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Syntheses of C_{18} dibenzocyclooctadiene lignan derivatives as anti-HBsAg and anti-HBeAg agents

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Abstract—(+)-Gomisin K_3 (**1**) and kadsurarin (**2**) were isolated from *Schizandra arisanensis* and *Kadsura matsudai*, respectively, and a series of C_{18} dibenzocyclooctadiene lignan analogues (**5**–**20**) derived from **1** and **2** were synthesized. Esterified derivatives of **1** and **2** were evaluated for inhibitory activity against human type B hepatitis with surface antigen (HBsAg) and e antigen (HBeAg). Most of the analogues (**5**–**8**, **10**, **12**–**13**) derived from **1** exhibited higher anti-HBsAg effects and lower toxicity, and **6**, **7**, **8** and **12** also showed higher anti-HBeAg activity. Among these active C_{18} dibenzocyclooctadiene lignan analogues, the lignan with a but-3-enoyl group (**6**) exhibited the most active inhibition.

1. Introduction

Several C_{18} dibenzocyclooctadiene lignans have been isolated from plants of the Schizandraceae and these have exhibited some pharmacological effects such as anti-oxidant, anti-hepatitis, anti-hepatotoxic and anti-lipid peroxidative activities.[1–5] In the course of our search for bioactive constituents from Schizandraceous plants (*Schizandra arisanensis* Hayata and *Kadsura matsudai* Hayata) in Taiwan, a series of C_{18} dibenzocyclooctadiene lignans (schizanrins B-H) and C_{19} homolignans have been isolated.[6–9] Preliminary bioassay data revealed that several of the C_{18} dibenzocyclooctadiene lignans exhibited anti-HBeAg and anti-HBsAg effects, while the C_{19} homolignans were cytotoxic. These results prompted our further search for compounds active against human type B hepatitis by modification of C_{18} dibenzocyclooctadiene lignans. (+)-Gomisin K_3 (**1**),[9] which showed moderate anti-HBeAg and anti-HBsAg effects, served as starting material. We report herein several lignan esters (**5**–**13**) derived from (+)-gomisin K_3 (**1**) in an attempt to increase anti-hepatitis activity and decrease toxicity. A previous paper revealed that lignans, such as kadsurarin (**2**), with substituents at C-9, might have decreased anti-hepatitis activity.[9] Thus, the syntheses of kadsurarin derivatives with various functional groups at C-9 were also established.

2. Results and discussion

EtOH extracts of the dried stems of *K. matsudai* and *S. arisanensis* were successively extracted with *n*-hexane, EtOAc and BuOH, respectively. Repeated column chromatography of the EtOAc extract of *S. arisanensis* yielded (+)-gomisin K_3 (**1**). The EtOAc extract of *K. matsudai* yielded kadsurarin (**2**).

In previous research, (+)-gomisin K_3 (**1**), gomisin B (**3**) and gomisin G (**4**) showed similar activity in anti-HBeAg and anti-HBsAg effects, whereas (+)-gomisin K_3 (**1**) also displayed the strongest toxicity.[9] Comparison of the structures of **1**, **3** and **4** indicated that the free hydroxy group at C-14 only found in **1** was correlated with increased toxicity (Fig. 1). Based on this evidence,

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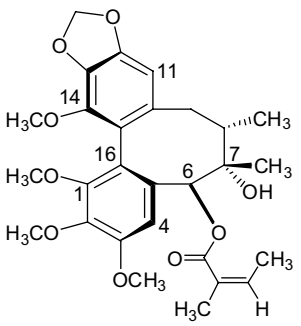
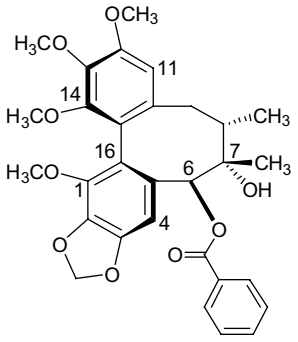


gomisin B (3)



gomisin G (4)

Figure 1. Structures of gomisin B (**3**) and gomisin G (**4**).

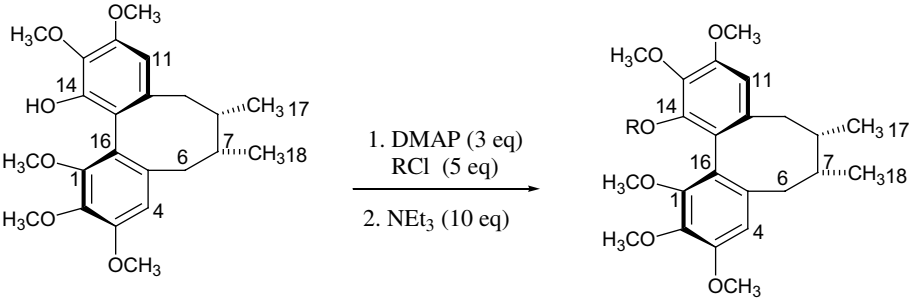
acylation and sulfonylation of **1** were carried out in attempts to decrease the toxicity.

Acylation and sulfonylation of **1** proceeded at room temperature in good yields using dimethylaminopyridine (DMAP) to activate the various acyl chlorides or sulfonyl chlorides and adding NEt_3 to quench the generated acid. The reagents, acyl chlorides (R = Ac, Bz, crotonoyl, 4-methoxycarbonylbenzoyl, 4-nitrobenzoyl, 2-furoyl, 2-thenoyl) and sulfonyl chlorides (R = Ms, Ts), were employed in excess amounts to ensure complete reaction. It was noted that crotonoyl substituent was not yielded when **1** reacted with crotonoyl chloride. On the contrast, a but-3-enoyl group in **6** was found in this reaction. The results of acylation and sulfonylation of **1** are summarized in Scheme 1.

The another C_{18} dibenzocyclooctadiene lignan, kadsurarin (**2**), structurally similar to gomisin B (**3**) except for the C-9 acetate in **2**, had no anti-HBeAg or anti-HBsAg activity.[9] Gomisin B (**3**), gomisin G (**4**) and (+)-gomisin K_3 (**1**) all lack ester substitution at C-9. These preliminary results motivated us to prepare various C-9 esters of **2** to study structure–activity relationships.

