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Cytotoxic Diterpenoid Pseudodimers from the Korean Sponge *Phorbas gukhulensis*

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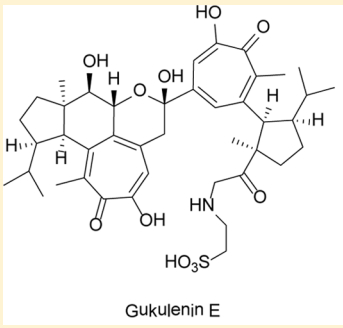
Supporting Information

ABSTRACT: Four new cytotoxic diterpenoid pseudodimers (**2**–**5**), along with a previously reported one, gukulenin A (**1**), were isolated from the marine sponge *Phorbas gukhulensis* collected off the coast of Gagu-do, Korea. These novel compounds, designated gukulenins C–F (**2**–**5**), were determined by extensive spectroscopic analyses to be pseudodimers of the gagunins, like gukulenin A. The termini of the tropolone-containing side chains in gukulenins C–E (**2**–**4**) were found to have diverse modifications involving acetamides or taurine, whereas gukulenin F (**5**) was formed from **1** by the ring-opening of a cyclic hemiketal. The relative and absolute configurations were assigned by Murata's and modified Snatzke's methods using a HETLOC experiment and a CD measurement of a dimolybdenum complex, respectively. All of these compounds exhibited significant cytotoxicity against the K562 and A549 cell lines.



Gukulenin E

Phorbas gukhulensis

Sponges have been the most prolific sources of biologically active and structurally unique secondary metabolites.[1] Despite their wide geographic distribution in the marine environment, investigations of the thickly encrusting sponges of the genus *Phorbas* (order Poecilosclerida, suborder Myxilina, family Hymedesmiidae) began much later than investigations of other sponge genera. However, since the discovery of the phorbazoles in 1994,[2] novel metabolites have been continuously isolated from these animals. The most notable example might be the phorboxazoles, highly functionalized macrolides that exhibit potent cytostatic activity.[3] Subsequently, compounds with diverse biogenetic origins and functionalities have been reported, primarily by the Molinski and Capon research groups. These compounds include amaranzoles,[4] phorbasides,[5] phorbasins,[6,7b] phorbasterones,[8] and amaroxocanes.[9] More recently, natural products with a new skeletal class with diverse biogenetic origins have been isolated from these animals. Ansellone A,[10] a new skeletal sesterterpenoid, and muironolide A,[11] a tetrachloro-polyketide, are examples of compounds that have been isolated from Pacific *Phorbas* sponges. *Phorbas* sponges are also abundant around Gagu-do, a small island in southwestern Korea. Despite their highly limited geographical distribution, Korean *Phorbas* sponges are very prolific sources of terpenoids with novel carbon skeletons and diverse functionalities. These terpenoids include the phorbasins,[6e,f] gagunins,[7] phorbaketals,[12] phorbasones,[13,14] and phorone A.[14] These compounds have been shown to exhibit potent and diverse bioactivities, such as the inhibition of the yeast-to-hypha transition,[6] induction of calcium deposition[13] and osteoblast differentiation,[13] suppression of NO production,[14f] and cytotoxicity,[7] which have attracted significant biomedical interest.

During the course of our previous work on the gagunins,[7] unusual upfield methyl signals (δ_H 0–0.5) in ^{1}H NMR spectra of gagunin-containing chromatographic fractions captured our attention. However, due to the extremely low concentrations and instability of the compounds, we did not attempt to obtain more information. In our continuing search for bioactive substances from marine organisms in Korea, we re-collected *Phorbas gukhulensis* specimens, and the ^{1}H NMR data showed the same upfield signals. Furthermore, the organic extract of these specimens exhibited significant cytotoxicity toward the human leukemia cell line K562 (LC_{50} < 1 μg/mL) and was lethal to brine shrimp (LC_{50} 36 ppm). The persistence and significant bioactivity of these compounds prompted us to investigate this sponge in detail. We describe herein the

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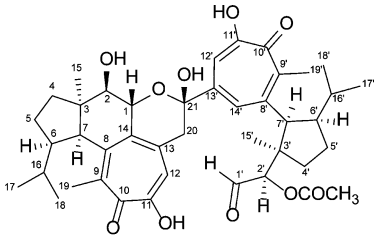
isolation and structure determination of four new gukulenin compounds, gukulenins C–F (**2**–**5**), along with gukulenin A (**1**), that exhibited acute cytotoxicity against both the K562 and A549 cell lines. All of the new compounds have the same pseudodimeric gagunin-like skeleton as congener **1**, which was recently isolated from the same sponge.[15] However, these compounds differ structurally from **1** by the diverse functionalities modifying the cyclopentane ring or by the absence of the cyclic hemiketal.



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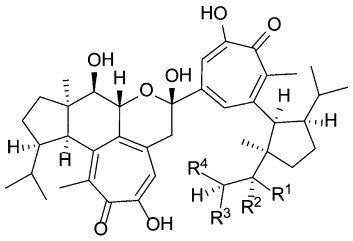
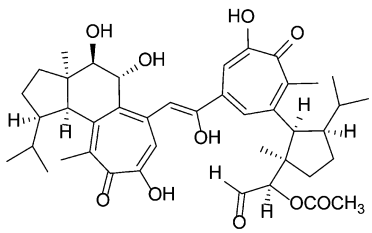


2 R^1 = OH, R^2 = H, R^3 = OCH_3, R^4 = $NHCOCH_3$
3 R^1 = OH, R^2 = H, R^3 = OH, R^4 = $NHCOCH_3$
4 R^1R^2 = O, R^3 = H, R^4 = $NH(CH_2)_2SO_3H$



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RESULTS AND DISCUSSION

A combination of mass and NMR spectroscopy and the comparison of the spectroscopic data with those published in the literature readily identified compound **1** as gukulenin A. The absolute and relative configurations of this compound were also assigned to be the same as those in the literature by a comparison of the NMR spectra and specific rotations and by a NOESY analysis.

