Benzoylphloroglucinol Derivatives from *Hypericum scabrum*

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Nine new polyprenylated benzoylphloroglucinol derivatives, hyperibones A−I (**1**−**9**), were isolated from the aerial parts of the Uzbekistan medicinal plant *Hypericum scabrum*. Their structures were determined mainly on the basis of spectroscopic evidence (2D NMR and HRMS). Compounds **1**, **2**, and **4** showed mild in vitro antibacterial activity against methicillin-resistance *Staphylococus aureus* (MRSA) and methicillin-sensitive *Staphylococus aureus* (MSSA).

The recent widespread interest in the antidepressant activity of *Hypericum parforatum* (St. John's wort, Clusiaceae) has encouraged the investigation of secondary metabolites from *Hypericum* species, many of which are biologically active compounds with an acylphloroglucinol moiety.[1,2] *Hypericum scabrum* (Clusiaceae) is one of the most popular medicinal herbs in Uzbekistan and is used in the treatment of numerous disorders such as liver, gall bladder, intestinal, and heart disease, rheumatism, and cystitis.[3,4] The volatile oil constituents of *H. scabrum* have been studied,[5] but a chemical investigation of its polar constituents has not been conducted. As part of our continuing study of the chemical constituents of medicinal plants in Uzbekistan,[6,7] we have examined the aerial parts of *H. scabrum* and isolated nine new polyprenylated benzoylphloroglucinol derivatives (**1**−**9**), which we have named hyperibones A (**1**), B (**2**), C (**3**), D (**4**), E (**5**), F (**6**), G (**7**), H (**8**), and I (**9**). In this paper, we describe the isolation, structural elucidation, and antibacterial activity of some of the isolated compounds.

Results and Discussion

The methanol extract of air-dried aerial parts of *Hypericum scabrum* was partitioned between H_2O and EtOAc, and the EtOAc extract was separated by column chromatography (CC) to afford nine new compounds, **1**−**9**.

Hyperibone A (**1**), obtained as a colorless oil, showed hydroxy and carbonyl bands at 3448 and 1723 cm^{-1} in IR spectrum, and the UV spectrum indicated the presence of an aromatic moiety (283 and 247 nm). The ^{13}C NMR spectrum (Table 1) showed signals due to three carbonyls (δ_C 206.7, 193.4, and 187.9), a benzene ring (δ_C 136.9, 128.3 × 2, 128.1 × 2, and 132.3), eight methyls, four methylenes, four methines, of which one bore an oxygen atom (δ_C 93.2), and eight quaternary carbons. The ^1H NMR spectrum also showed the presence of eight singlet methyls and an aromatic ring. The positive HRFABMS of compound **1** gave the quasi-molecular ion peak at *m/z* 519.3118 $[M + H]^+$, suggesting the molecular formula of $C_{33}H_{42}O_5$. The analysis of 2D NMR spectra using HMQC and HMBC techniques enabled the assignment of ^1H and ^{13}C NMR signals. The foregoing data indicated that **1** was a benzoylphloroglucinol derivative that contained four isoprene units. Many types of benzoylphloroglucinol derivatives have been isolated from *Hypericum*, *Clusia*, and *Garcinia* species,[8−11] and the ^{13}C NMR spectrum of **1** is very similar to that of scrobiculatone A;[11] the clearest difference between them is the chemical shifts at C-17, -18, and -19. This suggested that the core bicyclic system of **1** was the same as that in scrobiculatone A and that these differed only with regard to the structure of the side chain attached at C-3. In the HMBC spectrum of **1**, the correlations of the proton signals at δ_H 1.33 and 1.23 (H-20 and H-21) with the carbon signals at δ_C 93.2 (C-18) and 72.0 (C-19), and the proton signal at δ_H 2.95 (H-17a) with the carbon signals at δ_C 175.8 (C-4),

Table 1. ^{13}C NMR Data for Compounds **1**−**9** ($CDCl_3$)

