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ORIGINAL ARTICLE

Naphthacemycins, novel circumventors of β-lactam resistance in MRSA, produced by *Streptomyces* sp. KB-3346-5. II. Structure elucidation

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Seventeen new compounds, naphthacemycins A_1–A_{11}, B_1–B_4 and C_1–C_2, were isolated from a cultured broth of *Streptomyces* sp. KB-3346-5 during screening for circumventors of β-lactam resistance in methicillin-resistant *Staphylococcus aureus*. Their structures were elucidated by spectroscopic studies, including NMR and X-ray crystallographic analysis. The naphthacemycin A series has a new skeleton displaying a 7-phenylnaphthacene-5,6,11(12*H*)-trione. In contrast, the quinone moiety of the A series is changed to dehydroxyquinol in the B series and to a semiquinone-like structure in the C series.
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INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of untreatable and potentially fatal hospital-associated infections. Community-acquired MRSA has also become a serious public health issue.[1] MRSA is resistant to β-lactam antibiotics and usually resistant to most other classes of antibiotics. There are a few antibiotics used for MRSA, for example, vancomycin, teicoplanin, arbekacin, linezolid, daptomycin and tigecycline, but microorganism strains resistant to these compounds are increasingly being reported. In the course of screening for new anti-MRSA compounds, we found cyslabdan, which enhances the activity of imipenem (a carbapenem type β-lactam antibiotic) against MRSA.[2,3] Further screening for microbial metabolites that circumvent the β-lactam resistance of MRSA led us to find the naphthacemycins A_1–A_{11} (**1–11**), B_1–B_4 (**12–15**) and C_1–C_2 (**16–17**) (Scheme 1), reported as KB-3346-5 substances in the patent by our group,[4] from a cultured broth of *Streptomyces* sp. KB-3346-5.[5] Here we describe the structure elucidation of the naphthacemycins, based on NMR study and X-ray crystallographic analysis.

RESULTS AND DISCUSSION

Physicochemical properties

Naphthacemycins (**1–17**) were purified from a cultured broth of *Streptomyces* sp. KB-3346-5, isolated from a soil sample collected in Okinawa Prefecture, Japan.[5] They are red powders. Compounds **2**, **4-6**, **8**, **11** and **14–17** have one chlorine atom, while **7** and **10** have two chlorine atoms. The IR spectra showed they all have carbonyl (1600–1720 cm^{-1}) and hydroxyl (3350–3440 cm^{-1}) groups. UV spectra were observed at 274–280, 302–306 and 362–408 nm in the naphthacemycin A series (**1–11**), at 246–249, 287–288, 352–354 and 414–417 nm in the naphthacemycin B series (**12–15**), and 248 and 410 nm in the naphthacemycin C series (**16–17**). Naphthacemycins are soluble in chloroform, ethyl acetate and methanol and insoluble in *n*-hexane and H_2O.

Structure elucidation of the naphthacemycin A series

The molecular formula of naphthacemycin A_9 (**9**) was established as $C_{30}H_{26}O_8$ by HR-FAB-MS. ^1H and ^{13}C NMR spectra and HSQC analysis revealed the presence of 30 carbons, including six methyl, six sp^2 methine, one sp^3 quaternary and 17 sp^2 quaternary carbons (Table 1). The long-range couplings of HMBC correlation revealed fragments I and II (Figure 1) as follows; the cross peaks from H-1 (δ_H 6.55) to C-2 (δ_C 163.9), C-3 (δ_C 101.6), C-4a (δ_C 110.0) and C-12a (δ_C 154.7), from H-3 (δ_H 6.25) to C-1 (δ_C 105.5), C-2, C-4 (δ_C 165.0) and C-4a, and from 4-OH (δ_H 12.86) to C-3, C-4 and C-4a indicated a 2,3,5-trisubstituted phenol (ring A). The cross peaks from H_3-13 (δ_H 1.77) and H_3-14 (δ_H 1.83) to C-11a (δ_C 155.4), C-12 (δ_C 39.2) and C-12a indicated a dimethyl moiety being connected to C-12a via C-12. This was confirmed by the coupling between H-1 and C-12, and thus the fragment I was established. An oxygen atom is suggested to be attached to C-2 by its chemical shift. The cross peaks from H-8 (δ_H 6.99) to C-6a (δ_C 125.8), C-7 (δ_C 140.1), C-9 (δ_C 162.6) and C-10 (δ_C 109.5), from H-10 (δ_H 7.52) to C-6a, C-8 (δ_C 124.6), C-9, C-10a (δ_C 135.8) and C-11 (δ_C 185.7), and from 9-OCH_3 (δ_H 3.94) to C-9 indicated a 3,4,5-trisubstituted anisole (ring D). The cross peaks from H-17 (δ_H 6.39) to C-15 (δ_C 120.9), C-16 (δ_C 156.7), C-18 (δ_C 159.9) and

