Norlignans, Acylphloroglucinols, and a Dimeric Xanthone from *Hypericum chinense*

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Received July 2, 2010

Two new norlignans, hyperiones A (**1**) and B (**2**), three new acylphloroglucinols, aspidinol C (**3**) and hyperaspidinols A (**5**) and B (**6**), the known compound aspidinol D (**4**), and the symmetrical dimeric xanthone hyperidixanthone (**7**) were isolated from *Hypericum chinense*. Their structures were established by spectroscopic analysis. In an antibacterial assay using a panel of multidrug-resistant (MDR) strains, compounds **3** and **4** exhibited promising activity against the NorA efflux protein overexpressing MDR *Staphylococcus aureus* strain SA-1199B with a minimum inhibitory concentration (MIC) of 2 μg/mL (8.4 μM) and 4 μg/mL (16.8 μM), respectively. The positive control antibiotic norfloxacin showed activity at MIC 32 μg/mL (100 μM).

There is an urgent need to develop new classes of antibacterial agents to combat bacterial multidrug resistance (MDR), which can contribute to the failure of many classes of antibiotics in clinical therapy. Multidrug-resistant *Staphylococcus aureus* infections, particularly those caused by methicillin-resistant *S. aureus* (MRSA), have been a major threat to public health in hospitals and the community over the past decade. Despite the development of new compounds such as linezolid, MRSA infections remain a considerable concern due to the few agents that can be used in their treatment. In 2002, MRSA strains fully resistant to vancomycin were isolated in the United States.[1] Resistance to linezolid has also been reported in some patients followed by prolonged antibiotic treatment in the United States.[2] Therefore, there is a need to develop new classes of antibiotics to fight the problem of drug resistance. The genus *Hypericum* is a valuable source of naturally occurring inhibitors of bacterial growth, including compounds with activity against MDR and MRSA.[3] As part of our ongoing research on plant metabolites with activity against *S. aureus* antibiotic-resistant variants,[4] we have studied the chemistry and antibacterial activity of extracts of *Hypericum chinense* L. (Hypericaceae). This herb is widespread in China and has long been used for the treatment of fever, sepsis, acute laryngo-pharyngitis, conjunctivitis, hepatitis, and snake bite.[5] Former research on its chemical constituents resulted in the characterization of pharmacologically active compounds such as the antimicrobial compounds chinensins I and II,[6] the anti-HIV agents biyouyanagin A[7] and biyouyanagin B,[8] and several substituted xanthones.[9,10] A series of spiro-lactones, hyperolactones A−D, were also discovered.[8,11,12]

Our current phytochemical investigation of *H. chinense* has resulted in the isolation of two new norlignans (**1** and **2**), three new acylphloroglucinols (**3**−**6**), a known compound (**4**), and a symmetrical dimeric xanthone (**7**). Against a panel of multidrug-resistant strains, two acylphloroglucinols (**3** and **4**) showed promising activity against the NorA overexpressing MDR *Staphylococcus aureus* strain SA-1199B.

Results and Discussion

Powdered leaves of *H. chinense* were extracted with 95% EtOH, and the extract was separated into petroleum ether-soluble, EtOAc-soluble, and MeOH-soluble fractions. Repeated chromatography on the EtOAc-soluble fraction yielded compounds **1**−**6**, sesamin, and betulinic acid. Similarly, the EtOAc-soluble fraction of the roots afforded compound **7**.

Compound **1** was obtained as colorless oil. HREIMS (*m*/*z* 340.0950) indicated a molecular formula of $C_{19}H_{16}O_6$, which was supported by its ^{1}H and ^{13}C NMR spectra (Table 1). The ^{13}C NMR spectrum revealed a carbonyl [δ 196.7 (C-7)], which was coupled to two aromatic protons [δ 7.47 (d, J = 1.8 Hz, H-2) and 7.55 (dd, J = 8.2 Hz, J = 1.8 Hz, H-6)] in the HMBC spectrum. These aromatic hydrogens formed a piperonyl group with the remaining protons at δ 6.87 (d, J = 8.2 Hz, H-5) and the methylenedioxy protons downfield at δ 6.13 (s, OCH_2O-10). The two methylenedioxy protons correlated with the carbons at δ 148.3 (C-3) and 152.0 (C-4), confirming a 3,4-methylenedioxyphenyl (piperonyl) ketone system.[11] Similarly, another 3′,4′-methylenedioxyphenyl moiety was established by the ^{1}H and ^{13}C NMR, DEPT, and HMBC data. These two fragments were confirmed by the EIMS, which exhibited fragment ion peaks at *m*/*z* 149 (base peak) and 121, corresponding to the acylpiperonyl ion $[ArCO]^+$ and piperonyl ion $[Ar]^+$ fragments, generated due to bond cleavage at C-7/C-8 and C-1′/C-7′, respectively.[13]