	1	2	3	4	5	6	7	8	9
C-1	79.2	79.3	78.9	78.9	78.6	77.8	78.8	68.1	67.5
C-2	187.9	188.0	187.5	194.3	194.2	194.3	193.4	177.2	176.5
C-3	118.5	117.7	118.9	112.4	112.7	117.0	116.1	119.1	118.1
C-4	175.8	176.3	175.5	175.1	174.9	173.0	172.4	196.6	194.7
C-5	55.6	56.0	55.3	60.6	60.4	60.1	60.3	61.6	61.8
C-6	39.9	39.5	39.6	39.6	39.4	39.1	39.4	42.5	39.1
C-7	43.5	43.9	46.1	43.1	45.8	45.6	42.9	48.0	45.9
C-8	47.9	48.2	49.3	48.3	47.7	47.7	48.2	46.8	46.6
C-9	206.7	206.4	205.8	204.9	204.9	204.2	205.1	206.3	206.4
C-10	193.4	193.5	193.1	193.5	193.2	193.5	193.6	26.3	26.4
C-11	136.9	136.9	136.7	136.8	136.6	137.0	137.1	92.9	93.3
C-12	128.3	128.0	128.2	128.3	128.5	128.3	128.2	71.2	71.2
C-13	128.1	128.4	128.3	128.5	128.3	128.1	128.1	26.7	26.7
C-14	132.3	132.2	132.4	132.4	132.5	132.3	132.2	24.2	24.0
C-15	128.1	128.0	128.3	128.5	128.3	128.1	128.1	192.0	192.4
C-16	128.3	128.4	128.2	128.3	128.5	128.3	128.2	137.2	137.4
C-17	27.3	27.6	27.3	25.0	24.9	22.3	22.3	129.2	129.6
C-18	93.2	92.7	93.3	86.0	86.0	120.6	120.8	128.8	128.8
C-19	72.0	72.1	71.9	143.6	143.6	133.0	133.7	133.8	133.6
C-20	23.3	24.0	23.5	113.6	113.5	26.0	25.9	128.8	128.8
C-21	26.2	25.2	26.1	19.2	19.3	18.0	18.0	129.2	129.6
C-22	29.3	29.1	29.2	30.0	30.0	30.5	30.5	30.8	30.8
C-23	120.5	118.5	120.2	90.6	90.2	90.4	90.3	119.1	119.5
C-24	134.9	135.5	135.1	71.0	70.9	71.1	71.3	135.4	134.9
C-25	26.1	25.9	26.2	27.8	27.8	26.9	26.9	26.3	26.2
C-26	18.3	18.2	18.4	24.1	24.2	24.2	24.1	18.3	18.3
C-27	27.2	27.0	128.0	27.0	127.6	127.7	26.7	130.0	29.8
C-28	122.6	122.7	137.8	122.4	138.1	138.1	122.5	137.6	124.2
C-29	133.7	133.9	82.2	133.8	82.3	82.2	132.8	81.9	133.6
C-30	26.1	26.2	24.4[a]	26.1	24.4	24.4	26.1	25.3	26.0
C-31	18.1	19.9	24.4[a]	18.1	24.7	24.7	18.1	24.7	18.2
C-32	16.1	16.0	16.5	16.5	16.9	17.0	16.5	25.1	23.9
C-33	23.9	23.9	24.7	23.3	23.9	23.8	23.3	26.8	27.3

[a] Overlapping signals.

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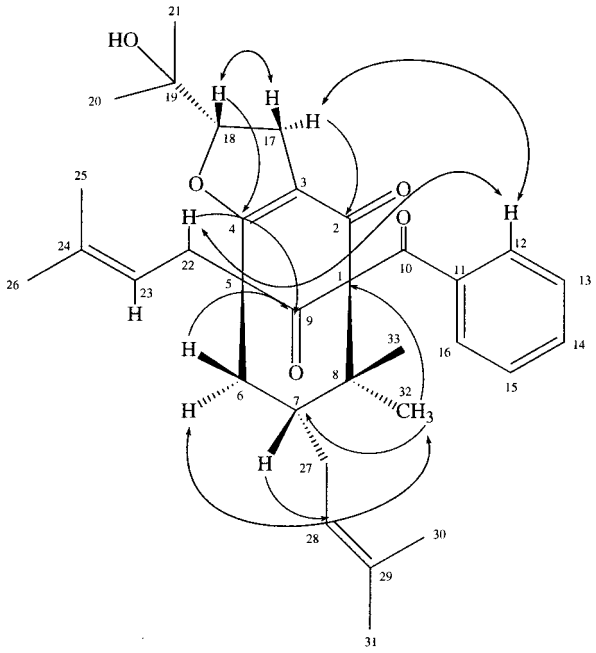

Figure 1. Significant long-range ^{1}H, ^{13}C NMR correlations and NOE correlations observed by HMBC and NOESY for **1**.

187.9 (C-2), and 72.0 (C-19), indicated that the structure of the side chain is $-CH_2-CH(O-)-C(CH_3)_2OH$. The correlation of the proton signal at δ_H 4.85 (H-18) with the carbon signal at δ_C 175.8 (C-4) suggested that a dihydrofuran ring was formed between C-4 and C-18. The relative configuration of **1** was revealed by an NOE experiment: the correlation of the proton signals at δ_H 7.55 (H-12 and H-16) with those at δ_H 2.95 (H-17a) and 5.05 (H-23) suggested that the benzoyl moiety and C-5 linked to the 3-methyl-2-butenyl side chain has an α-orientation. The correlation of the proton signal at δ_H 2.95 (H-17a) with those at δ_H 1.33 and 1.23 (H-20 and H-21) and of the proton signal at δ_H 3.05 (H-17b) with that at δ_H 4.85 (H-18) indicated that the 2-propanol group should have an α-orientation. The cyclohexanone ring adopts a chair conformation, since the proton (H-6a) showed NOE correlation with methyl protons (H-32) and a coupling constant (J = 4.0 Hz) to methine protons (H-7), which indicated that the 3-methyl-2-butenyl side chain on C-7 was α-equatorial.[12] Thus, the structure of hyperibone A (**1**) was assigned as shown in Figure 1.

Hyperibone B (**2**), $C_{33}H_{42}O_5$, has the same molecular formula as **1**, and their ^{13}C NMR spectra (Table 1) are very similar. The ^{1}H NMR spectrum of **2** is similar to that of **1** except for the signals assignable to H-17. 2D NMR spectral data also suggested that **1** and **2** have the same skeleton. Thus, **2** was deduced to be a C-18 epimer of **1**. In the NOESY spectrum of **2**, the proton signal at δ_H 2.93 (H-17b) was correlated with those at δ_H 1.30 and 1.24 (H-20 and H-21), the proton signal at δ_H 2.99 (H-17a) was correlated with that at δ_H 4.83 (H-18), and the proton signal at δ_H 2.00 (H-6b) was correlated with that at δ_H 1.24 (H-20 or H-21). On the basis of these correlations, the 2-propanol group on C-18 was assigned a β-orientation. Thus, the structure of hyperibone B (**2**) was assigned as shown in Figure 2.