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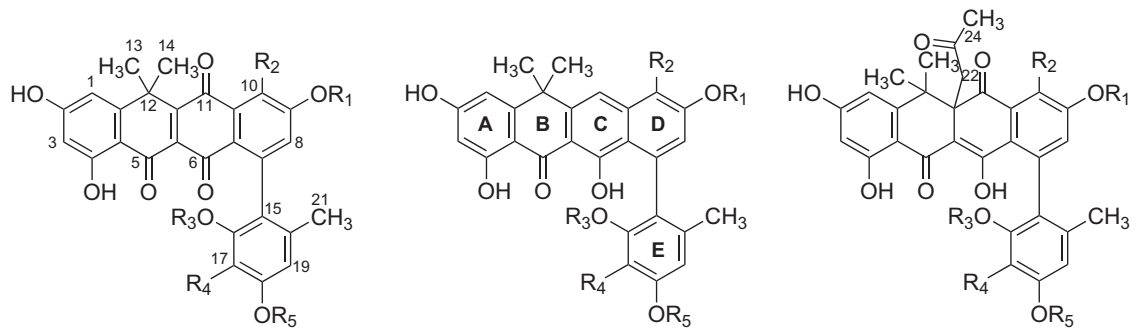

Naphthacemycin A series (**1**-**11**) | Naphthacemycin B series (**12**-**15**) | Naphthacemycin C series (**16** and **17**)

	R_1	R_2	R_3	R_4	R_5
Naphthacemycin A_1 (**1**)	H	H	CH_3	H	H
Naphthacemycin A_2 (**2**)	H	H	CH_3	Cl	H
Naphthacemycin A_3 (**3**)	CH_3	H	CH_3	H	H
Naphthacemycin A_4 (**4**)	CH_3	Cl	CH_3	H	H
Naphthacemycin A_5 (**5**)	H	H	CH_3	Cl	CH_3
Naphthacemycin A_6 (**6**)	CH_3	H	CH_3	Cl	H
Naphthacemycin A_7 (**7**)	CH_3	Cl	CH_3	Cl	H
Naphthacemycin A_8 (**8**)	CH_3	Cl	CH_3	H	CH_3
Naphthacemycin A_9 (**9**)	CH_3	H	CH_3	H	CH_3
Naphthacemycin A_{10} (**10**)	CH_3	Cl	CH_3	Cl	CH_3
Naphthacemycin A_{11} (**11**)	CH_3	H	CH_3	Cl	CH_3
Naphthacemycin B_1 (**12**)	H	H	H	H	H
Naphthacemycin B_2 (**13**)	H	H	CH_3	H	H
Naphthacemycin B_3 (**14**)	H	H	CH_3	Cl	H
Naphthacemycin B_4 (**15**)	H	Cl	CH_3	H	H
Naphthacemycin C_1 (**16**)	CH_3	Cl	CH_3	H	H
Naphthacemycin C_2 (**17**)	CH_3	H	CH_3	Cl	CH_3

Scheme 1 Structures of naphthacemycins A_1–A_{11} (**1**–**11**), B_1–B_4 (**12**–**15**) and C_1–C_2 (**16**–**17**).

