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Schisanwilsonins A–G and related anti-HBV lignans from the fruits of *Schisandra wilsoniana*

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ABSTRACT

Seven new dibenzocyclooctane lignans, schisanwilsonins A–G (1–7), were isolated from the fruits of *Schisandra wilsoniana*, together with five known lignans (8–12). The structures of these new compounds were elucidated by spectroscopic methods including 2D-NMR techniques. The 12 lignans were tested for anti-hepatitis B virus (HBV) activity in vitro. Schisanwilsonin D (4), schisantherin C (9), deoxyschizandrin (10) and (+)-gomisin K_3 (11) showed anti-HBV activity. 9 exhibited the most potent anti-HBV activity with potency against HBsAg and HBeAg secretion by 59.7% and 34.7%, respectively, at 50 μg/mL.

Dibenzocyclooctane lignans, mostly isolated from the Schisandraceae plants,[1] are important natural products with many beneficial pharmacological effects, such as calcium antagonism, anti-lipid peroxidation, and antitumor effect.[2–4] Several dibenzocyclooctane lignans isolated from *Kadsura interior* and *Schisandra rubriflora* were found to show strong antiviral effect against human immunodeficiency virus (HIV).[5–7] It's interesting that some dibenzocyclooctane lignans obtained from *Kadsura induta*, *Kadsura japonica*, and *Schisandra. arisanensis* were also reported to be active against hepatitis B virus (HBV).[8–10] The fruits of *Schisandra wilsoniana* A. C. Smith (Schisandraceae) are used in Chinese folk medicine as a substitute for 'wu-wei-zi' to treat hepatitis, and the Et_2O extract was found to show anti-HBV activity. In our continuing effort to search for anti-HBV agents from Chinese herbs, a phytochemical investigation on the fruits of this plant[11] led to the isolation and characterization of seven new dibenzocyclooctane lignans,[12] schisanwilsonins A–G (**1**–**7**), along with five known lignans (**8**–**12**), which were identified as schisantherin A (**8**),[13] schisantherin C (**9**),[13] deoxyschizandrin (**10**),[14] (+)-gomisin K_3 (**11**),[15] and gomisin H (**12**),[16] respectively, by comparison of their physical and spectroscopic data with those reported. This paper reports the isolation and structure elucidation of the new compounds, as well as the in vitro anti-HBV activity of these isolated lignans.

Schisanwilsonin A (**1**), white powder, had molecular formula $C_{28}H_{34}O_9$ on the basis of HREIMS data (*m*/*z* 514.2209). The UV and NMR spectra indicated that **1** was a dibenzocyclooctane-type lignan.[14] The ^{1}H NMR spectrum of **1** (Table 1) showed the presence of four MeO groups at δ_H 3.52, 3.69, 3.89 and 3.91 (3H each, s), and a methylenedioxy (OCH_2O) moiety at δ_H 5.87 (1H, d, J = 1.4 Hz) and 5.90 (1H, d, J = 1.4 Hz) on aromatic ring. The O-atoms of the methylenedioxy moiety were attached to C-12 (δ_C 148.7) and C-13 (δ_C 134.5), on the basis of HMBC correlations of the proton signal at δ_H 6.46 (H-11) and OCH_2O resonances with C-12 and C-13 (Fig. 2). Four MeO groups were thus connected to C-1, C-2, C-3 and C-14, respectively, and which were confirmed by HMBC.

The EIMS fragments at *m*/*z* 414 ([M−C_4H_7COOH]$^+$), 83 ($C_5H_7O^+$) and 55 ($C_4H_7^+$) suggested the presence of an angeloyl (=(*Z*)-2-methylbut-2-enoyl; Ang) group, as confirmed by the ^{1}H NMR signals at δ_H 6.00 (1H, m, H-3′), 1.86 (3H, d, J = 7.0 Hz, Me-4′) and 1.38 (3H, s, Me-5′), along with the corresponding ^{13}C NMR signals (Table 2) at δ_C 166.1 (C-1′), 127.1 (C-2′), 140.4 (C-3′), 15.8 (C-4′) and 19.6 (C-5′).[16] The HMBC correlations of H-6 at δ_H 5.63 with C-1′ at δ_C 166.1, as well as the ROESY cross-peak between H-4 and H-6 indicated that a β-AngO group was located at C-6.