The remaining bridge assignment was established as a tetrahydrofuran ring according to the NMR spectroscopic data. A signal at δ 4.86 (dd, J = 9.8 Hz, J = 5.8 Hz, H-7′), which in the HMBC spectrum was coupled to C-2′ and C-6′, was assigned to the benzylic oxygen-bearing carbon C-7′ (δ 82.0). The correlation between H-7′ and H-8′a (δ 2.51, m) and H-8′b (δ 2.30, m) shown in the ^{1}H−^{1}H COSY spectrum indicated a C-7′ and C-8′ (δ 38.3) linkage. The multiplet signals centered at δ 4.28 (H-9a) and 4.17 (H-9b) were assigned to the oxygen-bearing carbon C-9 (δ 70.3). The multiplet centered at δ 4.09 (m, H-8), assigned to C-8 at δ 47.0, displayed a correlation with C-7, C-9, and C-8′, indicating a tetrahydrofuran moiety of cyclo[C8−C9−O−C7′−C8′], with C-8 connected to the C-7 carbonyl and C-7′ to the phenyl ring at C-1′. This structure was therefore a norlignan compound with a $C_6C_3-C_2C_6$ skeleton. NOE experiments (Table 1) indicated a *cis*-configuration between H-8 and H-7′, which were both close to H-9a and H-8′a in space. Thus, the relative configuration of **1** was elucidated as shown, and this compound was given the trivial name hyperione A.

Compound **2** was obtained as a white, amorphous powder. The molecular formula $C_{19}H_{16}O_6$ was indicated by HREIMS, the same as that of **1**. Analyzing the NMR data, compound **2** was deduced to have the same planar structure as **1**. However, **1** and **2** showed different R_f values in TLC, and their ^{1}H NMR spectra revealed an obvious disparity in the splitting of H-9a, H-9b, and H-7′. Therefore, **2** was presumed to be a stereoisomer of **1** with the differences at the bridging tetrahydrofuran ring. In the ^{1}H NMR spectrum, an NOE between H-8 and H-7′ was observed. The H-8 proton showed no enhancement when H-7′ was irradiated, and *vice versa*. Furthermore, according to the NOE experiments, H-8 was close to

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10.1021/np1004483
Published on Web 11/02/2010

Table 1. NMR Spectroscopic Data for Hyperiones A (**1**) and B (**2**)

	1 ($CDCl_3$)				**2** (acetone-d_6)			
no.	δ_C, mult.		δ_H (J in Hz)	HMBC (C no.)	δ_C, mult.		δ_H (J in Hz)	HMBC (C no.)
1	131.1	C			132.1	C		
2	108.2	CH	7.47, d (1.8)	3, 4, 6, 7	108.7	CH	7.48, d (1.8)	3, 4, 6, 7
3	148.3	C			149.3	C		
4	152.0	C			152.9	C		
5	108.0	CH	6.87, d (8.2)	1, 3, 4	108.6	CH	6.98, d (8.2)	1, 3
6	124.7	CH	7.55, dd (8.2, 1.8)	2, 4, 7	125.7	CH	7.70, dd (8.2, 1.8)	2, 4, 7
7	196.7	C			198.1	C		
8[a]	47.0	CH	4.07, m	7, 9, 8′	46.9	CH	4.28, m	9
9	70.3	CH_2	(a) 4.28, m	7, 8, 7′, 8′	71.0	CH_2	(a) 4.40, t (8.2)	7, 8, 7′, 8′
			(b) 4.17, m	7, 8, 7′, 8′			(b) 3.95, dd (8.2, 6.3)	7
1′	135.1	C			137.5	C		
2′	106.7	CH	6.93, d (1.8)	4′, 6′, 7′	107.0	CH	6.92, d (1.6)	4′, 6′, 7′
3′	147.7	C			148.6	C		
4′	147.1	C			147.7	C		
5′	108.0	CH	6.76, d (7.8)	1′, 3′	108.5	CH	6.80, d (7.8)	1′, 3′
6′	119.7	CH	6.84, dd (7.8, 1.8)	2′, 4′, 7′	120.0	CH	6.87, dt (7.8, 1.6)	2′, 4′, 7′
7′[b]	82.0	CH	4.86, dd (9.8, 5.8)	2′, 6′	81.4	CH	4.83, t (7.8, 7.0)	2′, 6′
8′	38.3	CH_2	(a) 2.51, m	7, 8, 9, 7′	39.0	CH_2	(a) 2.60, m	7, 8, 9
			(b) 2.30, m	7, 7′			(b) 2.05, m	7′
10	101.9	OCH_2O	6.13, s	3, 4	103.1	OCH_2O	6.18, s	3, 4
9′	101.0	OCH_2O	5.96, s	3′, 4′	101.9	OCH_2O	5.99, s	3′, 4′

[a] NOE of H-8 (**1**): H-2, H-6, H-7′, H-9a, H-8′a; NOE of H-8 (**2**): H-2, H-6, H-8′b. [b] NOE of H-7 (**1**): H-2′, H-6′, H-8, H-9a, H-8′a; NOE of H-7 (**2**): H-2′, H-6′, H-8′a.