C-19 (δ_C 106.9), from H-19 (δ_H 6.44) to C-15, C-17 (δ_C 96.4) and C-18, from H_3-21 (δ_H 2.06) to C-15, C-19 and C-20 (δ_C 137.0), from 16-OCH_3 (δ_H 3.65) to C-16, and from 18-OCH_3 (δ_H 3.80) to C-18 indicated a 2-monosubstituted 3,5-dimethoxytoluene (ring E). The coupling between H-8 and C-15 suggested a connection of rings D and E at C-7 and C-15, respectively, and thus the fragment II was established. Among the remaining three carbons, C-5 (δ_C 185.0) had couplings with H-1 and H-3 and C-6 (δ_C 182.6) had a coupling with H-10. They are considered to be $^4J_{CH}$ W-couplings, and C-5 and C-6 are suggested to bond to C-4a and C-6a, respectively (Figure 2). The chemical shifts of C-6 (δ_C 182.6) and C-11 (δ_C 185.7) indicated that they form a quinone. If the remaining C-5a (δ_C 135.5) bonds to C-5 and C-11a to form ring B, and C-5a–C-6 and C-11–C-11a bondings form ring C, the naphthacenequinone structure is constructed. C-2 is suggested to be hydroxylated by the molecular formula. The carbon connections unrevealed by HMBC were clarified by INADEQUATE analysis (Table 1). Thus the structure of **9** was elucidated as shown in Scheme 1. It has a 7-phenylnaphthacene-5,6,11 (12*H*)-trione skeleton. Most structurally related compounds, tetarimycin A[6] and fasamycins[7], were recently reported by Brady and co-workers as antibacterial agents. The structure was confirmed by X-ray crystallographic analysis as shown in Figure 3. The naphthacenequinone forms a planar skeleton.

The molecular formula of naphthacemycin A_8 (**8**) was established as $C_{30}H_{25}ClO_8$ by HR-FAB-MS, and this suggested that **8** was chlorinated analog of **9**. ^1H and ^{13}C NMR spectra of **8** were similar to those of **9**, except for ring D (Table 1). The HMBC correlations from H-8 (δ_H 6.93) to C-6a (δ_C 127.1), C-7 (δ_C 137.4), C-9 (δ_C 158.4) and C-10 (δ_C 120.4) and from 9-OCH_3 (δ_H 3.95) to C-9 were observed, and C-10 was a quaternary carbon (Figure 4). Thus, the structure of **8** was elucidated as 10-chloro-**9**, which was confirmed by X-ray crystallographic analysis (Figure 5). In contrast to **9**, the naphthacenequinone of **8** bent about 40° at two quinone carbonyl atoms of ring C.

The structures of the other naphthacemycin A series were elucidated by comparison with NMR data of **8** or **9** or other A series compounds (Supplementary Tables S1 and S2) and analyses of HMBC correlations (Supplementary Figure S1).

Structure elucidation of the naphthacemycin B series

The molecular formula of naphthacemycin B_1 (**12**) was established as $C_{27}H_{22}O_7$ by HR-FAB-MS, which indicated **12** was 28 mass units (CO) less than **1**. ^1H and ^{13}C NMR spectra and HSQC analysis revealed the presence of 27 carbons, including three methyl, seven sp^2 methine, one sp^3 quaternary and sixteen sp^2 quaternary carbons (Table 1). The structure was elucidated by long-range couplings of HMBC correlation (Figure 4). The cross peaks from H-1 (δ_H 6.65) to

Table 1 NMR spectroscopic data for 8, 9, 12 and 17[a]

