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Brominated Aromatic Furanones and Related Esters from the Ascidian *Synoicum* sp.

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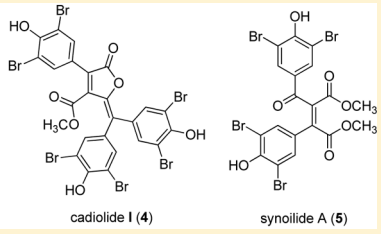
Supporting Information

ABSTRACT: Nine new compounds, tris-aromatic furanones (**1**, **2**, **3a**, **3b**, and **4**) and related bis-aromatic diesters (**5a**, **5b**, **6a**, and **6b**), are described from the ascidian *Synoicum* sp. collected off the coast of Chuja-do, Korea. The structures of these compounds, designated as cadiolides E and G–I (**1**–**4**) and synoilides A and B (**5** and **6**), were determined by extensive spectroscopic analyses. The absolute configuration at the asymmetric center of cadiolide G (**2**) was assigned by ECD analysis. Of these new compounds, cadiolide I and the synoilides possess unprecedented carbon skeletons. Several of these compounds exhibited significant inhibition against diverse bacterial strains as well as moderate inhibition against the enzymes sortase A, isocitrate lyase, and Na^+/K^+-ATPase.



Ascidians (phylum Chordata, class Ascidiacea) produce a wide variety of bioactive secondary metabolites biogenetically derived from amino acids.[1] Rubrolides, halogenated bis-aromatic furanones, are proposed to be biosynthesized from phenylalanine or tyrosine.[2] Such rubrolides isolated from ascidians of the genera *Botryllus*,[3] *Ritterella*,[2] and *Synoicum*[4,5] exhibited significant antibacterial, cytotoxic, and anti-inflammatory activities and inhibitory activity against protein phosphatases. Meanwhile, cadiolides derived from a specimen of the genus *Botryllus* are brominated tris-aromatic furanones structurally reminiscent of rubrolides.[3] Their bioactivity, however, remains unknown.

During the course of a search for bioactive metabolites from Korean marine invertebrates, we encountered the dark red ascidian *Synoicum* sp. off the coast of Chuja-do, southern Korea, whose organic extract exhibited moderate cytotoxicity (LD_{50} 39.0 μg/mL) against the A549 cell line. Subsequent chemical investigation of this animal led to the isolation of eudistomins Y_2–Y_7, antimicrobial β-carboline alkaloids, as the major constituents.[6] However, the marginal cytotoxicity of these compounds as well as the ^{1}H NMR spectra of moderately polar chromatographic fractions suggested the presence of another group of metabolites as minor constituents. Herein, we report the isolation and structure determination of nine new aromatic furanones and related diesters, which are divided into two structural classes and designated as cadiolides E, G, and I (**1**, **2**, and **4**), *Z*/*E*-cadiolides H (**3a** and **3b**), and *Z*/*E*-synoilides A and B (**5a**, **5b**, **6a**, and **6b**). The carbon skeletons of cadiolide I and the synoilides are heretofore unreported to the best of our knowledge. Several of these compounds exhibited significant inhibition against diverse bacterial strains and moderate inhibition against the enzymes sortase A, isocitrate lyase, and Na^+/K^+-ATPase.

RESULTS AND DISCUSSION

Reversed-phase HPLC performed on the moderately polar fractions from the extract yielded six compounds (**1**–**6**). However, spectroscopic analyses of these compounds revealed that **3**, **5**, and **6** were indeed interconverting isomeric mixtures, e.g., **3a** and **3b**. Repeated efforts to separate the isomers under diverse chromatographic conditions were unsuccessful;[5] thus, nine compounds are described in total (**1**, **2**, **3a**, **3b**, **4**, **5a**, **5b**, **6a**, and **6b**).

Cadiolide E (**1**)[7] was isolated as an amorphous solid, whose composition was determined to be $C_{24}H_{11}Br_5O_6$ by HRFABMS analysis. The ^{13}C NMR data of this compound showed all of the carbon signals in the downfield region, suggesting a highly aromatic nature that is also consistent with the high degree of unsaturation deduced from the molecular formula (Table 1). After matching all of the protons and proton-bearing carbons by gHSQC analysis, these aromatic moieties were traced by a combination of COSY and gHMBC spectroscopy experiments.

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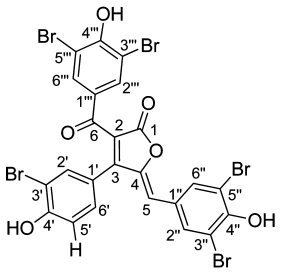
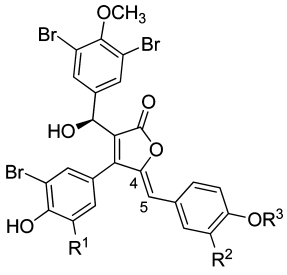
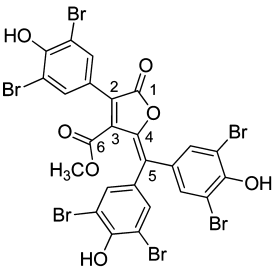