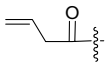
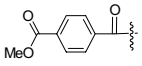
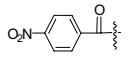
Kadsurarin (**2**) was deacetylated with 10% NaOH in EtOH to give the hydrolyzed product **14** in 87% yield (Scheme 2). Compound **14** was then reacted with various 4-substituted benzoic acid chlorides (R = H, Me, F, Cl, NO_2, CO_2Me) to yield compounds **15**–**20** (Scheme 3). Due to steric hindrance in these nucleophilic acyl substitution reactions, the tertiary free hydroxy group of **14** (at C-7) did not undergo acylation even though excess acyl chloride was used.

The structure–activity relationships of derivatives **5**–**13** of (+)-gomisin K_3 (**1**) were evaluated for anti-HBsAg and anti-HBeAg activity (Table 1). Except for **9**, derivatives **5**–**12** displayed strong inhibition (>56.4%) at 25 and 50 μg/mL in the anti-HBsAg test. Compounds **6**, **7**, **8** and **11** showed strong inhibition (>53.8%) against HBsAg at 10 μg/mL. In the anti-HBeAg assay, **6** also exhibited strong inhibition, and **8** and **13** displayed medium inhibition at responding concentrations as shown in Table 1. Bioassay results revealed that most of C_{18} lignans with esterified substituents at C-14 had the higher

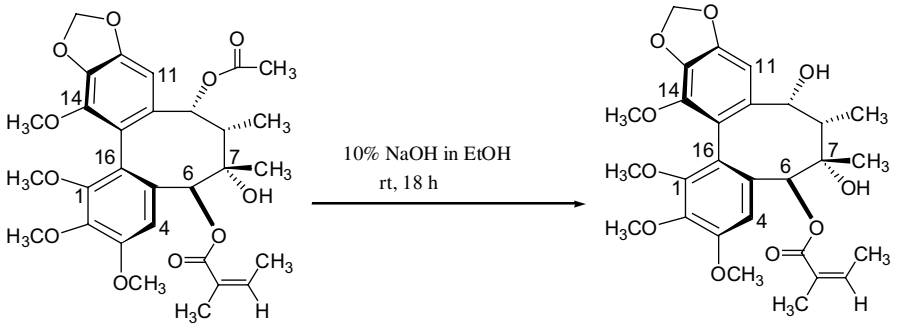


(+)-gomisin K_3 (1)

5-13

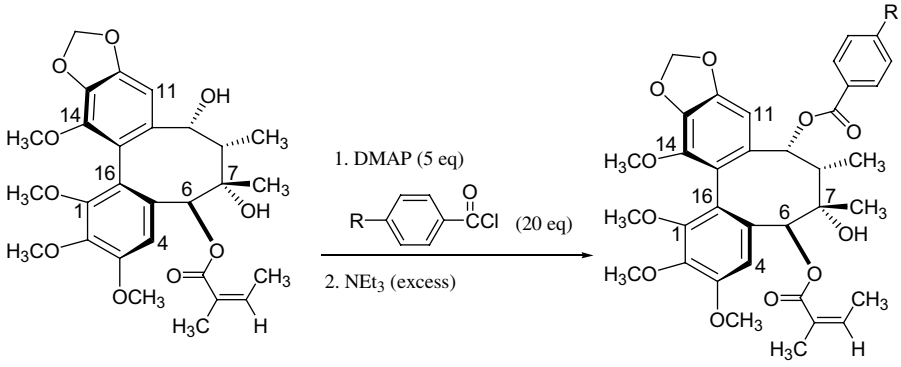
Compound no.	R	Yield (%)
5	Ac	56
6		68
7	Bz	65
8		68
9		64
10	2-Furoyl	81
11	2-Thenoyl	65
12	Ms	60
13	Ts	53

Scheme 1. The acylation and sulfonylation of **1** with various acyl chlorides and sulfonyl chlorides.



2 **14** (87%)

Scheme 2. Deacetylation of **2**.



14 **15-20**

Compound no.	R	Yield (%)
15	H	75
16	Me	66
17	F	98
18	Cl	75
19	NO_2	99
20	CO_2Me	82

Scheme 3. The acylation of **14** with various benzoic acid chlorides.

activity than those of hydroxyl group at C-14. Notably, the C_{18} lignans with esterified but-3-enoyl group as **6** displayed the most inhibition in both anti-HBsAg and anti-HBeAg test. Moreover, comparing with the toxicity of **1**, the obvious decrease of toxicity for the derivatives **5**–**12** was found. These evidences imply that the hydroxyl group at C-14 position in the C_{18} dibenzocyclooctadiene lignans would play a committed role for the activities. Not only the hydroxyl group at C-14, but also at C-1, such as previously isolated schizarins C, D and E showed toxicity and moderate anti-HBeAg effects.[8] These findings also agree the importance of C-1 position in the lignans for the mentioned activities. Unexpectedly, kadsurarin (**2**) and its derivatives **15**–**20** were only partially soluble in DMSO, there are no available data for the evaluation of **15**–**20** in the anti-HBsAg and anti-HBeAg assays. These evidences motivate us to check the other reported inactive lignans[9] as schizarins F, G and H, which have an acetate group at C-9, together with the results that the active lignans are lacking substituents at C-9, corroborating that the additional substituents at C-9 in the C_{18} dibenzocyclooctadiene lignans might eliminate the activities due to the lower solubility.

3. Conclusion

To summarize, a series of esterified derivatives of (+)-gomisin K_3 (**1**) and kadsurarin (**2**) were synthesized and evaluated for anti-HBsAg and anti-HBeAg. All of (+)-gomisin K_3 derivatives (**5**–**13**) exhibited higher positive activity against human B-type hepatitis and lower toxicity than **1**. These results indicated that the substituents at C-14 in the C_{18} dibenzocyclooctadiene lignans do have an effect on inhibition and toxicity. The aromatic hydroxyl group at C-14 or C-1 that both might play an important role for the activities was concluded.

