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Antibacterial Butenolides from the Korean Tunicate *Pseudodistoma antinboja*

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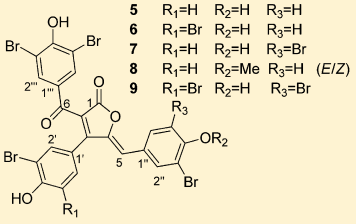
Supporting Information

ABSTRACT: Six new (**1**, **2**, and **5**−**8**) and three known (**3**, **4**, and **9**) butenolide metabolites were isolated from the tunicate *Pseudodistoma antinboja* by activity-guided fractionations. The structures were elucidated by combined NMR and MS spectroscopic methods. These compounds were evaluated for their antibacterial activity, and most of them exhibited moderate to significant activity that selectively targeted Gram-positive strains and did not exhibit cytotoxicity in the MTT assay at 100 μM. Cadiolides **5**−**9** in particular exhibited significant antibacterial activity that was comparable to or even better than those of marketed drugs such as vancomycin and linezolid against all of the drug-resistant strains tested.



Natural products-derived antibiotics have played a pivotal role in the treatment of bacterial infections and diseases since their discovery in the early and mid 20th century. However, with the widespread use of broad-spectrum antibiotics, enormous selection pressures have been placed on bacterial populations, provoking the evolution of resistance mechanisms and thus causing the emergence of resistant bacterial strains. The resistance of Gram-positive pathogens to antibiotics is a great concern because these bacteria pose a continuous and serious threat to public health. One Gram-positive pathogen of significance in public health, *Staphylococcus aureus*, is currently the most frequent cause of nosocomial bacteremia and skin/wound infections and the second most frequent cause of nosocomial lower respiratory infections. Despite the importance of antibiotics, many pharmaceutical companies have reduced or completely eliminated their antibiotics research and development efforts for economic reasons. Most of the natural product-derived antibacterial compounds currently undergoing clinical evaluation are based on well-known antibiotic templates. Therefore, there is an urgent and continuing need to develop new antibiotics, preferably ones that are structurally different from extant ones, to combat the worldwide public health crisis.

Marine tunicates (phylum Chordata) are widely recognized as a prolific source of structurally diverse nitrogen-containing secondary metabolites. One major group of tunicate metabolites is peptides that contain phenylalanine- or tyrosine-derived residues. A second major group of tunicate metabolites is the alkaloids. Marine tunicates have also yielded a small number of non-nitrogenous metabolites produced through diverse biosynthetic pathways. However, more significant than their interesting structures, tunicate metabolites exhibit a wide spectrum of pharmacological activities, such as anticancer, antiviral, and antifungal activities, and antibacterial activity against Gram-positive bacteria.[1−3] Among tunicate metabolites, ecteinascidin 743 (marketed under the trade name Yondelis) and dehydrodidemnin B (also known as aplidine) are noteworthy examples.[4]

As a part of our ongoing project directed toward the search for antibacterial metabolites from marine tunicates, we analyzed the colonial tunicate *Pseudodistoma antinboja* (order Aplousobranchia, family Pseudodistomidae). Bioassay-guided fractionation using various chromatographic techniques resulted in the isolation of six new (**1**, **2**, and **5**−**8**) and three known (**3**, **4**, and **9**) non-nitrogenous metabolites that are responsible for the antibacterial properties of the EtOAc extract. These compounds are structurally related to rubrolides[5,6] and cadiolides,[7] respectively. Rubrolides A, B, and C have been reported to possess potent antibiotic activity.[5] Cytotoxicity,[6] aldose reductase inhibitory activity,[8] inhibitory activity against protein phosphatases 1 and 2A,[5] and anti-inflammatory activity[9] have been observed for other rubrolide compounds, making these compounds worthwhile targets for synthetic studies.[10−15] However, only two compounds with a cadiolide carbon skeleton have been reported from a natural source, and another two have been reported as synthetic intermediates.[16] There are

