Structure-activity Relationships of Stemphones, Potentiators of Imipenem Activity against Methicillin-resistant *Staphylococcus aureus*

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Abstract From a further purification study, four new stemphones D to G were isolated along with previously reported stemphones B and C from the culture broth of *Aspergillus* sp. FKI-2136. Twenty-one derivatives were semisynthetically prepared from stemphones C, E and G. Potentiation of imipenem activity against methicillin-resistant *Staphylococcus aureus* (MRSA) by all the stemphones including natural and semisynthetic ones was compared to study the structure-activity relationships. Derivatives with a free hydroxy or an *O*-acyl residue having a C2 to C5 carbon length at C-4 held the potentiating activity, but those with a longer acyl residue lost the activity. The presence of an oxo or a free hydroxy residue at C-10 is important for the potentiating activity because introduction of an alkyl or acyl residue at this position resulted in a loss of activity. Among them, stemphone E exhibited the most potent potentiation of imipenem activity against MRSA and the lowest cytotoxic activity against Jurkat cells.

Keywords stemphones, imipenem potentiator, methicillin-resistant *Staphylococcus aureus*, MRSA, fungal metabolite

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA), a

major and widespread pathogen in hospitals, has developed resistance to many other antibiotics [1, 2]. It is therefore important and necessary to find new antimicrobial agents and to devise new measures that are effective against MRSA infection. During the course of our screening for potentiators of imipenem activity against MRSA from microorganisms, stemphones B and C (Fig. 1) were previously discovered from the culture broth of *Aspergillus* sp. FKI-2136 [3]. From further precise analysis of the culture broth, new stemphones D, E, F and G (Fig. 1) were discovered. Furthermore, twenty-one derivatives (Fig. 2) were prepared semisynthetically from stemphones C, E and G. In this study, isolation, structural elucidation of new stemphones, preparation of the derivatives and the structure-activity relationships are described.

Materials and Methods

General Experimental Procedures

Fungal strain *Aspergillus* sp. FKI-2136 was used for production of stemphones [3]. Stemphones B and C and cochlioquinone D were obtained from an 11-day old culture broth of *Aspergillus* sp. FKI-2136 as previously reported [3]. Stemphones D and E were obtained from the 7-day old culture broth, and stemphones F and G were from the 15-day old culture broth as described below. For detection of stemphone analogs in culture broths, the samples (ethyl

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Fig. 1 Structures of stemphones B to G, and cochlioquinone D.

acetate extracts) were dissolved in methanol and analyzed by an HP1100 system (Hewlett Packard) under the following conditions: column, Symmetry (2.1×150 mm, Waters); flow rate, 0.2 ml/minute; mobile phase, a 20-minute linear gradient from 30% CH₃CN to 70% CH₃CN containing 0.05% H₃PO₄ and a continuous 5-minute linear gradient from 70% CH₃CN to 100% CH₃CN containing 0.05% H₃PO₄; detection, UV at 210 nm. Stemphones D, E, F and G were eluted as peaks with retention times of 17.3, 14.5, 11.7, 18.5 minutes, respectively.

UV spectra were recorded on a spectrophotometer (DU640, Beckman). IR spectra were recorded on a Fourier transform infrared spectrometer (FT-710, Horiba). Optical rotations were measured with a digital polarimeter (DIP-1000, JASCO). FAB-MS and HRFAB-MS spectra were recorded on a mass spectrometer (JMS-DX300, JEOL and JMS-AX505 HA, JEOL, respectively). The various NMR spectra were measured with a spectrometer (XL-400, Varian).

Isolation of Stemphones D to G

Stemphones D and E were isolated from the 7-day old culture broth of the fungus. The whole broth (4.9 liters) was centrifuged at 3000 rpm for 10 minutes to separate the mycelium and supernatant. After treating the mycelium

with Me₂CO (1.5 liters), the extracts were filtered and concentrated to remove Me₂CO. The resulting aqueous solution and the supernatant were mixed and extracted with EtOAc. The extracts were dried over Na₂SO₄ and concentrated *in vacuo* to dryness to yield crude materials (6.4 g). The materials were dissolved in CHCl₃, applied on a silica gel column (60 g), and eluted stepwise with 100:0 (100 ml), 100:1 (200 ml), 50:1 (300 ml), 10:1 (300 ml), 5:1 (300 ml) and 1:1 (300 ml) of CHCl₃-CH₃OH solvents (v/v). The 50:1 fraction was concentrated to give a red-brown oily material (704 mg), which was subjected to a second silica gel column (30 g). The material was eluted with 100:0 (50 ml×2), 100:1 (30 ml×5), 50:1 (25 ml×10), 10:1 (30 ml×5) and 5:1 (30 ml×5) of CHCl₃-CH₃OH solvents (v/v). The fractions of the 50:1 solvent were collected and concentrated *in vacuo* to dryness to give pure stemphone E (318 mg) as a white powder. The 3rd and 4th fractions of the 10:1 solvent were collected and concentrated to give a yellow powder (30 mg). This powder dissolved in MeOH (2.0 ml) was centrifuged at 3000 rpm to remove MeOH-insoluble impurities. The soluble supernatant (17 mg) containing stemphone D was finally purified with preparative HPLC (column; PEGASIL ODS (Senshu Sci. Co.), 20×250 mm; solvent, 45% CH₃CN; detection, UV at 210 nm; flow rate, 6.0 ml/minute). Under

these conditions, stemphone D was eluted as a peak with a retention time of 64 minutes. The fractions were pooled and concentrated *in vacuo* to dryness to give pure stemphone D (1.2 mg) as a yellow powder (Fig. 3A).

Stemphones F and G were isolated from the 15-day old culture broth. The whole broth (0.10 liter) was centrifuged at 3000 rpm for 10 minutes to separate the mycelium and supernatant. After treating the mycelium with Me₂CO, the extracts were filtered and concentrated to remove Me₂CO. The resulting solution and the supernatant were mixed and

extracted with EtOAc. The extracts were dried over Na₂SO₄ and concentrated *in vacuo* to dryness to yield crude materials (0.50 g). The materials were dissolved in CHCl₃, applied on a silica gel column (2.3 g), and eluted stepwise with 100 : 0 (10 ml×4), 100 : 1 (10 ml×2), 50 : 1 (10 ml×3), 10 : 1 (10 ml×3), 5 : 1 (10 ml) and 1 : 1 (10 ml) of CHCl₃ - CH₃OH solvents (v/v). The 2nd and 3rd fractions of the 50 : 1 solvent were collected and concentrated to give a yellow oil (19.2 mg). The oil was finally purified with preparative HPLC (column; PEGASIL

A) C-4 derivatives

Stemphone	R ₁
C2	
C3a	
C3b	
C3c	
C3d	
C3e	

B) C-11 derivatives

Stemphone	R ₅
C1a	
C1b	
C1c	
C1d	
C1e	
C1f	
C1g	
C1h	

Fig. 2 Structures of stemphone derivatives.

A) C-4 derivatives, B) C-11 derivatives, C) Other derivatives.

C) Other derivatives

Fig. 2 (Continued)

Fig. 3 Chromatographic profiles of isolation of stemphones by HPLC.

(A) Stemphone D

Column, PEGASIL ODS 20×250 mm; solvent 45% aq CH₃CN; detection, UV at 210 nm; flow rate, 6.0 ml/minute; sample, 2.0 mg of active materials (obtained through second silica gel column chromatography) dissolved in 200 μ l MeOH was injected.

(B) Stemphone F

Column, PEGASIL ODS 20×250 mm; solvent 50% aq CH₃CN; detection, UV at 210 nm; flow rate, 6.0 ml/minute; sample, 2.0 mg of active materials (obtained through second silica gel column chromatography) dissolved in 200 μ l MeOH was injected.

(C) Stemphone G

Column, PEGASIL ODS 20×250 mm; solvent 70% aq CH₃CN; detection, UV at 210 nm; flow rate, 6.0 ml/minute; sample, 2.0 mg of active materials (obtained through second silica gel column chromatography) dissolved in 200 μ l MeOH was injected.

ODS, 20×250 mm; solvent, 50% CH₃CN; detection, UV at 210 nm; flow rate, 6.0 ml/minute). Under these conditions, stemphone F was eluted as a peak with a retention time of 18 minutes (Fig. 3B). The fraction was concentrated *in vacuo* to dryness to give pure stemphone F (10.3 mg) as a yellow powder. On the other hand, the 2nd fraction of the 100:1 solvent was collected and concentrated *in vacuo* to dryness to give a red-brown oil (28.2 mg), which was subjected to a second silica gel column (3.0 g). The material was eluted with 100:0 (1.0 ml×40), 100:1 (10 ml×4), 1:1 (40 ml) of CHCl₃-CH₃OH solvents (v/v). The 21st to 40th fractions of the 100:0 solvent were collected and concentrated to give a red-brown oil (14.7 mg). The oil was finally purified with preparative HPLC (column; PEGASIL ODS, 20×250 mm; solvent, 70% CH₃CN; detection, UV at 210 nm; flow rate, 6.0 ml/minute). Under these conditions, stemphone G was eluted as a peak with a retention time of 23.8 minutes (Fig. 3C). The fraction was concentrated *in vacuo* to dryness to give pure stemphone G (1.0 mg) as a yellow powder.