	8[b]		***9***[b]			***12***[c]		***17***[c]	
Position	δ_C	δ_H	δ_C	δ_H	*INADEQUATE*	δ_C	δ_H	δ_C	δ_H
1	105.4	6.54 (1H, s)	105.5	6.55 (1H, s)	2, 12a	107.1	6.65 (1H, s)	105.4	6.46 (1H, s)
2	164.3		163.9		1, 3	166.7		162.6	
3	101.3	6.27 (1H, s)	101.6	6.25 (1H, s)	2, 4	102.1	6.20 (1H, s)	102.1	6.26 (1H, s)
4	165.0		165.0		3, 4a	166.6		165.4	
4-OH		13.02 (1H, s)		12.86 (1H, s)			12.87 (1H, s)		12.52 (1H, s)
4a	109.2		110.0		4, 5, 12a	108.6		108.6	
5	183.8		185.0		4a, 5a	191.6		188.5	
5a	134.6		135.5		5, 6, 11a	107.6		107.6	
6	181.4		182.6		5a, 6a	160.1		168.8	
6a	127.1		125.8		6, 7, 10a	118.2		123.8	
6-OH							14.53 (1H, s)		14.74 (1H, s)
7	137.4		140.1		6a, 8, 15	140.8		139.3	
8	119.2	6.93 (1H, s)	124.6	6.99 (1H, s)	7, 9	122.5	6.72 (1H, s)	124.6	6.98 (1H, s)
9	158.4		162.6		8, 10	166.9		161.9	
9-OMe	56.8	3.95 (3H, s)	55.8	3.94 (3H, s)				55.8	3.94 (3H, s)
10	120.4		109.5	7.52 (1H, s)	9, 10a	110.1	7.05 (1H, s)	110.1	7.73 (1H, s)
10a	133.0		135.8		6a, 10, 11	142.9		137.3	
11	186.5		185.7		10a, 11a	116.1	7.36 (1H, s)	197.8	
11a	158.1		155.4		5a, 11, 12	146.5		53.2	
12	39.4		39.2		11a, 12a, 13, 14	39.7		43.3	
12a	154.3		154.7		1, 4a, 12	155.5		152.9	
13	28.5	1.76 (3H, s)	29.6	1.77 (3H, s)	12	34.5	1.91 (3H, s)	21.0	1.59 (3H, s)
14	30.8	1.85 (3H, s)	30.0	1.83 (3H, s)	12	34.7	1.70 (3H, s)	26.5	1.01 (3H, s)
15	120.3		120.9		7, 16, 20	124.5		130.2	
16	156.4		156.7		15, 17	155.4		153.1	
16-OMe	55.7	3.62 (3H, s)	55.7	3.65 (3H, s)				60.5	3.63 (3H, s)
17	96.3	6.39 (1H, s)	96.4	6.39 (1H, s)	16, 18	100.8	6.23 (1H, s)	113.5	
18	160.2		159.9		17, 19	157.5		154.7	
18-OMe	55.2	3.83 (3H, s)	55.2	3.80 (3H, s)				56.3	3.94 (3H, s)
19	106.9	6.46 (1H, s)	106.9	6.44 (1H, s)	18, 20	108.8	6.26 (1H, s)	108.6	6.61 (1H, s)
20	137.1		137.0		15, 19, 21	138.3		134.9	
21	20.7	2.08 (3H, s)	20.6	2.06 (3H, s)	20	20.7	1.88 (3H, s)	20.3	1.96 (3H, s)
22								52.4	2.89 (1H, d, 17.7), 3.52 (1H, d, 17.7)
23								205.4	
24								29.6	1.89 (3H, s)

[a]300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR.
[b]Solvent: $CDCl_3$.
[c]Solvent: acetone-d_6. δ_H (Int, mult, J in Hz).

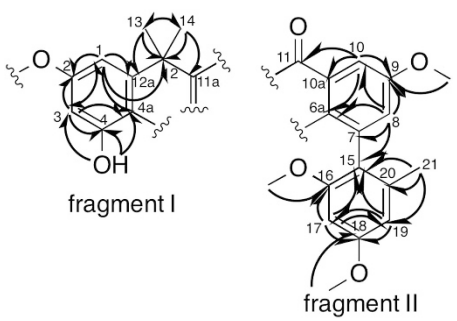

Figure 1 Partial structures of **9**.