Table 2. NMR Spectroscopic Data of Aspidinols C (**3**) and D (**4**)

	3 (acetone-d_6)				**4** ($CDCl_3$)			
no.	δ_C, mult.		δ_H (J in Hz)	HMBC (C no.)	δ_C, mult.		δ_H (J in Hz)	HMBC (C no.)
1	104.7	C			104.4	C		
2	162.8	C			159.8	C		
3	103.4	C			103.5	C		
4	163.5	C			163.0	C		
5	90.2	CH	6.13, s	1, 3, 4, 6	91.6	CH	5.97, s	1, 3, 4, 6
6	160.2	C			161.1	C		
7	6.5	CH_3	1.91, s	2, 3, 4	7.1	CH_3	2.01, s	2, 3, 4, 5
8	55.0	OCH_3	3.81, s	4	55.6	OCH_3	3.82, s	4
1′	205.8[a]	C			210.5	C		
2′	52.6	CH_2	2.96, 2H, d (6.7)	1′, 3′, 4′, 5′	46.0	CH	3.75, m (6.7)	1′, 3′, 4′, 5′
3′	25.0	CH	2.24, m (6.7)	2′, 4′, 5′	27.0	CH_2	(a) 1.83, m	1′, 2′, 4′, 5′
							(b) 1.42, m	1′, 2′, 4′, 5′
4′	22.2	CH_3	0.94, d (6.7)	2′, 3′, 5′	12.0	CH_3	0.92, t (7.4)	2′, 3′
5′	22.2	CH_3	0.94, d (6.7)	2′, 3′, 4′	16.7	CH_3	1.18, dd (7.1, 1.2)	1′, 2′, 3′
2-OH			13.63, s	2, 3			13.62, s	
6-OH			10.12, s				9.67, s	

[a] Signal shown in $CDCl_3$ but overlapped in acetone-d_6.

H-8′b, while H-7′ to H-8′a, which confirmed a *trans*-configuration, and **2** was given the trivial name hyperione B.

In the isolation, a lignan was obtained and identified as sesamin by its NMR and X-ray data. Considering the reference,[11] hyperione B (**2**) was therefore presumed to be derived from sesamin by losing a $-CH_2-$ moiety in the plant, and the analogue hyperione A (**1**) could be derived from asarinin, an epimer of sesamin (Scheme S1, Supporting Information).

Aspidinol C (**3**), yellow crystals, had the molecular formula $C_{13}H_{18}O_4$ (by HREIMS). Six aromatic carbon signals (δ_C 163.5, 162.8, 160.2, 104.7, 103.4, 90.2) were displayed in its ^{13}C NMR spectrum (Table 2), in which three signals were downfield and over δ_C 160, implying a phloroglucinol skeleton. The ^1H NMR spectrum showed two hydroxy groups and a methoxy group, which were likely to be the three substituents of a phloroglucinol skeleton. This conclusion was confirmed by HMBC correlations (Figure 1a) between these groups and the corresponding aromatic quaternary carbons. The aromatic methyl (δ_H 1.91, s) was coupled to OH-bearing C-2 (δ_C 162.8) and C-3 (δ_C 103.4) and to OCH_3-bearing C-4 (δ_C 163.5) and was therefore placed at position 3. The only aromatic proton (δ_H 6.13, s) was coupled to C-1, C-3, C-4, and C-6, and therefore it was placed at position 5, *para* to C-2. The positions of these substituents were further supported by their NOE

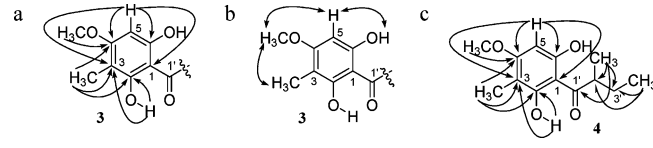


Figure 1. Key HMBC and NOE correlations: (a) key HMBC correlations of **3**; (b) key NOE correlations of **3**; (c) key HMBC correlations of **4**.

correlations (Figure 1b). The chain attached to C-1 included a carbonyl (C-1′), a methylene doublet (C-2), a methine septet (C-3), and a six-hydrogen methyl doublet (C-4 and C-5). The other aromatic hydroxyl group was placed at C-6 on the basis of its NOE spectrum (Figure 1b). Considering the coupling constant together with its $^1H-^1H$ COSY correlations, an isobutyl side chain was deduced [$-CH_2-CH-(CH_3)_2$]. In the HMBC spectrum, the methylene doublet was coupled to C-1′, and therefore an isopentanoyl chain was directly attached to C-1 of the phloroglucinol skeleton. The carbonyl group at C-1′ and the 2-OH formed an intramolecular H-bond. Furthermore, the base peak at m/z 181 $[M - C_4H_9]^+$ in the EIMS was attributable to the fragment generated from cleavage between C-1 and C-1′. Therefore, **3** was identified as 1-[2,6-dihydroxy-4-methoxy-3-methylphenyl]-3′-methylbutan-1′-one, a new

Table 3. NMR Spectroscopic Data of Hyperaspidinols A (**5**) and B (**6**)