Hyperibone C (**3**) has a molecular formula of $C_{33}H_{42}O_6$ based on HRFABMS (m/z 533.2896 $[M - H]^+$). The ^{13}C NMR (Table 1) and ^{1}H NMR spectral data of **3** were compared with those of the closely related **1**. 2D NMR spectral data also suggested that **1** and **3** have the same skeleton and differ only with regard to the side chain

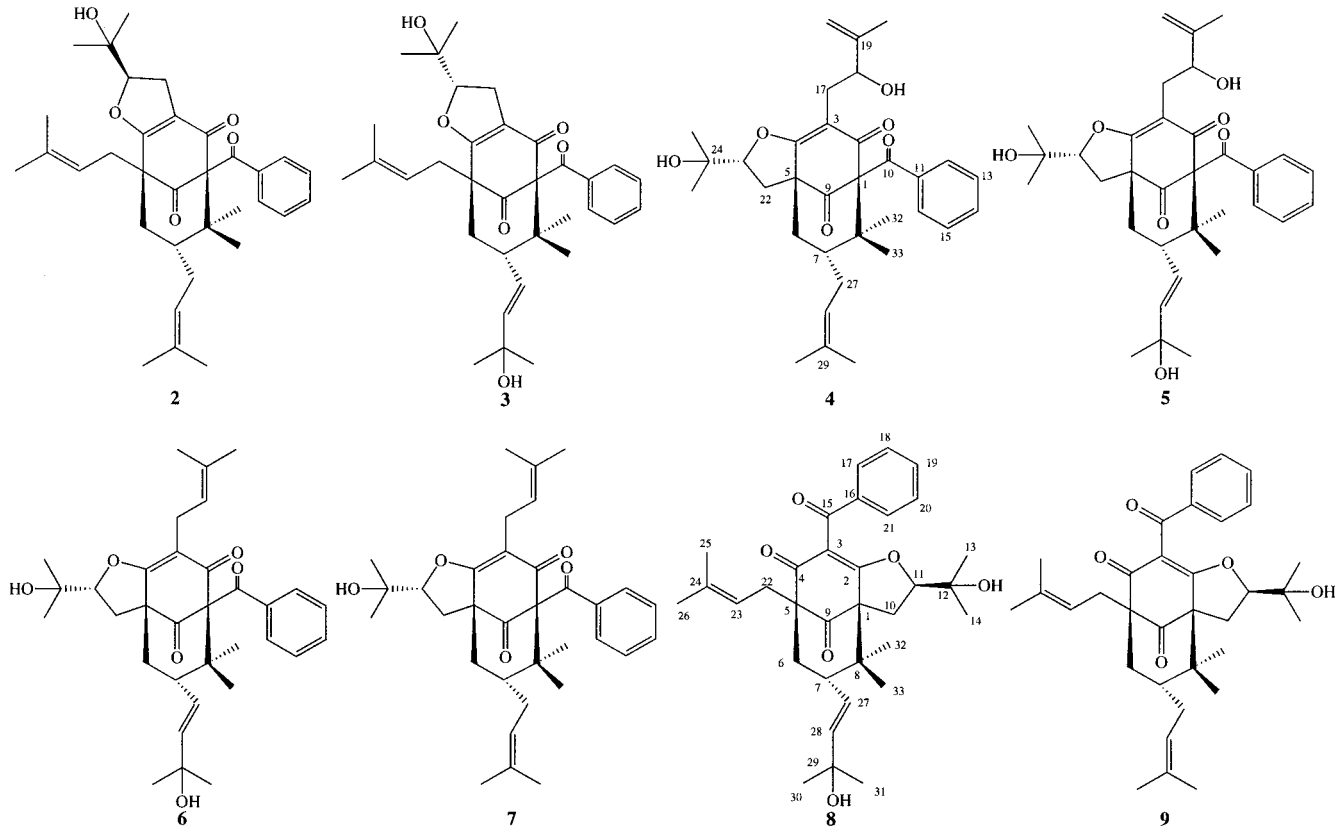

Figure 2. Compounds **2**–**9**.

attached to C-5 or -7. The $^1H-^1H$ COSY spectrum of **3** showed correlations of the proton signal at δ_H 5.56 (H-27) with the proton signals at δ_H 5.64 (H-28) and 2.46 (H-7). The HMBC spectrum showed correlations of the proton signals at δ_H 1.36 (H-30 and H-31) with the carbon signals at δ_C 137.8 (C-28) and 82.2 (C-29), and the proton signal at δ_H 5.64 (H-28) was correlated with the carbon signal at δ_C 46.1 (C-7). On the basis of these correlations, compound **3** has a 3-hydroxyl-3-methylbutenyl side chain at C-5 instead of the 3-methyl-2-butenyl side chain in **1**. In addition, a 3-hydroxyl-3-methylbutenyl side chain is attached to C-7. Therefore, the structure of hyperibone C (**3**) was assigned as shown in Figure 2.