Preparation of C-11 Derivatives (C1a~C1i)

Stemphone derivatives with a diversity at C-11 (C-11 derivatives) were prepared on the basis of the basic nucleophilic reaction [4]. To a solution of stemphone C (20 mg, 0.039 mmol) in pyridine (400 μl) 4-(dimethylamino) pyridine (5.0 mg, 0.040 mmol) and methanol (200 μl, 7.80 mmol) were added. After the reaction mixture was stirred at 60°C for 48 hours, EtOAc and distilled water were added. The organic layer was recovered, dried over anhydrous Na₂SO₄, filtered and concentrated to dryness to afford a yellow material. This material was purified by HPLC (column; PEGASIL ODS, 20×250 mm; solvent, 80% CH₃CN; detection, UV at 210 nm; flow rate, 6.0 ml/minute) to give 11-methoxy stemphone C (C1a, 40.4%) and 11-hydroxy stemphone C as yellow oils. Moreover, the other derivatives were obtained using the following alcohols; ethanol (C1b, 35.6%), 1-propanol (C1c, 29.9%), 2-propanol (C1d, 17.7%), 1-butanol (C1e, 17.5%), 2-butanol (C1f, 2.11%), 3-methyl-1-butanol (C1g, 24.9%) and 2-mercaptoethanol (C1i, 53.0%).

11-Methoxy stemphone C (C1a): ¹H-NMR (300 MHz, CDCl₃) δ 0.88 (s, 3H), 1.02 (d, 3H, *J*=8.0 Hz), 1.16 (s, 3H), 1.18 (s, 3H), 1.20, 1.86 (m, 2H), 1.28 (s, 3H), 1.41 (dd, 1H, *J*=13.0, 5.0 Hz), 1.48, 1.66 (m, 2H), 1.57, 1.76 (m, 2H), 1.62 (s, 3H), 1.63 (d, 3H, *J*=7.0 Hz), 1.80, 2.18 (m, 2H), 1.87 (s, 3H), 2.18, 2.48 (dd, 2H, *J*=19.0, 13.0 Hz, *J*=18.0, 5.0 Hz), 3.10 (dd, 1H, *J*=12.0, 4.0 Hz), 3.21 (dd, 1H, *J*=12.0, 3.0 Hz), 3.49 (dq, 1H, *J*=9.0, 8.0 Hz), 4.03 (s, 3H), 5.44 (d, 1H, *J*=9.0 Hz), 5.68 (q, 1H, *J*=7.0 Hz); ¹³C-

NMR (75 MHz, CDCl₃) δ 10.6, 12.2, 13.2, 15.0, 16.3, 20.8, 21.2, 21.3, 23.8, 25.2, 26.1, 31.5, 35.5, 36.8, 37.0, 46.7, 61.5, 71.8, 80.6, 81.7, 84.0, 84.8, 115.9, 126.2, 130.5, 132.4, 152.1, 156.6, 169.9, 181.1, 183.0; HR FAB-MS (*m/z*) found: 544.3016, calcd: 544.3036 (M⁺) for C₃₁H₄₄O₈.

11-Ethoxy stemphone C (C1b): ¹H-NMR (300 MHz, CDCl₃) δ 0.88 (s, 3H), 1.03 (d, 3H, *J*=7.5 Hz), 1.16 (s, 3H), 1.18 (s, 3H), 1.18, 1.88 (m, 2H), 1.27 (s, 3H), 1.42 (dd, 1H, *J*=13.0, 5.0 Hz), 1.43 (t, 3H, *J*=7.5 Hz), 1.48, 1.66 (m, 2H), 1.60, 1.78 (m, 2H), 1.62 (s, 3H), 1.63 (d, 3H, *J*=8.0 Hz), 1.86 (s, 3H), 1.90, 2.17 (m, 2H), 2.14, 2.52 (dd, 2H, *J*=19.0, 13.0 Hz, *J*=18.0, 5.0 Hz), 3.11 (dd, 1H, *J*=12.0, 4.0 Hz), 3.21 (dd, 1H, *J*=12.0, 3.0 Hz), 3.45 (dq, 1H, *J*=10.0, 8.0 Hz), 4.33 (q, 1H, *J*=7.5 Hz), 5.48 (d, 1H, *J*=10.0 Hz), 5.65 (q, 1H, *J*=8.0 Hz); ¹³C-NMR (75 MHz, CDCl₃) δ 10.9, 12.4, 13.4, 15.2, 16.1, 16.5, 21.0, 21.6, 21.7, 24.0, 25.4, 26.3, 31.8, 35.8, 37.0, 37.3, 46.9, 70.3, 72.1, 80.8, 81.9, 84.3, 85.1, 116.0, 126.3, 132.7, 133.0, 152.4, 156.5, 170.1, 182.4, 183.4; HR FAB-MS (*m/z*) found: 581.3097, calcd: 581.3090 (M+Na)⁺ for C₃₂H₄₆O₈Na.

11-(*n*-Propanoxy) stemphone C (C1c): ¹H-NMR (300 MHz, CDCl₃) δ 0.88 (s, 3H), 1.03 (d, 3H, *J*=8.0 Hz), 1.04 (t, 3H, *J*=8.0 Hz), 1.16 (s, 3H), 1.18 (s, 3H), 1.18, 1.87 (m, 2H), 1.27 (s, 3H), 1.42 (dd, 1H, *J*=13.0, 5.0 Hz), 1.46, 1.66 (m, 2H), 1.60, 1.78 (m, 2H), 1.62 (s, 3H), 1.63 (d, 3H, *J*=8.0 Hz), 1.84 (m, 2H), 1.86 (s, 3H), 1.88, 2.17 (m, 2H), 2.04, 2.48 (dd, 2H, *J*=18.0, 13.0 Hz, *J*=18.0, 5.0 Hz), 3.10 (dd, 1H, *J*=12.0, 4.0 Hz), 3.20 (dd, 1H, *J*=12.0, 3.0 Hz), 3.47 (dq, 1H, *J*=10.0, 8.0 Hz), 4.24 (m, 2H), 5.49 (d, 1H, *J*=10.0 Hz), 5.64 (q, 1H, *J*=7.5 Hz); ¹³C-NMR (75 MHz, CDCl₃) δ 10.6, 10.8, 12.4, 13.4, 15.3, 16.5, 21.0, 21.1, 21.6, 23.9, 24.0, 25.4, 26.3, 31.8, 35.8, 37.0, 37.3, 46.9, 72.1, 76.0, 80.8, 84.3, 85.1, 116.0, 126.4, 130.2, 132.7, 152.4, 156.6, 170.1, 182.4, 183.4; HR FAB-MS (*m/z*) found: 595.3250, calcd: 595.3247 (M+Na)⁺ for C₃₃H₄₈O₈Na.

11-(1-Methyl-ethoxy) stemphone C (C1d): ¹H-NMR (300 MHz, CDCl₃) δ 0.88 (s, 3H), 1.04 (d, 3H, *J*=7.0 Hz), 1.16 (s, 3H), 1.18 (s, 3H), 1.18, 1.87 (m, 2H), 1.28 (s, 3H), 1.35 (d, 3H, *J*=7.0 Hz), 1.37 (d, 3H, *J*=7.0 Hz), 1.42 (dd, 1H, *J*=13.0, 5.0 Hz), 1.48, 1.68 (m, 2H), 1.61 (s, 3H), 1.60, 1.78 (m, 2H), 1.63 (d, 3H, *J*=7.5 Hz), 1.85 (s, 3H), 1.88, 2.17 (m, 2H), 2.04, 2.48 (dd, 2H, *J*=18.0, 13.0 Hz, *J*=18.0, 5.0 Hz), 2.18 (m, 1H), 3.11 (dd, 1H, *J*=12.0, 4.0 Hz), 3.21 (dd, 1H, *J*=12.0, 3.0 Hz), 3.46 (dq, 1H, *J*=10.0, 7.0 Hz), 5.52 (d, 1H, *J*=10.0 Hz), 5.66 (q, 1H, *J*=8.0 Hz); ¹³C-NMR (75 MHz, CDCl₃) δ 10.8, 12.4, 13.4, 15.6, 16.5, 20.9, 21.4, 21.6, 23.4, 24.0, 25.4, 26.3, 32.3, 35.8, 37.0, 37.3, 46.9, 72.1, 80.8, 82.1, 84.3, 85.1, 115.8, 126.3, 132.6, 132.7, 152.5, 155.6, 169.9, 182.4, 183.6; HR FAB-MS (*m/z*)

found: 572.3348, calcd: 572.3249 (M^+) for $C_{33}H_{48}O_8$.

11-(*n*-Butoxy) stemphone C (C1e): 1H -NMR (300 MHz, $CDCl_3$) δ 0.88 (s, 3H), 0.98 (d, 6H, $J=7.0$ Hz), 1.03 (d, 3H, $J=7.5$ Hz), 1.16 (s, 3H), 1.18 (s, 3H), 1.18, 1.86 (m, 2H), 1.27 (s, 3H), 1.42 (dd, 1H, $J=13.0$, 5.0 Hz), 1.48, 1.64 (m, 2H), 1.51 (m, 2H), 1.58, 1.78 (m, 2H), 1.62 (s, 3H), 1.63 (d, 3H, $J=8.0$ Hz), 1.76 (m, 2H), 1.85 (s, 3H), 1.87, 2.18 (m, 2H), 2.04, 2.48 (dd, 2H, $J=18.0$, 13.0 Hz, $J=18.0$, 5.0 Hz), 3.10 (dd, 1H, $J=12.0$, 4.0 Hz), 3.20 (dd, 1H, $J=12.0$, 3.0 Hz), 3.46 (dq, 1H, $J=10.0$, 8.0 Hz), 4.28 (m, 2H), 5.48 (d, 1H, $J=10.0$ Hz), 5.64 (q, 1H, $J=8.0$ Hz); ^{13}C -NMR (75 MHz, $CDCl_3$) δ 10.8, 12.4, 13.4, 14.1, 15.3, 16.5, 19.3, 21.0, 21.4, 21.6, 24.0, 25.4, 26.3, 31.8, 32.6, 35.7, 37.0, 37.3, 46.9, 72.1, 74.2, 80.8, 82.0, 84.3, 85.1, 115.9, 126.3, 130.4, 132.7, 152.4, 156.6, 170.1, 182.4, 183.4; HR FAB-MS (m/z) found: 609.3417, calcd: 609.3403 ($M+Na$) $^+$ for $C_{34}H_{50}O_8Na$.