The IR absorption at 3442 cm^{-1} and the Me signals at δ_H1.13 (d, J = 7.0 Hz) and 1.08 (s) in the ^{1}H NMR spectrum suggested the presence of an OH group at C-6 or C-7.[17] The HMBC correlations of H-6 with C-7 at δ_C 75.4 and Me-18 at δ_C 19.2, as well as the ROESY cross-peaks observed between H-4 and Me-18, H-6 and Me-18 revealed that the β-OH group was located at C-7.

The circular dichroism (CD) spectrum of **1** showed a negative *Cotton* effect at 255 nm, and a positive one at 230 nm, in accord with a *S* biphenyl configuration.[18] The ROESY cross-peaks between H-4 and H-6, H-4 and Me-18, as well as H-6 and Me-18 (Fig. 3) indicated a 'twist-boat-chair' (TBC) conformation for the cyclooc-

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Table 1
^{1}H NMR (400 MHz) data of compounds **1–7** ($CDCl_3$, δ_H in ppm, J in Hz)

	1	2	3	4	5	6	7
4	6.71 (s)	6.70 (s)	6.77 (s)	6.71 (s)	6.67 (s)	6.66 (s)	6.79 (s)
6	5.63 (s)	5.68 (s)	5.84 (s)	5.76 (s)	5.63 (s)	5.58 (s)	5.93 (s)
8	1.98 (m)	2.01 (m)	2.17 (m)	2.078 (m)	1.95 (m)	1.94 (m)	2.22 (m)
9	2.22 (m)	2.24 (m)	2.31 (m)	2.26 (m)	2.23 (m)	2.21 (m)	2.34 (m)
11	6.46 (s)	6.50 (s)	6.57 (s)	6.68 (s)	6.48 (s)	6.47 (s)	6.78 (s)
17	1.13 (d, 7.0)	1.13 (d, 7.0)	1.18 (d, 7.0)	1.14 (d, 7.0)	1.11 (d, 7.0)	1.09 (d, 7.0)	1.18 (d, 7.0)
18	1.08 (s)	1.08 (s)	1.14 (s)	1.07 (s)	1.06 (s)	1.04 (s)	1.12 (s)
MeO-1	3.52 (s)	3.53 (s)	3.55 (s)	3.57 (s)	3.52 (s)	3.52 (s)	3.54 (s)
MeO-2	3.89 (s)	3.88 (s)	3.88 (s)	3.88 (s)	3.89 (s)	3.88 (s)	3.89 (s)
MeO-3	3.91 (s)	3.90 (s)	3.93 (s)	3.92 (s)	3.89 (s)	3.87 (s)	3.95 (s)
MeO-13				3.86 (s)			3.39 (s)
MeO-14	3.69 (s)	3.66 (s)	3.26 (s)	3.31 (s)	3.83 (s)	3.82 (s)	3.11 (s)
OCH_2O	5.87 (d, 1.4)	5.87 (d, 1.2)	5.72 (d, 1.5)		5.90 (d, 1.4)	5.90 (s)	
	5.90 (d, 1.4)	5.93 (d, 1.2)	5.80 (d, 1.5)		5.92 (d, 1.4)	5.91 (s)	
HO-12				5.71 (br s)			5.71 (br s)
2′					2.10 (m)	1.64 (s)	
3′	6.00 (m)	6.00 (m)	7.48 (d, 7.0)	5.97 (m)	0.90 (d, 7.0)		7.42 (d, 7.0)
4′	1.86 (d, 7.0)	1.67 (d, 7.0)	7.31 (t, 7.4)	1.61 (d, 7.0)	0.91 (d, 7.0)		7.29 (t, 7.4)
5′	1.38 (s)	1.58 (s)	7.50 (t, 7.4)	1.52 (s)			7.45 (t, 7.4)
6′			7.31 (t, 7.4)				7.29 (t, 7.4)
7′			7.48 (d, 7.0)				7.42 (d, 7.0)

tane ring.[19] From the above data, the structure of **1**[20] was elucidated as shown in Figure 1.

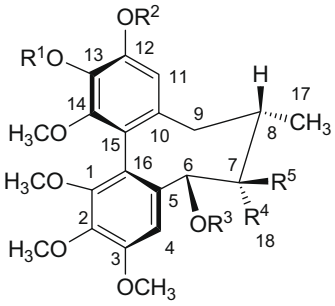
The IR, UV, CD and NMR data of compounds **2–7** were similar to those of **1**, indicating that **2–7** were all dibenzocyclooctane-type lignans.

