Four New Nonaoxygenated C_{18} Dibenzocylcooctadiene Lignans from *Kadsura philippinensis*

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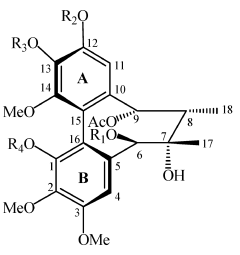
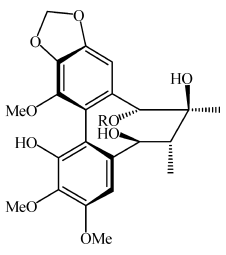
Four new nona-oxygenated C_{18} dibenzocyclooctadiene lignans, kadsuphilins C—F (1—4), were isolated from the EtOAc soluble portion of the alcoholic extract of the aerial parts of *Kadsura philippinensis*. The structures of 1—4 were elucidated on the basis of extensive spectroscopic analyses, including 2D NMR (HMQC, HMBC, and NOESY) experiments, comparison of the spectral data with those of the related metabolites. The stereochemistries of the biphenyl and octadiene moieties were deduced from circular dichorism (CD) and the NOESY spectra, respectively. The *in vitro* antiplatelet aggregation activity of metabolites 1—4 also have been evaluated.

Key words *Kadsura philippinensis*; Schizandraceae; dibenzocyclooctadiene lignan; antiplatelet aggregation assay

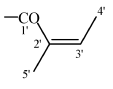
Plants belonging to genus *Kadsura* (Schizandraceae) have been used for long time in traditional Chinese medicine to treat several diseases and have been well known as a rich source of C_{18} dibenzocyclooctadiene lignans.[1—5] This type of lignans have been shown significant anti-HIV,[5] antioxidant,[6] antihepatotoxic,[7,8] antihepatitis,[9] cytotoxic,[10] antitumor-promotion,[11] and anti-lipid peroxidative activities.[12] Our previous investigation on the chemical constituents of *Kadsura philippinensis* ELMER, growing in the Southern islet of Taiwan, led to the isolation of twelve dibenzocyclooctadiene lignans including new kadsuphilins A and B, 6-*epi*-gomisin and 1-demethylkadsuphilin A from the EtOAc soluble portion of the alcoholic extact.[13] Further chromatographic separation and purification of the more polar fractions afforded additional four new polyoxygenated dibenzocylcooctadiene lignans, kadsuphilins C—F (**1**—**4**) and schizanrin G (**5**).[14] Their structures were elucidated on the bases of spectroscopic analyses, including 2D NMR experiments, comparison of the spectral data with those of the related metabolites. Their stereochemistries were assigned by CD and NOESY spectra. The *in vitro* antiplatelet aggregation activity of metabolites **1**—**5** also have been evaluated.



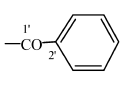
1: R_1 = benzoyl, $R_2 + R_3$ = CH_2, R_4 = H
3: R_1 = benzoyl, $R_2 = R_3$ = Me, R_4 = H
5: R_1 = angeloyl, $R_2 + R_3 = CH_2$, R_4 = H
6: R_1 = benzoyl, $R_2 = R_3 = R_4$ = Me



2: R = Ac
4: R = benzoyl



angeloyl



benzoyl

Results and Discussion

The EtOAc-soluble portion from the alcoholic extract of *Kadsura philippinensis* was subjected to flash column chromatography over silica gel. Eluted polar fractions were further isolated or purified using normal or reversed phase HPLC to afford the new kadsuphilins C—F (**1**—**4**) and schizanrin G (**5**).