(1-Methyl-*n*-propanoxy) stemphone C (C1f): 1H -NMR (300 MHz, $CDCl_3$) δ 0.88 (s, 3H), 1.00 (t, 3H, $J=7.0$ Hz), 1.03 (d, 3H, $J=8.0$ Hz), 1.16 (s, 3H), 1.18 (s, 3H), 1.20, 1.90 (m, 2H), 1.27 (s, 3H), 1.28 (d, 3H, $J=5.0$ Hz), 1.43 (m, 1H), 1.48, 1.64 (m, 2H), 1.53, 1.78 (m, 2H), 1.60 (s, 3H), 1.63 (d, 3H, $J=7.0$ Hz), 1.64, 1.78 (m, 2H), 1.82, 1.84 (m, 2H), 1.84 (s, 3H), 2.05, 2.50 (m, 2H), 2.52 (m, 1H), 3.10 (dd, 1H, $J=12.0$, 4.0 Hz), 3.21 (dd, 1H, $J=12.0$, 3.0 Hz), 3.46 (dq, 1H, $J=11.0$, 7.0 Hz), 5.52 (d, 1H, $J=10.0$ Hz), 5.65 (q, 1H, $J=7.0$ Hz); ^{13}C -NMR (75 MHz, $CDCl_3$) δ 10.0, 10.8, 12.4, 13.4, 15.5, 16.5, 20.5, 20.9, 21.4, 21.6, 24.0, 25.4, 26.3, 30.4, 32.3, 35.8, 37.0, 37.3, 46.9, 72.1, 80.7, 81.2, 82.1, 84.3, 85.1, 115.7, 126.3, 130.9, 132.7, 152.5, 155.7, 169.9, 182.3, 183.6; HR FAB-MS (m/z) found: 586.3487, calcd: 586.3506 (M^+) for $C_{34}H_{50}O_8$.

11-(3-Methyl-1-butoxy) stemphone C (C1g): 1H -NMR (300 MHz, $CDCl_3$) δ 0.88 (s, 3H), 0.97 (d, 6H, $J=7.0$ Hz), 1.03 (d, 3H, $J=7$ Hz), 1.16 (s, 3H), 1.18 (s, 3H), 1.21, 1.86 (m, 2H), 1.27 (s, 3H), 1.42 (dd, 1H, $J=13.0$, 5.0 Hz), 1.48, 1.64 (m, 2H), 1.58, 1.78 (m, 2H), 1.62 (s, 3H), 1.63 (d, 3H, $J=7.0$ Hz), 1.68 (m, 2H), 1.79, 2.18 (m, 2H), 1.82 (m, 1H), 1.86 (s, 3H), 2.08, 2.48 (dd, 2H, $J=18.0$, 13.0 Hz, $J=18.0$, 5.0 Hz), 3.10 (dd, 1H, $J=12.0$, 4.0 Hz), 3.20 (dd, 1H, $J=12.0$, 3.0 Hz), 3.45 (dq, 1H, $J=10.0$, 7.0 Hz), 4.30 (m, 2H), 5.48 (d, 1H, $J=10.0$ Hz), 5.64 (q, 1H, $J=7.0$ Hz); ^{13}C -NMR (75 MHz, $CDCl_3$) δ 10.8, 12.4, 13.4, 15.3, 16.5, 21.0, 21.5, 21.6, 22.8, 24.0, 25.1, 25.4, 26.3, 33.9, 35.8, 37.0, 37.3, 39.3, 46.9, 72.1, 73.0, 80.8, 82.0, 84.3, 85.1, 116.0, 126.4, 130.3, 132.7, 152.4, 156.6, 170.1, 182.4, 183.4; HR FAB-MS (m/z) found: 600.3675, calcd: 600.3662 (M^+) for $C_{35}H_{52}O_8$.

11-Hydroxy stemphone C (C1h): 1H -NMR (300 MHz, $CDCl_3$) δ 0.89 (s, 3H), 1.04 (d, 3H, $J=7.0$ Hz), 1.16 (s, 3H), 1.18 (s, 3H), 1.18, 1.87 (m, 2H), 1.30 (s, 3H), 1.42

(dd, 1H, $J=13.0$, 5.0 Hz), 1.48, 1.68 (m, 2H), 1.58, 1.78 (m, 2H), 1.62 (s, 3H), 1.63 (d, 3H, $J=8.0$ Hz), 1.86 (s, 3H), 1.87, 2.20 (m, 2H), 2.20, 2.52 (dd, 2H, $J=18.0$, 13.0 Hz, $J=18.0$, 5.0 Hz), 3.10 (dd, 1H, $J=12.0$, 4.0 Hz), 3.21 (dd, 1H, $J=12.0$, 3.0 Hz), 3.39 (dq, 1H, $J=11.0$, 7.0 Hz), 5.52 (d, 1H, $J=11.0$ Hz), 5.66 (q, 1H, $J=8.0$ Hz); ^{13}C -NMR (75 MHz, $CDCl_3$) δ 10.9, 12.5, 13.4, 14.7, 16.3, 21.1, 21.5, 21.6, 24.0, 25.4, 26.3, 31.5, 35.8, 37.0, 37.3, 46.8, 72.1, 81.4, 81.9, 84.2, 85.1, 113.3, 118.8, 126.3, 132.6, 151.8, 154.7, 170.3, 181.5, 182.4; HR FAB-MS (m/z) found: 553.2782, calcd: 553.2777 ($M+Na$) $^+$ for $C_{30}H_{42}O_8Na$.

11-(2-Mercapto-ethoxy) stemphone C (C1i): 1H -NMR (300 MHz, $CDCl_3$) δ 0.91 (s, 3H), 1.07 (d, 3H, $J=7.5$ Hz), 1.17 (s, 3H), 1.18 (s, 3H), 1.22, 1.98 (m, 2H), 1.23 (s, 3H), 1.52 (dd, 1H, $J=13.0$, 5.0 Hz), 1.62, 1.78 (m, 2H), 1.64 (s, 3H), 1.66 (d, 3H, $J=7.0$ Hz), 1.69 (s, 3H), 1.98, 2.04 (m, 2H), 2.40, 2.72 (dd, 2H, $J=19.0$, 13.0 Hz, $J=18.0$, 5.0 Hz), 2.82, 2.94 (m, 2H), 3.14 (dd, 1H, $J=12.0$, 4.0 Hz), 3.24 (dd, 1H, $J=12.0$, 3.0 Hz), 4.07 (dq, 1H, $J=10.0$, 7.5 Hz), 5.98 (d, 1H, $J=10.0$ Hz), 5.74 (q, 1H, $J=8.0$ Hz); ^{13}C -NMR (75 MHz, $CDCl_3$) δ 11.1, 12.3, 13.5, 15.4, 18.2, 21.2, 21.3, 21.6, 24.0, 25.5, 26.3, 35.7, 37.0, 37.9, 38.5, 39.0, 47.5, 47.5, 60.6, 72.2, 78.3, 81.7, 84.5, 85.1, 107.5, 107.6, 126.1, 129.4, 133.1, 138.0, 142.7, 149.4, 169.9; HR FAB-MS (m/z) found: 592.3089, calcd: 592.3070 (M^+) for $C_{32}H_{46}O_8S$.

Preparation of Deacetyl Stemphone C (C2)

Stemphone C (100 mg, 0.211 mmol) was reacted with lithium aluminium hydride (100 mg, 2.63 mmol) in diethylether (4.0 ml) at 0°C. After 15 minutes, the reaction was quenched with EtOAc and then washed with 1.0 N HCl. The organic layer was recovered, dried over anhydrous Na_2SO_4 , filtered and concentrated *in vacuo* to dryness. The residue was purified by preparative HPLC (column; PEGASIL ODS, 20×250 mm; solvent, 80% CH_3CN ; detection, UV at 210 nm; flow rate, 6.0 ml/minute) to afford 4-deacetyl stemphone C (C2, 42.1 mg, 42.2%).