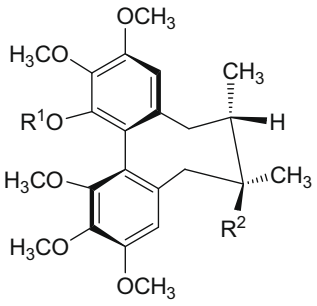
Schisanwilsonin B (**2**),[21] had molecular formula $C_{28}H_{34}O_9$ according to the HRESIMS data (m/z 537.2103, $[M+Na]^+$). The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) of **2** were quite similar to those of **1** except for the characteristic signals due to a tigloyl (Tig) group instead of the angeloyl group in **1**. The ^{1}H NMR signals at δ_H 6.00 (1H, m, H-3′), 1.67 (3H, d, J = 7.0 Hz, Me-4′) and 1.58 (3H, s, Me-5′), along with the corresponding ^{13}C NMR signals at δ_C 166.7 (C-1′), 127.6 (C-2′), 137.7 (C-3′), 14.2 (C-4′) and 11.5 (C-5′) indicated the presence of a Tig group.[22]

Schisanwilsonin C (**3**),[23] was assigned the molecular formula $C_{30}H_{32}O_9$ based on HREIMS (m/z 536.2045). Comparison of the NMR data of **3** with those of **1** indicated that the angeloyl group in **1** was replaced by a benzoyl (Bz) group in **3**. The EIMS fragments at m/z 414 ($[M-C_6H_5COOH]^+$), 105 ($C_6H_5CO^+$), and 77 ($C_6H_5^+$) suggested the presence of a benzoyl group, which was confirmed by the ^{1}H NMR signals at δ_H 7.31 (t, J = 7.0 Hz, H-4′,6′), 7.48 (d, J = 7.0 Hz, H-3′,7′), 7.50 (t, J = 7.0 Hz, H-5′), and ^{13}C NMR signals

Table 2
^{13}C NMR (100 MHz) data of compounds **1–7** ($CDCl_3$, δ in ppm)

	1	2	3	4	5	6	7
1	152.0 s	152.0 s	152.1 s	152.1 s	152.0 s	152.0 s	152.1 s
2	141.6 s	141.6 s	141.7 s	141.8 s	141.6 s	141.6 s	141.9 s
3	151.7 s	151.7 s	151.8 s	151.8 s	151.7 s	151.9 s	151.8 s
4	110.0 d	110.0 d	109.9 d	110.3 d	110.1 d	110.0 d	110.3 d
5	131.5 s	131.7 s	131.3 s	131.4 s	131.5 s	131.5 s	131.1 s
6	86.3 d	86.1 d	86.8 d	85.9 d	86.1 d	86.3 d	85.5 d
7	75.4 s	75.4 s	75.5 s	75.8 s	75.6 s	72.0 s	75.9 s
8	43.0 d	43.0 d	43.4 d	42.7 d	42.6 d	42.7 d	42.9 d
9	37.2 t	37.2 t	37.2 t	37.1 t	37.2 t	37.0 t	37.2 t
10	135.0 s	135.1 s	135.0 s	137.1 s	135.1 s	135.2 s	137.0 s
11	102.6 d	102.5 d	102.3 d	109.8 d	102.5 d	102.6 d	109.7 d
12	148.7 s	148.5 s	148.8 s	148.8 s	148.6 s	148.6 s	149.0 s
13	134.5 s	134.5 s	134.4 s	137.7 s	134.3 s	134.2 s	137.7 s
14	140.7 s	140.7 s	140.4 s	149.4 s	140.5 s	140.3 s	149.4 s
15	121.0 s	121.2 s	120.9 s	121.7 s	120.9 s	121.1 s	121.4 s
16	121.7 s	121.6 s	121.7 s	121.6 s	121.9 s	121.3 s	121.7 s
17	18.8 q	18.8 q	18.8 q	18.7 q	18.7 q	18.6 q	18.8 q
18	19.2 q	19.2 q	19.2 q	19.2 q	19.5 q	19.2 q	19.2 q
MeO-1	60.6 q	60.7 q	60.7 q	60.8 q	60.6 q	60.6 q	60.7 q
MeO-2	60.9 q	60.9 q	60.9 q	60.9 q	60.9 q	60.9 q	60.9 q
MeO-3	55.8 q	55.9 q	55.9 q	55.8 q	55.9 q	55.9 q	56.0 q
MeO-13				60.6 q			60.0 q
MeO-14	59.1 q	59.0 q	58.6 q	59.2 q	59.2 q	59.2 q	59.2 q
OCH_2O	100.6 t	100.5 t	100.5 t		100.6 t	100.6 t	
1′	166.1 s	166.7 s	165.2 s	166.7 s	176.3 s	170.0 s	165.2 s
2′	127.1 s	127.6 s	129.4 s	127.4 s	33.9 s	20.2 q	129.4 s
3′	140.4 d	137.7 d	129.6 d	138.5 d	18.9 d		129.6 d
4′	15.8 q	14.2 q	127.8 d	14.3 q	17.9 q		128.1 d
5′	19.6 q	11.5 q	133.0 d	11.5 q			133.1 d
6′			127.8 d				128.1 d
7′			129.6 d				129.6 d