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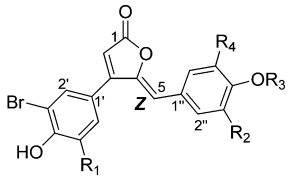
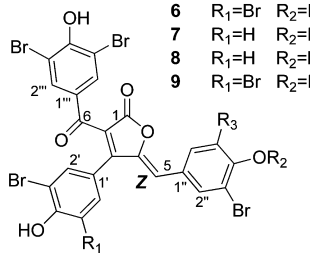
no previously published biological data for these compounds. In this study, metabolites **5**–**9** of the cadiolide class were found to exhibit *in vitro* antibacterial activities that are more potent or comparable to those of marketed drugs against all of the drug-resistant Gram-positive strains in our bioassay study. Herein we report the isolation and structural elucidation of these new metabolites and their antibacterial activities.



1 R_1=Br R_2=H R_3=Me R_4=H (**1a** = *E*; **1b** = *Z*)
2 R_1=H R_2=H R_3=Me R_4=H (*E*/*Z*)
3 R_1=Br R_2=Br R_3=H R_4=Br (*Z*)
4 R_1=H R_2=Br R_3=H R_4=Br (*Z*)



5 R_1=H R_2=H R_3=H
6 R_1=Br R_2=H R_3=H
7 R_1=H R_2=H R_3=Br
8 R_1=H R_2=Me R_3=H (*E*/*Z*)
9 R_1=Br R_2=H R_3=Br

RESULTS AND DISCUSSION

Rubrolide P (**1**) was isolated as a yellow, amorphous solid. It yielded an isotopic cluster of $[M + H]^+$ ion peaks at *m*/*z* 451/453/455 with intensities in a 1:2:1 ratio in the FAB mass spectrum, which is characteristic of a dibrominated compound. A molecular formula of $C_{18}H_{12}Br_2O_4$ was indicated by its HRFABMS data. Compound **1** always exhibited two sets of NMR signals with an integral ratio of 1:2 due to the rapid interconversion of its geometrical isomers in solution (Table 1). To simplify our description, the minor isomer is symbolized by **1a** and the major one by **1b**. The ^{13}C NMR spectrum of **1a** contained only 14 resonance signals, indicating that there were some elements of symmetry present in the molecule. In the ^1H NMR spectrum, only six proton signals were observed. The two doublet signals at δ 6.65 and 6.96, which were mutually coupled, arose from two pairs of adjacent aromatic protons on a *para*-disubstituted phenyl ring (H-3″/5″ and H-2″/6″). The three-proton singlet at δ 3.70 can be assigned to the methoxy group at C-4″. The two-proton singlet at δ 7.23 arose from two equivalent protons attached to two methine carbons situated *meta* to each other (H-2′/6′) on a symmetrically tetrasubstituted phenyl ring. On the basis of the comparison of these NMR data with those of related natural products and with calculated values, the presence of a 4-methoxyphenyl ring and a 3,5-dibromo-4-hydroxyphenyl ring was established.

The remaining fragment of **1a** had to account for an elemental composition of $C_5H_2O_2$ and four degrees of unsaturation. An unsaturated butenolide ring bearing an exocyclic trisubstituted double bond was evident based on the NMR data and IR absorptions and supplied the four degrees of unsaturation required for this fragment. Long-range correlations between the protons of the phenyl rings and nearby butenolide carbons in the HMBC experiment located the 3,5-dibromo-4-hydroxyphenyl residue and the 4-methoxyphenyl residue at C-3 and C-5, respectively.

A similar set of arguments led to the assignment of the NMR signals for the major isomer, **1b**. Compounds **1a** and **1b** have identical atomic connectivities. The difference in the structures of the two isomers is due to the different configuration at $\Delta^{4,5}$, which was defined by a series of NOE difference experiments. Irradiation of H-5 of **1b** induced NOEs for the H-2′/6′ and H-2″/6″ signals, indicating that the $\Delta^{4,5}$ olefin has the *Z* configuration in **1b**. In contrast, irradiation of H-5 of the *E* isomer (**1a**) resulted in an enhancement of only the H-2″/6″ signal. In addition, irradiation of H-2′/6′ in **1a** induced an enhancement at H-2″/6″, indicating that the two phenyl rings are much closer to each other in this compound, consistent with the *E* configuration at C-4/C-5. A comparison of the ^1H NMR data of **1a** with those of **1b** showed that H-2′/6′ and H-