The molecular formula of gukulenin C (**2**), a yellowish, amorphous solid, was deduced to be $C_{43}H_{59}NO_{10}$ based on the HRESIMS and ^{13}C NMR analyses. The NMR spectroscopic data for this compound were very similar to those for other gukulenins; the chemical shifts of the protons and the carbons of the 6,5-bicyclic moiety substituted with an isopropyl group and the tropolones were almost identical to those of **1**. A detailed examination of the NMR data showed that structural modification occurred at the terminal C-1′ and C-2′ of the tropolone-containing half of the molecule. That is, the aldehyde carbon at C-1′ (δ_C 199.0) of **1** was replaced by an oxymethine (δ_C 80.9) that coincided with the appearance of a methoxy group (δ_C 53.9) in the ^{13}C NMR data (Table 1). Although the presence of the same acetyl group was reflected in the carbon signals (δ_C 170.0 and 22.8), the mass data suggested the replacement of the −OAc group with −NHAc. Corresponding differences were observed in the ^1H NMR spectrum, in which signals attributed to a methoxy group and an oxymethine group appeared at δ_H 3.07 and 4.82, respectively (Table 2). The replacement of the C-2′ acetoxy moiety with a hydroxy group

Table 1. ^{13}C NMR (ppm, mult) Assignments for Compounds 2–5

position	**2**[a]	**3**[a]	**4**[a]	**5**[b]
1	72.1, CH	72.2, CH	72.1, CH	80.4, CH
2	75.3, CH	75.3, CH	75.6, CH	77.3, CH
3	43.9, C	44.0, C	43.9, C	43.9, C
4	33.3, CH_2	33.6, CH_2	33.1, CH_2	33.6, CH_2
5	26.8, CH_2	26.8, CH_2	26.6, CH_2	27.6, CH_2
6	51.1, CH	51.7, CH	51.2, CH	52.3, CH
7	55.3, CH	55.3, CH	55.5, CH	56.5, CH
8	147.5, C	147.4, C	147.4, C	149.7, C
9	133.3, C	133.2, C	133.1, C	135.1, C
10	169.0, C	168.8, C	168.7, C	169.2, C
11	166.7, C	166.9, C	167.0, C	167.9, C
12	122.6, CH	122.9, CH	123.0, CH	120.0, CH
13	140.2, C	140.4, C	140.2, C	137.5, C
14	138.6, C	138.7, C	138.6, C	128.2, C
15	30.1, CH_3	30.2, CH_3	30.2, CH_3	30.4, CH_3
16	26.4, CH	26.5, CH	26.3, CH	27.0, CH
17	23.7, CH_3	23.8, CH_3	23.8, CH_3	24.2, CH_3
18	18.7, CH_3	18.7, CH_3	18.6, CH_3	19.2, CH_3
19	18.8, CH_3	18.9, CH_3	18.9, CH_3	19.9, CH_3
20	47.3, CH_2	47.3, CH_2	46.4, CH_2	110.2, CH
21	97.2, C	97.3, C	97.3, C	157.4, C
1′	80.9, CH	73.1, CH	53.6, CH_2	197.6, CH
2′	74.5, CH	76.2, CH	207.1, C	82.7, CH
3′	52.9, C	53.0, C	61.4, C	50.8, C
4′	34.7, CH_2	34.8, CH_2	31.5, CH_2	35.2, CH_2
5′	29.1, CH_2	32.1, CH_2	28.2, CH_2	30.9, CH_2
6′	50.3, CH	50.2, CH	49.9, CH	51.2, CH
7′	56.3, CH	56.6, CH	55.4, CH	57.2, CH
8′	151.3, C	151.6, C	149.8, C	150.0, C
9′	140.8, C	141.1, C	140.3, C	141.6, C
10′	174.0, C	174.3, C	175.0, C	175.4, C
11′	163.3, C	162.8, C	162.8, C	163.5, C
12′	115.9, CH	115.8, CH	115.8, CH	114.1, CH
13′	147.4, C	146.8, C	148.7, C	136.4, C
14′	125.8, CH	125.7, CH	123.2, CH	124.7, CH
15′	22.9, CH_3	22.8, CH_3	28.7, CH_3	25.4, CH_3
16′	29.5, CH	29.6, CH	29.3, CH	30.7, CH
17′	21.3, CH_3	21.4, CH_3	21.8, CH_3	22.7, CH_3
18′	22.3, CH_3	22.3, CH_3	21.4, CH_3	22.2, CH_3
19′	18.2, CH_3	18.2, CH_3	18.2, CH_3	18.6, CH_3
1′-NHAlkyl	170.0, C	170.0, C	43.3, CH_2	
	22.8, CH_3	23.1, CH_3	46.3, CH_2	
1′-OMe	53.9, CH_3			
2′-OAc				169.8, C
				20.2, CH_3

[a]Data were obtained in DMSO-d_6 solutions at 125 (**2** and **4**) and 150 MHz (**3**). [b]Data were obtained in $CDCl_3$ solutions at 225 MHz.