The HRFABMS (*m*/*z* 533.2906 $[M - H]^+$) of **4** indicated a molecular formula of $C_{33}H_{42}O_6$. The ^{13}C NMR spectral data (Table 1) of **4** were similar to those of **1** except for C-2, -3, and -5 (**1**: δ_C 187.9, 118.5, and 55.6, **4**: δ_C 194.3, 112.4, and 60.6) and the side chain carbons (C-17−C-26). The ^1H NMR spectrum of **4** shows the presence of a 2,3-dihydroxy-3-methylbutane side chain [δ_H 2.93 and 1.90 (H-22), 4.67 (H-23) 1.49 and 1.22 (H-26 and H-25)] and a 2-hydroxy-3-methyl-3-butenyl side chain [δ_H 2.82 and 2.75 (H-17), 4.13 (H-18), 5.00 (H-20), and 1.78 (H-21). The HMBC spectrum of **4** showed correlations of the proton signals at δ_H 1.24 and 1.41 (H-33 and H-32) with the carbon signals at δ_C 78.9 (C-1), 48.3 (C-8), and 43.1 (C-7). The correlation of the proton signals at δ_H 2.82 and 2.75 (H-17) with the carbon signals at δ_C 194.3 (C-2) and 175.1 (C-4) suggested that the 2-hydroxy-3-methyl-3-butenyl side chain is linked to C-3. In the NOESY spectrum, the correlation of the proton signal at δ_H 4.67 (H-23) with that at δ_H 2.10 (H-6b) and the correlation between the proton signal at δ_H 1.64 (H-6a) and that at δ_H 1.24 (H-32) suggested that the dihydrofuran moiety is connected to C-5 and the 2-propanol group was assigned an α-orientation. The relative stereochemistry of the side chain at C-7 was determined to be α on the basis of the coupling constants of the proton signals at δ_H 2.10 (dd, J = 3.7 and 12.7 Hz) and 1.64 (t, J = 12.7 Hz). Therefore, the structure of hyperibone D (**4**) was assigned as shown in Figure 2.

Hyperibone E (**5**) showed a molecular ion peak at *m*/*z* 551.3083, corresponding to $[M + H]^+$ in HRFABMS, indicating a molecular formula of $C_{33}H_{42}O_7$. The comparison of the ^{13}C NMR (Table 1) and ^1H NMR spectral data of **5** with those of **4** revealed that the only difference is in the side chain at C-7, in that the 3-methyl-2-butenyl side chain in **4** was replaced by a 3-hydroxy-3-methyl-1-butenyl side chain in **5**. In the $^1H-^1H$ COSY spectrum, the correlation of the proton signal at δ_H 5.56 (H-27) with that at δ_H 2.50 (H-7) suggested that the 3-hydroxy-3-methyl-1-butenyl side chain is connected to C-7. Therefore, the structure of hyperibone E (**5**) was assigned as shown in Figure 2.

Hyperibone F (**6**) exhibited a quasi-molecular ion peak at *m*/*z* 533.2936 $[M - H]^+$) in the negative FABMS to give a molecular formula of $C_{33}H_{42}O_6$. The ^{13}C NMR (Table 1) and ^1H NMR spectral data of **6** were similar to those of **5**, except that the 2-hydroxy-3-methyl-3-butenyl side chain in **5** is replaced by a 3-methyl-2-butenyl side chain in **6**. In the HMBC spectrum, the correlation of δ_H 3.18 and 3.07 (H-17) with the carbon signals at δ_C 194.3 (C-2) and 173.0 (C-4) suggested that the 3-methyl-2-butenyl side chain is linked to C-3. Therefore, the structure of hyperibone F (**6**) was assigned as shown in Figure 2.

Hyperibone G (**7**) was assigned the molecular formula $C_{33}H_{42}O_5$ on the basis of HRFABMS (*m*/*z* 519.3128 $[M + H]^+$). The ^{13}C NMR (Table 1) and ^1H NMR spectral data of **7** were similar to those of **6** except that the 3-hydroxy-3-methyl-1-butenyl side chain in **6** is replaced by the 3-methyl-2-butenyl side chain in **7**. In the NOESY spectrum, the correlation of the proton signal at δ_H 4.64 (H-23) with that at δ_H 2.10 (H-6b) suggested that the dihydrofuran moiety is connected to C-5 and the isopropyl group was assigned an α-orientation. Therefore, the structure of hyperibone G (**7**) was assigned as shown in Figure 2.

Hyperibone H (**8**) was assigned the molecular formula $C_{33}H_{42}O_6$ on the basis of HRFABMS (*m*/*z* 533.2929 $[M - H]^+$). The ^{13}C NMR spectrum (Table 1) of **8** was similar to that of **6** except for the chemical shifts of C-1 (**8**: δ_C 68.1, **6**: δC 77.8) and C-10−21. In the HMBC the proton signals at δ_H 1.16 (H-32) and δ_H 1.14 (H-33) were correlated with the carbon signals at δ_C 68.1 (C-1), 48.0 (C-7), and 46.8 (C-8). In the NOESY spectrum, the proton signal at δ_H 1.16 was correlated with that at δ_H 4.52 (H-11). These correlations suggested that the dihydrofuran ring was formed through the hydroxyl group at C-2, and the 2-propanol group was assigned a β-orientation. On the other hand, the proton signal at δ_H 2.34 (H-6 or H-7) was correlated with the carbon signal at δ_H 130.0 (C-27). This correlation indicated that the 3-hydroxy-3-methyl-1-butenyl side chain is connected to C-7. Therefore, the structure of hyperibone H (**8**) was assigned as shown in Figure 2.

Hyperibone I (**9**) was assigned the molecular formula $C_{33}H_{42}O_5$ on the basis of HRFABMS (*m*/*z* 519.3118 $[M + H]^+$). The ^{13}C NMR (Table 1) and ^1H NMR spectral data of **9** were similar to those of **8** except that the 3-hydroxy-3-methyl-1-butenyl side chain in **8** is replaced by the 3-methyl-2-butenyl side chain in **9**. In the HMBC spectrum, the proton signal at δ_H 2.10 (H-6) was correlated with the carbon signal at δ_C 29.8 (C-27), and the proton signal at δ H 2.55 (H-22) was correlated with the carbon signals at δ_C 206.4 (C-9) and 194.7 (C-4), which suggested the structure of hyperibone I (**9**) as shown.