Table 1. ^1H NMR Data for Rubrolides P (1) and Q (2)[a]

	1 (*E*)[b]		**1** (*Z*)[b]		**2** (*E*)[c]		**2** (*Z*)[c]	
compd	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	167.6		168.2		169.1		169.7	
2	119.7	6.66, s	113.4	6.55, s	120.6	6.39, s	114.2	6.34, s
3	152.9		155.2		156.1		158.6	
4	145.5		145.3		148.9		148.0	
5	117.0	7.11, s	113.0	6.33, s	117.9	7.00, s	114.2	6.36, s
1′	123.5		124.3		125.4		124.9	
2′	132.2	7.23, s	132.4	7.80, s	134.7	7.23, d (2.0)	134.8	7.81, d (2.0)
3′	111.1		112.3		110.8		111.6	
4′	151.7		152.9		156.5		157.7	
5′	111.1		112.3		117.2	6.83, d (8.3)	118.1	7.22, d (8.4)
6′	132.2	7.23, s	132.4	7.80, s	130.4	7.07, dd (8.3, 2.0)	130.9	7.53, dd (8.4, 2.0)
1″	124.0		125.6		125.9		127.6	
2″/6″	131.5	6.96, d (8.4)	132.4	7.82, d (8.9)	133.1	6.98, d (8.4)	133.9	7.85, d (8.8)
3″/5″	113.4	6.65, d (8.4)	114.4	7.04, d (8.9)	114.9	6.65, d (8.4)	115.8	7.03, d (8.8)
4″	159.7		160.1		161.5		161.9	
4″-OMe	55.2	3.70, s	55.3	3.81, s	56.3	3.74, s	56.3	3.87, s

[a]500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR. [b]Spectra were recorded in DMSO-d_6. [c]Spectra were recorded in acetone-d_6.

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2″/6″ are considerably more shielded in the *E* isomer (**1a**) than in the *Z* isomer (**1b**) because the two phenyl rings are in closer proximity in the *E* isomer. Conversely, H-5 is less shielded in the *E* isomer because H-5 points out into space in the *E* isomer, whereas in the *Z* isomer, H-5 is close to the aromatic ring attached at C-3. These observations corroborated the assignment of the geometry at C-4/C-5.

Rubrolide Q (**2**, this compound has been reported from the South African tunicate *Synoicum globosum* as 3′-bromorubrolide F),[17] an amorphous yellow solid, gave a $[M + H]^+$ ion cluster at *m/z* 373/375 with intensities in a 1:1 ratio in the FABMS spectrum. Its molecular formula was established as $C_{18}H_{13}BrO_4$ on the basis of the HRFABMS data. Similar to the case of compound **1**, *E* and *Z* isomers of this compound exist in equilibrium, with the *Z* form predominating. The NMR data and IR absorptions suggested that **2** is a 5′-debromo derivative of **1** (Table 1).

On the basis of the combined spectroscopic analyses and the comparison of the spectroscopic data with those reported in the literature, compounds **3** and **4** were identified as rubrolides A[5] and J,[6] respectively. It should be mentioned that the color and NMR spectra of rubrolides and cadiolides described below vary with pH of the solvent. The minute amount of residual TFA from our experimental procedure makes the NMR data slightly different from those obtained for compounds isolated without acidic mobile phase additives.