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Table 2. ^{1}H NMR (δ, mult (J in Hz)) Assignments for Compounds 2−5

position	2[a]	3[a]	4[a]	5[b]
1	4.72, d (9.5)	4.72, d (9.6)	4.68, d (9.5)	4.92, d (9.7)
2	3.42, dd (9.5, 3.5)	3.42, dd (9.6, 3.5)	3.41, dd (9.5, 2.7)	3.93, d (9.7)
4	1.28, m	1.27, m	1.30, m	1.43, m
	2.14, ddd (12.8, 12.8, 6.6)	2.16, ddd (12.6, 12.6, 6.3)	2.16, ddd (12.3, 12.3, 6.6)	2.13, ddd (11.0, 11.0, 4.5)
5	1.38, m	1.39, m	1.35, m	1.41, m
	1.67, ddd (12.8, 12.8, 6.6)	1.67, ddd (12.6, 12.6, 6.3)	1.65, m	1.74, ddd (11.0, 11.0, 4.5)
6	2.32, m	2.32, m	2.32, m	2.36, m
7	3.69, d (9.5)	3.68, d (9.5)	3.70, d (9.4)	3.77, d (9.7)
12	6.85, s	6.89, s	6.96, s	7.05, s
15	1.31, s	1.30, s	1.34, s	1.42, s
16	1.04, m	1.04, m	1.03, qqd (6.6, 6.6, 4.6)	1.12, qqd (6.6, 6.6, 4.7)
17	0.56, d (6.6)	0.54, d (6.6)	0.59, d (6.6)	0.62, d (6.6)
18	0.23, d (6.6)	0.23, d (6.6)	0.17, d (6.6)	0.29, d (6.6)
19	2.43, s	2.43, s	2.44, s	2.56, s
20	3.11, d (15.3)	3.09, d (15.5)	3.06, d (15.3)	6.43, s
	3.16, ND[c]	3.17, ND[c]	3.25, d (15.3)	
1′	4.82, dd (9.6, 3.4)	5.05, m	4.14, d (18.4)	9.45, s
			3.86, d (18.4)	
2′	2.99, dd (5.6, 3.4)	2.89, dd (5.4, 3.8)		4.42, s
4′	1.37, m	1.42, m	1.68, m	1.82, m
	1.85, m	1.92, m	2.38, m	2.45, m
5′	1.24, m	1.25, m	0.89, m	1.99, m
	1.82, m	1.84, m	1.80, m	2.40, m
6′	2.09, m	2.08, m	2.11, m	2.38, m
7′	3.28, ND[c]	3.30, ND[c]	3.53, d (7.8)	3.65, s
12′	7.30, s	7.29, s	7.40, s	7.57, d (1.8)
14′	6.93, s	6.99, s	6.62, s	7.26, br s
15′	1.02, s	1.04, s	1.32, s	1.30, s
16′	0.74, m	0.74, m	0.59, m	1.33, m
17′	0.40, d (6.3)	0.40, d (6.3)	0.40, d (6.4)	0.65, d (6.3)
18′	0.51, d (6.3)	0.49, d (6.3)	0.42, d (6.4)	0.94, d (6.3)
19′	2.45, s	2.47, s	2.42, s	2.65, s
2-OH	4.70, d (3.5)	4.80, d (3.5)	4.95, d (2.7)	
21-OH	7.02, s	7.03, s	6.86, s	
1′-NH	7.62, d (9.6)	7.66, d (8.8)		
1′-NHAlkyl	1.86, s	1.78, s	2.96, t (6.4)	
			2.73, dt (13.1, 6.4)	
			2.63, dt (13.1, 6.4)	
1′-OH		5.30, d (5.1)		
1′-OMe	3.07, s			
2′-OH	4.58, d (5.6)	4.38, d (5.4)		
2′-OAc				2.01, s

[a]Data were obtained in DMSO-d_6 solutions at 500 (**2** and **4**) and 600 MHz (**3**). [b]Data were obtained in $CDCl_3$ solutions at 900 MHz. [c]Due to the severe overlapping with solvent, splitting patterns were undetected.

was evidenced by the signals at δ_H 4.58 and 2.99. In addition, the newly appearing signal at δ_H 7.62 was thought to correspond to the amide proton.

A combination of COSY, gHSQC, and gHMBC analyses not only supported this interpretation but also revealed the partial structure at C-1′ and C-2′. Sequential couplings among the protons at δ_H 4.58, 2.99, 4.82, and 7.62 in the COSY spectrum readily led to the identification of the side chain. The oxymethine carbon at δ_C 74.5 bearing the proton at δ_H 2.99 was placed at C-2′ by its long-range coupling with the H_3-15′ methyl protons at δ_H 1.02 in the gHMBC data. On the basis of the long-range correlations of the H-1′ proton (δ_H 4.82) with the C-2′ (δ_C 74.5), OMe (δ_C 53.9), and NHAc carbonyl (δ_C 170.0) carbons, a methoxy group and an acetamide moiety were determined to be present at C-1′ (Figure 1). The

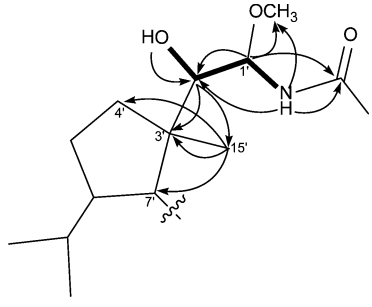

Figure 1. Selected gHMBC (arrows) and COSY (bold lines) correlations in the cyclopentane portion of compound **2**.

presence of the latter group was further supported by the correlation of the carbonyl carbon with the methyl protons at

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δ_H 1.86. The remaining portion of compound **2** was also identified to be the same as **1** by these 2-D NMR experiments, in particular, the gHMBC analysis, in which the correlations between the methyl protons and the neighboring carbons provided key information on the gukulenin skeleton of **2**. Thus, compound **2** was determined to be the 1′-deoxo-1′-methoxy-1′-*N*-acetyl-2′-deacetyl derivative of **1**.