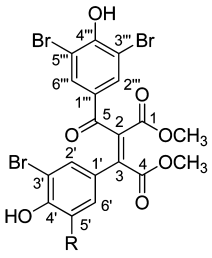
1



2 R^1=H, R^2=Br, R^3=H, 4*Z*
3a R^1=Br, R^2=H, R^3=CH_3, 4*Z*
3b R^1=Br, R^2=H, R^3=CH_3, 4*E*



4



5a R=Br, 2*Z*
5b R=Br, 2*E*
6a R=H, 2*Z*
6b R=H, 2*E*

Assignment of substituents on the aromatic moieties was accomplished by evaluating the chemical shifts of protons and carbons assisted by the molecular formula. The presence of three aromatic moieties, a 3-bromo-4-hydroxyphenyl and two 3,5-dibromo-4-hydroxyphenyl groups, was readily deduced by these experiments (Table 2).

The ^{13}C NMR data of **1** exhibited signals for six additional carbons at δ_C185.4 (C), 165.4 (C), 156.5 (C), 146.3 (C), 121.8 (C), and 113.8 (CH). The strong absorption band at 1743 cm^{-1} in the IR spectrum and the absorption maximum at 254 nm in the UV spectrum suggested a conjugated lactone and a conjugated ketone group for these carbons. Considering the remaining five degrees of unsaturation from the molecular formula, the lactone moiety was determined to be a furanone, which is consistent with the carbon chemical shifts of compounds with a similar moiety in the literature.[8]

Connectivity of these partial structures was determined by gHMBC experiments. First, the long-range couplings of an olefinic carbon at δ_C 156.5 with the protons at δ_H 7.21 (H-6′) and 7.50 (H-2′) suggested the attachment of the 3-bromo-4-hydroxyphenyl group at the *β*-position (C-3) of the furanone. Similarly, a series of couplings of the olefinic proton at δ_H 6.42 (H-5) with the aromatic (C-1″, C-2″, and C-6″) and furanone (C-3 and C-4) carbons suggested the connection of a 3,5-dibromo-4-hydroxyphenyl group at the *γ*-position of the furanone via an exomethylene group. Finally, the long-range couplings of the ketone carbon at δ_C 185.4 with the olefinic protons at δ_H 7.96 (2 H, H-2‴, and H-6‴) suggested the linkage between the ketone and the remaining 3,5-dibromo-4-hydroxyphenyl group. The connection of the ketone at C-2 of the furanone was deduced by evaluation of carbon chemical shifts, despite the lack of protons in the vicinity and the lack of direct gHMBC evidence. Thus, the structure of cadiolide E (**1**)

Table 1. ^{13}C NMR (ppm, mult) Assignments for Compounds 1−6

position	1[a]	2[b]	3a[b]	3b[b]	4[a]	5a[a]	5b[a]	6a[a]	6b[a]
1	165.4, C	168.8, C	168.9, C	168.3, C	166.3, C	163.6, C	164.0, C	163.7, C	164.8, C
2	121.8, C	139.3, C	139.0, C	139.1, C	126.3, C	132.7, C	139.3, C	129.5, C	137.3, C
3	156.5, C	151.5, C	150.1, C	147.1, C	138.6, C	140.8, C	138.7, C	143.8, C	140.6, C
4	146.3, C	146.7, C	145.8, C	146.6, C	142.7, C	166.1, C	165.2, C	166.9, C	166.1, C
5	113.8, CH	113.6, CH	115.6, CH	119.6, CH	123.6, C	187.5, C	187.4, C	188.4, C	187.5, C
6	185.4, C	66.9, CH	66.8, CH	67.0, CH	162.7, C				
1′	120.4, C	122.0, C	123.4, C	123.2, C	130.5, C	125.9, C	127.5, C	123.9, C	125.3, C
2′	133.8, CH	132.6, CH	132.4, CH	132.2, CH	134.4, CH	132.0, CH	132.7, CH	132.6, CH	133.4, CH
3′	109.7, C	110.8, C	110.3, C	109.7, C	111.5, C	111.9, C	111.3, C	109.7, C	109.1, C
4′	156.3, C	154.1, C	150.8, C	150.0, C	151.9, C	152.6, C	151.6, C	156.3, C	155.6, C
5′	116.3, CH	116.5, CH	110.3, C	109.7, C	111.5, C	111.9, C	111.3, C	116.6, CH	116.1, CH
6′	130.2, CH	129.8, CH	132.4, CH	132.2, CH	134.4, CH	132.0, CH	132.7, CH	129.3, CH	129.8, CH
1″	127.5, C	126.7, C	125.2, C	124.1, C	129.5, C				
2″	134.7, CH	134.4, CH	132.9, CH	131.0, CH	134.8, CH				
3″	112.0, C	110.9, C	114.5, CH	113.4, CH	111.7, C				
4″	152.1, C	153.7, C	161.1, C	160.1, C	152.1, C				
5″	112.0, C	116.5, CH	114.5, CH	113.4, CH	111.7, C				
6″	134.7, CH	132.1, CH	132.9, CH	131.0, CH	134.8, CH				
1‴	129.1, C	125.9, C	126.2, C	126.1, C	122.0, C	126.6, C	128.8, C	127.4, C	128.8, C
2‴/6‴	133.8, CH	130.4, CH	130.4, CH	130.4, CH	132.2, CH	133.1, CH	132.9, CH	133.1, CH	132.9, CH
3‴/5‴	111.6, C	118.3, C	118.3, C	118.4, C	111.8, C	112.3, C	112.0, C	112.2, C	112.1, C
4‴	156.3, C	153.6, C	153.7, C	153.8, C	152.5, C	157.9, C	156.3, C	157.3, C	156.5, C
1-OMe						53.2, CH_3	52.9, CH_3	53.2, CH_3	52.9, CH_3
4-OMe						53.3, CH_3	53.1, CH_3	53.2, CH_3	53.0, CH_3
6-OMe					52.6, CH_3				
4″-OMe			55.4, CH_3	55.4, CH_3					
4‴-OMe		60.6, CH_3	60.7, CH_3	60.6, CH_3					