4-Deacetyl stemphone C (C2): 1H -NMR (300 MHz, $CDCl_3$) δ 0.88 (s, 3H), 0.99 (d, 3H, $J=6.0$ Hz), 1.16 (s, 3H), 1.17 (s, 3H), 1.21, 1.88 (m, 2H), 1.27 (s, 3H), 1.43 (dd, 1H, $J=13.0$, 5.0 Hz), 1.47, 1.64 (m, 2H), 1.56, 1.77 (m, 2H), 1.61 (d, 3H, $J=8.0$ Hz), 1.63 (s, 3H), 1.78, 2.16 (m, 2H), 2.12, 2.52 (dd, 2H, $J=19.0$, 13.0 Hz, $J=19.0$, 5.0 Hz), 3.14 (dd, 1H, $J=13.0$, 4.0 Hz), 3.21 (dd, 1H, $J=12.0$, 3.0 Hz), 3.12 (m, 1H), 3.90 (d, 1H, $J=10.0$ Hz), 5.48 (q, 1H, $J=7.0$ Hz), 6.52 (s, 1H); ^{13}C -NMR (75 MHz, $CDCl_3$) δ 10.9, 12.4, 13.3, 16.6, 16.9, 21.0, 21.6, 24.0, 25.4, 26.3, 35.8, 37.0, 37.4, 46.8, 72.1, 80.7, 82.6, 84.3, 85.1, 118.1, 123.7, 132.7, 136.4, 149.4, 152.5, 182.8, 187.5; HR FAB-MS (m/z) found: 473.2896 ($M+H$) $^+$,

calcd: 473.2903 (M+H)⁺ for C₂₈H₄₀O₆.

Preparation of C-4 Derivatives from Deacetyl Stemphone C (C3a~C3e)

To a solution of deacetyl stemphone C (5.0 mg, 0.011 mmol) in CH₂Cl₂ (500 μl) propionic anhydride (15 μl, 0.11 mmol), triethylamine (15 μl, 0.11 mmol) and 4-(dimethylamino) pyridine (1.0 mg, 0.0080 mmol) were added, and the resulting solution was stirred at room temperature. After 10 hours, the reaction mixture was diluted with 1.0 N HCl and the aqueous phase was extracted with EtOAc. The organic layer was recovered, dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo* to dryness. The product was purified by preparative HPLC (column; PEGASIL ODS, 20×250 mm; solvent, 80% CH₃CN; detection, UV at 210 nm; flow rate, 6.0 ml/minute) to give 4-deacetyl-4-*O*-propionyl stemphone C (C3a, 2.57 mg, 44.9%) as a yellow powder. Moreover, other C-4 derivatives were obtained using the following acid anhydrides instead of propionic anhydride; *n*-butyric anhydride (C3b, 41.3%), *n*-valeric anhydride (C3c, 46.6%), *n*-hexanoic anhydride (C3d, 22.4%) and benzoic anhydride (C3e, 37.7%).

4-Deacetyl-4-*O*-propionyl stemphone C (C3a): ¹H-NMR (300 MHz, CDCl₃) δ 0.89 (s, 3H), 1.03 (t, 3H, *J*=8.0 Hz), 1.03 (d, 3H, *J*=7 Hz), 1.17 (s, 3H), 1.18 (s, 3H), 1.18, 1.84 (m, 2H), 1.28 (s, 3H), 1.42 (dd, 1H, *J*=13.0, 5.0 Hz), 1.42, 1.63 (m, 2H), 1.54, 1.72 (m, 2H), 1.58 (d, 3H, *J*=8.0 Hz), 1.60 (s, 3H), 1.78, 2.16 (m, 2H), 2.14, 2.52 (dd, 2H, *J*=19.0, 13.0 Hz, *J*=18.0, 5.0 Hz), 2.18 (m, 2H), 3.12 (dd, 1H, *J*=12.0, 4.0 Hz), 3.21 (dd, 1H, *J*=12.0, 3.0 Hz), 3.34 (m, 1H), 5.14 (d, 1H, *J*=9.0 Hz), 5.53 (q, 1H, *J*=7.0 Hz), 6.48 (s, 1H); ¹³C-NMR (75 MHz, CDCl₃) δ 9.4, 11.8, 12.4, 13.4, 16.7, 17.1, 21.0, 24.0, 25.4, 26.3, 28.0, 33.9, 35.8, 37.0, 37.4, 46.9, 72.1, 80.8, 81.6, 84.3, 85.1, 118.0, 125.1, 132.4, 132.7, 148.3, 152.5, 173.5, 181.6, 187.3; HR FAB-MS (*m/z*) found: 528.3087, calcd: 528.3094 (M⁺) for C₃₁H₄₄O₇.

4-Deacetyl-4-*O*-*n*-butyryl stemphone C (C3b): ¹H-NMR (300 MHz, CDCl₃) δ 0.84 (t, 3H, *J*=8.0 Hz), 0.88 (s, 3H), 1.03 (d, 3H, *J*=7.0 Hz), 1.16 (s, 3H), 1.18 (s, 3H), 1.19, 1.88 (m, 2H), 1.27 (s, 3H), 1.28, 1.54 (m, 2H), 1.43 (dd, 1H, *J*=13.0, 4.5 Hz), 1.48, 1.67 (m, 2H), 1.55, 1.75 (m, 2H), 1.59 (d, 3H, *J*=7.0 Hz), 1.60 (s, 3H), 1.78, 2.18 (m, 2H), 2.12, 2.51 (dd, 2H, *J*=19.0, 13.0 Hz, *J*=18.0, 4.5 Hz), 2.16 (m, 2H), 3.11 (dd, 1H, *J*=12.0, 4.0 Hz), 3.21 (dd, 1H, *J*=12.0, 3.0 Hz), 3.34 (m, 1H), 5.14 (d, 1H, *J*=9.0 Hz), 5.54 (q, 1H, *J*=7.0 Hz), 6.48 (s, 1H); ¹³C-NMR (75 MHz, CDCl₃) δ 11.8, 12.4, 13.4, 13.9, 16.7, 17.1, 18.7, 21.0, 24.0, 25.4, 26.3, 33.9, 35.8, 36.6, 37.0, 37.4, 46.9, 72.1, 80.7, 81.7, 84.3, 85.1, 118.0, 125.2, 132.4, 132.7, 148.3,

152.5, 172.7, 181.6, 187.3; HR FAB-MS (*m/z*) found: 542.3244 (M⁺), calcd: 542.3263 (M⁺) for C₃₂H₄₆O₇.

4-Deacetyl-4-*O*-*n*-valeryl stemphone C (C3c): ¹H-NMR (300 MHz, CDCl₃) δ 0.84 (t, 3H, *J*=8.0 Hz), 0.88 (s, 3H), 1.02 (d, 3H, *J*=7.0 Hz), 1.17 (s, 3H), 1.18 (s, 3H), 1.18, 1.88 (m, 2H), 1.20 (m, 2H), 1.28 (s, 3H), 1.41 (dd, 1H, *J*=13.0, 4.5 Hz), 1.47 (m, 2H), 1.47, 1.65 (m, 2H), 1.58, 1.78 (m, 2H), 1.59 (d, 3H, *J*=8.0 Hz), 1.61 (s, 3H), 1.78, 2.18 (m, 2H), 2.12, 2.51 (dd, 2H, *J*=19.0, 13.0 Hz, *J*=18.0, 4.5 Hz), 2.17 (m, 2H), 3.11 (dd, 1H, *J*=12.0, 4.0 Hz), 3.20 (dd, 1H, *J*=12.0, 3.0 Hz), 3.35 (dq, 1H, *J*=9.0, 7.0 Hz), 5.14 (d, 1H, *J*=9.0 Hz), 5.54 (q, 1H, *J*=7.0 Hz), 6.48 (s, 1H); ¹³C-NMR (75 MHz, CDCl₃) δ 11.8, 12.4, 13.4, 14.0, 16.7, 17.1, 21.0, 22.4, 24.0, 25.4, 26.3, 27.3, 33.9, 34.4, 35.8, 37.0, 37.4, 46.9, 72.1, 80.7, 81.7, 84.3, 85.1, 118.0, 125.2, 132.4, 132.7, 148.3, 152.5, 172.7, 181.6, 187.3; HR FAB-MS (*m/z*) found: 556.3391 (M⁺), calcd: 556.3400 (M⁺) for C₃₃H₄₈O₇.

4-Deacetyl-4-*O*-*n*-hexanoyl stemphone C (C3d): ¹H-NMR (300 MHz, CDCl₃) δ 0.85 (t, 3H, *J*=8.0 Hz), 0.88 (s, 3H), 1.02 (d, 3H, *J*=7.0 Hz), 1.16 (s, 3H), 1.18 (s, 3H), 1.20, 1.88 (m, 2H), 1.20 (m, 2H), 1.23 (m, 2H), 1.27 (s, 3H), 1.41 (dd, 1H, *J*=13.0, 4.5 Hz), 1.48 (m, 2H), 1.48, 1.65 (m, 2H), 1.57, 1.78 (m, 2H), 1.59 (d, 3H, *J*=7.0 Hz), 1.61 (s, 3H), 1.78, 2.16 (m, 2H), 2.08, 2.51 (dd, 2H, *J*=19.0, 13.0 Hz, *J*=18.0, 4.5 Hz), 2.16 (m, 2H), 3.11 (dd, 1H, *J*=12.0, 4.0 Hz), 3.20 (dd, 1H, *J*=12.0, 3.0 Hz), 3.35 (dq, 1H, *J*=9.0, 7.0 Hz), 5.13 (d, 1H, *J*=9.0 Hz), 5.54 (q, 1H, *J*=7.0 Hz), 6.48 (s, 1H); ¹³C-NMR (75 MHz, CDCl₃) δ 11.8, 12.4, 13.4, 14.2, 16.7, 17.2, 21.6, 22.6, 24.9, 25.4, 26.3, 31.4, 33.7, 34.7, 35.8, 37.0, 37.4, 46.9, 72.1, 80.7, 81.7, 84.3, 85.1, 118.0, 125.3, 132.4, 132.7, 148.4, 152.5, 172.9, 181.6, 187.3; HR FAB-MS (*m/z*) found: 570.3544 (M⁺), calcd: 570.3557 (M⁺) for C₃₄H₅₀O₇.