Cadiolide C (**5**) was isolated as an amorphous, yellow-orange solid. The molecular formula was established as $C_{24}H_{12}Br_4O_6$ on the basis of the HRFABMS data. The ^{1}H NMR spectrum contained two spin systems of three signals each at δ 7.07 (d, J = 8.3 Hz), 7.34 (dd, J = 8.3, 1.9 Hz), and 7.67 (d, J = 1.9 Hz) and at δ 7.13 (d, J = 8.5 Hz), 7.82 (dd, J = 8.5, 1.9 Hz), and 8.13 (d, J = 1.9 Hz), respectively, reminiscent of two 1,2,4-trisubstituted phenyl rings. The two-proton singlet at δ 8.04 is reminiscent of a symmetrically tetrasubstituted phenyl ring. Analysis of the ^{13}C NMR and HMBC data revealed the presence of two 3-bromo-4-hydroxy phenyl residues and a 3,5-dibromo-4-hydroxyphenyl residue. The IR absorption (1754 cm^{-1}) and the ^{13}C NMR signals at δ 166.4, 157.3, 147.3, 123.3, and 116.3 suggested that compound **5** contained, similarly to rubrolide P (**1**), a butenolide ring bearing an exocyclic trisubstituted double bond. The carbon signal at δ 186.4 and the carbonyl stretching band at 1705 cm^{-1} in the IR spectrum were indicative of the presence of a conjugated keto carbonyl group, which is attached to C-2. The carbon signals at δ 157.3 and 123.3 could be assigned to the olefinic carbons positioned β and α to this carbonyl group, respectively. The locations of the three phenyl rings were assigned based on HMBC correlations. The *Z* geometry at C-4/C-5 was deduced from a 2D NOESY experiment, which showed correlations from H-5 (δ 6.42) to H-2′ (δ 7.67) and H-6′ (δ 7.34) in addition to correlations from H-5 to H-2″ (δ 8.13) and H-6″ (δ 7.82).

The molecular formula of cadiolide D (**6**), an amorphous, orange solid, was determined to be $C_{24}H_{11}Br_5O_6$ based on the HRFABMS data. The ^{1}H NMR data revealed that compound **6** differed from **5** only in the replacement of the 3-bromo-4-hydroxyphenyl residue attached at C-3 in **5** with a 3,5-dibromo-4-hydroxyphenyl residue in **6**. The ^{13}C NMR, COSY, HSQC, and HMBC data for **6** were in complete agreement with this assignment.

Cadiolide E[18] (**7**) was isolated as an amorphous, brown solid, and a formula of $C_{24}H_{11}Br_5O_6$ was indicated by the HRFABMS data. This compound is a constitutional isomer of **6**, with the locations of the two phenyl residues at C-3 and C-5 exchanged. This structure can explain the similar multiplicity patterns and the different chemical shift values of the ^{1}H NMR signals of these two phenyl rings [**6**: δ 7.70 (s), 7.14 (d, J = 8.5 Hz), 7.84 (dd, J = 8.5, 1.9 Hz), and 8.14 (d, J = 1.9 Hz); **7**: δ 8.14 (s), 7.07 (d, J = 8.3 Hz), 7.34 (dd, J = 8.3, 2.0 Hz), and 7.67 (d, J = 2.0 Hz)]. This assignment was also supported by the HMBC experiment.

Cadiolide F (**8**) was isolated as an amorphous, yellow solid. It displayed two distinct HPLC peaks. Both peaks showed the same distinct peaks upon reinjection into the HPLC system as well as the same molecular formula of $C_{25}H_{14}Br_4O_6$ in the HRFABMS data when these peaks were collected separately. Both of these peaks exhibited two sets of NMR signals in an integral ratio of 1:2. These phenomena can be interpreted as reflective of the rapid interconversion of the *E*/*Z* geometrical isomers in solution. Compound **8** was obtained as a mixture of *E*/*Z* isomers, with the *Z* form predominating. The ^{1}H and ^{13}C NMR data, including those for a methoxy group of the *Z* isomer, showed that this compound is a monomethyl ether of cadiolide C (**5**). The location of the methoxy group at C-4″ was determined by the ROESY correlation between the methoxy protons (δ 3.99) and H-5″ (δ 7.24). Two other ROESY correlations, from H-5 to H-2′/6′ and H-2″/6″, established the *Z* configuration at $\Delta^{4,5}$. The *E* isomer showed a series of ^{1}H and ^{13}C NMR signals with the same splitting pattern but different chemical shifts compared with those of the *Z* isomer. In particular, H-5 is less shielded in the *E* isomer than it is in the *Z* isomer, and conversely, H-2′/6′ and H-2″/6″ are much more shielded in the *E* isomer than they are in the *Z* isomer. These shielding patterns are consistent with the geometrical assignment established by the ROESY experiments. Compound **9** was determined to be cadiolide B by comparison of its spectroscopic data with those reported previously.[7]