C-2 (δ_C 166.7), C-3 (δ_C 102.1) and C-4a (δ_C 108.6), from H-3 (δ_H 6.20) to C-1 (δ_C 107.1), C-2, C-4 (δ_C 166.6) and C-4a, and from 4-OH (δ_H 12.87) to C-3, C-4 and C-4a indicated a 2,3,5-trisubstituted phenol (ring A). The cross peaks from H_3-13 (δ_H 1.91) and H_3-14 (δ_H 1.70) to C-11a (δ_C 146.5), C-12 (δ_C 39.7) and C-12a (δ_C 155.5) indicated a dimethyl moiety connected to C-12a via C-12. This was confirmed by the coupling between H-1 and C-12. The cross peaks from H-8 (δ_H 6.72) to C-6a (δ_C 118.2), C-9 (δ_C 166.9) and C-10 (δ_C 110.1) and from H-10 (δ_H 7.05) to C-6a, C-8 (δ_C 122.5), C-9, C-10a (δ_C 142.9) and C-11 (δ_C 116.1) indicated a 3,4,5-trisubstituted phenol (ring D). The cross peaks from H-11 (δ_H 7.36) to C-5a (δ_C 107.6), C-6a and C-12 and from 6-OH (δ_H 14.53) to C-5a, C-6 (δ_C 160.1) and C-6a indicated a 2,3,5,6-tetrasubstituted phenol (ring C), which condensed with ring D and connected to ring A via C-12. Comparing NMR data and structures of **12** with **1**, it is appropriate to form 4,4-dimethylcyclohexadienone (ring B) by C-4a, 5, 5a, 11a, 12 and 12a, though C-5 had no coupling with neighboring protons. The resemblance of the NMR data also suggested that a hydroxyl residue is attached to C-2 of **12** as in **1**. The cross peaks from H-17 (δ_H 6.23) to C-15 (δ_C 124.5), C-16 (δ_C 155.4), C-18 (δ_C 157.5) and C-19 (δ_C 108.8), from H-19 (δ_H 6.26) to C-15, C-17 (δ_C 100.8) and C-18, and from H_3-21 (δ_H 1.88) to C-15, C-19 and C-20 (δ_C 138.3) indicated 2-monosubstituted

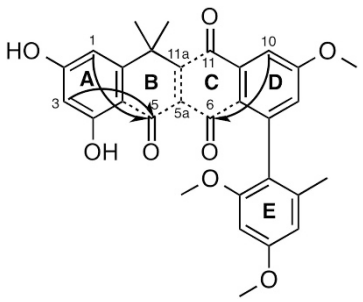

Figure 2 Structure elucidation of **9**.

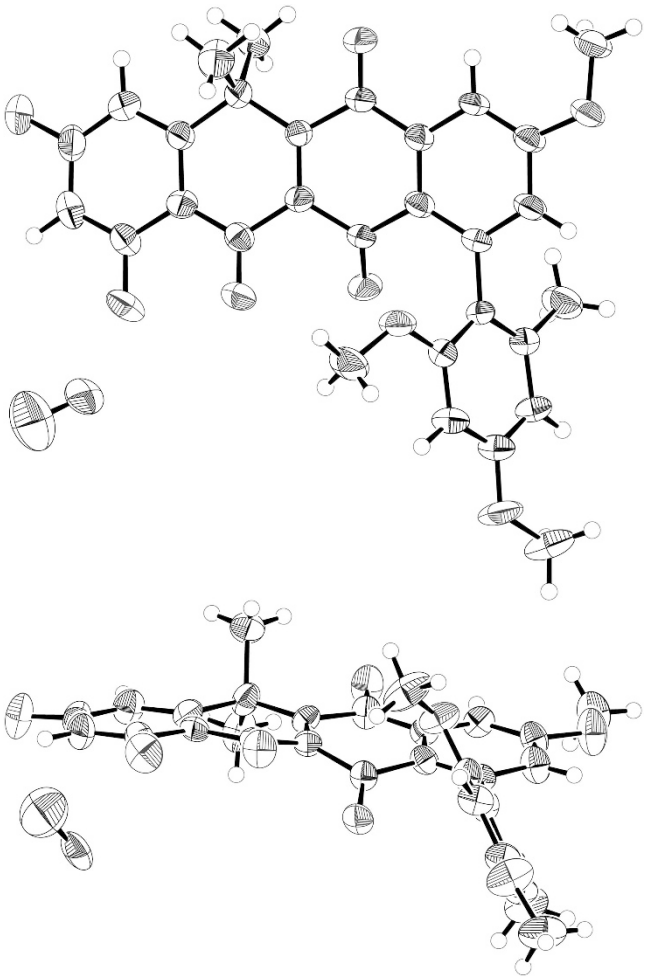

Figure 3 ORTEP (Oak Ridge thermal ellipsoid plot) plot of the X-ray crystallographic structure of **9**.

3,5-dihydroxytoluene (ring E). The coupling between H-8 and C-15 indicated that ring E connects to ring D at C-7. Thus, the structure of **12** was elucidated as 6,11-didehydro-O^{16}-demethyl-6,11-dideoxo-11-hydroxy-**1**.