Configurational assignments of the asymmetric centers in **2** were initially made based on coupling constant and NOESY analyses (Figure 2). The H-15 methyl proton at the ring

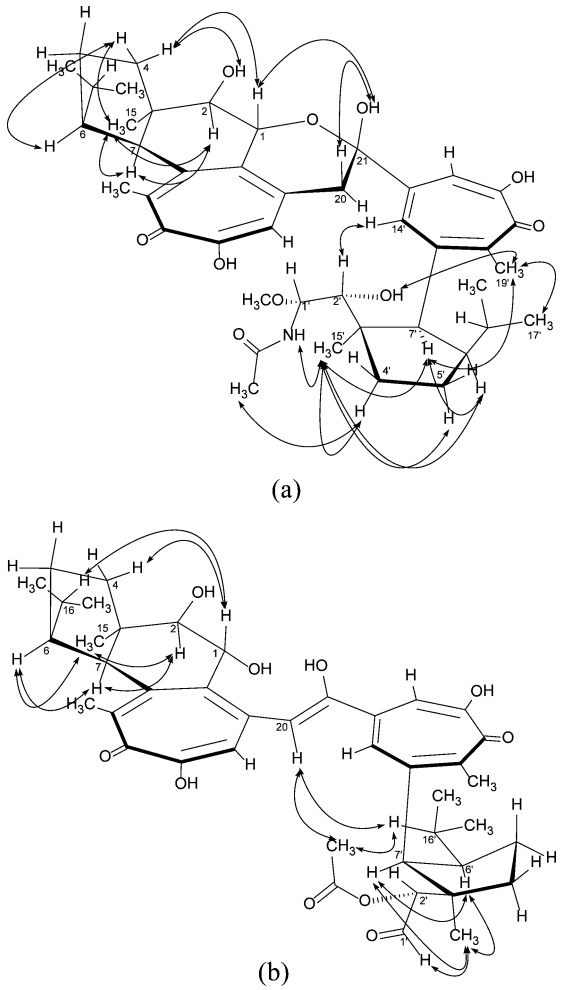


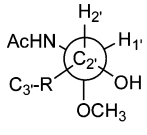
Figure 2. Selected NOESY correlations for compounds **2** (a) and **5** (b).

junction had NOESY cross-peaks with H-2, H-4a (δ_H 1.28), and H-7. Additional cross-peaks were observed at H-5a/H-6 and H-6/H-7. Together with the cross-peaks at H-2/H-7 and H-2/H-15, these data suggested a *cis* ring junction between the six- and five-membered rings and α-orientations for H-2, H-6, H-7, and H-15. The β-orientations of H-1 and 21-OH were also confirmed by cross-peaks at H-1/H-4b (δ_H 2.14) and H-1/21-OH, as well as by the large vicinal proton coupling constant ($J_{1,2}$ = 9.5 Hz) (Table 2). A three-dimensional molecular model of compound **2** revealed that the placement above the tropolone ring caused an abnormal upfield shift of the H_3-18 methyl protons (δ_H 0.23) due to a diamagnetic shielding effect. Similarly, the NOESY correlations of the H_3-15′ methyl protons with H-4′a (δ_H 1.37), H-5′a (δ_H 1.24), H-6′, and H-7′ along with the correlation of H-6′ with H-7′ confirmed the α-orientations of these protons on the five-membered ring. This interpretation was further supported by the cross-peaks at H_3-17′/H_3-19′ and H-2′/H-14′. The steric crowding caused by the substituents at C-1′ effectively prevents free rotation of the isolated five-membered ring about the C-7′/C-8′ bond. The unusual upfield shift of the carbinol proton H-2′ at δ_H 2.99 in the ^{1}H NMR spectrum might also be attributed to the steric crowding in this portion of the molecule.

Further NMR studies were performed to determine the relative configuration of compound **2** by using the hetero half-filtered TOCSY (HETLOC) experiment, from which the heteronuclear two- and three-bond coupling constants could be applied to the *J*-based method developed by Murata and co-workers.[16] For C-1′ and C-2′, the large and small two-bond heteronuclear couplings {^2J(C-2′, H-1′) = −3.66 Hz, ^2J (C-1′, H-2′) = −0.96} indicated a *gauche* orientation between the H-1′ and H-2′ that was also consistent with the small homonuclear coupling of ^3J(H-1′, H-2′) = 3.40 Hz. With these, further measurement of the small three-bond coupling of ^3J(H-2′, 1′-N) = 1.07 Hz allowed us to unequivocally identify the configurations depicted in Table 3. Even though the obtained values were slightly out of the reference ranges possibly due to the presence of a bulky amide group at C-1′, the trend was intact and acceptable for the conformational assignments. In addition, the NOE correlations at 1′-NH/H-15′ and 2′-OH/H-19′ were fully consistent with the coupling constant analysis and deduced the three-dimensional structure of the terminal side chain (Figure 2). Thus, the overall relative configurations of the asymmetric carbon centers of **2** were assigned as 1*R**, 2*R**, 3*R**, 6*R**, 7*R**, 21*S**, 1′*R**, 2′*R**, 3′*R**, 6′*R**, and 7′*R*.

The absolute configuration was assigned using the modified Snatzke's method[17] since the *in situ* complex of dimolybdenum tetraacetate [$Mo_2(OAc)_4$] with the C-1′ and C-2′ amide alcohol moiety of **2** could give a CD spectrum suitable for the determination of the absolute configuration. Compared with the CD spectrum of the reference Mo_2 complex,[17b] the

Table 3. $^3J_{H,H}$ and $^{2,3}J_{C,H}$ Values (Hz) at C-1′ and C-2′ in Compound 2 and Reference Values for a 1,2-Dioxygenated System[16]

configurational rotamer		(Hz)	reference (Hz)[a]	orientation
	^3J (H-1', H-2')	3.40	0 - 3, small	*gauche*
	^3J (H-2', 1'-N)	1.07	1 - 3, small	*gauche*
	^3J (C-3', H-1')	ND[b]	-	-
	^2J (C-2', H-1')	-3.66	-4 - -6, large	*gauche*[c]
	^2J (C-1', H-2')	-0.96	2 - 0, small	*anti*[c]

[a]Experimental value range in ref 16. [b]Not determined due to the quaternary nature of C-3′. [c]Oxygen functions on relevant carbons are *gauche* and *anti* to their vicinal protons.

conclusion was made that C-1′ and C-2′ both have *R* configurations with a negative Cotton effect (CE) at 295 nm and a positive CE at 338 nm (Supporting Information). A previous study on gukulenin A (**1**) used Mosher's method to determine that the absolute configuration at C-2 is *R*, and this assignment was in turn utilized to assign the absolute configuration of the whole molecule.[15] Our CD-based results on the configuration of **2** are consistent with the previously reported assignment. Furthermore, a comparison of the specific rotations ($[\alpha]_D^{25}$ = −19.1 and −4.8 for **1** and **2**, respectively) also supported that gukulenin C (**2**) has the same absolute configuration, except for C-1′, as gukulenin A (**1**).