The compounds were evaluated for their antibacterial activity by the 2-fold microtiter broth dilution method.[19] Most of these compounds displayed moderate to significant antibacterial activity against all of the four Gram-positive strains tested (*Staphylococcus aureus* ATCC 6538, *Kocuria rhizophila* (*Micrococcus luteus*) ATCC 9341, *Staph. epidermidis* ATCC12228, and *Bacillus subtilis* ATCC 6633) (Table 4). However, they did not show inhibitory activity toward the Gram-negative bacteria including *Escherichia coli* ATCC 11775, *Salmonella typhimurium*

Table 2. ^{1}H NMR Data for Cadiolides C−F (5−8) (acetone-d_6, 500 MHz)[a]

position	5	6	7	8 (*E*)	8 (*Z*)
5	6.42, s	6.50, s	6.45, s	7.16, s	6.47, s
2′	7.67, d (1.9)	7.70, s	7.67, d (2.0)	7.20, d (2.0)	7.66, d (1.9)
5′	7.07, d (8.3)		7.07, d (8.3)	6.78, d (8.3)	7.14, d (8.4)
6′	7.34, dd (8.3, 1.9)	7.70, s	7.34, dd (8.3, 2.0)	7.00, dd (8.3, 2.0)	7.34, dd (8.4, 1.9)
2″	8.13, d (1.9)	8.14, d (1.9)	8.14, s	7.19, d (1.9)	8.20, d (1.9)
5″	7.13, d (8.5)	7.14, d (8.5)		6.82, d (8.5)	7.24, d (8.6)
6″	7.82, dd (8.5, 1.9)	7.84, dd (8.5, 1.9)	8.14, s	7.11, dd (8.5, 1.9)	7.95, dd (8.6, 1.9)
4″-OMe				3.84, s	3.99, s
2‴/6‴	8.04, s	8.06, s	8.05, s	8.03, s	8.05, s

[a]Multiplicities and coupling constants are in parentheses.

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Table 3. ^{13}C NMR Data for Cadiolides C–F (5–8) (acetone-d_6, 125 MHz)

position	5	6	7	8 (*E*)	8 (*Z*)
1	166.4	166.2	166.1	166.6	166.5
2	123.3	124.1	123.8	123.3	123.3
3	157.3	156.7	157.5	153.9	157.3
4	147.3	147.0	148.2	148.9	147.9
5	116.3	117.0	114.5	119.0	115.7
6	186.4	186.1	186.4	186.4	186.4
1′	122.7	123.7	122.4	123.3	122.6
2′	135.0	134.2	135.1	134.5	135.0
3′	110.9	111.7	110.9	110.7	110.0
4′	157.2	153.4	157.2	156.7	157.5
5′	117.5	111.7	117.5	117.0	117.6
6′	131.2	134.2	131.3	130.4	131.2
1″	127.7	127.6	129.0	126.7	128.4
2″	136.9	137.0	135.7	135.6	136.4
3″	110.9	111.0	111.9	111.7	112.6
4″	156.7	156.8	152.8	157.3	158.1
5″	117.8	117.8	111.9	112.6	113.5
6″	133.0	133.1	135.7	131.9	133.2
4″-OMe				56.9	57.0
1‴	130.9	131.6	131.4	130.8	130.9
2‴	134.9	134.9	134.9	134.9	134.9
3‴	111.8	111.4	111.5	111.8	111.8
4‴	157.4	156.3	156.4	157.6	157.7
5‴	111.8	111.4	111.5	111.8	111.8
6‴	134.9	134.9	134.9	134.9	134.9

ATCC 14028, and *Klebsiella pneumoniae* ATCC 4352. Cadiolides **5**–**9** exhibited significant antibacterial activity against all of the drug-resistant Gram-positive strains (Table 5).