Table 1. Anti-HBsAg and anti-HBeAg effects of compounds **5**–**13**

Compound no	Concn, μg/mL	HBsAg[a] (inhibition %)	HBeAg[a] (inhibition, %)	AST[b] (inhibition, %)
(+)-Gomisin K3 (**1**)	50	61.7	20.0	19.5
	25	28.4	16.1	12.6
	10	−1.1	5.2	15.9
5	50	79.6	19.5	5.9
	25	56.4	16.8	4.0
	10	26.1	−2.7	8.5
6	50	84.6	66.6	5.0
	25	81.4	52.9	13.7
	10	57.9	27.9	−0.1
7	25	60.6	13.6	−12.1
	10	58.0	18.0	−2.4
8	50	64.0	41.9	2.5
	25	60.5	44.7	0.6
	10	53.8	32.4	11.7
9	50	34.5	13.2	−16.7
	25	34.0	7.7	−14.3
	10	31.6	17.3	−9.5
10	50	68.1	14.2	−8.2
	25	66.3	8.2	−7.5
	10	40.9	−7.2	2.3
11	50	57.7	13.2	−12.5
	25	57.6	15.5	−12.4
	10	54.4	13.5	−6.6
12	25	60.9	24.4	−11.0
	10	23.2	8.0	−10.5
13	10	32.7	34.8	9.1
DMSO	2.5 μL/mL	3.9 ± 0.7	2.2 ± 7.8	13.3 ± 0.9

[a] Active: 25–35% (moderate inhibition), 35–45% (medium inhibition), >45% (strong inhibition); inactive: <25% inhibition.

[b] AST were explained in the text (see Anti-HBsAg and anti-HBeAg test).

Moreover, due to the lower solubility of **15**–**20** along with the other inactive analogues, the decrease of anti-HBsAg and anti-HBeAg resulting from the substituents at C-9 in the C_{18} dibenzocyclooctadiene lignans was also deduced.

Of those synthesized active derivatives, **6** displayed the most potent efficacy for anti-HBsAg and anti-HBeAg and provided the available basis for further investigation as a human B-type hepatitis drug.

4. Experimental

Infrared spectra were recorded with an FT-IR Analect RFX-65 spectrometer. ^{1}H and ^{13}C NMR spectra were measured for samples in $CDCl_3$ with a Bruker AC-400 FT-NMR spectrometer at 400 and 100 MHz, respectively. Electron impact (EI)-MS was performed on a JEOL SX-102A instrument. Silica gel (Merck 70–230 mesh) was used for column chromatography, and precoated silica gel (Merck $60_{F\text{-}254}$) plates were used for TLC. HPLC was accomplished on an SPD-6AV liquid chromatograph using a preparative C_{18} column. Melting points were determined on a Fisher-Johns apparatus and were uncorrected. All reagents were of reagent grade.

4.1. Plant materials

The stems of *K. matsudai* Hayata (NRICM-KKM-002) were collected in July 2000 in Pi-Tong County, and the stems of *S. arisanensis* (NRICM-KKM-0021) were collected in May 2002 in Taipei County, Taiwan. These two voucher samples were stored at the Herbarium of National Research of Chinese Medicine, Taipei.

4.2. Extraction and isolation

The dried stems of *K. matsudai* Hayata (6.2 kg) and *S. arisanensis* Hayata (6.9 kg) were extracted exhaustively with EtOH. The crude syrup was extracted five times with hexane. The respective EtOH layers of *K. matsudai* and *S. arisanensis* were partitioned with EtOAc–H_2O (1:1) three times to give EtOAc and H_2O layers. The EtOAc extract of *S. arisanensis* was chromatographed on a silica gel column with *n*-hexane/EtOAc (8:1, 6:1, 4:1, 2:1, 1:1, EtOAc) to give 14 fractions. Fraction 11 was further purified by HPLC (C_{18}, 250 × 10mm, MeOH–H_2O = 7:3) to furnish **1** (356 mg).[10] The EtOAc extract of *K. matsudai* was evaporated in vacuo and then was chromatographed on a silica gel column with *n*-hexane/EtOAc (8:1, 6:1, 4:1, 2:1, 1:1, EtOAc) to give 12 fractions. After recrystallization, compound **2** (194 mg) was obtained from fraction 6.[9]

4.3. 9-Hydroxy-gomisin B (14)

A mixture of **2** (83 mg, 0.14 mmol) and 10% NaOH in EtOH (10 mL) was stirred at room temperature for 18 h. The solution was neutralized by 10% aqueous HCl and extracted with CH_2Cl_2. The organic solution was dried ($MgSO_4$) and evaporated to give pure product **14**. White solid (67 mg, 87%); IR (film) 3487 (OH), 1713 (ester), 1622 (aromatic) cm^{-1}; ^{1}H NMR δ 1.27 (3H, s), 1.30 (3H, d, J = 7.2 Hz), 1.34 (3H, dq, J = 1.2, 1.2 Hz), 1.81 (3H, dq, J = 7.2, 1.2 Hz), 1.93 (1H, q, J = 7.2 Hz), 3.67 (3H, s), 3.68 (3H, s), 3.83 (3H, s), 3.85 (3H, s), 4.79 (1H, s), 5.61 (1H, s), 5.85 (1H, d, J = 0.8 Hz), 5.89 (1H, d, J = 0.8 Hz), 5.95 (1H, dq, J = 7.2, 1.2 Hz), 6.25 (1H, s), 6.72 (1H, s); ^{13}C NMR δ 15.6, 17.6, 19.7, 29.0, 43.2, 55.7, 59.0, 60.0, 60.0, 74.2, 83.8, 85.1, 100.8, 101.6, 110.7, 120.1, 120.5, 127.0, 131.0, 135.1, 135.9, 139.7, 140.7, 141.0, 148.5, 150.9, 151.8, 165.8; EIMS (relative intensity) m/z 530 (M^+, 3), 516 (4), 514 (6), 513 (29), 512 (100), 492 (4), 480 (4), 454 (7), 441 (12), 440 (26), 430 (7); exact mass calcd for $C_{28}H_{34}O_{10}$ m/z 530.2152, EIHRMS m/z 530.2147.