	5 ($CDCl_3$)				**6** ($CDCl_3$)			
no.	δ_C, mult.		δ_H (J in Hz)	HMBC(C no.)	δ_C, mult.		δ_H (J in Hz)	HMBC (C no.)
1	108.1	C			107.6	C		
2	163.0	C			163.0	C		
3	112.1	C			112.1	C		
4	162.3	C			162.2	C		
5	104.0	C			104.0	C		
6	153.2	C			152.9	C		
7	206.9	C			211.7	C		
8	53.3	CH_2	(a) 3.14, dd (16.0, 5.7)	7, 9, 10, 11	45.8	CH_2	4.00, m	7, 9, 11
			(b) 2.83, dd (16.0, 7.7)	7, 9, 10, 11				
9	24.9	CH	2.20, m	8, 10, 11	27.7	CH	(a) 1.82, m	7, 10, 11
							(b) 1.35, m	7, 10, 11
10	22.7	CH_3	0.84, d (6.7)	8, 9, 11	11.5	CH_3	0.77, dd (7.8, 7.3)	8, 9
11	22.4	CH_3	0.81, d (6.7)	8, 9, 10	15.6	CH_3	1.12, dd (6.8, 2.4)	7, 8, 9, 10
12	8.4	CH_3	2.12, s	2, 3, 4, 5	8.5	CH_3	2.18, s	2, 3, 4
13	60.3	OCH	3.71, s	4	60.2	OCH_3	3.77, s	4
14	19.5	CH_2	(a) 2.95, d (17.0)	4, 5, 6, 15, 18	19.3	CH_2	(a) 3.01, dd (16.3, 2.0)	4, 5, 6, 15, 16, 18
			(b) 2.67, dd (17.0, 5.3)	4, 5, 6, 15, 16			(b) 2.74, dd (16.3, 6.8)	5, 6, 15, 16
15	44.3	CH	2.87, m	5, 16	44.5	CH	2.93, m	5, 14, 16
16	37.7	CH_2	(a) 2.40, m	15, 18	37.7	CH_2	(a) 2.47, m	15, 18
			(b) 1.84, m	14, 15, 17, 1″			(b) 1.88, m	14, 17, 1″
17	81.6	CH	5.24, dd (16.0, 7.7)	1″, 2″, 6″	81.8	CH	5.31, m	16, 1″, 2″, 5″
18	109.3	C			109.4	C		
1′	135.5	C			135.5	C		
2′	106.3	CH	7.01, d (1.8)	18, 4′, 6′	106.2	CH	7.08, d (1.5)	3′, 6′
3′	147.8	C			147.7	C		
4′	147.8	C			147.7	C		
5′	108.0	CH	6.79, d (7.5)	6′	107.9	CH	6.85, d (overlapped)	
6′	119.0	CH	7.00, dd (7.9, 1.8)	18, 2′, 4′	119.0	CH	7.06, dd (6.8, 1.5)	18, 2′, 3′, 4′
7′	101.3	CH	5.98, d (1.3)	3′, 4′	101.2	CH	6.03, s	3′, 4′
1″	135.1	C			135.1	C		
2″	106.6	OCH_2O	6.78, d (overlapped)	17, 4′, 6	106.5	OCH_2O	6.83, d (overlapped)	
3″	147.1	C			147.7	C		
4″	147.1	C			147.0	C		
5″	108.0	CH	6.70, d (7.9)	1″, 3″, 4″	107.9	CH	6.75, d (7.9)	
6″	119.4	CH	6.75, d (7.9)	17, 2″, 4″	119.4	CH	6.80, dd (7.9, 1.3)	
7″	101.0	OCH_2O	5.91, d (1.3)	3″, 4″	101.0	OCH_2O	5.96, s	3″, 4″
2-OH			13.87, s	1, 2, 3			13.80, s	1, 2, 3

compound of the acylphloroglucinol type, which was given the trivial name aspidinol C.

Compound **4** showed fragment ion peaks similar to those of **3** in the EIMS, and the base peak at 181. Its ^1H and ^{13}C NMR spectra indicated the same skeleton as **3**, with the exception of the C-1 side chain. The substituent at C-1 included a methine multiplet (C-2′), a methylene multiplet (C-3′), a methyl triplet (C-4′), and a methyl doublet (C-5′). By examining the coupling constants, the triplet methyl was placed next to the methylene. In the $^1H-^1H$ COSY spectrum, the methylene was coupled to the methine, which was coupled to the methyl doublet. In the HMBC spectrum the methylene multiplet was coupled to C-2′ and C-5′, while the methyl doublet was coupled to C-1′, C-2′, and C-3′. Therefore, a 2-methylbutanoyl side chain was deduced, which was directly attached to C-1 of the aspidinol skeleton to form an intramolecular hydrodren bond with the 2-OH. This compound was isolated from *Eucalyptus pulverulenta* in 1984.[14] Here we report its complete NMR assignments for the first time (Table 2) and have given it the trivial name aspidinol D.

Compound **5** was obtained as pale yellow oil. The HRESIMS ($[M + H]^+$ 561.21066, calcd for $C_{32}H_{33}O_9$ 561.21191) suggested a molecular formula of $C_{32}H_{32}O_9$, consistent with its ^1H and ^{13}C NMR spectroscopic data (Table 3). Further inspection of the spectra indicated signals similar to those of **1** and **3** combined, with signals reminiscent of an aspidinol skeleton (resembling that of **3**) and two methylenedioxyphenyl groups (resembling those in compound **1**). However, **5** was not a simple mixture of the two compounds mentioned above, but it was a complex single compound formed by combination of the two aspidinol moieties. This was supported by its TLC and HPLC chromatograms taken together with spectroscopic evidence from its HMBC spectrum.