The molecular formula of a related compound, gukulenin D (**3**), was deduced to be $C_{42}H_{57}NO_{10}$ by HRESIMS analysis. The NMR data for this compound were very similar to those for **2**, with the replacement of a methoxy group (δ_H 3.07, δ_C 53.9) by a hydroxy group (δ_H 5.30) being the most noticeable difference in the NMR data. These spectroscopic differences were readily identified in the combined 2-D NMR analyses, including the analysis of the sequential couplings in the COSY spectrum among the proton signals at 2′-OH (δ_H 4.38), H-2′ (δ_H 2.89), H-1′ (δ_H 5.05), 1′-OH (δ_H 5.30), and 1′-NH (δ_H 7.66); the remaining signals were identical to those for **2**. NOESY data and specific rotations ($[\alpha]_D^{25}$ −4.8 and −19.2 for **2** and **3**, respectively) as well as the biogenetic considerations of **3** as an *O*-demethyl derivative of **2** led us to assign a configuration for **3** identical with those of **1** and **2**: 1*R*, 2*R*, 3*R*, 6*R*, 7*R*, 21*S*, 1′*R*, 2′*R*, 3′*R*, 6′*R*, and 7′*R*. Thus, gukulenin D (**3**) was determined to be the 1′-*O*-demethyl derivative of gukulenin C (**2**). The structural similarity between **2** and **3** brought the possibility of the former as a methylated artifact of the latter compound during the isolation process. However, this possibility was excluded by LC-ESIMS analysis of the extract using MeCN, in which peaks of both **2** and **3** were clearly observed.

The molecular formula of gukulenin E (**4**) was determined to be $C_{42}H_{57}NO_{11}S$, significantly different from that of the other metabolites, on the basis of the HRESIMS analysis and the ^{13}C NMR data. A preliminary examination of the ^1H and ^{13}C NMR spectra of this compound revealed that the substituents at the C-1′ and C-2′ positions were notably different from those of the other gukulenins; the oxymethine and *N*-acetamide groups were absent, and three upfield methylenes and a ketone carbonyl were present (Tables 1 and 2). In addition, the strong absorption bands at 1203 and 1039 cm^{-1} in the IR spectrum revealed the presence of a sulfonic acid functionality.

These spectroscopic changes were verified by the combined 2-D NMR analyses. The gHMBC correlation with the H_3-15′ methyl protons at δ_H 1.32 placed the ketone carbonyl carbon at δ_C 207.1 at C-2′. Similarly, an isolated methylene carbon was located at C-1′ based on the long-range correlations of its protons at δ_H 4.14 and 3.86 with the C-2′ ketone carbon. In addition, two remaining methylenes (δ_C 43.3, δ_H 2.96, 2 H; δ_C 46.3, δ_H 2.73 and 2.63) were found to be directly connected to each other based on the strong proton−proton couplings among the protons and the gHMBC correlations between these groups. Additional gHMBC correlations with the H-1′ methylene connected these two methylene moieties to C-1′ via a secondary amine that was deduced from the chemical shifts of the protons. Finally, the sulfonic acid group whose presence was indicated by the molecular formula and IR data was placed at the other end of the two-methylene portion of the molecule. Thus, a taurine moiety was found to be connected at C-1′ of the carbon framework. The comparison of the NMR and specific rotation data, in conjunction with the NOESY analysis, yielded the same 1*R*, 2*R*, 3*R*, 6*R*, 7*R*, 21*S*, 3′*R*, 6′*R*, and 7′*R* configuration as found for the other gukulenins. Thus, gukulenin E (**4**) was determined to be a taurine-containing diterpene pseudodimer of the gukulenin class. A literature survey revealed that taurine-containing terpenoids,[6c−e,18] fatty acids,[19] and aromatic compounds[20] have recently been isolated from marine sponges.

The molecular formula of gukulenin F (**5**) was established to be $C_{42}H_{54}O_{10}$, identical with that of **1**, on the basis of the HRESIMS results and the interpretation of the ^{13}C NMR data. During the course of our structural determination using NMR experiments, the highly unstable nature of this compound in DMSO-d_6 was overcome by using $CDCl_3$. Despite the change in the NMR solvent, the ^1H and ^{13}C NMR data for **5** were very similar to those for **1**, including the signals for the acetaldehyde moiety at the C-1′ terminal. However, the detailed examination of the ^{13}C NMR data revealed that the carbon signals of the cyclic hemiketal moiety (δ_C 46.6 and 96.7 for C-20 and C-21, respectively) of **1** were replaced by those of a trisubstituted double bond at δ_C 157.4 and 110.2. Corresponding differences were also observed in the ^1H NMR data, in which a signal corresponding to a new olefinic proton appeared at δ_H 6.43. These spectroscopic changes were readily accommodated by the cleavage of the cyclic hemiketal to an enol-ether group. The crucial evidence was provided by the gHMBC data, in which long-range couplings were found between the newly formed H-20 olefinic protons and the neighboring C-12, C-14, C-21, and C-13′ carbons at δ_C 120.0, 128.2, 157.4, and 136.4, respectively. Also supporting this interpretation were the long-range couplings of the H-12′ and H-13 aromatic protons with the C-21 enol-ether carbon. Furthermore, the bathochromic shifts in the UV maxima were thought to be attributed to the extended conjugated system containing the enol-ether: **1**−**4**, 355−360 and 372−373 nm; **5**, 383 and 419 nm. The configuration at the newly formed C-20 asymmetric double bond was assigned as *Z* on the basis of the cross-peak at H-20/H-16′ in the NOESY data (Figure 2). On the basis of the results of the extensive NOESY analysis and the specific rotations, as well as the biogenetic relationships with other gukulenins, the absolute configurations at the remaining asymmetric carbon centers were proposed to be 1*R*, 2*R*, 3*R*, 6*R*, 7*R*, 2′*R*, 3′*R*, 6′*R*, and 7′*R*, the same as in **1**. Thus, gukulenin F (**5**) was determined to be a new enol-ether-containing derivative of the gukulenin class.