4-Deacetyl-4-*O*-benzoyl stemphone C (C3e): ¹H-NMR (300 MHz, CDCl₃) δ 0.85 (s, 3H), 1.10 (d, 3H, *J*=6.0 Hz), 1.15 (s, 3H), 1.17 (s, 3H), 1.12, 1.84 (m, 2H), 1.24 (s, 3H), 1.26 (dd, 1H, *J*=13.0, 5.0 Hz), 1.45, 1.62 (m, 2H), 1.58, 1.76 (m, 2H), 1.59 (d, 3H, *J*=8.0 Hz), 1.70 (s, 3H), 1.75, 2.16 (m, 2H), 2.03, 2.42 (dd, 2H, *J*=19.0, 13.0 Hz, *J*=19.0, 5.0 Hz), 3.06 (dd, 1H, *J*=13.0, 4.0 Hz), 3.18 (dd, 1H, *J*=12.0, 3.0 Hz), 3.12 (dq, 1H), 5.34 (d, 1H, *J*=7.0 Hz), 5.67 (q, 1H, *J*=7.0 Hz), 6.55 (s, 1H), 7.40 (t, 2H, *J*=5.0 Hz), 7.54 (t, 1H, *J*=5.0 Hz), 7.90 (d, 2H, *J*=5.0 Hz); ¹³C-NMR (75 MHz, CDCl₃) δ 11.7, 12.4, 13.4, 16.6, 16.9, 21.0, 21.6, 24.0, 25.4, 26.3, 33.5, 35.8, 36.9, 37.4, 46.7, 72.1, 80.7, 83.3, 84.2, 85.1, 118.1, 125.9, 128.6, 128.6, 129.9, 129.9, 130.4, 132.3, 132.5, 133.1, 148.5, 152.5, 165.5, 181.8, 187.2; HR FAB-MS (*m/z*) found: 576.3087 (M)⁺, calcd: 576.3087 (M⁺) for C₃₅H₄₄O₇.

Preparation of Methoxy Derivatives (E1 and G1)

To a solution of stemphone E (5.3 mg, 0.010 mmol) in MeOH (340 μ l) TMS-diazomethane (170 μ l, 0.10 mmol) was added. After stirring at 40°C for 24 hours, the reaction mixture was concentrated *in vacuo* to dryness to give a brown material. This material was purified by preparative HPLC to give 10-methoxy stemphone E (E1, 1.0 mg, 19%) as a white powder. Similarly, 7,10-dimethoxy stemphone G (G1) was prepared from stemphone G.

10-Methoxy stemphone E (E1): $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ 1.00 (s, 3H), 1.08 (d, 3H, $J=7.0$ Hz), 1.15 (s, 3H), 1.21 (s, 3H), 1.25 (s, 3H), 1.56, 1.92 (m, 2H), 1.60, 1.80 (m, 2H), 1.64 (d, 3H, $J=8.0$ Hz), 1.67 (s, 3H), 1.80 (s, 3H), 1.92 (m, 2H), 2.35 (d, 1H, $J=10.0$ Hz), 3.43 (dq, 1H, $J=10.0, 7.0$ Hz), 3.60 (dd, 1H, $J=13.0, 4.0$ Hz), 3.74 (dd, 1H, $J=13.0, 5.0$ Hz), 4.12 (brt, 1H), 5.06 (d, 1H, $J=10.0$ Hz), 5.45 (d, 1H, $J=10.0$ Hz), 5.67 (q, 1H, $J=8.0$ Hz), 6.27 (s, 1H); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) δ 11.3, 13.3, 13.4, 17.6, 21.4, 22.3, 23.9, 25.4, 26.6, 28.4, 34.0, 37.8, 40.8, 46.9, 55.7, 63.5, 71.0, 72.0, 77.3, 79.7, 80.9, 82.5, 101.2, 112.2, 125.6, 128.6, 132.8, 137.9, 140.1, 150.3, 170.4; HR FAB-MS (m/z) found: 563.3153 ($\text{M}+\text{H}$) $^+$, calcd: 563.3153 ($\text{M}+\text{H}$) $^+$ for $\text{C}_{31}\text{H}_{47}\text{O}_9$.

7,10-Dimethoxy stemphone G (G1): $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ 1.05 (d, 3H, $J=7$ Hz), 1.13 (s, 3H), 1.15 (s, 3H), 1.19 (s, 3H), 1.46, 2.01 (m, 2H), 1.48 (s, 3H), 1.58, 1.82 (m, 2H), 1.65 (d, 3H, $J=9.0$ Hz), 1.66 (s, 3H), 1.79 (s, 3H), 2.05 (m, 2H), 3.35 (s, 1H), 3.60 (dd, 1H, $J=13.0, 4.0$ Hz), 3.69 (dd, 1H, $J=13.0, 5.0$ Hz), 3.79 (s, 3H), 3.84 (s, 3H), 4.75 (brt, 1H, $J=6.0$ Hz), 5.34 (d, 1H, $J=9.5$ Hz), 5.69 (q, 1H, $J=9.0$ Hz), 6.28 (s, 1H); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) δ 11.2, 13.4, 13.5, 18.6, 21.4, 21.9, 24.0, 25.0, 26.3, 29.7, 34.5, 37.7, 40.2, 53.7, 56.2, 61.1, 70.0, 72.0, 76.0, 79.6, 82.5, 82.7, 100.7, 110.9, 126.2, 132.5, 140.7, 144.7, 153.5, 155.9, 170.1, 193.0; HR FAB-MS (m/z) found: 575.3220 ($\text{M}+\text{H}$) $^+$, calcd: 575.3226 ($\text{M}+\text{H}$) $^+$ for $\text{C}_{32}\text{H}_{47}\text{O}_9$.

Preparation of 7,10-Diacetyl Stemphone C (C4)

To a solution of stemphone C (10 mg, 0.019 mmol) in MeOH (500 μ l) sodium borohydride (5.5 mg, 0.145 mmol) was added, and the resulting solution was stirred at room temperature. After 10 minutes, the mixture was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 (500 μ l), and acetic anhydride (15 μ l, 0.11 mmol), triethylamine (15 μ l, 0.16 mmol) and 4-(dimethylamino)pyridine (1.0 mg, 0.0080 mmol) were added to the solution. The resulting mixture was stirred at 40°C for 10 hours, and diluted with 1.0 N HCl, and the aqueous phase was extracted with EtOAc. The organic layer was recovered, dried over anhydrous Na_2SO_4 , filtered and concentrated *in*

vacuo to dryness. The residue was purified by preparative HPLC (column; PEGASIL ODS, 20 $\times$ 250 mm; solvent, 80% CH_3CN ; detection, UV at 210 nm; flow rate, 6.0 ml/minute) to give 7,10-diacetyl stemphone C (C4, 1.4 mg, 14.0%) as a white powder.

7,10-Diacetyl-stemphone C (C4): $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ 0.88 (s, 3H), 1.01 (d, 3H, $J=7.5$ Hz), 1.17 (s, 3H), 1.19 (s, 3H), 1.20 (s, 3H), 1.22, 1.84 (m, 2H), 1.44 (m, 1H), 1.44, 1.66 (m, 2H), 1.60 (s, 3H), 1.61 (d, 3H, $J=7.0$ Hz), 1.63, 1.80 (m, 2H), 1.74, 2.00 (m, 2H), 1.83 (s, 3H), 2.28, 2.48 (m, 2H), 2.29 (s, 3H), 2.30 (s, 3H), 3.13 (dd, 1H, $J=13.0, 4.0$ Hz), 3.21 (dd, 1H, $J=13.0, 3.0$ Hz), 3.23 (m, 1H), 5.22 (d, 1H, $J=10.0$ Hz), 5.59 (q, 1H, $J=7.0$ Hz), 6.50 (s, 1H); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) δ 11.5, 12.3, 13.4, 17.8, 18.1, 20.7, 20.7, 21.0, 21.1, 21.6, 23.9, 25.4, 26.4, 34.6, 35.6, 36.8, 37.8, 46.7, 72.1, 80.8, 82.5, 84.5, 85.0, 111.2, 114.5, 125.7, 132.4, 136.1, 145.8, 146.8, 168.8, 169.0, 170.5; HR FAB-MS (m/z) found: 623.3230, calcd: 623.3196 ($\text{M}+\text{Na}$) $^+$ for $\text{C}_{34}\text{H}_{48}\text{O}_9\text{Na}$.

Preparation of 7,10,12,19,23-Pentaacetyl Stemphone E (E4)

To a solution of stemphone E (10 mg, 0.011 mmol) in CH_2Cl_2 (500 μ l) acetic anhydride (15 μ l, 0.16 mmol), triethylamine (15 μ l, 0.11 mmol) and 4-(dimethylamino)pyridine (1.0 mg, 0.0080 mmol) were added, and the resulting solution was stirred at 40°C for 10 hours. The reaction mixture was diluted with 1.0 N HCl, and the aqueous phase was extracted with EtOAc. The organic layer was recovered, dried over anhydrous Na_2SO_4 , filtered and concentrated *in vacuo* to dryness. The product was purified by preparative HPLC (column; PEGASIL ODS, 20 $\times$ 250 mm; solvent, 80% CH_3CN ; detection, UV at 210 nm; flow rate, 6.0 ml/minute) to give 7,10,12,19,23-pentaacetyl stemphone E (E4, 6.4 mg, 64%) as a white powder.