[a]Recorded in DMSO-d_6. [b]Recorded in $CDCl_3$.

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Table 2. ^{1}H NMR (δ, mult (J in Hz)) Assignments for Compounds 1−6

position	1[a]	2[b]	3a[b]	3b[b]	4[a]	5a[a]	5b[a]	6a[a]	6b[a]
5	6.42, s	5.86, s	5.90, s	7.06, s	5.86, s				
6		5.59, s	5.61, s	5.53, s					
2′	7.50, d (1.8)	7.37, br s	7.33, s	6.79, s	7.51, s	7.33, s	7.65, s	7.28, d (2.2)	7.55, d (2.2)
5′	6.97, d (8.4)	7.10, br s						6.87, d (8.4)	7.00, d (8.4)
6′	7.21, dd (8.4, 1.8)	7.10, br s	7.33, s	6.79, s	7.51, s	7.33, s	7.65, s	7.03, dd (8.4, 2.2)	7.19, dd (8.4, 2.2)
2″	8.14, s	7.92, d (1.9)	7.73, d (7.8)	6.80, d (7.5)	7.45, s				
3″			6.92, d (7.8)	6.54, d (6.5)					
5″		7.03, d (8.5)	6.92, d (7.8)	6.54, d (6.5)					
6″	8.14, s	7.63, dd (1.9, 8.5)	7.73, d (7.8)	6.80, d (7.5)	7.45, s				
2‴/6‴	7.96, s	7.42, s	7.41, s	7.39, s	7.67, s	7.79, s	8.05, s	7.78, s	7.98, s
1-OMe						3.73, s	3.56, s	3.71, s	3.56, s
4-OMe						3.84, s	3.45, s	3.85, s	3.49, s
6-OMe					3.32, s				
4″-OMe			3.85, s	3.72, s					
4‴-OMe		3.84, s	3.83, s	3.84, s					
6-OH		ND[c]	6.26, s	6.04, s					
4′-OH	10.96, s	ND	ND	ND	ND	ND	ND	11.08, br s	10.90, br s

[a]Recorded in DMSO-d_6. [b]Recorded in $CDCl_3$. [c]Not detected.

was determined to be a tris-aromatic furanone metabolite whose spectroscopic data were in good agreement with those of cadiolides A and B.[3] The configuration at the C-4 asymmetric double bond was assigned as *Z* on the basis of the cross-peak at H-5/H-2′ (H-6′) in the NOESY data.

The molecular formula of cadiolide G (**2**) was deduced to be $C_{25}H_{16}Br_4O_6$ by HRESIMS analysis. The NMR data of this compound were similar to those of **1**, revealing the cadiolide feature, namely, three aromatic rings and a furanone moiety. The most conspicuous difference in the NMR data was the reduction of the C-6 carbonyl of **1** to a secondary alcohol (δ_C 66.9, δ_H 5.59). Also evident from the NMR and mass data was the appearance of a methoxy group (δ_C 60.6, δ_H 3.84). In addition, the NMR data suggested one less bromine in an aromatic moiety. These differences were traced by combined 2-D NMR analyses. After defining each aromatic ring by COSY, gHSQC, and gHMBC data, their attachments to the furanone moiety were determined from the gHMBC correlations between the key protons and neighboring carbons. That is, the reduction of the C-6 carbonyl to a secondary alcohol and the attachment of the 3,5-dibromo-4-methoxyphenyl group were deduced by a series of long-range correlations: H-6/C-1, C-2, C-3, C-1‴, and C-2‴(C-6‴); H-2‴(H-6‴)/C-6. The 3-bromo-4-hydroxyphenyl group was also determined to be attached at C-5 by the long-range correlations H-5/C-1″ and C-2″ (C-6″) and H-2″(H-6″)/C-5. The remaining 3-bromo-4-hydroxyphenyl moiety was determined to be intact at C-1′ from the correlation H-2′(H-6′)/C-3. Thus, the structure of cadiolide G (**2**) was found to be a new hydroxy-containing cadiolide.