Four hyperibone compounds (**1**−**4**) isolated from the aerial parts of *H. scabrum* were screened for antibacterial activity by the disc-diffusion test against methicillin-sensitive *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA). Compounds **1**, **2**, and **4** showed mild activity.

Experimental Section

General Experimental Procedures. NMR (400 MHz for ^1H NMR, 100 MHz for ^{13}C NMR, both use TMS as internal standard) were measured on a Bruker AM 400 spectrometer, and MS spectra were measured on a JEOLJMS-DX-303 and SX-102A instruments; CC: silica gel 60 (Merck); HPLC: GPC (shodex H-2001, 2002, $CHCl_3$), silica gel (YMC-pack SIL-06 SH-043-5-06, 250 × 20 mm, Hibar RT 250-25 Si 60), ODS (YMC-R-ODS-5, Yamamura). IR spectra were recorded on a JASCO Fourier transform infrared spectrometer (FT/IR-420), and UV spectra were recorded on a UV2100 UV−vis recording spectrometer (Shimadzu). Optical rotations were measured with a JASCO DIP-370 digital polarimeter.

Plant Material. The dried aerial parts of *Hypericum scabrum* (2.1 kg) were collected in June 1998 in Chimgan, Uzbekistan. Herbarium speciments (ESM-3910) were deposited in the herbarium of the Academy of Sciences, Institute of Botany and Botanical Garden, Uzbekistan.

Extraction and Isolation. The aerial parts of *H. scabrum* (2.1 kg) were crushed and extracted three times with MeOH at 60 °C. The MeOH extracts were concentrated in vacuo to give a residue (520 g), which was partitioned between EtOAc and H_2O. The EtOAc layer was concentrated to give a residue (100 g), which was loaded on a silica gel column and eluted with different solvents of increasing polarity (*n*-hexanes−EtOAc; EtOAc−MeOH) to give 31 fractions (A−Y, Z1−Z6).

Fraction N (1.6 g) was applied to a silica gel column with $CHCl_3$−MeOH (97:3) as an eluent to give 16 fractions (N1−N16). Fraction N5 (392 mg) was subjected to HPLC (GPC, $CHCl_3$) separation to give 9 fractions (N5.1−N5.9). Fraction N5.4 (297 mg) was chromatographed on silica gel (*n*-hexanes−EtOAc, 1:2) to give 14 fractions (N5.4.1−N5.4.14). Fraction N5.4.6 (73 mg) was purified by HPLC (silica, *n*-hexanes−EtOAc, 1:1) to give **1** (16 mg) and **9** (2 mg). Fraction N5.4.13 (31 mg) was applied to an HPLC column (silica, *n*-hexanes−EtOAc, 1:1) to give **3** (7 mg) and **8** (5 mg). Fractions N6 −N8 (443 mg) were isolated by GPC ($CHCl_3$) to give 10 fractions (N6.1−N6.10). Fraction N6.3 (270 mg) was purified by HPLC (silica, *n*-hexanes−EtOAc, 1:2) to give 11 fractions (N6.3.1−N6.3.11). Fraction N6.3.10 (51 mg) was further purified by preparative TLC ($CHCl_3$−MeOH, 98:2) to give **2** (17 mg). Fraction M (1.2 g) was applied to a silica gel column with $CHCl_3$−MeOH (99:1) as eluent to give 8 fractions (M1−M8). Fraction M5 (575 mg) was subjected to HPLC (GPC, $CHCl_3$) separation to give 6 fractions (M5.1−M5.6). Fraction M5.3 (460 mg) was chromatographed on silica gel (*n*-hexanes−EtOAc, 1:1) to give 12 fractions Fraction M5.3.2 (233 mg) was applied to an HPLC column (ODS, acetone−H_2O, 9:1) to give **4** (8 mg) and **7** (9 mg). Fractions M5.3.4−M5.3.6 (80 mg) were isolated by HPLC (silica, *n*-hexanes−EtOAc, 1:1) to give **5** (2 mg) and **6** (5 mg).

Hyperibone A (1): colorless oil; $[\alpha]_D$ −37.7° (*c* 1.1, $CHCl_3$); IR (KBr) ν_{max} cm^{-1} 3448, 2974, 2931, 1723, 1699, 1618, 1375, 1224; UV (MeOH) λ_{max} nm (log ϵ) 283 (3.9), 247 (4.1); HRFABMS *m/z* 519.3118 $[M + H]^+$ (calcd for $C_{33}H_{43}O_5$, 519.3110). ^1H NMR ($CDCl_3$) δ_H 7.55 (2H, d, *J* = 7.7 Hz, H-12 and H-16), 7.40 (1H, t, *J* = 7.7 Hz, H-14), 7.27 (2H, t, *J* = 7.7 Hz, H-13 and H-15), 5.05 (1H, br s, H-23), 5.00 (1H, br s, H-28), 4.85 (1H, dd, *J* = 7.4, 10.3 Hz, H-18), 3.05 (1H, dd, *J* = 10.3, 14.9 Hz, H-17b), 2.95 (1H, dd, *J* = 7.4, 14.9 Hz, H-17a), 2.54 (2H, m, H_2-22), 2.18 (1H, m, H-27), 2.02 (1H, dd, *J* = 4.0, 12.9 Hz, H-6b), 1.76 (1H, m, H-27), 1.72 (3H, s, H_3-30), 1.70 (7H, s, H-7, H_3-25, H_3-26), 1.59 (3H, s, H_3-31), 1.50 (1H, t, *J* = 12.9 Hz, H-6a), 1.41 (3H, s, H_3-33), 1.33 and 1.23 (each 3H, s, H_3-20 and H_3-21), 1.14 (3H, s, H_3-31); ^{13}C NMR data, Table 1.