Structure elucidation of the naphthacemycin C series

The molecular formula of naphthacemycin C_2 (**17**) was established as $C_{33}H_{31}ClO_9$ by HR-FAB-MS, which indicated **17** was 58 mass units (C_3H_6O) more than **11**. ^1H and ^{13}C NMR spectra and HSQC analysis revealed the presence of 33 carbons, including seven methyl, one

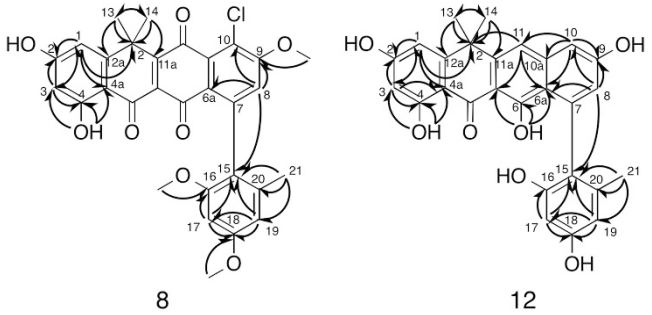

Figure 4 Structure elucidation of **8** and **12** by HMBC.

sp^3 methylene, five *sp*^{2} methine, two *sp*^{3} quaternary and eighteen *sp*^{2} quaternary carbons (Table 1). The structure was elucidated by long-range couplings of HMBC correlation (Figure 6). The cross peaks from H-1 (δ_H 6.46) to C-2 (δ_C 162.6), C-3 (δ_C 102.1) and C-4a (δ_C 108.6), from H-3 (δ_H 6.26) to C-1 (δ_C 105.4), C-2, C-4 (δ_C 165.4) and C-4a, and from 4-OH (δ_H 12.52) to C-3, C-4 and C-4a indicated a 2,3,5-trisubstituted phenol (ring A). The cross peaks from H_3-13 (δ_H 1.59) and H_3-14 (δ_H 1.01) to C-11a (δ_C 53.2), C-12 (δ_C 43.3) and C-12a (δ_C 152.9) indicated a dimethyl moiety was connected to C-12a via C-12. This was confirmed by the coupling between H-1 and C-12. The cross peaks from H-8 (δ_H 6.98) to C-6a (δ_C123.8), C-9 (δ_C 161.9) and C-10 (δ_C 110.1), H-10 (δ_H 7.73) to C-6a, C-8 (δ_C 124.6), C-9, C-10a (δ_C 137.3) and C-11 (δ_C 197.8), and from 9-OCH_3 (δ_H 3.94) to C-9 indicated a 3,4,5-trisubstituted anisole (ring D). The cross peaks from 6-OH (δ_H 14.74) to C-5a (δ_C 107.6), C-6 (δ_C 168.8) and C-6a and from H_2-22 (δ_H 2.89, 3.52) to C-5a, C-11 and C-11a indicated a 2,3,5,6-tetrasubstituted 4-hydroxy-2,4-cyclohexadienone (ring C), condensed with ring D and connected to ring A via C-12. The cross peaks from H_3-24 (δ_H 1.89) to C-22 (δ_C 52.4) and C-23 (δ_C 205.4) and from H_2-22 to C-23 indicated that a 2-oxopropyl residue was attached to C-11a. Comparing NMR data and structures of **17** with **11**, it is appropriate to form 4,4-dimethyl-2-cyclohexenone (ring B) by C-4a, 5, 5a, 11a, 12 and 12a, though C-5 had no coupling with neighboring protons. The chemical shifts of ring E of **17** is quite similar to **11**, which suggests ring E of **17** is 2-monosubstituted 4-chloro-3,5-dimethoxytoluene. This was confirmed by the cross peaks from H-19 (δ_H 6.61) to C-15 (δ_C 130.2), C-17 (δ_C 113.5) and C-18 (δ_C 154.7), from H_3-21 (δ_H 1.96) to C-15, C-19 (δ_C 108.6) and C-20 (δ_C 134.9), from 16-OCH_3 (δ_H 3.63) to C-16 (δ_C 153.1), and from 18-OCH_3 (δ_H 3.94) to C-18. The coupling between H-8 and C-15 indicated that ring E connects to ring D at C-7. Thus, C-11a of **11** was substituted with a 2-oxopropyl group and the C-6 ketone was reduced in **17**.