1 $R^1+R^2=CH_2$, R^3=Ang, $R^4=CH_3$, R^5=OH

2 $R^1+R^2=CH_2$, R^3=Tig, $R^4=CH_3$, R^5=OH

3 $R^1+R^2=CH_2$, R^3=Bz, $R^4=CH_3$, R^5=OH

4 $R^1=CH_3$, R^2=H, R^3=Tig, $R^4=CH_3$, R^5=OH

5 $R^1+R^2=CH_2$, R^3=iso-Buty, $R^4=CH_3$, R^5=OH

6 $R^1+R^2=CH_2$, R^3=Ac, $R^4=CH_3$, R^5=OH

7 $R^1=CH_3$, R^2=H, R^3=Bz, $R^4=CH_3$, R^5=OH

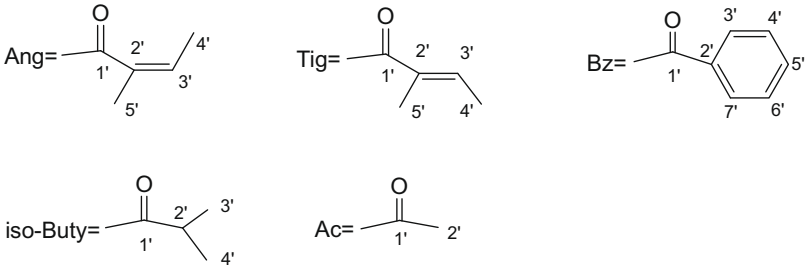
8 $R^1+R^2=CH_2$, R^3=Bz, R^4=OH, $R^5=CH_3$

9 $R^1+R^2=CH_2$, R^3=Tig, R^4=OH, $R^5=CH_3$

10 $R^1=CH_3$, R^2=H

11 R^1=H, R^2=H

12 R^1=H, R^2=OH



(Arbitary numbering. For systematic names, see References and Notes)

Figure 1. Structures of compounds **1**–**12**.

at δ_C 165.2 (C-1′), 129.4 (C-2′), 129.6 (C-3′,7′), 127.8 (C-4′,6′) and 133.0 (C-5′).[24]

Schisanwilsonin D (**4**)[25] had molecular formula $C_{28}H_{36}O_9$ (HRE-SIMS). Comparison of the NMR data of **4** with those of **2** indicated that the OCH_2O group in **2** was replaced by a MeO and an OH groups in **4**. Lacking any ROESY correlations observed between H-11 and MeO suggested that the OH group was attached to C-12.[24]

Schisanwilsonin E (**5**)[26] had molecular formula $C_{27}H_{34}O_9$ (HRE-SIMS). Comparison of the NMR data of **5** with those of **1** indicated that the Ang group in **1** was replaced by an isobutyryl (*iso*-Buty) group in **5**. Fragment ions at *m*/*z* 414 ($[M-C_3H_7COOH]^+$), 71 $(C_4H_7O)^+$ and 43 $(C_3H_7)^+$ in the EIMS spectrum suggested that there was an isobutyryl group in **5**, which was confirmed by the NMR signals at δ_H 2.10 (1H, m, H-2′), 0.90 (3H, d, *J* = 7.0 Hz, H-3′), 0.91

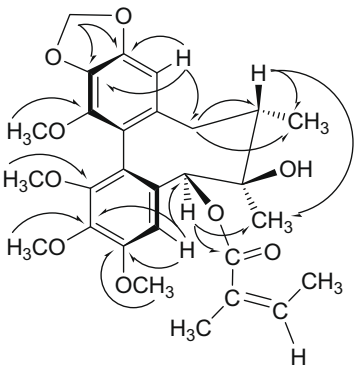


Figure 2. Key HMBC correlations of **1**.