Hyperibone B (2): colorless oil; $[\alpha]_D$ −20.8° (*c* 0.5, $CHCl_3$); IR (KBr) ν_{max} cm^{-1} 3430, 2974, 2930, 1724, 1698, 1619, 1447, 1224, 1186; UV (MeOH) λ_{max} nm (log ϵ) 283 (4.0), 247 (4.0); HRFABMS *m/z* 519.3051 $[M + H]^+$ (calcd for $C_{33}H_{43}O_5$, 519.3110); ^1H NMR ($CDCl_3$) δ_H 7.54 (2H, d, *J* = 7.4 Hz, H-12 and H-16), 7.40 (1H, t, *J* = 7.4 Hz, H-14), 7.27 (2H, t, *J* = 7.4 Hz, H-13 and H-15), 5.10 (1H, t, *J* = 7.7 Hz, H-23), 4.98 (1H, t, *J* = 7.2 Hz, H-28), 4.83 (1H, dd, *J* = 8.4, 10.4 Hz, H-18), 2.99 (1H, dd, *J* = 10.4, 14.2 Hz, H-17a), 2.93 (1H, dd, *J* = 8.4, 14.2 Hz, H-17b), 2.60 (1H, dd, *J* = 7.7, 14.3 Hz, H-22), 2.45 (1H, dd, *J* = 7.7, 14.3 Hz, H-22), 2.18 (1H, m, H-27), 2.00 (1H, dd, *J* = 3.5, 13.0 Hz, H-6b), 1.70 (4H, s, H-27 and H_3-30), 1.67 (7H, s, H-7, H_3-25 and H_3-26), 1.58 (3H, s, H_3-31), 1.52 (1H, t, *J* = 13.0 Hz, H-6a), 1.40 (3H, s, H_3-33), 1.30 and 1.24 (each 3H, s, H_3-20 and H_3-21), 1.13 (3H, s, H_3-32); ^{13}C NMR data, Table 1.

Hyperibone C (3): colorless oil; $[\alpha]_D$ −27.3° (*c* 0.3, $CHCl_3$); IR (KBr) ν_{max} cm^{-1} 3438, 2976, 2931, 1724, 1698, 1619, 1448, 1372, 1225; UV (MeOH) λ_{max} nm (log ϵ) 280 (3.9), 247 (4.0); HRFABMS *m/z* 533.2896 $[M - H]^+$ (calcd for $C_{33}H_{41}O_6$, 533.2903); ^1H NMR ($CDCl_3$) δ_H 7.54 (2H, d, *J* = 7.7 Hz, H-12 and H-16), 7.40 (1H, t, *J* = 7.7 Hz, H-14), 7.27 (2H, t, *J* = 7.7 Hz, H-13 and H-15), 5.64 (1H, d, *J* = 16.0 Hz, H-28), 5.56 (1H, dd, *J* = 7.6, 16.0 Hz, H-27), 5.07 (1H, br s, H-23), 4.87 (1H, dd, *J* = 7.4, 10.4 Hz, H-18), 3.04 (1H, dd, *J* = 10.4, 15.0 Hz, H-17b), 2.97 (1H, dd, *J* = 7.4, 15.0 Hz, H-17a), 2.58 (2H, m, H_2-22), 2.46 (1H, m, H-7), 1.90 (2H, m, H_2-6), 1.77 (3H, s, H_3-25), 1.71 (3H, s, H_3-26), 1.36 (6H, s, H_3-30 and H_3-31), 1.34 (6H, s, H_3-21 and H_3-33), 1.24 (3H, s, H_3-20), 1.12 (3H, s, H_3-32); ^{13}C NMR data, Table 1.

Hyperibone D (4): colorless oil; $[\alpha]_D$ −61.9° (*c* 0.7, $CHCl_3$); IR (KBr) ν_{max} cm^{-1} 3435, 2976, 2929, 1728, 1696, 1624, 1448, 1372, 1223; UV (MeOH) λ_{max} nm (log ϵ) 272 (4.0), 248 (4.0); HRFABMS *m/z* 533.2906 $[M - H]^-$ (calcd for $C_{33}H_{41}O_6$, 533.2903); ^1H NMR ($CDCl_3$) δ_H 7.53 (2H, d, *J* = 7.3 Hz, H-12 and H-16), 7.38 (1H, t, *J* = 7.3 Hz, H-14), 7.33 (2H, t, *J* = 7.3 Hz, H-13 and H-15), 5.00 (3H, m, H_2-20 and H-28), 4.67 (1H, dd, *J* = 6.3, 9.8 Hz, H-23), 4.13 (1H, dd, *J* = 3.4, 10.3 Hz, H-18), 2.93 (1H, dd, *J* = 9.8, 13.1 Hz, H-22a), 2.82 (1H, dd, *J* = 10.3, 14.1 Hz, H-17), 2.75 (1H, dd, *J* = 3.4, 14.1 Hz, H-17), 2.18 (1H, m, H-27), 2.10 (1H, dd, *J* = 3.7, 12.7 Hz, H-6b), 1.90 (1H, dd, *J* = 6.3, 13.1 Hz, H-22b), 1.78 (3H, s, H_3-21), 1.70 (2H, m, H-7 and H-27), 1.64 (1H, t, *J* = 12.7 Hz, H-6a), 1.62 (3H, s, H_3-30), 1.49 (3H, s, H_3-25 or H_3-26), 1.48 (3H, s, H_3-31), 1.41 and 1.24 (each 3H, s, H_3-32 and H_3-33), 1.22 (3H, s, H_3-25 or H_3-26); ^{13}C NMR data, Table 1.