Previous studies on the bioactive metabolites isolated from *Phorbas* showed that these compounds have diverse actions. They exhibit cytotoxicity against various cancer cell lines[5−7,11,15] and have cytostatic,[3] antifungal,[3] and antioxidant[21] activities. Among these compounds, gukulenins A and B had significant cytotoxic activity against various cancer cell lines.[15] Our results also indicated that gukulenins A and C−F have potent cytotoxicity against the K562 and A549 cell lines (Table 4). It is noteworthy that gukulenin F, possessing an enol-ether residue, exhibited cytotoxicity against K562 that was 17-fold more potent than doxorubicin, as a positive control. However, none of these compounds have inhibitory activities against diverse bacterial strains (MIC > 100 μg/mL) as well as the enzymes sortase A, a pivotal enzyme for bacterial adhesion and invasion of host cells, isocitrate lyase, a key enzyme in fungal metabolism, and Na^+/K^+-ATPase (IC_{50} > 100 μM).

Table 4. Results of Cytotoxicity Tests[a]

	K562	A549
	LC_{50} (μM)	LC_{50} (μM)
1	0.26 ± 0.03	0.32 ± 0.04
2	0.12 ± 0.01	0.10 ± 0.02
3	0.44 ± 0.01	0.55 ± 0.03
4	0.32 ± 0.05	0.11 ± 0.04
5	0.04 ± 0.09	0.38 ± 0.02
doxorubicin	0.70 ± 0.07	0.52 ± 0.01

[a]Data expressed as the mean ± SD (n = 3).

In summary, four new tropolone-containing diterpenoid pseudodimers, gukulenin C–F (**2**–**5**), and one known compound, gukulenin A (**1**), were isolated from the Korean sponge *P. gukhulensis*. Their structures and configurations were determined using various spectroscopic analyses. Compounds **2**–**4** possess diversely modified side chains, and compound **5** possesses an enol-ether that presumably arises from opening of the hemiketal ring of **1**. All of these compounds exhibited significant cytotoxicity against the K562 and A549 cell lines.

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 spectropolarimeter using a 0.2 mm cell, and 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 (**5**), 600 and 150 MHz (**3**), and 500 and 125 MHz (**2** and **4**), respectively. High-resolution electrospray ionization (ESI) mass spectrometric data were obtained at the National Instrumentation Center for Environmental Management (Seoul, Korea) using a Thermo-Finnigan LTQ-Orbitrap instrument equipped with Dionex U-3000 HPLC system. Semipreparative high-performance liquid chromatography (HPLC) was performed on a Spectrasystem p2000 equipped with a refractive index detector (Spectrasystem RI-150) and UV/vis detector (Gilson UV/vis-151). All solvents used were spectroscopic grade or distilled from glass prior to use.

Animal Material. Specimens of *Phorbas gukhulensis* were collected by hand using scuba gear at a depth of 20–25 m off the shore of Gagu-do (Island), Korea, in July 2000. Later, additional specimens of the same sponge (sample number 06SH-4) were collected at Gagu-do, Korea, in July 2006. The samples were dark red, and the gross morphological features were identical with those previously identified.[7a] Although this sponge was originally reported as *Phorbas* sp., continuing studies on sponge taxonomy revealed that this animal was new to science, and it was named *Phorbas gukhulensis*.[22a] Oscules were rare, and the texture was very soft. The color of the living animal was dark red. In the skeleton, the megascleres took the form of tornotes (295–410 × 7–10 μm), small acanthostyles (145–185 × 8–10 μm), and large acanthostyles (300–420 × 9–11 μm), and the microscleres were isochelas (25–30 μm). This sponge had thicker megascleres than Pulitzer-Finali's specimen from the Bay of Naples.[22b] The growth form of this specimen was a thick mass, while that from Naples was encrusting. The specimens were deposited (registry number Spo. 37) at the Natural History Museum, Hannam University, Korea, under the curatorship of C.J.S.

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 (1180.0 g) were successively partitioned between H_2O (980.0 g) and *n*-BuOH (151.1 g); the latter fraction was repartitioned between H_2O–MeOH (15:85) (27.4 g) and *n*-hexane (119.4 g). An aliquot of the former layer (10.0 g) was separated by C_{18} reversed-phase vacuum flash chromatography using a sequential mixture of MeCN and H_2O as eluents (five fractions in an H_2O–MeCN gradient from 50:50 to 0:100), followed by acetone and finally EtOAc.

On the basis of the results of the ^{1}H NMR and cytotoxicity analyses, the fractions that eluted with H_2O–MeCN (30:70) (1.03 g) were reseparated by C_{18} reversed-phase vacuum flash chromatography using the same elution conditions as in the previous step. Among the five fractions, the fraction that eluted with H_2O–MeCN (30:70) (0.45 g) was chosen for separation. It was separated by semipreparative reversed-phase HPLC (YMC-ODS column, 10 × 250 mm; H_2O–MeCN, 50:50), yielding two peaks rich in compounds containing atypical upfield methyl signals in the ^{1}H NMR spectra. Further purification of the first peak by reversed-phase HPLC (YMC-ODS column, 4.6 × 250 mm; H_2O–MeCN, 70:30) provided compound **4**. The second peak under the same HPLC conditions provided, in order of elution, compounds **2** and **3**.