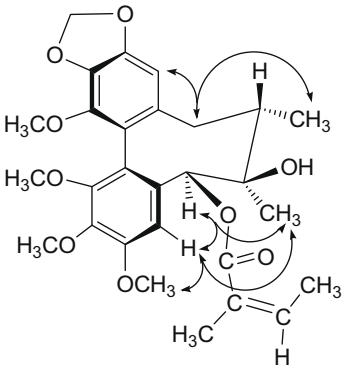


Figure 3. Key ROESY correlations of **1**.

Table 3
Anti-HBsAg and anti-HBeAg effects of compounds **1**–**12** in HepG2 2.2.15 cell line

	concn (μg/mL)	HBsAg (inhibition%)	HBeAg (inhibition%)		concn (μg/mL)	HBsAg (inhibition%)	HBeAg (inhibition%)
1	200	/	/	**7**	200	0	5.6
	100	/	/		100	0	3.4
	50	5.3	14.0		50	0	1.6
	25	0	0		25	0	0
2	200	/	/	**8**	200	/	/
	100	7.4	18.1		100	/	/
	50	0	9.2		50	23.9	0
	25	0	0.6		25	0	0
3	200	/	/	**9**	200	/	/
	100	/	/		100	/	/
	50	0	11.7		50	59.7	34.7
	25	0	11.6		25	22.1	16.9
4	200	/	/	**10**	200	50.3	15.9
	100	28.8	21.6		100	30.9	11.9
	50	19.5	16.5		50	8.8	0
	25	12.5	6.2		25	0	0
5	200	/	/	**11**	200	/	/
	100	0	2.0		100	/	/
	50	0	0		50	/	/
	25	0	0		25	45.0	0
6	200	/	/	**12**	200	11.6	1.6
	100	0	9.3		100	3.7	0
	50	0	6.7		50	0	0
	25	0	5.5		25	0	0
3TC[a]	200	28.6	34.5				

/ These compounds were cytotoxic. Cell damage was assessed by means of the MTT assay; cell growth inhibition ⩾25% was considered as cytotoxic.
[a] Positive control (3TC = Lamivudine).

(3H, d, J = 7.0 Hz, H-4′), and δ_C 176.3 (C-1′), 33.9 (C-2′), 18.9 (C-3′) and 17.9 (C-4′).

Schisanwilsonin F (**6**)[27] had molecular formula $C_{25}H_{30}O_9$ (HRESIMS). Comparison of the NMR data of **6** with those of **1** indicated that the Ang group in **1** was replaced by an acetyl (Ac) group in **6**. The fragment ions at m/z 414 $[M-CH_3COOH]^+$ and 43 (CH_3CO^+) implied the presence of an Ac group in **6**, as confirmed by the ^{1}H NMR signals at δ_H 1.64 (3H, s, H-2′) and ^{13}C NMR signals at δ_C 170.0 (C-1′), 20.2 (C-2′).

Schisanwilsonin G (**7**)[28] had molecular formula $C_{30}H_{34}O_9$ (HREIMS). Comparison of the NMR data of **7** with those of **3** indicated that the OCH_2O group in **3** was replaced by a MeO and an OH groups in **7**. Lacking any ROESY correlations observed between H-11 and MeO suggested that the OH group was attached to C-12.[24]