4.4. General procedure for the esterification and sulfonylation of (+)-gomisin K_3 (1) with various acyl chlorides or sulfonyl chlorides

A mixture of **1** (30 or 40 mg, 0.075 or 0.1 mmol), the dimethylaminopyridine (DMAP, 0.2 or 0.3 mmol) and acyl chloride (0.37 or 0.5 mmol) or or sulfonyl chlorides

(0.37 or 0.5 mmol) was stirred at room temperature in CH_2Cl_2 (10 mL) under nitrogen for several hours, and then the triethylamine (0.75 or 1.0 mmol) was added and stirred for 1–4 h. The CH_2Cl_2 and excess triethylamine were removed under vacumn, then the reaction mixture was added to CH_2Cl_2 (50 mL) and washed with a 5% aqueous Na_2CO_3 or $NaHCO_3$ (50 mL × 3). The organic layer was dried ($MgSO_4$) and evaporated. The crude product was purified by preparative TLC and HPLC using hexane/EtOAc (2:1) as the eluent, to afford products **5**–**13**.

4.4.1. 14-Acetyl gomisin K_3 (5). Pale yellow solid (18.4 mg, 56%); mp 151–152 °C; IR (film) 1770, 1597 cm^{-1}; ^1H NMR δ 0.74 (3H, d, J = 7.2 Hz), 0.96 (3H, d, J = 7.2 Hz), 1.74–1.76 (1H, m), 1.87–1.91 (1H, m), 1.94 (3H, s), 1.99 (1H, dd, J = 13.6, 1.62 Hz), 2.23 (1H, dd, J = 13.6, 9.8 Hz), 2.50 (1H, dd, J = 13.6, 2.4 Hz), 2.59 (1H, dd, J = 13.6, 7.6 Hz), 3.59 (3H, s), 3.81 (3H, s), 3.82 (3H, s), 3.85 (3H, s), 3.87 (3H, s), 6.48 (1H, s), 6.66 (1H, s); ^{13}C NMR δ 12.9, 20.4, 21.5, 33.7, 35.3, 39.1, 40.5, 55.8, 55.9, 60.7, 60.8 (×2), 107.3, 112.9, 120.7, 123.4, 133.9, 139.2, 139.4, 140.1, 142.2, 151.1, 151.3, 153.0, 168.4; EIMS (relative intensity) m/z 444 (M^+, 20), 403 (27), 402 (100), 370 (13), 356 (10), 355 (11); exact mass calcd for $C_{25}H_{32}O_7$ m/z 444.2148, EIHRMS m/z 444.2149.

4.4.2. 14-(But-3-enoyl)-gomisin K_3 (6). Pale yellow viscous liquid (13.9 mg, 68%); IR (film) 1760, 1597 cm^{-1}; ^1H NMR δ 0.74 (3H, d, J = 7.2 Hz), 0.96 (3H, d, J = 7.2 Hz), 1.74–1.76 (1H, m), 1.87–1.89 (1H, m), 1.99 (1H, dd, J = 12.8 Hz), 2.24 (1H, dd, J = 13.6, 9.6 Hz), 2.50 (1H, dd, J = 13.6, 2.0 Hz), 2.59 (1H, dd, J = 13.6, 7.2 Hz), 2.91 (1H, dd, J = 16.4, 6.8 Hz), 3.01 (1H, dd, J = 16.4, 6.8 Hz), 3.58 (3H, s), 3.81 (3H, s), 3.82 (3H, s), 3.85 (3H, s), 3.87 (3H, s), 4.96–5.03 (2H, m), 5.63–5.70 (1H, m), 6.47 (1H, s), 6.67 (1H, s); ^{13}C NMR δ 12.8, 21.5, 33.7, 35.3, 38.5, 39.0, 40.5, 55.9, 56.0, 60.6, 60.8, 60.9, 107.3, 113.0, 118.4, 120.7, 123.4, 129.7, 133.9, 139.3, 139.4, 140.1, 142.1, 151.1, 151.4, 153.1, 169.0; EIMS (relative intensity) m/z 470 (M^+, 14), 403 (25), 402 (100), 370 (14), 356 (9), 355 (12); exact mass calcd for $C_{27}H_{34}O_7$ m/z 470.2305, EIHRMS m/z 470.2300.

4.4.3. 14-Benzoyl gomisin K_3 (7). Pale yellow viscous liquid (32.9 mg, 65%); IR (film) 2934, 2871, 1738, 1598 cm^{-1}; ^1H NMR δ 0.78 (3H, d, J = 7.2 Hz), 0.98 (3H, d, J = 7.2 Hz), 1.75–1.78 (1H, m), 1.90–1.92 (1H, m), 2.02 (1H, dd, J = 13.2, 3.6 Hz), 2.23 (1H, dd, J = 13.2, 9.6 Hz), 2.54 (1H, dd, J = 13.6, 2.0 Hz), 2.63 (1H, dd, J = 13.6, 7.2 Hz), 3.50 (3H, s), 3.60 (3H, s), 3.76 (3H, s), 3.82 (3H, s), 3.90 (3H, s), 6.41 (1H, s), 6.72 (1H, s), 7.31 (2H, ddd, J = 7.6, 7.6, 1.2 Hz), 7.46 (1H, td, J = 7.6, 1.2 Hz), 7.95 (2H, dd, J = 7.6, 1.2 Hz); ^{13}C NMR δ 12.9, 21.6, 33.7, 35.3, 39.0, 40.6, 55.7, 56.0, 60.5, 60.6, 60.9, 107.4, 113.0, 120.8, 123.4, 128.1, 129.7, 129.9, 132.8, 133.9, 139.3, 139.6, 140.0, 142.3, 151.1, 151.4, 152.8, 164.1; EIMS (relative intensity) m/z 507 (35), 506 (M^+, 100), 402 (14), 401 (44), 371 (19), 370 (72), 355 (37); exact mass calcd for $C_{30}H_{34}O_7$ m/z 506.2305, EI-HRMS m/z 506.2307.