The 100% MeCN fraction (0.06 g) was separated by C_{18} reversed-phase semipreparative HPLC (H_2O–MeCN, 50:50) to yield, in order of elution, compounds **1** and **5**. Final purifications of these metabolites were performed by reversed-phase HPLC (H_2O–MeCN, 70:30). The purified metabolites were isolated in the following amounts: 10.0, 3.8, 1.1, 3.8, and 10.0 mg for **1**–**5**, respectively.

Gukulenin A (1): $[\alpha]_D^{25}$ −19.1 (*c* 0.35, MeCN); lit. $[\alpha]_D^{25}$ −64.7 (*c* 0.15, MeOH).[15]

Gukulenin C (2): yellow, amorphous solid; $[\alpha]_D^{25}$ −4.8 (*c* 0.35, MeCN); UV (MeCN) λ_{max} (log ε) 248 (3.66), 332 (3.44), 355 (3.49), 373 (3.46) nm; IR (ZnSe) ν_{max} 3352, 2956, 1731, 1666, 1243 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRESIMS *m*/*z* 750.4213 $[M + H]^+$ (calcd for $C_{43}H_{60}NO_{10}$, 750.4212).

Gukulenin D (3): yellow, amorphous solid; $[\alpha]_D^{25}$ −19.2 (*c* 0.20, MeCN); UV (MeCN) λ_{max} (log ε) 250 (3.28), 347 (2.81), 359 (2.80), 372 (2.77) nm; IR (ZnSe) ν_{max} 3340, 2954, 1705, 1681, 1242 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRESIMS *m*/*z* 677.3682 $[M - NHAc + H]^+$ (calcd for $C_{40}H_{53}O_9$, 677.3684).

Gukulenin E (4): yellow, amorphous solid; $[\alpha]_D^{25}$ −13.9 (*c* 0.55, MeCN); UV (MeCN) λ_{max} (log ε) 249 (3.74), 333 (3.77), 360 (3.77), 372 (3.76) nm; IR (ZnSe) ν_{max} 3309, 2957, 1712, 1203, 1039 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRESIMS *m*/*z* 784.3729 $[M + H]^+$ (calcd for $C_{42}H_{58}NO_{11}S$, 784.3725).

Gukulenin F (5): yellow, amorphous solid; $[\alpha]_D^{25}$ −21.2 (*c* 0.40, MeCN); UV (MeCN) λ_{max} (log ε) 248 (3.82), 329 (3.82), 383 (3.81), 419 (3.90) nm; IR (ZnSe) ν_{max} 3527, 2960, 1740, 1235, 1039 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRESIMS *m*/*z* 719.3761 $[M + H]^+$ (calcd for $C_{42}H_{55}O_{10}$, 719.3790).

Preparation of Mo_2 Complex of Compound 2. According to the reported procedure,[17] mixtures of 1:1.5 amide alcohol–$Mo_2(OAc)_4$ for **2** were subjected to CD measurements at concentration of 0.5 mg/mL in DMSO. The mixture was kept for 30 min to form a stable chiral metal complex, after which the CD spectrum was recorded. The observed sign of the diagnostic induced CD curve in the range of 260–400 nm was correlated to the absolute configuration of C-1′ and C-2′ in **2**.[17b]

Biological Assays. Cytotoxicity assays were performed in accordance with literature protocols.[23] The LC_{50} value was defined as the concentration that resulted in a 50% decrease in cell viability compared to that of control reactions in the absence of an inhibitor. The values (mean ± SD) were calculated from the dose–response curves of each test sample in three independent experiments (n = 3).

ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR spectra of compounds **2**–**5** and induced CD spectrum of the Mo_2 complex 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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REFERENCES

(1) Blunt, J. W.; Copp, B. R.; Keyzers, R. A.; Munro, M. H. G.; Prinsep, M. R. *Nat. Prod. Rep.* **2013**, *30*, 237−323 and earlier reports in the series.

(2) Rudi, A.; Stein, Z.; Green, S.; Goldberg, I.; Kashman, Y.; Benayahu, Y.; Schleyer, M. *Tetrahedron Lett.* **1994**, *35*, 2589−2592.

(3) (a) Searle, P. A.; Molinski, T. F. *J. Am. Chem. Soc.* **1995**, *117*, 8126−8131. (b) Searle, P. A.; Molinski, T. F.; Brzezinski, L. J.; Leahy, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 9422−9423. (c) Molinski, T. F. *Tetrahedron Lett.* **1996**, *37*, 7879−7880. (d) Fontana, C.; Incerti, M.; Moyna, G.; Manta, E. *Magn. Reson. Chem.* **2008**, *46*, 36−41. (e) Dalisay, D. S.; Molinski, T. F. *Org. Lett.* **2009**, *11*, 1967−1970.

(4) (a) Morinaka, B. I.; Masuno, M. N.; Pwalik, J. R.; Molinski, T. F. *Org. Lett.* **2007**, *9*, 5219−5222. (b) Morinaka, B. I.; Pawlik, J. R.; Molinski, T. F. *J. Org. Chem.* **2010**, *75*, 2453−2460.

(5) (a) Skepper, C. K.; MacMillan, J. B.; Zhou, G.-X.; Masuno, M. N.; Molinski, T. F. *J. Am. Chem. Soc.* **2007**, *129*, 4150−4151. (b) MacMillan, J. B.; Xiong-Zhou, G.; Skepper, C. K.; Molinski, T. F. *J. Org. Chem.* **2008**, *73*, 3699−3706. (c) Dalisay, D. S.; Molinski, T. F. *J. Nat. Prod.* **2010**, *73*, 679−682.