Hyperibone E (5): colorless oil; $[\alpha]_D$ −56.0° (*c* 0.2, $CHCl_3$); IR (KBr) ν_{max} cm^{-1} 3429, 2977, 2933, 1728, 1697, 1621, 1447, 1372, 1223; UV (MeOH) λ_{max} nm (log ϵ) 272 (4.1), 249 (4.2); HRFABMS *m/z* 551.3083 $[M + H]^+$ (calcd for $C_{33}H_{43}O_7$, 551.3009); ^1H NMR ($CDCl_3$) δ_H 7.53 (2H, d, *J* = 7.3 Hz, H-12 and H-16), 7.39 (1H, t, *J* = 7.3 Hz, H-14), 7.34 (2H, t, *J* = 7.3 Hz, H-13 and H-15), 5.65 (1H, d, *J* = 15.8 Hz, H-28), 5.56 (1H, dd, *J* = 7.8, 15.8 Hz, H-27), 5.03 (1H, s, H-20), 5.02 (1H, s, H-20), 4.66 (1H, dd, *J* = 6.1, 10.0 Hz, H-23), 4.15 (1H, m, H-18), 2.96 (1H, dd, *J* = 10.0, 13.1 Hz, H-22a), 2.78 (2H, m, H_2-17), 2.50 (1H, m, H-7), 2.00 (2H, m, H_2-6), 1.95 (1H, dd, *J* = 6.1, 13.1 Hz, H-22b), 1.79 (3H, s, H_3-21), 1.48 (3H, s, H_3-25 or H_3-26), 1.34 (6H, s, H_3-30 and H_3-31), 1.32 (3H, s, H_3-33), 1.24 (3H, s, H_3-32), 1.23 (3H, s, H_3-25 or H_3-26); ^{13}C NMR data, Table 1.

Hyperibone F (6): colorless oil; $[\alpha]_D$ −31.0° (*c* 0.2, $CHCl_3$); IR (KBr) ν_{max} cm^{-1} 3439, 2923, 2852, 1727, 1697, 1624, 1448, 1372, 1224; UV (MeOH) λ_{max} nm (log ϵ) 270 (3.9), 249 (4.1); HRFABMS *m/z* 533.2936 $[M - H]^-$ (calcd for $C_{33}H_{41}O_6$, 533.2903); ^1H NMR ($CDCl_3$) δ_H 7.46 (2H, d, *J* = 7.7 Hz, H-12 and H-16), 7.40 (1H, t, *J* = 7.7 Hz, H-14), 7.25 (2H, t, *J* = 7.7 Hz, H-13 and H-15), 5.64 (1H, dd, *J* = 16.0 Hz, H-28), 5.54 (1H, dd, *J* = 7.8, 16.0 Hz, H-27), 5.06 (1H, t, *J* = 6.4 Hz, H-18), 4.63 (1H, dd, *J* = 6.0, 10.8 Hz, H-23), 3.18 (1H, dd, *J* = 6.4, 14.1 Hz, H-17), 3.07 (1H, dd, *J* = 6.4, 14.1 Hz, H-17), 2.76 (1H, dd, *J* = 10.8, 13.3 Hz, H-22a), 2.46 (1H, m, H-7), 1.95 (2H, m, H_2-6), 1.91 (1H, dd, *J* = 6.0, 13.3 Hz, H-22b), 1.68 (6H, s, H_3-20 and H_3-21), 1.47 (3H, s, H_3-25 or H_3-26), 1.35 (6H, s, H_3-30 and H_3-31), 1.25 (3H, s, H_3-32), 1.23 (3H, s, H_3-25 or H_3-26); ^{13}C NMR data, Table 1.

Hyperibone G (7): colorless oil; $[\alpha]_D$ −29.3° (*c* 0.9, $CHCl_3$); IR (KBr) ν_{max} cm^{-1} 3511, 2929, 1728, 1697, 1626, 1448, 1372, 1223; UV (MeOH) λ_{max} nm (log ϵ) 273 (3.9), 248 (4.1); HRFABMS *m/z* 519.3128 $[M + H]^+$ (calcd for $C_{33}H_{43}O_5$, 519.3110); ^1H NMR ($CDCl_3$) δ_H 7.48 (2H, d, *J* = 7.7 Hz, H-12 and H-16), 7.38 (1H, t, *J* = 7.7 Hz, H-14), 7.20 (2H, t, *J* = 7.7 Hz, H-13 and H-15), 5.05 (1H, t, *J* = 7.4 Hz, H-18), 4.98 (1H, br s, H-28), 4.64 (1H, dd, *J* = 6.0, 10.0 Hz, H-23), 3.15 (1H, dd, *J* = 7.4, 14.4 Hz, H-17), 3.02 (1H, dd, *J* = 7.4, 14.4 Hz, H-17), 2.71 (1H, dd, *J* = 10.0, 13.0 Hz, H-22a), 2.20 (1H, m, H-27), 2.10 (1H, dd, *J* = 3.3, 12.5 Hz, H-6b), 1.89 (1H, dd, *J* = 6.0, 13.0 Hz, H-22b), 1.74 (1H, m, H-27), 1.72 (3H, s, H_3-30), 1.70 (1H, H-7), 1.64 (1H, H-6a), 1.68 (6H, s, H_3-20 and H_3-21), 1.60 (3H, s, H_3-31), 1.40 (6H, s, H_3-25 or H_3-26 and H_3-33), 1.41 (3H, s, H_3-33), 1.26 (3H, s, H_3-25 or H_3-26), 1.24 (3H, s, H_3-32); ^{13}C NMR data, Table 1.