Compounds **1**–**12** were tested for their antiviral activity against hepatitis B virus (HBV) in vitro,[29] and the results are summarized in Table 3. Compound **9,** at the concentration of 50 μg/mL, exhibited the most potent anti-HBV activity, with potency against HBsAg and HBeAg secretion by 59.7% and 34.7%, respectively, which was stronger than that of the positive control lamivudine (28.6% and 34.5% at 200 μg/mL). Compounds **10**, **11** and **4** inhibited HBsAg secretion by 50.3%, 45.0% and 28.8% at the concentrations of 200, 25 and 100 μg/mL, respectively. Compounds **1**–**3**, **5**–**8** and **12** displayed weaker or inactive effect on either HBsAg or HBeAg secretion. Compound **9** had a similar structure with **2** except for the orientation of OH group at C-7. **9** possessed an α-orientated OH while **2** had a β-OH at C-7. **9** exhibited strong anti-HBV activity, however, **2** was inactive. Thus, the α-OH at C-7 might be very important to the enhanced anti-HBV activity, which was supported by that of compounds **1**–**7** with β-OH at C-7 displayed no or weaker activity, and further strengthened by that of gomisin B and G with an α-orientated OH substitute at C-7 were also found to exhibit strong anti-HBV effect.[10] It seems that the β-Bz substituent at C-6 decreased the activity based on the structure and activity of compounds **8** and **9,** however, gomisin G with β-Bz substituent at C-6 was found to show strong anti-HBV effect.[10] Compound **8** had a similar structure with gomisin G except for the location of OCH_2O group, which was attached to C-12 and C-13 in **8**, and to C-2 and C-3 in gomisin G. Thus, the OCH_2O group on aromatic rings and the substitute group at C-6 would also influence the anti-HBV activity. For the lignans with an *R*-biphenyl configuration (**10**–**12**), the OH group on aromatic ring or in the cyclooctane ring seems to decrease the activity. Compound **12** with 7β-OH and 14-OH showed inactivity. Compound **11** with 14-OH showed weaker activity than **10**. However, to fully elucidate the structural determinants for anti-HBV activity of dibenzocyclooctane lignans, further investigations are necessary.