The molecular formula of naphthacemycin C_1 (**16**) was established as $C_{32}H_{29}ClO_9$ by HR-FAB-MS, which indicated **16** was 14 mass units (CH_2) less than **17**. ^1H and ^{13}C NMR spectra of **16** were quite similar to those of **17**, except for rings D and E (Supplementary Table S3). The H-10 signal of **17** disappeared in **16** and the cross peaks of HMBC from H-8 (δ_H 7.01) to C-6a (δ_C 126.7), C-9 (δ_C 158.6) and C-10 (δ_C 120.8) and from 9-OCH_3 (δ_H 4.03) to C-9 were observed, which indicated that a chlorine was attached to the C-10 of ring D (Figure 6). The 18-OCH_3 signal of **17** was also absent in **16** and an H-17 (δ_H 6.37) signal appeared in **17**. The cross peaks from H-17 (δ_H 6.37) to C-15 (δ_C 123.7), C-16 (δ_C 158.1), C-18 (δ_C 158.4) and C-19 (δ_C 109.4), from H-19 (δ_H 6.39) to C-15, C-17 (δ_C 97.4) and C-18, from H_3-21 (δ_H 2.01) to C-15, C-19 and C-20 (δ_C 137.0), and from 16-OCH_3 (δ_H 3.64) to C-16 indicated a ring E structure of 2-monosubstituted 3,5-dimethoxytoluene. Thus **16** was 10-

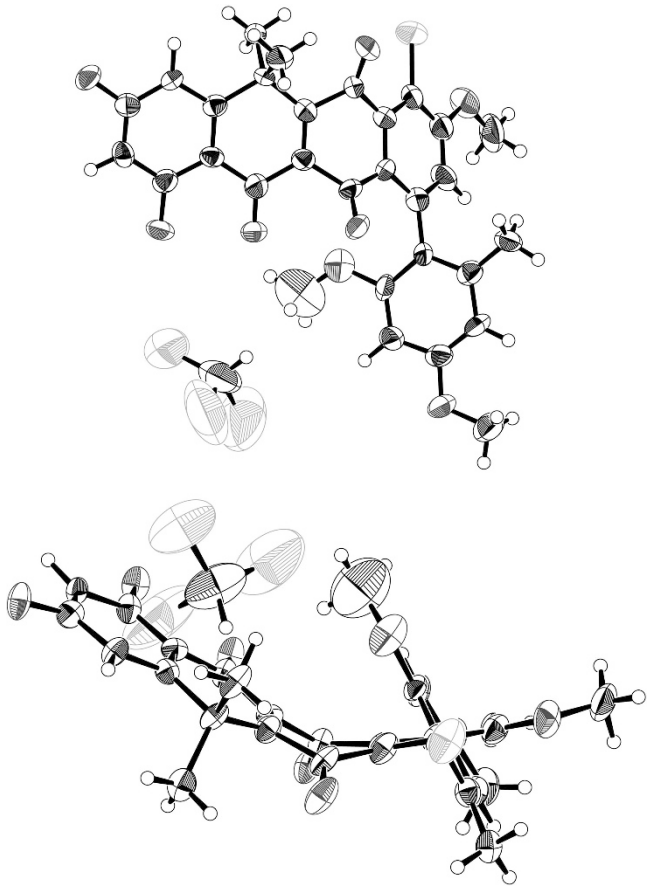

Figure 5 ORTEP plot of the X-ray crystallographic structure of **8**.

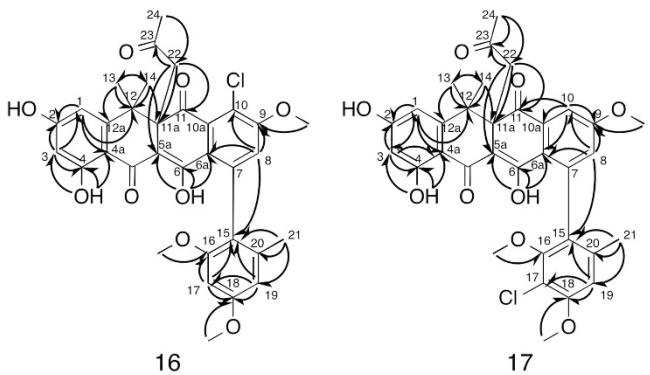

Figure 6 Structure elucidation of **16** and **17** by HMBC.

chloro-17-dechloro-O[18]-demethyl-**17**. We have not elucidated the configurations of **16** and **17** yet.