4.4.4. 14-(4-Methoxycarbonylbenzoyl)-gomisin K_3 (8). Pale yellow viscous liquid (37.9 mg, 68%); IR (film) 1728, 1597 cm^{-1}; ^1H NMR δ 0.78 (3H, d, J = 7.2 Hz), 0.97 (3H, d, J = 7.2 Hz), 1.75–1.78 (1H, m), 1.90–1.91 (1H, m), 2.02 (1H, dd, J = 13.6, 2.8 Hz), 2.30 (1H, dd, J = 13.6, 9.8 Hz), 2.53 (1H, dd, J = 13.6, 1.6 Hz), 2.63 (1H, dd, J = 13.6, 7.6 Hz), 3.47 (3H, s), 3.59 (3H, s), 3.75 (3H, s), 3.82 (3H, s), 3.93 (3H, s), 3.95 (3H, s), 6.41 (1H, s), 6.72 (1H, s), 7.96–8.01 (4H, m); ^{13}C NMR δ 12.8, 21.5, 33.6, 35.3, 39.0, 40.5, 52.3, 55.7, 56.0, 60.5, 60.6, 60.9, 107.5, 113.1, 120.7, 123.2, 129.3, 129.8, 133.5, 133.8, 134.0, 139.3, 139.5, 140.0, 142.1, 151.1, 151.4, 152.9, 163.3, 166.1; EIMS (relative intensity) m/z 565 (37), 564 (M^+, 100), 444 (18), 403 (23), 402 (90), 401 (44); exact mass calcd for $C_{32}H_{36}O_9$ m/z 564.2359, EI-HRMS m/z 564.2364.

4.4.5. 14-(4-Nitrobenzoyl)-gomisin K_3 (9). Yellow solid (34.9 mg, 64%); mp 142–143 °C; IR (film) 1745, 1598, 1529 cm^{-1}; ^1H NMR δ 0.78 (3H, d, J = 7.2 Hz), 0.98 (3H, d, J = 7.2 Hz), 1.74–1.79 (1H, m), 1.89–1.94 (1H, m), 2.02 (1H, dd, J = 13.6, 2.8 Hz), 2.28 (1H, dd, J = 13.6, 9.8 Hz), 2.53 (1H, dd, J = 13.6, 1.6 Hz), 2.64 (1H, dd, J = 13.6, 7.2 Hz), 3.45 (3H, s), 3.64 (3H, s), 3.76 (3H, s), 3.82 (3H, s), 3.91 (3H, s), 6.43 (1H, s), 6.74 (1H, s), 8.10 (2H, dd, J = 7.2, 2.0 Hz), 8.16 (2H, dd, J = 7.2, 2.0 Hz); ^{13}C NMR δ 12.8, 21.5, 33.6, 35.3, 39.0, 40.5, 55.7, 56.0, 60.5, 60.7, 60.9, 107.6, 113.3, 120.5, 122.9, 123.3, 130.9, 134.1, 135.2, 139.3, 139.4, 140.1, 141.8, 150.4, 151.0, 151.5, 152.9, 162.2; EIMS (relative intensity) m/z 552 (35), 551 (M^+, 100), 402 (10), 401 (19), 371 (15), 370 (59); exact mass calcd for $C_{30}H_{33}NO_9$ m/z 551.2155, EI-HRMS m/z 551.2153.

4.4.6. 14-(2-Furoyl)-gomisin K_3 (10). Pale yellow viscous liquid (30.1 mg, 81%); IR (film) 1746, 1596, 1573 cm^{-1}; ^1H NMR δ 0.76 (3H, d, J = 7.2 Hz), 0.96 (3H, d, J = 7.2 Hz), 1.74–1.77 (1H, m), 1.89–1.90 (1H, m), 2.02 (1H, dd, J = 13.6, 4.0 Hz), 2.30 (1H, dd, J = 13.6, 9.6 Hz), 2.52 (1H, dd, J = 13.6, 1.6 Hz), 2.61 (1H, dd, J = 13.6, 7.6 Hz), 3.58 (3H, s), 3.65 (3H, s), 3.79 (3H, s), 3.84 (3H, s), 3.89 (3H, s), 6.39 (1H, dd, J = 3.2, 1.0 Hz), 6.42 (1H, s), 6.71 (1H, s), 7.10 (1H, d, J = 3.2 Hz), 7.46 (1H, d, J = 1.0 Hz); ^{13}C NMR δ 12.8, 21.5, 33.7, 35.4, 39.0, 40.5, 55.7, 56.0, 60.6, 60.7, 60.9, 107.3, 111.7, 113.2, 118.6, 120.5, 123.5, 133.9, 139.2, 139.6, 140.0, 141.4, 144.0, 146.3, 151.1, 151.3, 152.9, 155.9; EIMS (relative intensity) m/z 497 (39), 496 (M^+, 100), 402 (36), 401 (22), 371 (18), 370 (66), 356 (11), 355 (35); exact mass calcd for $C_{28}H_{32}O_8$ m/z 496.2097, EI-HRMS m/z 496.2101.