Kadsuraphilin C (**1**) had a molecular formula $C_{31}H_{32}O_{11}$, as deduced from its HR-ESI-MS (*m/z* 581.2020, $[M+H]^+$) and NMR spectral data (Tables 1, 2). The IR spectrum suggested the presence of hydroxyl (ν_{max} 3564, 3450 cm^{-1}) and ester (1744, 1721 cm^{-1}) functionalities in the molecule. The UV spectrum showed λ_{max} (MeOH) at 219, 257, and 280 nm while its CD spectrum exhibit strong negative cotton effect at λ_{max} 251 nm, designating **1** as a dibenzocyclooctadiene lignan[1—5] with an *S*-biphenyl configuration.[11,15—18] The ^{1}H-NMR spectrum of **1** showed signals of two aromatic protons of a biphenyl moiety [δ_H 6.70 and 6.56 (1H each, s)]. The signals appeared at δ_H 5.78 and 5.62 (1H each, d, J=1.2 Hz); 3.96, 3.93, and 3.40 (3H each, s) indicated the presence of one methylenedioxyl and three methoxyls, respectively, as substituents on the biphenyl rings. One tertiary methyl [δ_H 1.41 (3H, s)] bound to an oxygenated carbon (δ_C 74.1, C, C-7) and one secondary methyl [δ_H 1.34 (3H, d, J=7.0 Hz)] bound to methine carbon (δ_C 43.2, d, C-8), as revealed from the HMBC spectrum, were also found on the cyclooctadiene ring. Moreover, the ^{1}H- and ^{13}C-NMR spectral data of **1** (Tables 1, 2) indicated the presence of an acetyl and a benzoyl moieties. This was further supported by the ion peaks appeared in the EI-MS spectrum at *m/z* 520 $(M-AcOH)^+$ and 398 $(M-AcOH-$benzoic acid$)^+$. From the HMBC spectra of **1**, it was found that one oxymethine proton at δ_H 5.86 (1H, s) exhibited $^1H/^{13}C$ long range correlations with a benzoyl carbonyl carbon (δ_C 164.8, C), a biphenyl methine carbon C-4 (δ_C 107.3, CH, C-4), and C-7. Similarly, another oxymethine proton at δ_H 5.69 (1H, s) was correlated with an acetyl carbonyl carbon (δ_C 168.9, C), a biphenyl carbon (δ_C 102.3,

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Table 1. ^{1}H-NMR Data for Compounds **1**—**4**

No	**1**[a]	**2**[a]	**3**[a]	**4**[b]
H-4	6.70 s	6.28 s	6.72 s	6.37 s
H-6	5.86 s	4.93 d (6.0)[c]	5.99 s	5.02 br d (5.5)
H-7		2.38 dd (7.2, 6.0)		2.49 dd (7.5, 5.5)
H-8	2.34 q (7.0)		2.38 q (7.0)	
H-9	5.69 s	5.53 s	5.81 s	5.84 s
H-11	6.56 s	6.67 s	6.69 s	6.79 s
H-17	1.41 s	0.92 d (7.2)	1.39 s	1.10 d (7.5)
H-18	1.34 d (7.0)	1.26 s	1.36 d (7.0)	1.39 s
OCH_2	5.62 d (1.2)	5.97d (1.2)		5.98 s
	5.78 d (1.2)	6.01 d (1.2)		6.02 s
1-OH	5.62 s	5.70 br s	5.75 s	5.37 s
1-OMe				
2-OMe	3.93 s	3.92 s	3.94 s	3.49 s
3-OMe	3.96 s	3.89 s	3.97 s	3.94 s
12-OMe			4.07 s	
13-OMe			3.35 s	
14-OMe	3.40, s	3.91 s	3.24 s	3.89 s
3′	7.49 d (7.5)		7.37 d (7.2)	7.35 d (7.5)
4′	7.33 t (7.5)		7.27 t (7.2)	7.26 t (7.5)
5′	7.52 t (7.5)		7.48 t (7.2)	7.44 t (7.5)
6′	7.33 t (7.5)		7.27 t (7.2)	7.26 t (7.5)
7′	7.49 d (7.5)		7.37 d (7.5)	7.37 d (7.5)
Ac	1.56 s	1.56 s	1.58 s	

a) Spectra recorded at 300 MHz in $CDCl_3$. *b*) 500 MHz in $CDCl_3$. *c*) The *J* values are in Hz in parentheses.