7,10,12,19,23-Pentaacetyl-methoxy stemphone E (E4): $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ 1.01 (d, 3H, $J=7.5$ Hz), 1.12 (s, 3H), 1.27 (s, 3H), 1.42 (s, 3H), 1.44 (s, 3H), 1.60 (s, 3H), 1.62 (d, 3H, $J=7.0$ Hz), 1.62, 1.74 (m, 2H), 1.64, 1.88 (m, 2H), 1.84 (m, 2H), 1.83 (s, 3H), 1.96 (s, 3H), 2.03 (s, 3H), 2.09 (s, 3H), 2.22 (s, 3H), 2.31 (s, 3H), 2.59 (d, 1H, $J=10.0$ Hz), 3.20 (dq, 1H, $J=10.0, 7.5$ Hz), 3.68 (dd, 1H, $J=13.0, 4.0$ Hz), 3.87 (dd, 1H, $J=13.0, 3.0$ Hz), 4.59 (brt, 1H, $J=3.0$ Hz), 5.16 (d, 1H, $J=10.0$ Hz), 5.60 (q, 1H, $J=7.0$ Hz), 6.21 (d, 1H, $J=10.0$ Hz), 6.46 (s, 1H); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) δ 11.3, 12.5, 13.4, 17.6, 20.7, 21.3, 21.6, 21.7, 21.7, 21.7, 22.3, 22.6, 23.0, 25.0, 25.6, 35.2, 37.2, 39.7, 44.5, 65.8, 67.0, 73.2, 77.1, 77.7, 80.8, 82.8, 113.5, 116.8, 126.1, 132.2, 136.3, 137.5, 146.6, 146.9, 168.5, 169.5, 170.4, 170.5, 170.6, 172.7; HR FAB-MS (m/z)

found: 781.3405, calcd: 781.3411 (M+Na)⁺ for C₄₀H₅₄O₁₄Na.

Preparation of 12-Dehydroxy-13-dehydro Stemphone E and 12-Dehydroxy-13-dehydro Stemphone B (E2 and E3)

Stemphone E (50 mg) was dehydrated by heating at 60°C for 1 hour in CHCl₃. Two main products were purified with preparative HPLC (column; PEGASIL ODS, 20×250 mm; solvent, 80% CH₃CN; detection, UV at 210 nm; flow rate, 6.0 ml/minute) to give 12-dehydroxy-13-dehydro stemphone E (E2, 12.0 mg, 71.0%) and 12-dehydroxy-13-dehydro stemphone B (E3, 2.3 mg, 14.0%) as white and red powders, respectively.

12-Dehydroxy-13-dehydro stemphone E (E2): ¹H-NMR (300 MHz, CDCl₃) δ 1.02 (d, 3H, *J*=7.5 Hz), 1.14 (s, 3H), 1.18 (s, 3H), 1.22 (s, 3H), 1.64 (d, 3H, *J*=7.0 Hz), 1.67 (s, 3H), 1.70 (m, 2H), 1.80, 2.01 (m, 2H), 1.82 (s, 3H), 1.96, 2.12 (m, 2H), 3.45 (dq, 1H, *J*=10.0, 7.5 Hz), 3.64 (dd, 1H, *J*=13.0, 4.0 Hz), 3.86 (dd, 1H, *J*=13.0, 3.0 Hz), 4.28 (brt, 1H), 5.41 (d, 1H, *J*=10.0 Hz), 5.66 (q, 1H, *J*=7.0 Hz), 6.22 (s, 1H), 6.60 (s, 1H); ¹³C-NMR (75 MHz, CDCl₃) δ 11.0, 13.2, 17.6, 20.5, 21.3, 23.8, 24.0, 26.3, 26.6, 27.9, 34.6, 37.1, 44.3, 69.3, 71.9, 74.5, 77.9, 78.9, 82.8, 105.9, 108.8, 113.7, 125.7, 130.0, 132.5, 136.6, 138.3, 139.5, 144.2, 170.9 HR FAB-MS (*m/z*) found: 531.2896 (M+H)⁺, calcd: 531.2980 (M+H)⁺ for C₃₀H₄₃O₈.

12-Dehydroxy-13-dehydro stemphone B (E3): ¹H-NMR (300 MHz, CDCl₃) δ 0.98 (d, 3H, *J*=7.5 Hz), 1.08 (s, 3H), 1.10 (s, 3H), 1.15 (s, 3H), 1.53 (d, 3H, *J*=7.0 Hz), 1.56 (s, 3H), 1.70 (m, 2H), 1.64, 1.80 (m, 2H), 1.70, 2.01 (m, 2H), 1.96, 2.20 (m, 2H), 1.88 (s, 3H), 3.27 (dq, 1H, *J*=10.0, 7.5 Hz), 3.55 (dd, 1H, *J*=13.0, 4.0 Hz), 3.77 (dd, 1H, *J*=13.0, 3.0 Hz), 4.22 (brt, 1H), 5.10 (d, 1H, *J*=10.0 Hz), 5.49 (q, 1H, *J*=7.0 Hz), 6.34 (s, 1H), 6.42 (s, 1H); ¹³C-

NMR (75 MHz, CDCl₃) δ 11.5, 13.2, 17.1, 20.8, 21.1, 23.9, 24.0, 26.3, 26.6, 28.2, 34.1, 36.7, 44.8, 69.1, 71.8, 73.8, 77.2, 78.9, 81.0, 81.5, 111.5, 117.0, 125.3, 131.6, 131.8, 143.5, 149.0, 149.3, 170.0, 180.8, 184.6 HR FAB-MS (*m/z*) found: 529.2723 (M+H)⁺, calcd: 529.2720 (M+H)⁺ for C₃₀H₄₁O₈.

Assay for Potentiation of Imipenem Activity against MRSA

Assay for potentiation of imipenem activity against MRSA was carried out by the paper disk method and the liquid microdilution method as previously reported [3].

Assay for Cytotoxicity

Cytotoxicity of stemphones to Jurkat cells was evaluated by the MTT assay [5]. Cells (2×10⁴ cells/100 μl/well) were cultured in 96-well plates (Corning) in the presence of stemphones at the indicated concentrations at 37°C for 48 hours after which they received MTT solution (SIGMA, 10 μl to each well, 5.5 mg/ml in phosphate buffered saline (PBS)) and incubated at 37°C for 4 hours. A 90-μl aliquot of the extraction solution (40% (v/v) *N,N*-dimethylformamide, 2.0% (v/v) CH₃COOH, 20% (w/v) SDS and 0.03 N HCl) was added to each well, and the cells were incubated at room temperature for 2 hours. Cytotoxicity was determined by measuring optical density at 550 nm with a plate reader (Bio-Tek instruments).

Results

Structural Elucidation

Physico-chemical properties of the isolated stemphones are summarized in Table 1. Stemphone D showed similar UV absorption maxima at 265 nm to stemphones B and C,

Table 1 Physico-chemical properties of stemphones D, E, F and G

	Stemphone D	Stemphone E	Stemphone F	Stemphone G
Appearance	Yellow powder	White powder	Yellow powder	Yellow powder
Molecular weight	530	548	604	546
Molecular formula	C ₃₀ H ₄₂ O ₈	C ₃₀ H ₄₄ O ₉	C ₃₃ H ₄₈ O ₁₀	C ₃₀ H ₄₂ O ₉
HRFAB-MS (<i>m/z</i>)				
Calcd.:	553.2777 (M+Na) ⁺	549.2986 (M+H) ⁺	627.3146 (M+Na) ⁺	545.2734 (M-H) ⁻
Found:	553.2788	549.2977	627.3151	545.2751
UV (MeOH) λ _{max} nm (ε)	203 (17442), 265 (8284), 396 (678)	204 (34447), 292 (4370), 356 (403)	201 (11669), 244 (6427), 288 (3603), 361 (387)	212 (10647), 242 (4368), 282 (7644), 380 (2457)
[α] _D ²⁶	+93.0° (c=0.1, MeOH)	+100.8° (c=0.1, MeOH)	+148.9° (c=0.1, MeOH)	+72.5° (c=0.1, MeOH)
IR (KBr) ν _{max} (cm ⁻¹)	3442, 2973, 1735, 1643, 1604	3434, 2929, 1718, 1629	3438, 2967, 1658, 1629, 1623	3428, 2925, 1729, 1639