4.4.7. 14-(2-Thenoyl)-gomisin K_3 (11). Pale yellow viscous liquid (25 mg, 65%); IR (film) 1732, 1596 cm^{-1}; ^1H NMR δ 0.76 (3H, d, J = 7.2 Hz), 0.97 (3H, d, J = 7.2 Hz), 1.75–1.77 (1H, m), 1.89–1.91 (1H, m), 2.03 (1H, d, J = 13.6 Hz), 2.32 (1H, dd, J = 13.6, 9.6 Hz), 2.53 (1H, dd, J = 13.6, 2.0 Hz), 2.62 (1H, dd, J = 13.6, 7.6 Hz), 3.56 (3H, s), 3.63 (3H, s), 3.78 (3H, s), 3.84 (3H, s), 3.89 (3H, s), 6.43 (1H, s), 6.71 (1H, s), 6.98 (1H, dd, J = 5.2, 3.6 Hz), 7.45 (1H, dd, J = 5.2, 1.2 Hz), 7.72 (1H, dd, J = 3.6, 1.2 Hz); ^{13}C NMR δ 12.8, 21.6, 33.7, 35.3, 39.0, 40.6, 55.8, 56.0, 60.6 (×2),

60.9, 107.3, 113.2, 120.7, 123.6, 127.5, 132.5, 132.9, 133.9, 134.0, 139.2, 139.7, 140.0, 141.9, 151.1, 151.4, 152.9, 159.4; EIMS (relative intensity) *m/z* 513 (35), 512 (M^+, 100), 402 (19), 401 (51), 371 (20), 370 (78), 369 (12), 356 (10), 355 (36); exact mass calcd for $C_{28}H_{32}O_7S$ *m/z* 512.1869, EIHRMS *m/z* 512.1868.

4.4.8. 14-(Methanesulfonyl)-gomisin K_3 (12). Pale yellow viscous liquid (21.4 mg, 60%); IR (film) 1596 cm^{-1}; ^1H NMR δ 0.72 (3H, d, J = 7.2 Hz), 0.97 (3H, d, J = 7.2 Hz), 1.78–1.79 (1H, m), 1.90–1.92 (1H, m), 2.05 (1H, d, J = 13.6 Hz), 2.30 (1H, dd, J = 13.6, 9.8 Hz), 2.50 (1H, dd, J = 13.6, 1.6 Hz), 2.61 (1H, dd, J = 13.6, 7.6 Hz), 2.62 (3H, s), 3.63 (3H, s), 3.82 (3H, s), 3.86 (3H, s), 3.88 (3H, s), 3.92 (3H, s), 6.52 (1H, s), 6.71 (1H, s); ^{13}C NMR δ 12.5, 21.7, 33.6, 35.3, 39.1 (×2), 40.4, 55.9, 56.0, 60.7, 60.9, 61.2, 107.2, 114.0, 120.6, 124.1, 134.3, 139.6, 140.4, 140.4, 141.5, 151.1, 151.5, 153.6; EIMS (relative intensity) *m/z* 480 (M^+, 29), 371 (28), 370 (100), 355 (18); exact mass calcd for $C_{24}H_{32}O_8S$ *m/z* 480.1818, EIHRMS *m/z* 480.1815.

4.4.9. 14-(Toluene-4-sulfonyl)-gomisin K_3 (13). Pale yellow viscous liquid (29.5 mg, 53%); IR (film) 1598 cm^{-1}; ^1H NMR δ 0.73 (3H, d, J = 7.2 Hz), 0.98 (3H, d, J = 7.2 Hz), 1.75–1.77 (1H, m), 1.88–1.90 (1H, m), 1.96 (1H, d, J = 13.6 Hz), 2.32 (1H, dd, J = 13.6, 9.6 Hz), 2.34 (3H, s), 2.40 (1H, dd, J = 13.6, 1.2 Hz), 2.57 (1H, dd, J = 13.6, 7.2 Hz), 3.46 (3H, s), 3.62 (3H, s), 3.85 (3H, s), 3.85 (3H, s), 3.87 (3H, s), 6.34 (1H, s), 6.69 (1H, s), 7.06 (2H, d, J = 8.2 Hz), 7.37 (2 H, d, J = 8.2 Hz); ^{13}C NMR δ 12.3, 21.5, 21.8, 33.6, 35.5, 38.9, 40.5, 55.7, 56.0, 60.4, 60.5, 61.0, 107.0, 114.2, 120.8, 124.0, 127.4, 129.0, 134.0, 134.9, 139.2, 139.9, 140.8, 141.5, 143.6, 150.7, 151.5, 153.3; EIMS (relative intensity) *m/z* 557 (32), 556 (M^+, 100), 402 (49), 401 (91); exact mass calcd for $C_{30}H_{36}O_8S$ *m/z* 556.2131, EIHRMS *m/z* 556.2123.

4.5. General procedure for the esterification and sulfonylation of 9-hydroxy-gomisin B (14) with various acyl chlorides or sulfonyl chlorides

A mixture of **14** (30 mg, 0.057 mmol), dimethylaminopyridine (DMAP, 34 mg, 0.28 mmol) and acyl chloride (1.1 mmol) or sulfonyl chlorides (1.1 mmol) was stirred at room temperature in CH_2Cl_2 (10 mL) under nitrogen for several hours, and then the triethylamine (0.25 mL) was added and stirred until the start compound was consumed completely. The CH_2Cl_2 and excess triethylamine were removed under vacumn, then the reaction mixture was added to CH_2Cl_2 (50 mL) and washed with a 5% aqueous Na_2CO_3 or $NaHCO_3$ (50 mL × 3). The organic layer was dried ($MgSO_4$) and evaporated. The crude product was purified by preparative TLC and HPLC using hexane/EtOAc (2:1) as the eluent, to afford products **15**–**20**.

4.5.1. 9-Benzoyloxy-gomisin B (15). White solid (26.8 mg, 75% yield), mp 223–225 °C; IR (film) 3568, 1716, 1596 cm^{-1}; ^1H NMR δ 1.29 (3H, d, J = 7.4 Hz), 1.37 (3H, s), 1.39 (3H, dq, J = 1.6, 1.6 Hz), 1.83 (3H, dq, J = 7.2, 1.6 Hz), 2.24 (1H, q, J = 7.4 Hz), 3.12 (3H, s), 3.43 (3H, s), 3.68 (3H, s), 3.98 (3H, s), 5.76 (1H, s), 5.89 (1H, d, J = 1.4 Hz), 5.90 (1H, d, J = 1.4 Hz), 5.96 (1H, dq, J = 7.2, 1.6 Hz), 6.04 (1H, s), 6.52 (1H, s), 6.89 (1H, s), 7.20–7.27 (4H, m), 7.43–7.44 (1H, m); ^{13}C NMR δ 15.6, 17.0, 19.7, 28.9, 43.3, 56.0, 59.1, 59.9, 60.0, 74.1, 83.9, 84.8, 101.0, 102.3, 110.5, 120.8, 122.2, 127.0, 128.4, 128.7, 129.1, 129.7, 132.5, 133.4, 135.9, 139.7, 141.0, 141.6, 148.7, 151.5, 152.1, 164.9, 165.7; EIMS (relative intensity) *m/z* 634 (M^+, 100), 628 (13), 616 (18), 592 (15), 580 (16), 578 (15), 566 (44), 542 (28), 535 (15), 534 (19), 530 (27), 528 (15); exact mass calcd for $C_{35}H_{38}O_{11}$ *m/z* 634.2414, EIHRMS *m/z* 634.2408.