Table 2. ^{13}C-NMR Data for Compounds **1**—**4**[a]

No	**1**	**2**	**3**	**4**
1	147.1 (C)[b]	147.4 (C)	147.1 (C)	147.8 (C)
2	134.9 (C)	134.5 (C)	135.4 (C)	134.7 (C)
3	150.6 (C)	150.2 (C)	150.5 (C)	150.4 (C)
4	107.3 (CH)	106.1 (CH)	107.8 (CH)	106.0 (CH)
5	130.2 (C)	134.1 (C)	129.8 (C)	133.7 (C)
6	85.4 (CH)	82.8 (CH)	85.1 (CH)	82.9 (CH)
7	74.1 (C)	47.4 (CH)	74.3 (C)	47.3 (CH)
8	43.2 (CH)	74.6 (C)	43.0 (CH)	74.9 (C)
9	83.6 (CH)	85.2 (CH)	83.8 (CH)	85.9 (CH)
10	133.4 (C)	130.4 (C)	134.7 (C)	130.2 (C)
11	102.3 (CH)	109.3 (CH)	107.0 (CH)	107.5 (CH)
12	149.2 (C)	148.3 (C)	153.4 (C)	148.2 (C)
13	136.0 (C)	136.6 (C)	141.4 (C)	136.5 (C)
14	140.5 (C)	140.7 (C)	150.7 (C)	140.5 (C)
15	118.8 (C)	119.6 (C)	119.9 (C)	119.8 (C)
16	115.7 (C)	115.6 (C)	116.3 (C)	115.5 (C)
17	29.0 (CH_3)	15.9 (CH_3)	28.9 (CH_3)	15.9 (CH_3)
18	17.3 (CH_3)	29.4 (CH_3)	17.3 (CH_3)	29.2 (CH_3)
OCH_2O	101.1 (CH_2)	101.4 (CH_2)		101.3 (CH_2)
1-OMe				
2-OMe	60.8 (CH_3)	60.9 (CH_3)	60.9 (CH_3)	60.4 (CH_3)
3-OMe	55.9 (CH_3)	55.9 (CH_3)	56.0 (CH_3)	56.0 (CH_3)
12-OMe			56.2 (CH_3)	
13-OMe			60.1 (CH_3)	
14-OMe	59.1 (CH_3)	59.8 (CH_3)	60.5 (CH_3)	59.7 (CH_3)
1′	164.8 (C)		164.9 (C)	165.3 (C)
2′	129.7 (C)		129.1 (C)	129.5 (C)
3′	129.4 (CH)		129.4 (CH)	129.4 (CH)
4′	127.9 (CH)		128.2 (CH)	128.0 (CH)
5′	132.9 (CH)		133.4 (CH)	132.7 (CH)
6′	127.9 (CH)		128.2 (CH)	128.0 (CH)
7′	129.4 (CH)		129.4 (CH)	129.4 (CH)
Ac	20.1 (CH_3)	20.2 (CH_3)	20.2 (CH_3)	
	168.9 (C)	169.3 (C)	168.9 (C)	

a) Spectra recorded at 75 MHz in $CDCl_3$ at 25 °C. *b*) Multiplicity deduced by DEPT.

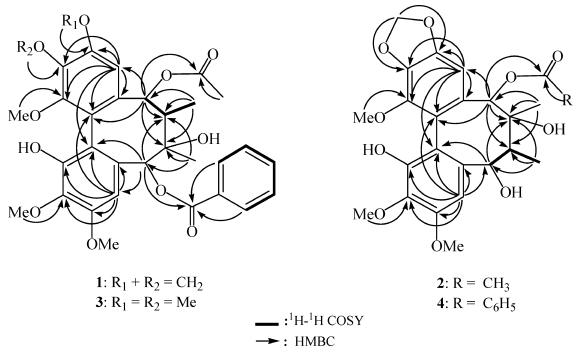

Fig. 1. ^{1}H–^{1}H COSY and HMBC Correlations for **1**—**4**

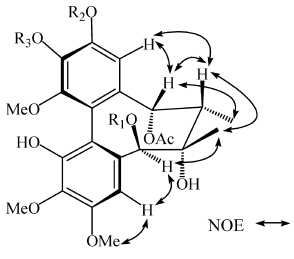

Fig. 2. Key NOESY Correlations for Kadsuphilins C (**1**) and E (**3**)

