; Wells, R. J.; Blount, J. F. *Aust. J. Chem.* **1978**, *31*, 2685.
9. Kobayashi, J.; Madono, T.; Shigemori, H. *Tetrahedron Lett.* **1995**, *36*, 5589.
10. Iguchi, K.; Sahashi, A.; Kohno, J.; Yamada, Y. *Chem. Pharm. Bull.* **1990**, *38*, 1121.
11. A structure–activity relationship study on nakijiquinone analogs as tyrosine kinase inhibitor was performed, please see: Kissau, L.; Stahl, P.; Mazitschek, R.; Giannis, A.; Waldmann, H. *J. Med. Chem.* **2003**, *46*, 2917.