4.5.2. 9-(4-Methyl-benzoyloxy)-gomisin B (16). White solid (24.1 mg, 66%), mp 162–164 °C; IR (film) 3576, 1720, 1612 cm^{-1}; ^1H NMR δ 1.28 (3H, d, J = 7.4 Hz), 1.36 (3H, s), 1.39 (3H, dq, J = 1.6, 1.6 Hz), 1.83 (3H, qd, J = 7.2, 1.6 Hz), 2.23 (1H, q, J = 7.4 Hz), 2.31 (3H, s), 3.12 (3 H, s), 3.47 (3H, s), 3.68 (3H, s), 3.99 (3H, s), 5.75 (1H, s), 5.89 (1H, d, J = 1.4 Hz), 5.90 (1H, d, J = 1.4 Hz), 5.96 (1H, dq, J = 7.2, 1.6 Hz), 6.04 (1H, s), 6.52 (1H, s), 6.89 (1H, s), 7.03 (2H, d, J = 8.2 Hz), 7.09 (2H, d, J = 8.2 Hz); ^{13}C NMR δ 15.6, 17.0, 19.7, 21.5, 28.9, 43.3, 56.0, 59.1, 59.9, 60.0, 74.1, 83.6, 84.8, 101.0, 102.4, 110.6, 120.8, 122.3, 126.0, 127.0, 129.1, 129.1, 129.7, 132.6, 135.9, 139.7, 141.0, 141.6, 144.2, 148.7, 151.5, 152.1, 165.0, 165.7; EIMS (relative intensity) *m/z* 648 (M^+, 100), 576 (7), 549 (9), 548 (38), 530 (4); exact mass calcd for $C_{36}H_{40}O_{11}$ *m/z* 648.2571, EIHRMS *m/z* 648.2570.

4.5.3. 9-(4-Fluoro-benzoyloxy)-gomisin B (17). White solid (35.9 mg, 98%), mp 182–184 °C; IR (film) 3581, 1719, 1602, 1506 cm^{-1}; ^1H NMR δ 1.28 (3H, d, J = 7.6 Hz), 1.37 (3H, s), 1.38 (3H, dq, J = 1.6, 1.6 Hz), 1.83 (3H, qd, J = 7.2, 1.6 Hz), 2.24 (1H, q, J = 7.6 Hz), 3.14 (3H, s), 3.53 (3H, s), 3.69 (3H, s), 3.97 (3H, s), 5.74 (1H, s), 5.89 (1H, d, J = 1.4 Hz), 5.90 (1H, d, J = 1.4 Hz), 5.97 (1H, dq, J = 7.2, 1.6 Hz), 6.04 (1H, s), 6.51 (1H, s), 6.88 (1H, s), 6.90–6.94 (2H, m), 7.19–7.22 (2H, m); ^{13}C NMR δ 15.6, 17.0, 19.7, 28.9, 43.3, 56.0, 59.1, 59.9, 60.1, 74.1, 83.9, 84.7, 101.0, 102.3, 110.5, 115.6 (1C, d, J = 22 Hz), 120.7, 122.3, 125.0 (1C, d, J = 3 Hz), 126.9, 129.7, 131.6 (1C, d, J = 9 Hz), 132.4, 135.9, 139.8, 141.0, 141.6, 148.8, 151.5, 152.1, 163.9, 165.6, 165.9 (1C, d, J = 255 Hz); EIMS (relative intensity) *m/z* 652 (M^+, 100), 566 (12), 553 (10), 552 (34), 542 (9); exact mass calcd for $C_{35}H_{37}FO_{11}$ *m/z* 652.2320, EIHRMS *m/z* 652.2315.

4.5.4. 9-(4-Chloro-benzoyloxy)-gomisin B (18). White solid (28.4 mg, 75%), mp 155–158 °C; IR (film) 3581, 1716, 1622, 1594 cm^{-1}; ^1H NMR δ 1.30 (3H, d, J = 7.2 Hz), 1.39 (3H, s), 1.41 (3H, dq, J = 1.6, 1.6 Hz), 1.86 (3H, qd, J = 7.2, 1.6 Hz), 2.27 (1H, q, J = 7.4 Hz), 3.18 (3H, s), 3.54 (3H, s), 3.71 (3H, s), 4.01 (3H, s), 5.76 (1H, s), 5.92 (2H, s), 6.00 (1H, dq, J = 7.2, 1.6 Hz), 6.06 (1H, s), 6.54 (1H, s), 6.91 (1H, s), 7.15 (2H, d, J = 8.8 Hz), 7.25 (2H, d, J = 8.8 Hz); ^{13}C NMR δ 15.6, 17.0, 19.7, 28.9, 43.3, 56.0, 59.0, 59.9, 60.0, 74.0, 84.0, 84.6, 101.0, 102.3, 110.4, 120.6, 122.2, 126.9, 127.2, 128.7, 129.7, 130.4, 132.2, 135.9, 139.8, 139.9, 140.9, 141.5, 148.7,

151.4, 152.1, 164.1, 165.6; EIMS (relative intensity) *m/z* 670 (39), 668 (M^+, 100), 652 (5), 596 (5), 570 (20), 569 (8), 568 (47), 566 (6); exact mass calcd for $C_{35}H_{37}ClO_{11}$ *m/z* 668.2024, EIHRMS *m/z* 668.2025.