Through careful examination of the ^1H and ^{13}C NMR and HMBC spectra, the carbons and protons of the aspidinol skeleton and two methylenedioxyphenyl groups were unambiguously assigned by direct reference to compounds **1** and **3**. The two protons H-5 and 6-OH in **3** were assumed to be substituted by alkyl groups in **5**, since no such signals were displayed in the ^1H NMR spectrum of **5**. The bridge between the three moieties deduced above consisted of two methylene groups (δ_C 37.7, 19.5), two methine groups (δ_C 81.6, 44.3), a quaternary carbon (δ_C 109.3), which were observed in the DEPT spectrum, and two oxygen atoms left according to the given molecular formula.

The $^1H-^1H$ COSY spectrum (Table 3) provided the evidence of how these saturated carbons were bonded: H-17 of a methine was coupled to H_2-16a/b, which were both coupled to H-15, with the latter coupling to H_2-14a/b. In the HMBC spectrum, H_2-14a/b were coupled to C-4, C-5, and C-6 of the aspidinol skeleton. The H-17 proton (δ_H 5.24, q), which was attached to an oxygen-bearing carbon, was coupled to three aromatic carbons (C-1′, C-2′, and C-6′) from one of the methylenedioxyphenyl groups. H-14b and H-16b, together with H-2′ and H-6′ from the other methylenedioxyphenyl group, were all coupled to a downfield quaternary carbon (δ_C 109.3, C-18), which, from its downfield nature, was attached to two oxygens (Figure 2a). Considering the degree of unsaturation as 17, the structure was deduced as shown. The ROESY spectrum elucidated a *cis*-configuration of H-15 and H-17. Compound **5** was therefore identified as a new acylphloroglucinol derivative, and it was given the trivial name hyperaspidinol A.

Compound **6** was obtained as pale yellow oil. The molecular formula of $C_{32}H_{33}O_9$ was assigned by the HRESIMS $[M + H]^+$, and its ^1H and ^{13}C NMR spectra closely resembled those of **5**. The spectroscopic data of the side chain resembled that of **4**, instead of

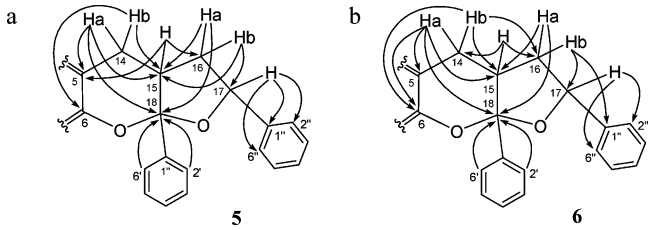


Figure 2. Key HMBC correlations: (a) key HMBC correlations of **5**; (b) key HMBC correlations of **6**.

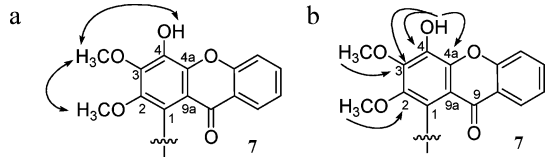


Figure 3. Key NOE and HMBC correlations: (a) key NOE correlations of **7**; (b) key HMBC correlations of **7**.

the isopentanoyl signals seen in compound **3**. Analysis of the HMBC spectra (Figure 2b) confirmed that the differences between **5** and **6** were solely due to the presence of a 2-methylbutanoyl side chain (in **6**) rather than a 3-methylbutanoyl side chain seen in **5**. The structure of compound **6** is, therefore, as shown, and it was named hyperaspidinol B.

Compound **7** was obtained as yellow crystals, and the molecular formula $C_{30}H_{22}O_{10}$ was determined by an accurate mass measurement of 542.1210 (calcd for $C_{30}H_{22}O_{10}$, 542.1213) in the HREIMS. The EIMS showed a molecular ion peak at *m*/*z* 542 $[M]^+$ and an obvious peak at *m*/*z* 271, which was presumably due to one-half of the molecule. The ^{1}H NMR spectrum showed four aromatic protons of one benzene ring, two methoxy groups, and one hydroxy group. The ^{13}C NMR data suggested a typical substituted xanthone skeleton[15] with two methoxy carbons and 12 aromatic carbons. These data indicated a structure consisting of two identical substituted xanthone moieties. Two methoxy groups and the one hydroxy group were attached to the substituted benzene ring, with the remaining free position (C-1) being the point of attachment to the other xanthone.

The locations of these substituents were supported by NOE experiments for the protons of compound **7** (Figure 3a). There existed a correlation between 2-OCH_3 and 3-OCH_3 (δ_H 3.43 and 3.95), respectively, in the NOE spectrum, and further radiation on the 3-OCH_3 resulted in an enhancement of the OH proton at δ_H 9.98 (4-OH). Therefore the 3-OCH_3 group (δ_H 3.95) had an *ortho* relationship with the other groups. The bond between the two xanthones was then expected to be either at C-1 or at C-4. The bonding position at C-1 was suggested by correlations between the 4-OH proton and C-3, C-4, and C-4a in the HMBC spectrum of **7**, and this linkage was further supported by the existence of C-9a at δ_C 115.8. This structure was also supported by its molecular ion (542 $[M]^+$) and the main fragment ions of cleavage in the EIMS (Figure 4). Compound **7** possessed a symmetrical dimeric xanthone skeleton and was therefore identified as 1-[4′-hydroxy-2′,3′-dimethoxy-1′-xanthonyl]-4-hydroxy-2,3-dimethoxyxanthone, with the trivial name hyperidixanthone.