It seems that the compounds of the cadiolide family present a new class of antibiotics active against Gram-positive bacteria. Although both cadiolides and rubrolides share the same 3-aryl-4-arylmethylenefuranone unit, the former is distinguished by an additional 2-ketoaryl substituent and a different carbon skeleton. It is obvious that, generally, all the cadiolides exhibited activities better than or comparable to (especially against drug-resistant strains) compound **3**, which was reported to have antibiotic activity (the antibacterial activity of compound **4** has not been reported previously). These data suggest that the benzoyl substituent at C-2 may be beneficial for stable and potent antibacterial activity. The most potent compound in the cadiolide series was **5**, with MICs against Gram-positive bacteria ranging from 0.2 to 3.1 μg/mL and with MICs against drug-resistant Gram-positive strains ranging from 0.13 to 0.5 μg/mL. The positions of the bromines appear to be important. The phenolic group is also likely responsible for the good activity because methylation of the phenolic group led to a reduction in the overall activity, as exemplified by compound **8**, which is less active than **5**, and by compounds **1** and **2**, which displayed only weak to modest antibacterial activity. The 4′,4″,4‴-trimethylation of **5** caused the complete loss of its activity. The analysis of the structure–activity relationships of these compounds may provide useful information on the structural requirements for antibacterial activity against Gram-positive bacteria, including drug-resistant strains. In addition, it should be noted that none of these compounds showed significant cytotoxicity in the MTT assay at 100 μM. Therefore, cadiolides could serve as new lead compounds for the development of antibiotics for the treatment of bacterial infections caused by Gram-positive bacteria such as *Staphylococcus aureus*. The cadiolides may also provide a tool for identifying new drug templates, perhaps with a novel mechanism of action, which would be significant for the development of novel antibiotics because the cadiolides possess a totally different scaffold from the benchmark antibiotics.

EXPERIMENTAL SECTION

General Experimental Procedures. UV spectra were obtained in MeOH using a Scinco UVS-2100 spectrophotometer. IR spectra were measured by a Thermo Electron Corp. Nicolet 570 spectrometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance DPX-500 instrument. Chemical shifts were reported with reference to the respective solvent peaks and residual solvent peaks (δ_H 2.05, δ_C 29.9 and 206.7 for acetone-d_6). FABMS spectra were measured on a JEOL JMS-AX505WA mass spectrometer. HPLC was performed with a Synersi Fusion-RP column (250 × 10 mm, 4 μm, 80 Å) using a Younglin M 720 UV detector.

Animal Material. The tunicate was collected with the aid of scuba gear at a depth of 10–15 m off the shore of Tong-Yeong City, South Sea, Korea, in July 2005. The fresh specimens were immediately frozen and kept at −24 °C until chemically investigated. The specimen was taxonomically identified as *Pseudodistoma antinboja* (order Aplousobranchia, family Pseudodistomidae) by one of the authors (B.J.R.). A voucher specimen (BYD-25) was deposited in the Center for Marine Natural Products and Drug Discovery, Seoul National University, Seoul, Korea.

Extraction and Isolation. The lyophilized specimens (dry wt 336 g) were cut into small pieces and extracted three times with 50% MeOH in CH_2Cl_2 (700 mL) at room temperature. These extracts were combined and partitioned three times between *n*-hexane (500 mL) and MeOH (500 mL). Then, the MeOH-soluble layer was further partitioned between EtOAc (500 mL) and H_2O (500 mL) three times. The EtOAc extract (1.15 g) was active in the antibacterial assay and was subjected to silica flash column chromatography, eluting with a step gradient of EtOAc and *n*-hexane, to afford 10 fractions. Active fractions were further separated by reversed-phase HPLC (Synersi Fusion-RP, 250 × 10 mm, 4 μm, 80 Å, 210 nm), eluting with 75% CH_3CN in H_2O (0.0025% TFA), to afford compounds **1**–**9** as amorphous solids. The overall purified metabolites were isolated in the following amounts: 2.0, 2.3, 1.9, 2.6, 20.6, 17.0, 19.4, 3.3, and 22.3 mg for **1**–**9**, respectively.

Rubrolide P (1): yellow, amorphous solid; UV (MeOH) λ_{max} (log ε) 254 (3.89), 360 (4.00) nm; IR (film) ν_{max} 1736, 1602, 1510, 1468, 1255, 1175 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 1; LRFABMS *m*/*z* 451/453/455 $[M + H]^+$; HRFABMS *m*/*z* 450.9185 (calcd for $C_{18}H_{13}{}^{79}Br_2O_4$, 450.9181).