Table 2 ^1H - and ^{13}C -NMR chemical shifts of stemphones

Position	Stemphone B		Stemphone C		Stemphone D	
	δ_{C}	δ_{H} (J Hz)	δ_{C}	δ_{H} (J Hz)	δ_{C}	δ_{H} (J Hz)
1	13.2 q	1.61 d, br (7.0)	13.1 q	1.59 d, br (7.0)	12.8	1.60 d (7.0)
2	125.3 d	5.56 q, br (7.0)	124.9 d	5.53 q (7.0)	125.0	5.55 q (7.0)
3	131.7 s	—	132.1 s	—	132.4	—
4	81.4 d	5.15 d (8.5)	81.5 d	5.14 d (8.5)	81.6	5.14 d (9.0)
5	34.2 d	3.30 dq (8.5, 7.0)	33.5 d	3.34 dq (8.5, 7.0)	33.4	3.34 dq (9.0, 7.5)
6	148.8 s	—	148.0 s	—	148.0	—
7	181.0 s	—	181.3 s	—	181.3	—
8	151.5 s	—	152.3 s	—	152.4	—
9	117.8 s	—	117.8 s	—	117.7	—
10	188.5 s	—	187.1 s	—	187.2	—
10-OH						
11	132.6 d	6.47 s	132.4 d	6.48 d (1.0)	132.4	6.48 s
12	62.3 d	4.90 d (11.0)	16.4 t	2.11 dd (19.0, 13.0) 2.52 dd (19.0, 5.0)	16.1	2.12, 2.50 m
12-OH ^a	—	3.42, br	—	—	—	—
13	45.4 d	2.23 d (11.0)	46.6 d	1.43 dd (13.0, 5.0)	38.9	2.12 m
14	83.8 s	—	80.5 s	—	81.0	—
15	37.1 t	1.88 m, 2.08 m	37.2 t	1.79 m, 2.18 m	36.7	1.82 m, 2.15 m
16	25.0 t	1.58 m, 1.86 m	25.2 t	1.63 m, 1.80 m	24.9	1.66 m, 1.82 m
17	76.2 d	3.68 dd (13.0, 4.0)	84.0 d	3.12 dd (12.0, 4.0)	75.9	3.70 dd (12.0, 3.5)
18	40.8 s	—	35.5 s	—	39.7	—
19	70.7 d	4.10 t, br (3.0)	36.8 t	1.21 m, 1.86 m	69.3	3.90 t, br (3.0)
19-OH ^a	—	4.13, br	—	—	—	—
20	28.2 t	1.60 m, 1.96 m	21.3 t	1.48 m, 1.66 m	29.9	1.48 m, 2.00 m
21	79.6 d	3.57 dd (13.0, 3.0)	84.9 d	3.21 dd (12.0, 3.0)	78.9	3.58 dd (12.5, 3.0)
22	71.7 s	—	71.8 s	—	71.8	—
23	23.8 q	1.14 s	23.8 q	1.16 s	23.8	1.15 s
24	26.4 q	1.20 s	26.1 q	1.18 s	26.2	1.20 s
25	13.1 q	0.98 s	12.2 q	0.88 s	13.1	0.89 s
26	21.3 q	1.32 s	20.8 q	1.28 s	20.9	1.29 s
27	16.9 q	1.04 d (7.0)	17.0 q	1.02 d (7.0)	17.1	1.02 d (7.5)
28	11.6 q	1.62 s	11.6 q	1.62 s, br	11.6	1.61 s
29	169.7 s	—	169.7 s	—	169.9	—
30	21.2 q	1.94 s	21.1 q	1.93 s	21.1	1.94 s
31						
32						
33						

^a Exchangeable signals.

Table 2 (Continued)

Position	Stemphone E		Stemphone F		Stemphone G	
	δ_C	δ_H (J Hz)	δ_C	δ_H (J Hz)	δ_C	δ_H (J Hz)
1	12.9	1.62 d (6.0)	13.4	1.63 d (7.0)	13.4	1.62 d (7.0)
2	125.8	5.63 q (6.0)	127.5	5.70 q (7.0)	125.6	5.64 q (7.0)
3	132.5	—	131.5	—	132.5	—
4	83.1	5.34 d (10.0)	83.1	5.14 d (10.0)	82.5	5.36 d (10.0)
5	33.8	3.44 dq (9.0, 7.0)	36.2	3.00 dq (10.0, 7.0)	36.1	3.48 dq (10.0, 7.0)
6	128.8	—	162.0	—	140.5	—
7	136.5	—	71.9	—	135.6	—
8	139.7	—	169.1	—	144.2	—
9	111.0	—	110.1	—	107.0	—
10	147.4	—	187.5	—	133.8	—
10-OH						10.8 s
11	104.6	6.18 s	124.1	6.06 s	107.4	6.33 s
12	63.6	5.06 d (10.0)	62.4	4.79 d (10.5) 2.52 dd (19.0, 5.0)	199.5	—
12-OH ^a	—	—	—	—	—	—
13	46.5	2.32 d (10.0)	46.4	2.06 d (10.5)	53.6	3.49 s
14	80.4	—	83.5	—	84.1	—
15	37.6	1.88 m	37.4	1.60, 1.96 m	37.4	2.38 m
16	25.1	1.58 m, 1.80 m	25.2	1.58, 1.82 m	24.9	1.66, 1.86 m
17	76.6	3.70 dd (13.0, 4.0)	76.7	3.66 dd (13.0, 4.5)	75.9	3.74 dd (13.0, 4.0)
18	40.8 s	40.5	—	40.9	—	40.3 —
19	70.7 d	4.14 t br (3.0)	70.9	4.11 t, br (3.5)	70.0	4.76 t, br (3.0)
19-OH ^a	—	—	—	—	—	—
20	28.1	1.60 m, 1.92 m	28.4	1.58, 1.94 m	29.7	1.50, 2.06 m
21	79.5	3.58 dd (13.0, 3.0)	80.0	3.56 dd (13.0, 3.0)	79.6	3.61 dd (13.0, 3.0)
22	72.0	—	72.1	—	72.0	—
23	23.7	1.13 s	23.8	1.13 s	24.0	1.16 s
24	26.0	1.18 s	26.5	1.18 s	26.4	1.20 s
25	13.1	0.97 s	13.3	0.95 s	13.3	1.12 s
26	22.1	1.24 s	21.8	1.28 s	22.5	1.48 s
27	17.6	0.99 d (7.5)	20.1	0.99 d (7.0)	17.0	1.09 d (7.0)
28	10.9	1.64 s	11.1	1.61 s	11.5	1.64 s
29	171.4	—	169.4	—	170.2	—
30	21.2	1.78 s	21.3	1.94 s	21.3	1.84 s
31			46.6	2.50 d (15.5) 2.94 d (15.5)		
32			209.9	—		
33			33.5	2.30 s		

^a Exchangeable signals.

while stemphones E, F and G had different patterns in UV absorption. The IR absorption at $3442\sim 2929$ and $1735\sim 1604\text{ cm}^{-1}$ suggested the presence of hydroxy and carbonyl groups in the structures.

Stemphone D: The molecular formula was determined to be $\text{C}_{30}\text{H}_{42}\text{O}_8$ on the basis of HRFAB-MS measurement, indicating that stemphone D contained one less oxygen atom relative to stemphone B. The ^{13}C -NMR spectrum (in CDCl_3) showed 30 resolved signals, which were classified into eight methyl carbons, four methylene carbons, two sp^3 methine carbons, two sp^2 methine carbons, four oxygenated sp^3 methine carbons, one sp^3 quaternary carbon, two oxygenated sp^3 quaternary carbons, four sp^2 quaternary carbons and three carbonyl carbons by analysis of the DEPT spectra. The ^1H -NMR spectrum (in CDCl_3)

displayed 40 proton signals. The connectivity of proton and carbon atoms was established by the ^{13}C - ^1H HMQC spectrum (Table 2). These spectral data and physico-chemical properties showed that stemphone D has the same skeleton as stemphones B and C [3]. Furthermore, the

Fig. 4 Key HMBC of stemphone E.

Fig. 5 Key HMBC of stemphone F.

Fig. 6 Key HMBC of stemphone G.

Scheme 1 Semisynthetic strategy for stemphone C-4 and C-11 derivatives.

(a) LiAlH_4 , Et_2O , 0°C ; (b) $(\text{R}_1\text{CO})_2\text{O}$, DMAP, TEA, CH_2Cl_2 ; (c) R_2OH , DMAP, pyridine, 60°C .

methylene proton signals (δ 2.12, 2.50) were observed instead of the methine proton signal of H-12 (δ 4.90) of stemphone B, indicating that stemphone D is 12-dehydroxy stemphone B. Thus, the structure of stemphone D was elucidated as shown in Fig. 1.

Stemphone E: The molecular formula of stemphone E is $C_{30}H_{44}O_9$, indicating that two hydrogen atoms are bigger than stemphone B. The 1H -NMR spectrum of stemphone E was almost identical to that of stemphone B except for the absence of the quinone carbonyl signals in stemphone E. Instead, five quaternary carbons (δ 111.2, 128.8, 136.5, 139.7, 147.4) and one methine carbon (δ 104.6) were observed, indicating the presence of a quinol residue instead of a quinone residue. This was also supported by the physico-chemical properties of the UV absorption (292 nm) and the appearance (white powder) (Table 1) [5]. Furthermore, the ^{13}C - 1H long range couplings of 2J and 3J in the ^{13}C - 1H HMBC spectrum revealed the following linkage of quinol carbons (Fig. 4). The cross peaks were observed from H-5 (δ 3.44) to C-6 (δ 128.8), C-7 (δ 136.5) and C-11 (δ 104.6), from H-11 (δ 6.18) to C-6, C-7 and C-9 (δ 111.0), and from H-12 (δ 5.06) to C-8 (δ 139.7), C-9 and C-10 (δ 147.4). The chemical shifts of the quinol moiety for stemphone E were comparable to that of isocochlioquinone A [6]. Taken together, the structure of stemphone E was elucidated as shown in Fig. 1.