Cadiolide G (**2**) possesses an asymmetric carbon center at C-6. Determination of the absolute configuration at this center was first attempted by Mosher's method, in which MTPA esterification was performed on a dimethoxy analogue to protect the phenolic hydroxy groups and to remove the effect of additional MTPA adducts in these positions. However, the MTPA esters of **2** exhibited scattered diamagnetic proton shifts, possibly due to the severe spatial crowding between the C-1′ and C-1‴ phenyl rings (Figure S1). We next attempted to resolve this problem using electronic circular dichroism (ECD) calculations using time-dependent density functional theory (TDDFT).[9] The ECD profiles of the 6*S*- and 6*R*-cadiolide G (**2S** and **2R**) are shown in Figure 1. The experimental CD

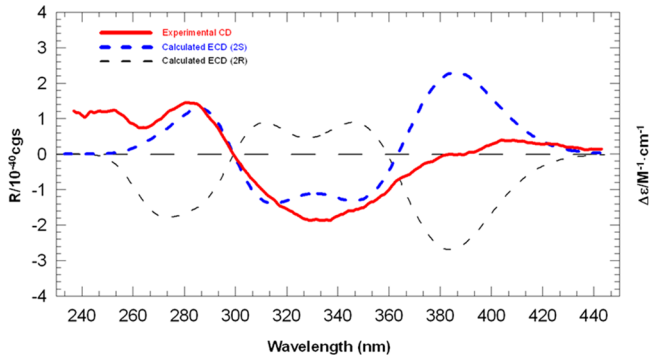

Figure 1. Experimental CD (bold solid line, in MeOH) and calculated ECD (**2R**, dotted line; **2S**, bold dotted line at B3LYP/def-SV(P)) spectra of compound **2**. For computational calculations, see Supporting Information, S42.

profile of **2**, with the positive and negative Cotton effects at 284 and 339 nm, respectively, matched well with **2S**, thus allowing the assignment of the 6*S* configuration for **2**.

Compounds **3a** and **3b** were isolated as an interconverting mixture (**3a**:**3b** = 2:1 in $CDCl_3$). The negative HRESIMS analysis showed a single quasi-molecular ion cluster at m/z 744.7716 ($C_{26}H_{17}Br_4O_6$), thus suggesting an isomeric nature for these compounds. Due to the significant difference of their concentrations, all of the signals in the ^{1}H and ^{13}C NMR data were readily assignable to each isomer (Tables 1 and 2). The NMR data of these compounds were similar to those of **2**, revealing the cadiolide features for both: three aromatic rings and a furanone moiety. The most noticeable difference in the NMR data was the appearance of additional methoxy groups (δ_C 61−55, δ_H 3.9−3.7). Also determined from the ^{1}H and ^{13}C NMR spectra was the change of substitution patterns in the aromatic rings. These changes were readily defined using combined 2-D NMR analyses, which resulted in the placements of 3,5-dibromo-4-hydroxyphenyl and 4-methoxyphenyl moi-

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eties at C-1′ and C-1″, respectively, while the 3,5-dibromo-4-methoxyphenyl moiety of **2** was intact at C-1‴.

Although the 2-D NMR data of **3a** and **3b** exhibited identical proton−proton and carbon−proton correlations throughout the entire molecules, their ^{1}H and ^{13}C NMR data differ noticeably (Tables 1 and 2). The significant shifts at C-3−C-5 in the ^{13}C NMR data suggested isomerization at the C-4 double bond, and the widely spread shifts in the ^{1}H NMR data could be attributed to the concomitant conformational change. This interpretation was confirmed by NOESY experiments, in which the crucial cross-peak was observed at H-5/H-2′ (H-6′) for **3a**, assigning the 4*Z* configuration. In contrast, the 4*E* configuration was assigned for **3b** on the basis of a cross-peak at H-2′ (H-6′)/H-2″ (H-6″). Thus, the structures of compounds **3a** and **3b**, designated to be *Z*- and *E*-cadiolide H, respectively, were determined to be 6*S*-hydroxy-trisaromatic furanones of the cadiolide class.

The molecular formula of cadiolide I (**4**) was determined to be $C_{25}H_{12}Br_6O_7$ by HRESIMS analysis. Although the presence of three 3,5-dibromo-4-oxygenated benzene rings was readily identified using ^{1}H and ^{13}C NMR spectra, detailed examination of these data showed that signals of carbons and protons at the furanone and in the vicinity of the furanone changed significantly. First, the carbon signals for the C-6 ketone (**1**) or alcohol (**2** and **3**) were replaced by a conjugated ester or acid carbon at δ_C 162.7. The C-5 olefinic methine was replaced by a quaternary carbon. In addition, signals for the methoxy group shifted significantly (δ_C 52.6, δ_H 3.32) from those of the aromatic methoxy groups (δ_C 55−61, δ_H 3.7−3.9) in other compounds, thereby suggesting the change of functionality (Tables 1 and 2).