Rubrolide Q (3′-bromorubrolide F, 2): yellow, amorphous solid; UV (MeOH) λ_{max} (log ε) 253 (3.87), 344 (4.04) nm; IR (film) ν_{max} 1736, 1602, 1510, 1301, 1255, 1175 cm^{-1}; ^1H NMR data, see Table 1;

Table 4. Antibacterial Activities (MIC μg/mL) against Non-Drug-Resistant Strains of Compounds 1–9

tested strain	gentamycin	1	2	3	4	5	6	7	8	9
Staphylococcus aureus ATCC 6538	<0.02	>50	>50	3.1	3.1	0.4	6.3	3.1	12.5	3.1
Staphylococcus epidermidis ATCC12228	<0.02	50	25	1.6	0.8	0.4	0.8	6.3	6.3	3.1
Kocuria rhizophila ATCC 9341	1.6	6.3	3.1	1.6	6.3	0.2	1.6	0.2	3.1	3.1
Bacillus subtilis ATCC 6633	0.05	50	50	1.6	1.6	3.1	6.3	3.1	12.5	3.1

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Table 5. Antibacterial Activities (MIC μg/mL) against MSSA and MRSA Strains of Compounds 1−9

microorganisms	platensimycin	linezolid	vancomycin	daptomycin	1	2	3	4	5	6	7	8	9
CCARM 0027[a]	4	4	0.5	16	>32	>32	4	2	0.5	0.5	2	4	2
CCARM 0204[a]	4	2	0.25	4	>32	>32	4	2	0.25	0.5	2	2	1
CCARM 0205[a]	4	2	<0.13	2	>32	>32	4	1	0.25	0.25	4	1	2
CCARM 3640[a]	8	4	0.25	16	>32	>32	8	2	<0.13	0.25	0.25	1	1
CCARM 3089[b]	8	2	1	>32	>32	>32	8	4	0.5	0.5	1	2	1
CCARM 3090[b]	8	4	1	>32	>32	>32	8	2	0.25	0.25	1	1	1
CCARM 3634[b]	8	4	0.5	>32	>32	>32	8	2	0.5	1	2	2	1
CCARM 3635[b]	8	4	1	>32	>32	>32	8	2	<0.13	0.25	0.5	1	0.5

[a]Methicillin-sensitive *Staphylococcus aureus* (MSSA). [b]Methicillin-resistant *Staphylococcus aureus* (MRSA).

^{13}C NMR data, see Table 1; LRFABMS *m/z* 373/375 $[M + H]^+$; HRFABMS *m/z* 373.0071 (calcd for $C_{18}H_{14}{}^{79}BrO_4$, 373.0075).

Cadiolide C (5): yellow-orange, amorphous solid; UV (MeOH) λ_{max} (log ε) 254 (4.36), 358 (4.33), 421 (4.04) nm; IR (film) ν_{max} 1754, 1705, 1644, 1579, 1298 cm^{-1}; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; LRFABMS *m/z* 464/466/468, 634/636/638/640, 713/715/717/719/721 $[M + H]^+$, 735/737/739/741/743 $[M + Na]^+$; HRFABMS *m/z* 712.7447 (calcd for $C_{24}H_{13}{}^{79}Br_4O_6$, 712.7446).

Cadiolide D (6): orange, amorphous solid; UV (MeOH) λ_{max} (log ε) 218 (4.00), 256 (3.79), 385 (3.93) nm; IR (film) ν_{max} 1742, 1707, 1660, 1567, 1382 cm^{-1}; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; LRFABMS *m/z* 541/543/545/547, 790/792/794/796/798/800 $[M + H]^+$, 812/814/816/818/820/822 $[M + Na]^+$; HRFABMS *m/z* 790.6533 (calcd for $C_{24}H_{12}{}^{79}Br_5O_6$, 790.6551).