Hyperibone H (8): colorless oil; $[\alpha]_D$ +12.4° (*c* 0.4, $CHCl_3$); IR (KBr) ν_{max} cm^{-1} 3429, 2977, 2933, 1728, 1697, 1621, 1447, 1372, 1223; UV (MeOH) λ_{max} nm (log ϵ) 252 (4.1); HRFABMS *m/z* 533.2929 $[M - H]^-$ (calcd for $C_{33}H_{41}O_6$, 533.2903); ^1H NMR ($CDCl_3$) δ_H 7.67 (2H, d, *J* = 7.3 Hz, H-17 and H21), 7.53 (1H, t, *J* = 7.3 Hz, H-19), 7.39 (2H, t, *J* = 7.3 Hz, H-18 and H-20), 6.31 (1H, dd, *J* = 8.6, 15.5 Hz, H-27), 5.47 (1H, d, *J* = 15.5 Hz, H-28), 5.08 (1H, t, *J* = 7.6 Hz, H-23), 4.52 (1H, dd, *J* = 6.6, 9.6 Hz, H-11), 2.75 (1H, dd, *J* = 9.6, 14.0 Hz, H-10b), 2.55 (1H, dd, *J* = 7.6, 14.3 Hz, H-22), 2.39 (1H, dd, *J* = 7.6, 14.3 Hz, H-22), 2.34 (2H, m, H-6a and H-7), 2.23 (1H, d, *J* = 12.6 Hz, H-6b), 2.12 (1H, dd, *J* = 6.6, 14.0 Hz, H-10a), 1.68 (3H, s, H_3-25), 1.62 (3H, s, H_3-26), 1.35 and 1.33 (each 3H, s, H_3-30 and H_3-31), 1.16 (3H, s, H_3-32), 1.14 (3H, s, H_3-33), 1.08 and 1.04 (each 3H, s, H_3-13 and H_3-14); ^{13}C NMR data, Table 1.

Hyperibone I (9): colorless oil; $[\alpha]_D$ +13.3° (*c* 0.3, $CHCl_3$); IR (KBr) ν_{max} cm^{-1} 3429, 2973, 2927, 1731, 1676, 1621, 1449, 1373, 1219; UV (MeOH) λ_{max} nm (log ϵ): 251 (4.2); HRFABMS *m/z* 519.3118 $[M + H]^+$ (calcd for $C_{33}H_{41}O_5$, 519.3110); ^1H NMR

($CDCl_3$) δ_H 7.68 (2H, d, J = 7.3 Hz, H-17 and H21), 7.53 (1H, t, J = 7.3 Hz, H-19), 7.40 (2H, t, J = 7.3 Hz, H-18 and H-20), 5.08 (1H, t, J = 6.8 Hz, H-23), 4.87 (1H, br s, H-28), 4.50 (1H, dd, J = 6.5, 10.0 Hz, H-11), 2.75 (1H, dd, J = 10.0, 14.0 Hz, H-10b), 2.55 (2H, m, H_2-22), 2.43 (2H, m, H_2-27), 2.16 (1H, dd, J = 6.5, 14.0 Hz, H-10a), 2.10 (2H, m, H_2-6), 1.70 (1H, m, H-7), 1.68 (6H, s, H_3-25 and H_3-30), 1.64 (3H, s, H_3-26), 1.57 (3H, s, H_3-31), 1.22 (3H, s, H_3-32), 1.14 (3H, s, H_3-33), 1.12 (6H, s, H_3-13 and H_3-14); ^{13}C NMR data, Table 1.

Preparation of Bacterial Cells. *S.aureus* strain No. 5, a clinical isolate (MRSA), and ATCC 6538 (MSSA) strains were from laboratory stock cultures. After culturing all strains on Mueller-Hinton agar (Difco, Detroit, MI), the cells were resuspended in Mueller-Hinton broth (Difco) to give 105 colony-forming units/mL; the resuspended cells were then incubated.[13]

Determination of Antibacterial Activity. During extraction and purification, disc-diffusion tests were performed with Whatman AA disks (6 mm) containing the test compounds (**1**−**4**, each 10 μg), positive control sample (tetracycline and quercetin, each 10 μg), and DMSO as a control. The disks were placed on Mueller-Hinton agar inoculated with 105 colony-forming units/mL of MRSA and MSSA.[13] The zone of inhibition was determined after incubation at 37 °C for 24 h. The disk that contained DMSO showed no zone of inhibition. Zone diameter (average 10 disks for each compounds) for MRSA: **1**, 9.5 mm; **2**, 9.2 mm; **3**, 6.0 mm; **4**, 9.3 mm; tetracycline, 34.0 mm; quercetin, 8.0 mm. Zone diameter (average 10 disks for each compounds) for MSSA: **1**, 9.0 mm; **2**, 9.1 mm; **3**, 6.0 mm; **4**, 9.0 mm; tetracycline, 34.0 mm; quercetin, 8.0 mm.

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