These spectroscopic changes were further elucidated by combined 2-D NMR experiments. The aromatic moieties were found to be three identical 3,5-dibromo-4-hydroxyphenyl groups by these experiments. The connectivity of groups with the furanone moiety was determined by gHMBC experiments in which parameters were repeatedly optimized for the crucial three- and four-bond couplings between the neighboring atoms. As summarized in Figure 2, the H-2‴ (H-6‴) aromatic proton

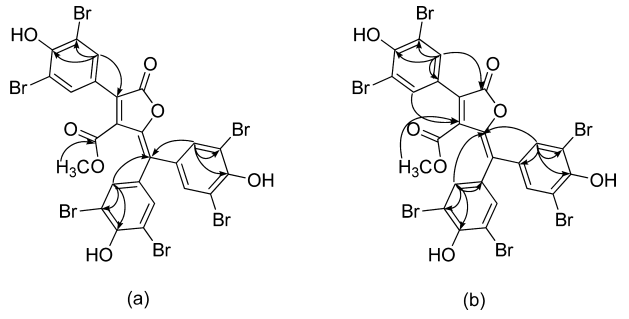

Figure 2. Selected gHMBC correlations (J_{CH} = 8 Hz, a) and D-HMBC correlations (J_{CH} = 1 Hz, b) for compound **4**.

exhibited long-range couplings with the carbons at δ_C 166.3, 138.6, and 126.3. The significant enhancement of correlations under the experimental conditions for a smaller coupling constant (D-HMBC experiment, J_{CH} = 1 Hz) than normal (gHMBC experiment, J_{CH} = 8 Hz) indicated that the former two carbons are connected to the aromatic protons with four-bond distances. The remaining carbon at δ_C 126.3 exhibited weaker intensity correlation and thus must be located three bonds away (Figure 2).[10] These results secured the placement of the carbons at C-1 (δ_C 166.3), C-2 (δ_C 126.3), and C-3 (δ_C 138.6), respectively, of the furanone moiety as well as the attachment of the 3,5-dibromo-4-hydroxyphenyl group at C-2. Similarly, a long-range correlation of a carbonyl carbon at δ_C 162.7 with a methoxy proton at δ_H 3.32 revealed the presence of a carbomethoxy group; its attachment at C-3 of the furanone was determined by another correlation in the D-HMBC data.

In contrast to other cadiolides, both of the 3,5-dibromo-4-hydroxyphenyl groups were found to be directly attached to an olefinic quaternary carbon at δ_C 123.6 based on gHMBC data. The extension of this linkage to another olefinic carbon at δ_C 142.7 was determined using the D-HMBC data (Figure 2). The presence of a furanone moiety accommodating the latter carbon at C-4 coincided well with both the molecular formula and a strong absorption band at 1737 cm^{-1} in the IR data. Thus, the structure of cadiolide I (**4**) was determined to be a tris-aromatic furanone with a heretofore unreported carbon skeleton.

In addition to the cadiolides, another group of compounds was also isolated and structurally elucidated. Compounds **5a** and **5b** were obtained as an interconverting mixture (**5a**:**5b** = 3:2 in DMSO-d_6). The HRFABMS data showed a single quasi-molecular ion cluster at m/z 672.7353, thus suggesting $C_{19}H_{12}Br_4O_7$ as the molecular formula for these isomeric compounds. Due to the noticeable difference in their concentrations, NMR signals were easily interpreted for each compound. The NMR data of these compounds differed significantly from those of cadiolides; the loss of signals for a phenyl group was the most noticeable difference. The significant changes (1726 cm^{-1}) in the IR data also suggested that the furanone moiety of the cadiolides was modified in these compounds. Detailed examination of the ^{13}C and ^{1}H NMR data in conjunction with the COSY and gHSQC analyses revealed the presences of two 3,5-dibromo-4-hydroxyphenyls, three carbonyls, one tetrasubstituted double bond, and two methoxy groups (Tables 1 and 2).

Given this information, the connectivity among the partial structures was deduced by HMBC experiments. The direct attachments of separate phenyl groups to a carbonyl carbon (δ_C 187.5 for **5a**, δ_C 187.4 for **5b**) and to an olefinic carbon (δ_C 140.8 for **5a**, δ_C 138.7 for **5b**) were revealed by the characteristic three-bond couplings between these carbons and neighboring protons in gHMBC data. The individual connections of two carbonyl carbons (δ_C 166.1 and 163.6 for **5a**, δ_C 165.2 and 164.0 for **5b**) to two methyl groups, thus forming two carbomethoxy groups, was also determined in the same gHMBC experiment. The assembly among these partial structures was accomplished by D-HMBC analysis with carbon proton coupling optimized for 1 Hz. In these data, the carbomethoxy groups were connected to C-2 and C-3 of the tetrasubstituted double bond by four-bond correlations between the olefinic carbons and methoxy protons (Figure 3). Similarly, the attachment of an aromatic moiety at C-3 was established by a long-range coupling between the C-4 carbonyl carbon and the H-2′ (H-6′) aromatic proton. Although not directly supported by HMBC data, the linkage between C-2 and C-5 was deduced from the molecular formula and from the chemical shifts of C-2 (δ_C 132.7 and 139.3 for **5a** and **5b**, respectively). Overall, these HMBC analyses determined that **5a** and **5b** possessed an unreported carbon skeleton.