Acknowledgments

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11. Plant material: the fruits of *Schisandra wilsoniana* were collected in August of 2005 at Heqing, Yunnan, China. The identity of the plant material was verified by Professor Dao-Feng Chen at Fudan University, and a voucher specimen (DFC-MWH20050801) has been deposited in the Herbarium of Materia Medica, Department of Pharmacognosy, School of Pharmacy, Fudan University, Shanghai, People's Republic of China.
12. Extraction and isolation: the dried and powdered material (8 kg) was extracted exhaustively with 95% aq EtOH at rt. The filtrate was evaporated in vacuo to give a residue (1600 g), a portion of which (1500 g) was suspended in H_2O (1.5 L) and partitioned with Et_2O (3×1 L). The resulting etheral soln. was concentrated to yield a residue (400 g), part of which (200 g) was subjected to column chromatography on silica gel eluted successively with petroleum ether (PE)-acetone (50:1, 30:1, 20:1, 10:1, 4:1, 3:1, 3:2, 1:1) and acetone to yield fractions 1–9. Fraction 2 (35 g) was subjected to repeated silica gel CC with PE–acetone (30:1) to give **10** (10 g). Fraction 4 (14 g) was applied to repeated silica gel CC with PE–EtOAc (6:1) to afford **11** (40 mg). Fraction 5 (20 g) was applied to repeated silica gel CC with PE–acetone (5:1), followed by CC on RP_{18} gel with MeOH–H_2O (7:3–8:2) to give five sub-fractions (Fr. 5a–e). Fr. 5a (200 mg) was subjected to prep. TLC with PE–EtOAc (3:1) to give **9** (35 mg). Frs. 5b (120 mg), 5c (30 mg), 5d (20 mg) and 5e (60 mg) were applied to this same procedure to yield **1** (10 mg) and **5** (4 mg), **8** (4 mg), **3** (5 mg), **2** (20 mg), respectively. Fraction 6 (23 g) was applied to repeated silica gel CC with PE–acetone (4:1), followed by CC on RP_{18} gel with MeOH–H_2O (6:4–7:3), and prep. TLC with PE–EtOAc (3:1) to give **12** (15 mg), **7** (6 mg), **6** (5 mg) and **4** (20 mg).
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20. Analytical data for **1** ((a*S*, 5*S*, 6*S*, 7*S*)-5,6,7,8-tetrahydro-1,2,3,13-tetramethoxy-6,7-dimethyl-5-[(2*Z*)-methylbut-2-enoyl]oxybenzo[3,4]cycloocta[1,2-*f*][1,3]benzodioxol-6-ol): white powder, $[\alpha]_D^{22}$ −17.5 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 221 (4.18), 290 (3.27) nm; CD (*c* 0.04, MeOH) $\Delta\varepsilon_{230}$ = +33.5, $\Delta\varepsilon_{255}$ = −29.1; IR (CH_2Cl_2) ν_{max} 3442, 2930, 2850, 1713, 1595, 1460, 1331, 1108, 1047 cm^{-1}; for ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) data, see Tables 1 and 2; EIMS *m*/*z* 514 $[M]^+$ (28), 83 (100), 55 (91), 343 (79), 342 (73), 300 (70), 414 (60), 312 (41), 43 (37), 514 (28); HREIMS *m*/*z* 514.2209 (calcd for $C_{28}H_{34}O_9$, 514.2203).
21. Analytical data for **2** ((a*S*, 5*S*, 6*S*, 7*S*)-5,6,7,8-tetrahydro-1,2,3,13-tetramethoxy-6,7-dimethyl-5-[(2*E*)-methylbut-2-enoyl]oxybenz-o[3,4]cyclooctа[1,2-*f*][1,3]benzodioxol-6-ol.): white powder, $[\alpha]_D^{22}$ −47.8 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.83), 285 (3.68) nm; CD (*c* 0.04 MeOH) $\Delta\varepsilon_{230}$ = +0.2, $\Delta\varepsilon_{245}$ = −26.9; IR ν_{max} (CH_2Cl_2) 3435, 2932, 2853, 2360, 1707, 1459, 1266, 1128, 1048, 735 cm^{-1}; for ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) data, see Tables 1 and 2; EIMS *m*/*z* 514 $[M]^+$ (6), 55 (100), 43 (92), 83 (81), 57 (69), 69 (60), 41 (56), 44 (44), 97 (42), 414 (32); HRESIMS *m*/*z* 537.2103 ($[M+Na]^+$) (calcd for $C_{28}H_{34}O_9Na$ $[M+Na]^+$, 537.2101).
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23. Analytical data for **3** ((a*S*, 5*S*, 6*S*, 7*S*)-5,6,7,8-tetrahydro-1,2,3,13-tetramethoxy-6,7-dimethyl-5-[benzoyl]oxybenzo[3,4]cyclooctа[1,2-*f*][1,3]benzodioxol-6-ol.): white powder, $[\alpha]_D^{22}$ −70.5 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 217 (4.78) nm; CD (*c* 0.04 MeOH) $\Delta\varepsilon_{222}$ = −4.6, $\Delta\varepsilon_{241}$ = −28.9; IR ν_{max} (CH_2Cl_2) 3445, 2930, 2850, 2349, 1716, 1597, 1456, 1108, 713 cm^{-1}; for ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) data, see Tables 1 and 2; EIMS *m*/*z* 536 $[M]^+$ (7), 105 (100), 414 (32), 77 (31), 343 (28), 43 (25), 342 (23), 44 (17), 312 (16); HREIMS *m*/*z* 536.2045 (calcd for $C_{30}H_{32}O_9$, 536.2046).