Stemphone F: The molecular formula of stemphone F is $C_{33}H_{48}O_{10}$. The 1H -NMR spectrum was similar to that of stemphone B except for the presence of a methyl proton signal (δ 2.30) and methylene proton signals (δ 2.50, 2.94) in stemphone F. Moreover, the signals of a methyl carbon (δ 33.5), a methylene carbon (δ 46.6), an oxyquaternary carbon (δ 71.9) and a carbonyl carbon (δ 209.9) were newly observed by the analysis of the ^{13}C -NMR spectrum instead of the absence of a quinone carbonyl carbon (C-7) in stemphone B. Furthermore, ^{13}C - 1H HMBC experiments gave the following linkage (Fig. 5). The cross peaks were observed from H-33 (δ 2.30) to C-31 (δ 46.6) and C-32 (δ 209.9), and from H-31 (δ 2.50, 2.94) to C-7 and C-8, indicating that a propanone residue was attached to the C-7 position. And the chemical shift of C-7 (δ 71.9) showed that a hydroxyl group was also attached to this position. Furthermore, the chemical shifts of stemphone F were comparable to those of cochlioquinol II [7]. Taken together, stemphone F was elucidated as shown in Fig. 1.

Stemphone G: The molecular formula is $C_{30}H_{42}O_9$, indicating that two hydrogen atoms were smaller than stemphone E. The 1H -NMR spectrum of stemphone G resembled that of stemphone E except that the methine proton (δ 5.06) in stemphone E was not observed. Accordingly, a carbonyl carbon signal (δ 199.5) was

Table 3 MIC of imipenem against MRSA in the presence of stemphones and cytotoxic effect of stemphones on Jurkat cells [3, 5]

In combination with	MIC of IPM (ng/ml)	Potential Ratio (None/stemphone)	IC ₅₀ (μ g/ml)
None	16000	1	—
Stemphone B	30	533	2.13
C	30	533	0.43
C1a	1000	16	13.7
C1b	16000	1	5.75
C1c	16000	1	8.51
C1d	2000	8	16.2
C1e	16000	1	9.12
C1f	16000	1	12.2
C1g	16000	1	20.6
C1h	1000	16	15.1
C1i	16000	1	2.82
C2	60	266	0.78
C3a	60	266	0.50
C3b	60	266	1.35
C3c	250	64	1.50
C3d	16000	1	0.56
C3e	250	64	0.95
C4	16000	1	34.8
D	60	266	1.81
E	30	533	3.64
E1	4000	4	26.2
E2	30	533	4.44
E3	30	533	0.59
E4	16000	1	>100
F	60	266	2.69
G	16000	1	23.7
G1	16000	1	>100
Cochlioquinone D	8000	2	0.070

Concentration of stemphones is 4.0 μ g/ml. IPM, imipenem.

observed in stemphone G instead of the C-12 methine carbon of stemphone E, showing the presence of an oxo residue at C-12 in stemphone G. Furthermore, cross peaks were observed from H-13 (δ 3.49) to C-9 (δ 107.0), C-12 (δ 199.5), C-14 (δ 84.1), C-15 (δ 37.4), C-17 (δ 75.9) and C-18 (δ 40.3) by the analysis of ^{13}C - 1H HMBC experiments (Fig. 6), revealing that an oxo residue was attached to the C-12 position. In addition, a low field of the hydroxy proton signal (δ 10.8) in the 1H -NMR spectrum indicated a hydrogen bond between the C-12 carbonyl oxygen and the H-10 hydroxy proton. Taken together, these data indicated that stemphone G was 12-oxo stemphone E as shown in Fig. 1.

Synthesis of Stemphone Derivatives

Semisynthetic strategy for C-4 and C-11 derivatives is summarized in Scheme 1.

Stemphone C-4 derivatives: First, reduction of the acetyl residue at C-4 of stemphone C with LiAlH_4 yielded stemphone C2 at 42.2%. Then, stemphone C2 was acylated with corresponding anhydrous acids to give stemphones C3a (44.9%), C3b (41.3%), C3c (46.6%), C3d (22.4%) and C3e (37.7%).

Stemphone C-11 derivatives: Stemphone C was treated with methanol and TEA to yield stemphone C1a (40.4%). Similarly, stemphone C was treated with corresponding alcohols and DMAP in pyridine to afford C1b (35.6%), C1c (29.9%), C1d (17.7%), C1e (17.5%), C1f (2.11%), C1g (24.9%) and C1i (53.0%).

Potential of Imipenem Activity against MRSA by Stemphones

By the paper disk assay, stemphones and the derivatives alone showed almost no anti-MRSA activity at least up to $50 \mu\text{g}/6 \text{ mm}$ disk as well as stemphones B and C. However, stemphones D, E, E1, E2, E3, F, C2, C3a, C3b and C3c showed anti-MRSA activity on the MHA plate containing imipenem ($10 \mu\text{g}/\text{ml}$) with an inhibition zone of 25, 22, 15, 22, 20, 20, 20, 16, 16 and 17 mm at $10 \mu\text{g}/6 \text{ mm}$ disk, respectively. Under the same conditions, stemphones B and C showed anti-MRSA activity with an inhibition zone of 20 and 22 mm as reported previously [3]. Thus, the findings

indicate that these stemphones potentiated the imipenem activity against MRSA. Cochlioquinone D and stemphones C1h and C3e seemed to be weak potentiators with an inhibition zone of 10 mm. On the other hand, the other stemphones showed almost no potentiating activity with no inhibition zone on the MHA plate containing imipenem.

Next, the potentiating activity of all stemphones was confirmed by the liquid microdilution method. First, anti-MRSA activity of these stemphones alone was studied by this method. Their MIC values were calculated to be over $16 \mu\text{g}/\text{ml}$ (*cf.* MIC of stemphone C is $16 \mu\text{g}/\text{ml}$ [3]). Therefore, the concentrations of stemphones were set up at $4.0 \mu\text{g}/\text{ml}$ to test the potentiation activity. The results of potentiation of imipenem activity against MRSA by the stemphones are summarized in Table 3. As previously reported [3], stemphones B and C enhanced the imipenem activity over 500-fold. Stemphones D, E, E2, E3, F, C2, C3a and C3b showed almost the same potentiating activity as stemphones B and C (266~533 folds) followed by stemphones C3c and C3e (64 folds). Stemphones C1a, C1d and C1h were moderate potentiators (8~16-fold). The 12 other compounds had almost no potentiating activity. These data are fundamentally comparable to those obtained from the paper disk method as described above.

Cytotoxicity against Jurkat Cells

Cytotoxic effect of all stemphones on Jurkat cells is summarized in Table 3. Among them, cochlioquinone D

Fig. 7 Summary of structure-activity relationships.

showed the most potent cytotoxicity with an IC_{50} of $0.070\ \mu\text{g/ml}$, while stemphones E1 and G1 had almost no cytotoxicity ($IC_{50} > 100\ \mu\text{g/ml}$). Stemphones B, E, E2 and E3 showed moderate cytotoxicity (IC_{50} , $2.13\sim 4.44\ \mu\text{g/ml}$) and the strongest potentiation of imipenem activity against MRSA (533 folds, Table 3).

Discussion

Seven structurally related compounds including six new stemphones B to G and known cochlioquinone D were isolated from the culture broth of *Aspergillus* sp. FKI-2136, but stemphone (stemphone A) itself was not detected and isolated from the culture. Since stemphone F has a propanone residue in the structure, it is plausible that stemphone F is produced by the treatment of stemphone B with acetone. However, no product was obtained by the treatment of stemphone B with acetone.

Compounds structurally related to stemphones and cochlioquinones have various biological activities [8~10]. As previously reported, we found a new biological activity, namely an activity potentiating the β -lactam imipenem activity against MRSA [3]. In this study, the potentiating activity of all the stemphones (natural and semisynthetic ones) was tested to investigate the structure-activity relationships (Fig. 7). Regarding the C-4 position (R_1), *O*-acyl derivatives with a C2 to C5 carbon length retained the potentiating activity, but derivatives with a longer acyl residue drastically decreased the activity, indicating that the moderate lipophilicity at C-4 is important for the activity. Regarding the C-11 position (R_5), introduction of most *O*-alkyl residues resulted in a decrease of the activity. Only hydroxy and *O*-methyl showed very weak activity. Thus, no modification at this position is preferable for the potentiating activity. Furthermore, regarding the 7,10-quinone part, it is replaceable by 7,10-hydroquinone or 7-hydroxy-7-propanone-10-oxo. However, activity was lost when a methyl or acetyl residue was introduced to the C-10 hydroxy group. Regarding the C-12 (R_3) and C-19 (R_4) positions, the potentiating activity was retained regardless of introduction of a hydroxy residue in these positions. Among these stemphones, it was concluded that stemphone E is the most selective compound, showing the most potent potentiation of imipenem activity against MRSA (533-fold) and the lowest cytotoxic activity against Jurkat cells. This information about the structure-activity relationships would lead to a new type of anti-infective agent as an imipenem potentiator against MRSA.

Several natural products have previously been reported to potentiate β -lactam activity against MRSA [11~16],

possibly due to inhibition of penicillin-binding proteins 2 (PBP2') and suppression of β -lactamase activity. Our preliminary experiments on the mechanism of action suggested that stemphones showed almost no effect on PBP2' (data not shown). The mechanism of the potentiating activity by stemphones remains to be determined. This study on the structure-activity relationships of stemphones could be useful for preparing a probe to analyze the target molecule. Further investigations on the mechanism of action are now in progress.

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