Cadiolide E (7): brown, amorphous solid; UV (MeOH) λ_{max} (log ε) 231 (4.23), 258 (4.23), 363 (4.38), 486 (3.76) nm; IR (film) ν_{max} 1744, 1703, 1564, 1294 cm^{-1}; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; LRFABMS *m/z* 541/543/545/547, 790/792/794/796/798/800 $[M + H]^+$, 812/814/816/818/820/822 $[M + Na]^+$; HRFABMS *m/z* 790.6530 (calcd for $C_{24}H_{12}{}^{79}Br_5O_6$, 790.6551).

Cadiolide F (8): yellow, amorphous solid; UV (MeOH) λ_{max} (log ε) 222 (4.08), 255 (4.01), 352 (4.18) nm; IR (film) ν_{max} 1753, 1680, 1640, 1599, 1270 cm^{-1}; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; LRFABMS *m/z* 727/729/731/733/735 $[M + H]^+$, 749/751/753/755/757 $[M + Na]^+$; HRFABMS *m/z* 726.7618 (calcd for $C_{25}H_{15}{}^{79}Br_4O_6$, 726.7602).

Preparation of 4′,4″,4‴-Trimethylated Cadiolide C. Dimethyl sulfate (4 mL) was added to the acetone solution (5 mL) containing compound **5** (5 mg) and anhydrous K_2CO_3 (10 mg). The resulting solution was refluxed at 50 °C for 24 h. After cooling to room temperature, the solvent was removed *in vacuo*. The residue was dissolved in distilled water (3 mL) and diethyl ether (3 mL), and the aqueous layer was further extracted twice with diethyl ether. After evaporation of the solvent under reduced pressure, the residue was chromatographed over silica gel (hexane−EtOAc = 9.5:0.5) to give the desired product (4.2 mg). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.32 (2H, s, H-2‴/6‴), 8.12 (1H, d, *J* = 2.1 Hz, H-2″), 7.88 (1H, dd, *J* = 8.2, 2.1 Hz, H-6″), 7.86 (1H, d, *J* = 2.0 Hz, H-2′), 7.55 (1H, dd, *J* = 8.4, 2.0 Hz, H-6′), 6.97 (1H, d, *J* = 8.2 Hz, H-5″), 6.90 (d, 1H, *J* = 8.4 Hz, H-5′), 6.85 (1H, s, H-6), 3.99 (3H, s, 4″-OMe), 3.98 (3H, s, 4‴-OMe), 3.93 (3H, s, 4′-OMe); ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.3, 166.4, 159.4, 158.2, 157.7, 157.2, 147.9, 143.3, 136.4, 135.4, 134.7, 133.2, 130.8, 130.7, 130.2, 127.8, 126.6, 125.6, 118.7, 112.1, 111.9, 111.1, 60.8, 56.5, 56.3.

Bioassay Procedures. The following seven microorganisms were obtained from the stock culture collection at the American Type Culture Collection (Maryland): *Staphylococcus aureus* ATCC 6538, *Kocuria rhizophila* (*Micrococcus luteus*) ATCC 9341, *Staph. epidermidis* ATCC12228, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 11775, *Salmonella typhimurium* ATCC 14028, and *Klebsiella pneumoniae* ATCC 4352. The following eight drug-resistant strains were obtained from the stock Culture Collection of Antimicrobial Resistant Microorganisms (Seoul Women's University): *Staph. aureus* CCARM 0027, *Staph. aureus* CCARM 0204, *Staph. aureus* CCARM 0205, *Staph. aureus* CCARM 3640, *Staph. aureus* CCARM 3089, *Staph. aureus* CCARM 3090, *Staph. aureus* CCARM 3634, and *Staph. aureus* CCARM 3635. The antibacterial activity was determined by the 2-fold microtiter broth dilution method.[17] Dilutions of the test compounds dissolved in DMSO were added to each well of a 96-well microtiter plate containing a fixed volume of Mueller Hinton broth (Difco) (final 0.64% DMSO). Each well was inoculated with an overnight culture of bacteria (5×10^5 cfu/mL), and the plate was incubated at 37 °C for 24 h. The minimum inhibitory concentration (MIC) was taken as the concentration at which no growth was observed.

■ ASSOCIATED CONTENT

Supporting Information

Full characterization details for the compounds **3**, **4**, and **9** and NMR spectra for compounds **1**−**9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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