4.5.5. 9-(4-Nitro-benzoyloxy)-gomisin B (19). Light yellow solid (38.1 mg, 99%), mp 103–106 °C; IR (film) 3584, 2941, 1722, 1598, 1530, 1463, 1411, 1348, 1252, 1105, 1046, 1014, 948, 836, 720 cm^{-1}; ^1H NMR δ 1.28 (3H, d, J = 7.2 Hz), 1.37 (3H, s), 1.39 (3H, dq, J = 1.6, 1.6 Hz), 1.83 (3H, qd, J = 7.2, 1.6 Hz), 2.25 (1H, q, J = 7.2 Hz), 3.19 (3H, s), 3.53 (3H, s), 3.69 (3H, s), 3.97 (3H, s), 5.73 (1H, s), 5.89 (1H, d, J = 1.4 Hz), 5.90 (1H, d, J = 1.4 Hz), 5.98 (1H, dq, J = 7.2, 1.6 Hz), 6.03 (1H, s), 6.50 (1H, s), 6.88 (1H, s), 7.37 (2H, d, J = 8.8 Hz), 8.07 (2H, d, J = 8.8 Hz); ^{13}C NMR δ 15.6, 17.0, 19.7, 29.0, 43.4, 56.0, 59.0, 60.0, 60.1, 74.0, 84.5, 84.8, 101.0, 102.2, 110.3, 120.6, 122.0, 123.4, 126.8, 129.6, 130.1, 131.8, 134.2, 135.9, 139.9, 140.9, 141.4, 148.8, 150.6, 151.4, 152.2, 163.2, 165.5; EIMS (relative intensity) *m/z* 680 (36), 679 (M^+, 100), 580 (10), 579 (32), 566 (7); exact mass calcd for $C_{35}H_{37}NO_{13}$ *m/z* 679.2265, EI-HRMS *m/z* 679.2262.

4.5.6. 9-(4-Methoxycarbonyl-benzoyloxy)-gomisin B (20). White solid (32.1 mg, 82%), mp 106–108 °C; IR (film) 3582, 1722, 1596 cm^{-1}; ^1H NMR δ 1.28 (3H, d, J = 7.2 Hz), 1.37 (3H, s), 1.38 (3H, dq, J = 1.6, 1.6 Hz), 1.82 (3H, qd, J = 7.2, 1.6 Hz), 2.24 (1H, q, J = 7.2 Hz), 3.16 (3H, s), 3.42 (3 H, s), 3.68 (3H, s), 3.89 (3H, s), 3.98 (3H, s), 5.73 (1H, s), 5.89 (2H, s), 5.96 (1H, dq, J = 7.2, 1.6 Hz), 6.02 (1H, s), 6.51 (1H, s), 6.88 (1H, s), 7.27 (2H, d, J = 8.0 Hz), 7.89 (2H, d, J = 8.0 Hz); ^{13}C NMR δ 15.6, 17.0, 19.7, 28.9, 43.3, 52.4, 56.0, 59.0, 59.9, 60.0, 74.0, 84.4, 84.6, 101.0, 102.2, 110.4, 120.6, 122.0, 126.8, 129.0, 129.5, 129.6, 132.1, 132.5, 134.2, 135.8, 139.8, 140.9, 141.4, 148.7, 151.4, 152.1, 164.1, 165.6, 165.8; EIMS (relative intensity) *m/z* 692 (M^+, 100), 662 (11), 661 (27), 593 (22), 592 (74), 558 (16); exact mass calcd for $C_{37}H_{40}O_{13}$ *m/z* 692.2469, EIHRMS *m/z* 692.2472.

4.6. Anti-HBsAg and anti-HBeAg test

The assays for in vitro anti-viral activity against hepatitis B virus (HBV) were performed according to our previously described procedure.[9] Briefly, the HBV-producing cell line MS-G2 was plated into 24-well flat-bottomed tissue culture plates at a density of 3×10^5 cells/mL/well. After an overnight stay to ensure that the cells were properly attached, the cells were challenged by test compounds. DMSO alone was added to each culture as solvent control. All tested pure compounds were dissolved in DMSO at a concentration of 1, 5, 10 and 20 μg/mL, respectively. The concentration of DMSO in the media was maintained at no more than 2.5 μL/mL, to ensure that it did not affect the growth of MS-G2 cells. Subsequently, the culture media was collected at 3 days for anti-viral assay. Then, the HBsAg and HBeAg values were analyzed as anti-viral indicators using the ELISA assay (Enzyme-Linked Immunosorbent Assay) (Instrument: DYNATECH MR 7000 at 490 nm) to evaluate the anti-viral effects for the test samples. The percentage inhibition (%) was calculated by comparing with the control group. Inhibition between 25% and 35% was defined as moderate inhibition, 35% and 45% as medium inhibition, and >45% as strong inhibition, while an inhibition percentage below 25% was defined as inactive.

4.6.1. Cell line and cell culture. A HBV DNA integrated HCC cell line, MS-G2, kindly provided by Dr. Max Essex,[11] was established from a hepatoblastoma derived cell line, HepG2, by transfection with two copies of the entire HBV genome. The MS-G2 cell line secreted HBV containing viral DNA and a DNA polymerase activity. The MS-G2 cells were cultured in RPMI-1640 (GIBCO, BRL, Grand Island, NY) medium supplemented with 10% fetal calf serum, 100 IU/mL penicillin, 100 μg/mL streptomycin, 2 mmol/L L-glutamine, 1% nonessential amino acids and 2.5 mg/mL fungizone. Exponentially growing cultures were maintained in a humidified atmosphere of 5% CO_2 at 37 °C. Under these condition the plating efficiency was above 95%.

4.6.2. Cytotoxic assay. Cell damage was tested by AST (aspartate transaminase) Fuji kit. AST values higher than 25 IU/L served as an indication of cell damage or lysis, as described previously.[11]

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