Circumvention of β-lactam resistance in MRSA

The circumvention of β-lactam resistance in MRSA was measured by enhancement of imipenem activity on MRSA using the paper disk method. Naphthacemycins alone showed no antibacterial activity against a clinically isolated MRSA strain K24 at 0.01–1 μg per disk. When the agar plates contained 10 μg ml^{-1} of imipenem, which also did not affect the growth of MRSA, 0.01–1 μg per disks of naphthacemycins showed inhibition zones (Table 2). Compounds **3** and **5–10** inhibited the growth of MRSA at 0.01 μg per disk in the presence of imipenem. Among them, **6** and **10** showed the largest inhibition zones (11 mm) at 0.01 μg per disk. The detailed activity of naphthacemycins against MRSA will be reported in an accompanying paper.[5]

Table 2 Enhancement of imipenem anti-MRSA activity by naphthacemycins

	Inhibition zone diameter (mm) by 6 mm paper disk				
	Agar plate containing imipenem (10 μg ml^{-1})				
	Amount of compound (μg per disk)				
Compound	*0.01*	*0.03*	*0.1*	*0.3*	*1*
1	–	–	–	10	16
2	–	10	16	17	22
3	9	14	18	19	21
4	–	10	11	13	19
5	9	15	17	21	25
6	11	15	19	21	23
7	10	11	14	19	21
8	9	12	13	15	16
9	7	13	15	17	17
10	11	12	12	14	15
11	–	10	11	16	16
12	–	–	–	10	14
13	–	8	11	15	17
14	–	13	14	14	16
15	–	9	10	15	18
16	–	9	11	15	17
17	–	–	7	10	17

CONCLUSION

In conclusion, 17 new compounds designated naphthacemycins were isolated from the culture broth of *Streptomyces* sp. KB-3346-5. They are circumventors of β-lactam resistance and enhanced imipenem activity against β-lactam resistant MRSA. Naphthacemycins are 1-phenylnaphthacene antibiotics produced by *Streptomyces* sp. Many naphthacene type compounds have been isolated from actinomycetes, but most of them have partially unsaturated rings, such as tetracyclines and anthracyclines. Some highly unsaturated naphthacene compounds have been reported to be produced by actinomycetes, such as tetracenomycin D, galtamycin and tetracenoquinocin.[8–10] A biosynthetic intermediate of tetracycline, pretetramid and an aglycone of anthracycline, η-pyrromycinone, are classified in the latter.[11,12] Naphthacemycins A and B series also belong to the latter group, but they are the first compounds having a 1-phenylnaphthacene skeleton isolated from a natural origin.

METHODS

General experimental procedure

^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Varian XL-300 spectrometer. Chemical shifts are shown in δ values (p.p.m.) relative to the solvents (acetone-d_6 at 2.05 p.p.m. for ^{1}H NMR and at 29.8 p.p.m. for ^{13}C NMR; $CDCl_3$ at 7.26 p.p.m. for ^{1}H NMR and at 77.0 p.p.m. for ^{13}C NMR; DMSO-d_6 at 2.50 p.p.m. for ^{1}H NMR and at 39.5 p.p.m. for ^{13}C NMR). INADEQUATE experiment of **9** (170 mg) was carried out for 100 h by 125 MHz NMR using methanol-d_4 as a solvent. Mass spectrometry was conducted on a JEOL JMS-AX505 HA spectrometer. The UV and IR spectra were measured with a Hitachi U-2810 spectrophotometer and a Horiba FT-710 Fourier transform infrared spectrometer, respectively. Optical rotations were recorded on a JASCO model DIP-1000 polarimeter.

Assay of antibacterial activity

Measurement of inhibition zone of naphthacemycins, with or without imipenem, to evaluate circumvention activity of β-lactam resistance were carried out by paper disk method, as reported previously.[3]

Data availability

The X-ray crystallographic data have been deposited at the Cambridge Crystallographic Data Centre (CCDC) under deposition numbers CCDC 1536220 (**8**) and CCDC 1536223 (**9**).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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