Betulinic acid was also isolated and identified by comparing its MS and NMR data with those in the literature.[16,17]

The isolates were tested for antibacterial activity against several resistant *S. aureus* strains, and only **3** and **4**, with the simple aspidinol skeleton, showed promising activity against all of the strains. Compounds **5** and **6**, possessing a more complex skeleton, exhibited no inhibitory activity (Table 4). Among the tested strains, SA-1199B possesses the NorA efflux protein, which confers resistance to certain fluoroquinolones and quaternary ammonium antiseptics. Against this strain, both **3** (2 μg/mL, 8.4 μM) and **4** (4 μg/mL, 16.8 μM) were more active than the control antibiotic norfloxacin (32 μg/mL, 100 μM). For MDR strain XU212, which possesses the TetK efflux transporter and is resistant to both tetracycline and methicillin, **4** showed inhibitory activity comparable to norfloxacin (8 μg/mL, 33.2 μM). A hospital epidemic MRSA,[18] EMRSA-16, was much more sensitive to **3** and **4** than to norfloxacin (Table 4). However, **3** and **4** showed moderate activity but were less active than the positive control antibiotic against the standard *S. aureus* strain ATCC 25923, the erythromycin-resistant strain RN4220 that carries the MsrA macrolide efflux protein, and the drug-resistant strain EMRSA-15.[19] These findings are encouraging, and further investigation of aspidinol as a template is suggested.

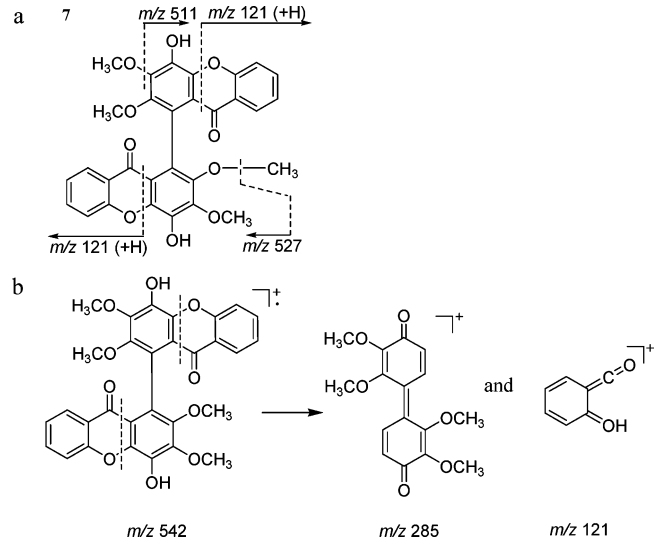


Figure 4. Generation of main fragment ions in the EIMS of **7**.

Table 4. MICs (μg/mL) of **3**, **4**, a Mixture of **5** and **6**, and Norfloxacin

compound	SA1199B	XU212	ATCC25943	RN4220	EMRSA-15	EMRSA-16
3	2	32	8	16	8	4
4	4	8	8	8	4	4
5 and **6**	>128	>128	>128	>128	>128	>128
norfloxacin	32	8	0.5	0.5	0.5	128

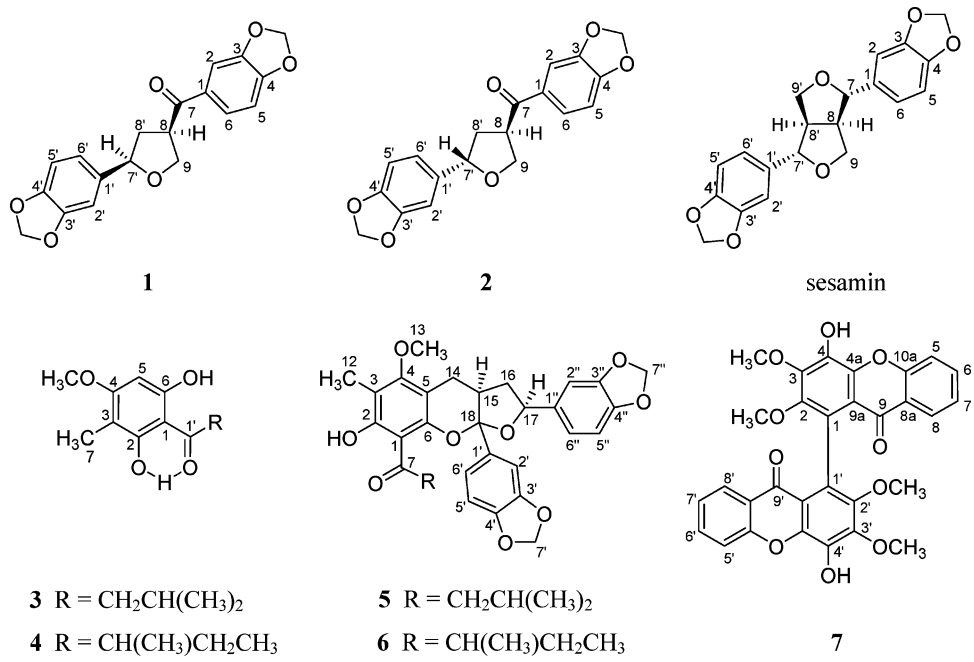
Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO P-1020 polarimeter. IR spectra were recorded using an Avatar 360 ESP FTIR spectrophotometer, and UV spectra on a Hitachi U-2900 spectropolarimeter. ^{1}H and ^{13}C NMR spectra were obtained on a Varian Mercury Plus 400 MHz spectrometer. EIMS were obtained on an Agilent 5973N MSD spectrometer, and HREIMS on an IonSpec 4.7 T FTMS. Column chromatography (CC) was carried out with silica gel (10−40 μm, Qingdao Marine Chemical Plant). Fractions obtained from CC were monitored by TLC (silica gel plate HGF254, 10−40 μm, Qingdao Marine Chemical Plant, Yantai, China; RP-18 plates, E. Merck Co. Ltd.). The developed TLC plates were visualized by spraying with 20% H_2SO_4 followed by heating.