Despite the same carbon−proton and proton−proton correlations for **5a** and **5b** in the 2-D NMR data, their chemical shifts differ significantly. This difference in chemical

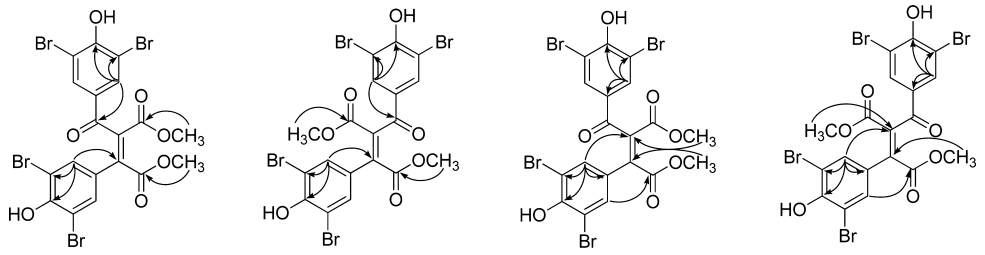

Figure 3. Selected gHMBC correlations (J_{CH} = 8 Hz, a and b) and D-HMBC correlations (J_{CH} = 1 Hz, c and d) for compound **5a** (a and c) and **5b** (b and d).

Table 3. Results of Bioactivity Tests[a]

	MIC (μg/mL)						IC_{50} (μM)			LC_{50} (μM)	
	Gram(+) bacteria			Gram(−) bacteria							
compound	A	B	C	D	E	F	SrtA	ICL	Na^+/K^+-ATPase	K562	A549
1	3.1	1.6	0.8	1.6	3.1	>100	78.8	8.9	2.5	>100	40.4
2	3.1	12.5	3.1	0.8	3.1	>100	>120	49.8	10.3	>100	>100
3	6.3	1.6	3.1	3.1	3.1	>100	>120	>100	13.5	50.8	38.5
4	0.8	0.8	0.8	1.6	6.3	>100	>120	10.8	5.0	51.2	52.7
5	100	50	100	50	100	>100	>120	26.3	28.0	53.7	19.2
6	>100	>100	>100	>100	>100	>100	>120	47.6	145.4	49.7	>100
ampicillin	0.4	0.4	0.4	0.4	1.6	6.3					
*p*HMB[b]							105.9				
3-NP[c]								53.9			
ouabain									5.8		
doxorubicin										4.7	1.7

[a]A: *Staphylococcus aureus* (ATCC 6538p), B: *Bacillus subtilis* (ATCC 6633), C: *Kocuria rhizophila* (NBRC 12708), D: *Salmonella enterica* (ATCC 14028), E: *Proteus hauseri* (NBRC 3851), F: *Escherichia coli* (ATCC 35270). [b]*para*-Hydroxymercuribenzoic acid. [c]3-Nitropropionic acid.

shift behavior could be attributed to the configurational difference at the C-2 double bond (Tables 1 and 2). The NOESY data for the mixture displayed cross-peaks at H-2′ (H-6′)/4-OMe and H-2‴(H-6‴)/1-OMe for both compounds. However, a crucial cross-peak at H-2′ (H-6′)/1-OMe was observed only in **5b**, thus assigning the 2*Z* and 2*E* configurations for **5a** and **5b**, respectively. Thus, the structures of **5a** and **5b**, designated to be *Z*- and *E*- synoilide A, respectively, were determined to be bis-aromatic diesters of a new structural class related to the rubrolides, bis-aromatic furanones from a *Botryllus* ascidian.[3]

Similar compounds **6a** and **6b** were isolated as an interconverting mixture (**6a**:**6b** = 6:1 in DMSO-d_6) and determined to have the composition $C_{19}H_{13}Br_3O_7$ by HRFABMS analysis. Besides the replacement of a bromine atom with a hydrogen, the ^1H and ^{13}C NMR data of these compounds were very similar to those of **5a** and **5b**. The combined 2-D NMR analyses confirmed this, and the newly apparent 3-bromo-4-hydroxyphenyl group was placed at C-3, while the 3,5-dibromo-4-hydroxyphenyl groups of **5a** and **5b** were intact in these compounds. On the basis of NOESY data, the structural difference between **6a** and **6b** was defined at the configuration of the C-2 double bond. Thus, compounds **6a** and **6b**, designated as *Z*- and *E*-synoilide B, respectively, were determined to be bis-aromatic diesters.