(6) (a) Vuong, D.; Capon, R. J. *J. Nat. Prod.* **2000**, *63*, 1684−1685. (b) McNally, M.; Capon, R. J. *J. Nat. Prod.* **2001**, *64*, 645−647. (c) Zhang, H.; Capon, R. J. *Org. Lett.* **2008**, *10*, 1959−1962. (d) Zhang, H.; Major, J. M.; Lewis, R. J.; Capon, R. J. *Org. Biomol. Chem.* **2008**, *6*, 3811−3815. (e) Lee, H.-S.; Park, S. Y.; Sim, C. J.; Rho, J.-R. *Chem. Pharm. Bull.* **2008**, *56*, 1198−1200. (f) Lee, S.-H.; Jeon, J.-e.; Ahn, C.-H.; Chung, S.-C.; Shin, J.; Oh, K.-B. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 3141−3148.

(7) (a) Rho, J.-R.; Lee, H.-S.; Sim, C. J.; Shin, J. *Tetrahedron* **2002**, *58*, 9585−9591. (b) Jang, K. H.; Jeon, J.-e.; Ryu, S.; Lee, H.-S.; Oh, K.-B.; Shin, J. *J. Nat. Prod.* **2008**, *71*, 1701−1707.

(8) Masuno, M. N.; Pawlik, J. R.; Molinski, T. F. *J. Nat. Prod.* **2004**, *67*, 731−733.

(9) Morinaka, B. I.; Pawlik, J. R.; Molinski, T. F. *J. Nat. Prod.* **2009**, *72*, 259−264.

(10) Daoust, J.; Fontana, A.; Merchant, C. E.; de Voogd, N. J.; Patrick, B. O.; Kieffer, T. J.; Andersen, R. J. *Org. Lett.* **2010**, *12*, 3208−3211.

(11) Dalisay, D. S.; Morinaka, B. I.; Skepper, C. K.; Molinski, T. F. *J. Am. Chem. Soc.* **2009**, *131*, 7552−7553.

(12) (a) Rho, J.-R.; Hwang, B. S.; Sim, C. J.; Joung, S.; Lee, H.-Y.; Kim, H.-J. *Org. Lett.* **2009**, *11*, 5590−5593. (b) Wang, W.; Mun, B.; Lee, Y.; Reddy, M. V.; Park, Y.; Lee, J.; Kim, H.; Hahn, D.; Chin, J.; Ekins, M.; Nam, S.-J.; Kang, H. *J. Nat. Prod.* **2013**, *76*, 170−177.

(13) Rho, J.-R.; Hwang, B. S.; Joung, S.; Byun, M. R.; Hong, J.-H.; Lee, H.-Y. *Org. Lett.* **2011**, *13*, 884−887.

(14) Wang, W.; Lee, Y.; Lee, T. G.; Mun, B.; Giri, A. G.; Lee, J.; Kim, H.; Hahn, D.; Yang, I.; Chin, J.; Choi, H.; Nam, S.-J.; Kang, H. *Org. Lett.* **2012**, *14*, 4486−4489.

(15) Park, S. Y.; Choi, H.; Hwang, H.; Kang, H.; Rho, J.-R. *J. Nat. Prod.* **2010**, *73*, 734−737.

(16) Matsumori, N.; Kaneno, D.; Murata, M.; Nakamura, H.; Tachibana, K. *J. Org. Chem.* **1999**, *64*, 866−876.

(17) (a) Snatzke, G.; Wagner, U.; Wolff, H. P. *Tetrahedron* **1981**, *37*, 349−361. (b) Frelek, J.; Klimek, A.; Ruśkowska, P. *Tetrahedron: Asymmetry* **2002**, *13*, 2461−2469. (c) Frelek, J.; Klimek, A.; Ruskowska, P. *Curr. Org. Chem.* **2003**, *7*, 1081−1104.

(18) (a) Grube, A.; Assmann, M.; Lichte, E.; Sasse, F.; Pawlik, J. R.; Köck, M. *J. Nat. Prod.* **2007**, *70*, 504−509. (b) Hertiani, T.; Edrada-Ebel, R.; Ortlepp, S.; van Soest, R. W. M.; de Voogd, N. J.; Wray, V.; Hentschel, U.; Kozytska, S.; Müller, W. E. G.; Proksch, P. *Bioorg. Med. Chem.* **2010**, *18*, 1297−1311. (c) Gupta, P.; Sharma, U.; Schulz, T. C.; McLean, A. B.; Robins, A. J.; West, L. M. *J. Nat. Prod.* **2012**, *75*, 1223−1227.

(19) (a) Emura, C.; Higuchi, R.; Miyamoto, T. *Tetrahedron* **2006**, *62*, 5682−5685. (b) McCulloch, M. W. B.; Bugni, T. S.; Concepcion, G. P.; Coombs, G. S.; Harper, M. K.; Kaur, S.; Mangalindan, G. C.; Mutizwa, M. M.; Veltri, C. A.; Virshup, D. M.; Ireland, C. M. *J. Nat. Prod.* **2009**, *72*, 1651−1656.

(20) (a) Sandoval, I. T.; Davis, R. A.; Bugni, T. S.; Concepcion, G. P.; Harper, M. K.; Ireland, C. M. *Nat. Prod. Res.* **2004**, *18*, 89−93. (b) de Almeida Leone, P.; Carroll, A. R.; Towerzey, L.; King, G.; McArdle, B. M.; Kern, G.; Fisher, S.; Hooper, J. N. A.; Quinn, R. J. *Org. Lett.* **2008**, *10*, 2585−2588.

(21) Nguyen, T. D.; Nguyen, X. C.; Longeon, A.; Keryhuel, A.; Le, M. H.; Kim, Y. H.; Chau, V. M.; Bourguet-Kondracki, M.-L. *Tetrahedron* **2012**, *68*, 9256−9259.

(22) (a) Sim, C. J.; Kim, H. J. *Korean J. Biol. Sci.* **2004**, *8*, 135−137. (b) Pulitzer-Finali, G. *Boll. Mus. 1st Biol. Univ. Genova* **1997**, *45*, 7−89.

(23) (a) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55−63. (b) Ulukaya, E.; Ozdikicioglu, F.; Oral, A. Y.; Demirci, M. *Toxicol. in Vitro* **2008**, *22*, 232−239.