Plant Material. The dried whole herb of *Hypericum chinense* (8.5 kg) was purchased in Chaling County in Hunan Province, China, and separated into roots, stems, and leaves. A voucher specimen (No. HC-003) was deposited at the Natural Medicine Chemistry Laboratory of the School of Pharmacy, Fudan University. The plant was identified by Dr. Zhang Wen-Ju, Associate Professor at the Center of Biodiversity of the Biology School, Fudan University, China.

Extraction and Isolation. Dried leaves of *H. chinense* (2.26 kg) were chopped and then extracted with 95% ethanol (5 × 3.5 L) at 45 °C for 4 h to afford 437.8 g of extract. The extract was partitioned into petroleum ether (73.3 g), EtOAc (109.5 g), and MeOH fractions. The EtOAc fraction was subjected to CC over silica gel, eluting with a gradient from petroleum ether to EtOAc, and finally washed with MeOH to afford fractions 1−16. Fraction 7 (2.96 g) was rechromatographed

Chart 1



on a silica gel column eluting with petroleum−EtOAc to yield **1** (10.0 mg) and betulinic acid (20.0 mg). Fraction 6 was rechromatographed to yield **2** (19.0 mg), **3** (101.9 mg), and sesamin (32.8 mg). Compounds **4** (19.7 mg), **5** (3.0 mg), and **6** (6.7 mg) were obtained from fraction 4. Similarly, **7** (10.0 mg) was isolated from the roots of *H. chinense* (1.96 kg).

Bacterial Strains. *S. aureus* standard strain ATCC 25923 and tetracycline-resistant strain XU212, which possesses the TetK tetracycline efflux protein, were provided by Dr. Edet Udo.[20] Strain SA-1199B, which overexpresses the norA gene encoding the NorA MDR efflux pump, was the kind gift of Professor Glenn W. Kaatz.[21] Strain RN4220, which possesses the MsrA macrolide efflux protein, was provided by Dr. Jon Cove.[22]

Minimum Inhibitory Concentration (MIC) Assay. All strains were cultured on nutrient agar (Oxoid) and incubated for 24 h at 37 °C prior to MIC determination. The control antibiotic norfloxacin was obtained from Sigma Chemical Co. Mueller-Hinton broth (MHB; Oxoid) was adjusted to contain 20 and 10 mg/L of Ca^{2+} and Mg^{2+}, respectively. An inoculum density of 5×10^5 cfu of each *S. aureus* strain was prepared in normal saline (9 g/L) by comparison with a 0.5 McFarland turbidity standard. The inoculum (125 μL) was added to all wells, and the microtiter plate was incubated at 37 °C for 18 h. For MIC determination, 20 μL of a 5 mg/mL methanolic solution of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT; Sigma) was added to each of the wells and incubated for 20 min. Bacterial growth was indicated by a color change from yellow to dark blue. The MIC was recorded as the lowest concentration at which no growth was observed.

Hyperione A (1): colorless oil; $[\alpha]^{20}_D$ +195.6 (*c* 0.045, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 307 (1.07), 276 (1.15), 229 (2.54), 207 (2.08) nm; IR (CH_2Cl_2) ν_{max} 1670, 1603, 1503, 1488, 1441, 1248 cm^{-1}; ^1H NMR, ^{13}C NMR, HMBC, and NOE data, see Table 1; EIMS *m/z* 340 $[M]^+$ (34), 310 (1), 177 (13), 164 (54), 149 (100), 121 (25), 65 (18), 63 (10); HREIMS *m/z* $[M]^+$ 340.0950 (calcd for $C_{19}H_{16}O_6$, 340.0947).