Rubrolides exhibit diverse bioactivities.[2,4,5] The bioactivities of the cadiolides are, however, poorly known (they are inactive against the HCT-116 cell line).[3] In our research on the discovery of antimicrobial compounds from natural products, we used whole microbial cells and several target enzymes related to antimicrobial activities. Sortase A (SrtA) and isocitrate lyase (ICL) were selected as target enzymes since they are not found in mammals and play crucial roles in the virulence or survival of various human-pathogenic bacteria and fungi.[11,12] Na^+/K^+-ATPase plays a crucial role in cellular function, and the development of a new type of less toxic natural regulators of this pump is an attractive prospect.[13] In our assays, cadiolides E and G−I (**1**−**4**) displayed significant antibacterial activities against several Gram-positive and Gram-negative strains, while synoilides A (**5**) and B (**6**) exhibited either much weaker or no activity (Table 3).[14] These results suggest that the antibacterial activity of these compounds may be attributable to the presence of the furanone moiety. For cytotoxicity, all of the tested compounds were inactive against the K562 and A549 cell lines (LC_{50} > 10 μM).[15] Several of these compounds also exhibited significant inhibition against *Candida albicans*-derived isocitrate lyase and Na^+/K^+-ATPase and moderate activity against bacterial sortase A (Table 3).[13,16,17]

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 polarimeter using a 1 cm cell. UV spectra were acquired with a Hitachi U-3010 spectrophotometer. CD spectra were obtained on a JASCO J-715 using a 0.2 mm cell. IR spectra were recorded on a JASCO 4200 FT-IR spectrometer using a ZnSe cell. NMR spectra were recorded in DMSO-d_6 and $CDCl_3$ solutions containing Me_4Si as an internal standard on Bruker Avance 900 and 600 and Varian Gemini 2000 spectrometers. Proton and carbon NMR spectra were measured at 900 and 225 MHz (**3**) or 600 and 150 MHz (**1**, **2**, **4**, **5**, and **6**), respectively. High-resolution FAB

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mass spectrometric data were obtained at the Korea Basic Science Institute (Daegu, Korea) and acquired using a JEOL JMS 700 mass spectrometer with *meta*-nitrobenzyl alcohol as a matrix for the FABMS. High-resolution electrospray ionization source (ESI) mass spectroscopic data were acquired using a Bruker Daltonics micro-OTOF-Q II ESI-Qq-TOF spectrometer with MeOH as a solvent for the ESIMS. Low-resolution ESIMS data were recorded on an Agilent Technologies 6130 Quadrupole mass spectrometer with an Agilent Technologies 1200 series HPLC. Semipreparative HPLC was performed on a Spectrasystem p2000 equipped with a refractive index detector (Spectrasystem RI-150). All solvents used were spectroscopic grade or distilled from glass prior to use.

Animal Material. Specimens of *Synoicum* sp. (sample number 09CH-11) were collected by hand with scuba equipment at a depth of 20 m off the coast of Chuja-do, Korea, on November 4, 2009. The colonial tunicates were up to 20 mm high and up to 50 mm in maximum dimension, rounded, cushion-shaped, sessile, and fixed by a small part of the basal surface; the zooids were bright dark red when alive. Zooids formed a circular system of 1 mm in diameter with up to six to eight zooids around a central colonial cavity. Contracted zooids were nearly 7 mm long, of which the thorax was 1−1.5 mm and the posterior abdomen was at least three times that length. Each branchial siphon had six lobes, and the protruding atrial siphon had small pointed lobes around its posterior rim. The thorax was especially large, with 16−18 segmental rows; the gut loop was moderately short, and the stomach was large, nearly spherical, and smooth-walled. These morphological features indicated the specimen to belong to the genus *Synoicum* and, in particular, was very similar to *S. pulmonaria*, but the lack of gonads and larvae prevented adequate species identification. The voucher specimens were deposited at the Natural History Museum, Ehwa Womans University, under the curatorship of B.J.R.

Extraction and Isolation. Freshly collected specimens were immediately frozen and stored at −25 °C until use. Lyophilized specimens were macerated and repeatedly extracted with MeOH (3 L × 3) and CH_2Cl_2 (3 L × 2). The combined extracts (160.95 g) were successively partitioned between H_2O (145.12 g) and *n*-BuOH (15.83 g); the latter fraction was repartitioned between H_2O−MeOH (15:85) (12.27 g) and *n*-hexane (3.56 g). An aliquot of the former layer (5.70 g) was separated by C_{18} reversed-phase flash chromatography using sequential mixtures of MeOH and H_2O as eluents (six fractions in gradient, H_2O−MeOH, from 50:50 to 0:100), acetone, and finally EtOAc.

On the basis of the results of ^{1}H NMR and cytotoxicity analyses, the fractions eluted with H_2O−MeOH (50:50; 1.10 g) and H_2O−MeOH (10:90; 0.59 g) were chosen for separation. The fraction eluted with H_2O−MeOH (50:50) was separated by silica normal-phase flash chromatography using sequential mixtures of CH_2Cl_2 and MeOH as eluents (six fractions in gradient, CH_2Cl_2−MeOH, from 100:0 to 50:50) and finally MeOH. The fraction that eluted with CH_2Cl_2−MeOH (80:20; 0.33 g) was chosen for separation. It was separated by semipreparative reversed-phase HPLC (YMC ODS-A column, 10 mm × 250 mm; H_2O−MeOH, 20:80, with 0.01% TFA), yielding four peaks rich with secondary metabolites. Further purification of the first and second peak by reversed-phase HPLC (H_2O−MeCN, 65:35, with 0.01% TFA) provided compounds **5** and **6**, respectively, as inseparable isomeric mixtures (**5** for **5a** and **5b**; **6** for **6a** and **6b**). The other two peaks provided, in order of elution, compounds **1** and **4**, having high purity without further purification.