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25. Analytical data for **4** ((a*S*, 5*S*, 6*S*, 7*S*)-5,6,7,8-tetrahydro-1,2,3,11,12-pentamethoxy-10-*OH*-5-[(2*E*)-methylbut-2-enoyl]oxy-6,7-dimethyldibenzo[*a*, *c*]cyclooctа-6-ol.): white powder, $[\alpha]_D^{22}$ −53.5 (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ε) 214 (4.78) nm; CD (*c* 0.02 MeOH) $\Delta\varepsilon_{204}$ = +34.5, $\Delta\varepsilon_{233}$ = −28.7, $\Delta\varepsilon_{248}$ = −11.9, $\Delta\varepsilon_{252}$ = −12.2; IR ν_{max} (CH_2Cl_2) 3440, 2937, 2849, 1709, 1595, 1457, 1404, 1330, 1260, 1124, 1090, 735 cm^{-1}; for ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) data, see Tables 1 and 2; HRESIMS *m*/*z* 539.2262 ($[M+Na]^+$) (calcd for $C_{28}H_{36}O_9Na$ $[M+Na]^+$, 539.2257).
26. Analytical data for **5** ((a*S*, 5*S*, 6*S*, 7*S*)-5,6,7,8-tetrahydro-1,2,3,13-tetramethoxy-6,7-dimethyl-5-[isobutyryl]oxybenzodioxolo[3,4]cyclooctа[1,2-*f*][1,3]benzodioxol-6-ol.): white powder, $[\alpha]_D^{22}$ +20 (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.65), 290 (sh, 3.16) nm; CD (*c* 0.02 MeOH) $\Delta\varepsilon_{229}$ = +14.5, $\Delta\varepsilon_{255}$ = −6.4; IR ν_{max} (CH_2Cl_2) 3440, 2850, 1733, 1595, 1457, 1195, 713 cm^{-1}; for ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) data, see Tables 1 and 2; EIMS *m*/*z* 502 $[M]^+$ (6), 43 (100), 59 (61), 55 (54), 41 (53), 57 (52), 71 (42), 44 (42), 69 (40), 414 (16); HRESIMS *m*/*z* 525.2098 ($[M+Na]^+$) (calcd for $C_{30}H_{32}O_9Na$ $[M+Na]^+$, 525.2101).
27. Analytical data for **6** ((a*S*, 5*S*, 6*S*, 7*S*)-5,6,7,8-tetrahydro-1,2,3,13-tetramethoxy-6,7-dimethyl-5-[*Ac*]oxybenzo[3,4]cyclooctа[1,2-*f*][1,3]benzodioxol-6-ol.): white powder, $[\alpha]_D^{22}$ −32 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.34), 290 (sh, 3.24) nm; CD (*c* 0.04 MeOH) $\Delta\varepsilon_{229}$ = +53.9, $\Delta\varepsilon_{255}$ = −43.4; IR ν_{max} (CH_2Cl_2) 3445, 2935, 1734, 1596, 1463, 1377, 1268, 1047, 735 cm^{-1}; for ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) data, see Tables 1 and 2; EIMS *m*/*z* 474 $[M]^+$ (36), 414 (100), 343 (84), 342 (67), 43 (59), 312 (56), 300 (38), 313 (36); HRESIMS *m*/*z* 497.1784 ($[M+Na]^+$) (calcd for $C_{25}H_{30}O_9Na$ $[M+Na]^+$, 497.1788).
28. Analytical data for **7** ((a*S*, 5*S*, 6*S*, 7*S*)-5,6,7,8-tetrahydro-1,2,3,11,12-pentamethoxy-10-*OH*-5-benzoyloxyl-6,7-dimethyldibenzo[*a*,*c*]cyclooctа-6-ol.): white powder, $[\alpha]_D^{22}$ −58.5 (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ε) 217 (4.76), 260 (sh, 3.53) nm; CD (*c* 0.02 MeOH) $\Delta\varepsilon_{217}$ = +5.0, $\Delta\varepsilon_{238}$ = −17.4, $\Delta\varepsilon_{248}$ = −7.6, $\Delta\varepsilon_{254}$ = −8.3; IR ν_{max} (CH_2Cl_2) 3446, 2936, 2849, 2359, 1716, 1455, 1120, 1714 cm^{-1}; for ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) data, see Tables 1 and 2; EIMS *m*/*z* 538 $[M]^+$ (2), 105 (100), 77 (35), 122 (28), 43(12), 51 (10), 44 (9), 106 (7), 75 (7), 416 (4); HREIMS *m*/*z* 538.2197 (calcd for $C_{30}H_{34}O_9$, 528.2203).
29. Anti-HBV tests: drug stock solutions were prepared in DMSO and stored at −70 °C. Upon dilution into culture medium, the final DMSO concentration was <1% DMSO (v/v), a concentration without effect on cell replication. Cell culture and other procedures were the same as those reported previously (Wu, T.; Huang, H.; Zhou, P. *Zhongguo Bing Du Xue* **1998**, *13*, 45). A HepG2-derived human hepatablastoma cell line, 2.2.15, was used in this study, which was transfected with cloned HBV DNA to produce HBV particles. All stock cultures were grown in T-25 flasks containing the DMEM supplemented with 10% (v/v) fetal bovine serum, 0.03% (v/v) L-glutamine, 100 μg/mL penicillin, 100 μg/mL streptomycin, and 380 μg/mL G418 at 37 °C in a humidified atmosphere containing 5% CO_2. After the HepG2 2.2.15 cell suspensions seeded in 24-well microtiter plates were cultured for 48 h, they were incubated at 37 °C for 9 days in the presence of various concentrations of drugs (200, 100, 50, and 25 μg/mL, respectively) from DMSO-diluted stock, and the medium was refreshed every 3 days. Then the culture supernatants were harvested to detect the HBsAg and HBeAg secretion using diagnostic ELISA kits (Shanghai SIIC KEHUA Biotech Co., Ltd) as described in triplicate, and the SEM (standard error of the mean) of inhibition values varied no more than 5%. Cell damage was assessed using MTT assay.