Hyperione B (2): white, amorphous powder; $[\alpha]^{20}_D$ +48.0 (*c* 0.050, acetone); UV (MeOH) λ_{max} (log ε) 308 (1.06), 276 (1.20), 230 (2.56), 207 (2.04) nm; IR (CH_2Cl_2) ν_{max} 1672, 1604, 1503, 1488, 1441, 1248 cm^{-1}; ^1H NMR, ^{13}C NMR, HMBC, and NOE data, see Table 1; EIMS *m/z* 340 $[M]^+$ (20), 310 (1), 177 (20), 164 (100), 149 (72), 121 (19), 65 (13), 63 (7); HREIMS *m/z* $[M]^+$ 340.0946 (calcd for $C_{19}H_{16}O_6$, 340.0947).

Aspidinol C (3): yellow crystals, mp 155−158 °C; UV ($CHCl_3$) λ_{max} (log ε) 330 (3.30), 288 (3.40), 267 (3.42), 243 (2.65) nm; IR (film) ν_{max} 3321, 2956, 1644, 1591, 1520, 1471, 1434, 1242, 1133, 794, 473 cm^{-1}; ^1H NMR, ^{13}C NMR, and HMBC, see Table 2; EIMS *m/z* 238 $[M]^+$ (25), 223 (14), 205 (4), 196 (4), 181 (100), 154 (6), 138 (2), 69 (4); HREIMS *m/z* $[M]^+$ 238.1207 (calcd for $C_{13}H_{18}O_4$, 238.1205).

Aspidinol D (4): yellow crystals, mp 95−97 °C; $[\alpha]^{20}_D$ −11.5 (*c* 0.400, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 293 (3.26), 288 (3.33), 275 (3.26), 241 (2.10) nm; IR (film) ν_{max} 3387, 2963, 1635, 1585, 1412, 1229, 1145, 1097, 801 cm^{-1}; ^1H NMR, ^{13}C NMR, and HMBC data, see Table 2; EIMS *m/z* 238 $[M]^+$ (16), 223 (1), 181 (100), 138 (2), 69 (3), 65 (2), 55 (2), 41 (2).

Hyperaspidinol A (5): pale yellow oil; ^1H NMR, ^{13}C NMR, and HMBC data, see Table 3; ESI $[M + H]^+$ 561.2, $[M + Na]^+$ 583.1; HRESIMS *m/z* $[M + H]^+$ 561.21066 (calcd for $C_{32}H_{33}O_9$, 561.21191).

Hyperaspidinol B (6): pale yellow oil; IR ($CHCl_3$) ν_{max} 2924, 1612, 1504, 1489, 1442, 1248, 1125, 1038, 937, 811 cm-1; ^1H NMR, ^{13}C NMR, and HMBC data, see Table 3; ESI $[M + H]^+$ 561.2, $[M + Na]^+$ 583.1; HRESIMS *m/z* $[M + H]^+$ 561.21125 (calcd for $C_{32}H_{33}O_9$, 561.21191).

Hyperidixanthone (7): yellow crystals, mp 370−375 °C); UV (DMSO) λ_{max} (log ε) 315 (2.40), 281 (3.38), 270 (3.31) nm; IR (film) ν_{max} 3443, 2925, 1634, 1593, 1456, 1404, 1331, 1289, 1067, 951, 758 cm^{-1}; ^1H NMR (DMSO) δ_H (*J* in Hz) 9.96 (1H, s, OH-4/4′), 7.83 (1H, dd, 7.94, 1.83, H-8/8′), 7.79 (1H, dt, 7.80, 1.83, H-6/6′), 7.65 (1H, dd, 8.25, 0.70, H-5/5′), 7.32 (1H, dt, 7.18, 0.70, H-7/7′), 3.95 (3H, s, OCH_3-3/3′), 3.43 (3H, s, OCH_3-2/2′); ^{13}C NMR (DMSO) δ_C 175.7 (C-9/9′), 154.8 (C-10a/10a′), 146.5 (C-2/2′), 145.9 (C-3/3′), 143.9 (C-4a/4a′), 138.4 (C-4/4′), 134.8 (C-6/6′), 125.9 (C-8/8′), 123.9 (C-7/7′), 121.1 (C-8a/8a′), 117.7 (C-5/5′), 115.8 (C-9a/9a′), 109.7 (C-1/1′), 60.6 (OCH_3-3/3′), 60.0 (OCH_3−2/2′); EIMS *m/z* 542 $[M]^+$ (95), 527 $[M - CH_3]^+$ (5), 511 $[M - OCH_3]^+$ (100), 285 (71), 271 (24), 258 (25), 240 (23), 121 (9); HREIMS *m/z* $[M]^+$ 542.1210 (calcd for $C_{30}H_{22}O_{10}$, 542.1213).

Acknowledgment. This work was supported by the NSFC (30772630) and Royal Society International Joint Project/NSFC (JP091083, Sino-UK) and partly supported by National Drug Innovative Program of China (2009ZX09301-011). The authors are grateful to Ms. P. Yang and Ms. C. Y. Liu in Analysis Center, School of Pharmacy, Fudan University, for their technical support.

Supporting Information Available: Spectra of compounds **1**−**7** and Scheme S1 are available free of charge via the Internet at http://pubs.acs.org.

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NP1004483