The H_2O−MeOH (10:90) fraction (0.59 g) was separated by reversed-phase semipreparative HPLC (H_2O−MeOH, 20:80) to yield, in order of elution, compounds **2** and **3** (**3** as an inseparable isomeric mixture of **3a** and **3b**). Final purifications of these metabolites were then accomplished by reversed-phase HPLC (H_2O−MeOH, 20:80, with 0.01% TFA). The purified metabolites were isolated in the following amounts: 21.5, 7.1, 34.2, 9.9, 15.6, and 19.7 mg of **1**−**6**, respectively.

Cadiolide E (1): orange, amorphous solid; UV (MeOH) λ_{max} (log ε) 206 (2.80), 254 (2.28), 342 (2.36), 362 (2.35), 488 (2.37) nm; IR (ZnSe) ν_{max} 3459, 1743, 1680 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS *m*/*z* 794.6508 [M + H]$^+$ (calcd for $C_{24}H_{12}{}^{79}Br_3{}^{81}Br_2O_6$, 794.6512).

Cadiolide G (2): yellow, amorphous solid; $[\alpha]_D^{25}$ −27.4 (*c* 0.40, MeOH); UV (MeOH) λ_{max} (log ε) 206 (2.85), 252 (2.16), 368 (2.37) nm; IR (ZnSe) ν_{max} 3380, 1738, 1681 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRESIMS *m*/*z* 730.7545 [M − H]$^-$ (calcd for $C_{25}H_{15}{}^{79}Br_2{}^{81}Br_2O_6$, 730.7558).

***Z*/*E*-Cadiolide H (3a and 3b; 2:1 in $CDCl_3$):** yellow, amorphous solid; $[\alpha]_D^{25}$ −27.1 (*c* 0.50, MeOH); UV (MeOH) λ_{max} (log ε) 205 (2.79), 252 (2.22), 370 (2.26) nm; IR (ZnSe) ν_{max} 3459, 1736, 1689 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRESIMS *m*/*z* 744.7716 [M − H]$^-$ (calcd for $C_{26}H_{17}{}^{79}Br_2{}^{81}Br_2O_6$, 744.7715).

Cadiolide I (4): red, amorphous solid; UV (MeOH) λ_{max} (log ε) 208 (2.46), 268 (2.18), 490 (2.32) nm; IR (ZnSe) ν_{max} 3366, 1737, 1686 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRESIMS *m*/*z* 902.5540 [M − H]$^-$ (calcd for $C_{25}H_{11}{}^{79}Br_3{}^{81}Br_3O_7$, 902.5541).

***Z*/*E*-Synoilide A (5a and 5b; 3:2 in DMSO-d_6):** bright yellow, amorphous solid; UV (MeOH) λ_{max} (log ε) 208 (2.46), 248 (2.11), 312 (2.00), 354 (2.25), 456 (1.59) nm; IR (ZnSe) ν_{max} 3382, 1726, 1687 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS *m*/*z* 672.7353 [M + H]$^+$ (calcd for $C_{19}H_{13}{}^{79}Br_2{}^{81}Br_2O_7$, 672.7356).

***Z*/*E*-Synoilide B (6a and 6b; 6:1 in DMSO-d_6):** bright yellow, amorphous solid; UV (MeOH) λ_{max} (log ε) 208 (2.46), 248 (2.08), 312 (2.00), 354 (2.21), 456 (1.57) nm; IR (ZnSe) ν_{max} 3380, 1726, 1670 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS *m*/*z* 592.8271 [M + H]$^+$ (calcd for $C_{19}H_{14}{}^{79}Br_2{}^{81}Br_1O_7$, 592.8270).

Computational Chemistry. The ground-state geometries were optimized with density functional theory (DFT) calculations, using Turbomole at the basis set def-SV(P) for all atoms and the functional B3LYP. The ground states were further confirmed by the harmonic frequency calculation. The calculated ECD data corresponding to the optimized structures were obtained with TDDFT at the B3LYP functional. The CD spectra were simulated by overlapping Gaussian functions for each transition, where σ is the width of the band at height 1/e. Values ΔE_i and R_i are the excitation energies and rotatory strengths for transition *i*, respectively. In the current work, the value of σ was 0.10 eV.

$$\Delta\varepsilon(E) = \frac{1}{2.297 \times 10^{-39}} \frac{1}{\sqrt{2\pi\sigma}} \sum_i^A \Delta E_i R_i \mathrm{e}^{[-(E-\Delta E_i)^2/(2\sigma)^2]}$$

Biological Assays. Antimicrobial assays were performed according to the method described previously.[14] Cytotoxicity assays were performed in accordance with literature protocols.[15] Isocitrate lyase, sortase A, and Na^+/K^+-ATPase inhibition assays were performed according to previously described methods.[13,16,17]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C NMR spectra of compounds **1**−**6** and the energy-minimized structure of compound **2** are 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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■ NOTE ADDED AFTER ASAP PUBLICATION

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