CH, C-11), and C-8 (Fig. 1). Thus, the two oxymethine protons were assigned as H-6 and H-9, and the benzoate and acetate groups were thus located at C-6 and C-9 of the cyclooctadiene ring, respectively. Comparison of the ^{1}H- and ^{13}C-NMR spectral data of **1** (Tables 1, 2) with those of schizanrin F (**6**),[14] indicated that **1** is the 1-demethylated derivative of **6**. Furthermore, careful analyses of the correlations observed in the ^{1}H–^{1}H COSY, HMQC, and HMBC spectra confirmed the planar structure of **1**, including the arrangement of substituents on the biphenyl rings (Fig. 1). Beside the *S*-configuration of the biphenyl deduced previously from the CD spectrum, the stereochemistry of the cyclooctadiene moiety was disclosed from the NOE correlations (Fig. 2) observed in the NOESY spectrum of **1**. The strong NOE correlations observed between H-11 and H-9 and between H-4 and H-6 suggested the β-orientation of H-9 and the α-orientation of H-6, respectively. Therefore, the acetyl and benzoyl groups are located on the α- and β-face of the molecule, respectively. Moreover, the NOE interaction exhibited between H-9 and H-8, and between H-8 and CH_3-7 reflected the α-orinetation of both methyl and hydroxyl groups at C-8 and C-7, respectively. Lack of NOE response between H-4 and CH_3-7 further supported the β-orientation of the methyl group at C-7. Moreover, methylation of **1** yielded a methylated product which showed a similar ^{1}H-NMR spectral data with those of **6**.[14] From the above findings, the structure of kadsuaphilin C was unambiguously established as in **1**.

Kadsuphilin D (**2**) exhibited similar absorption maxima in the UV spectrum as those of **1** and strong negative cotton effect at λ_{max} 255 nm in the CD spectrum. Its molecular formula was established as $C_{24}H_{28}O_{10}$ by the HR-ESI-MS (*m/z* 499.1583, $[M+Na]^+$) and NMR data (Tables 1, 2). The presence of at least one hydroxyl (IR ν_{max} 3470 cm^{-1} and EI-MS *m/z* 458 $[M-H_2O]^+$) and ester (IR ν_{max} 1741 cm^{-1} and EI-MS *m/z* 476 $[M-H_2O-AcOH]$) functionalities were re-

vealed in **2**. Comparison of NMR spectral data of **2** with those of **1** (Tables 1, 2), revealed that **2** differs in the absence of benzoyl group with the concomitant upfield shift at position 6 ($\Delta\delta_H$ −0.93, $\Delta\delta_C$ −2.6 ppm) relative to that of **1**. Moreover, the HMBC correlations observed from the tertiary methyl protons (δ_H 1.26, 3H, s) to C-9 (δ_C 85.2, CH), from H-9 (δ_H 5.53, 1H, s) to the biphenyl carbon C-11 (δ_C 109.3, CH), from the secondary methyl protons (δ_H 0.92, 3H, d, J=7.2 Hz) to C-6 (δ_C 82.8, CH), and from H-6 (δ_H 4.93, 1H, d, J=6.0 Hz) to the biphenyl carbon C-4 (δ_C 106.1, CH) confined the tertiary and secondary methyl groups to be at positions 8 and 7, respectively, in contrast to those of **1**. From the above findings and the results of detailed analyses of correlations observed in the ^{1}H–^{1}H COSY, HMBC, and HMBC spectra of **2**, the gross structure of kadsuphilin D was established. Beside the *S*-configuration of the biphenyl rings, the NOE correlations observed in the NOESY spectrum of **2** revealed the stereochemistries at C-6, C-9 to be the same as in case of **1**. The NOE interactions observed between CH_3-8 and CH_3-7, CH_3-7 and H-6, and H-6 and H-4 designated the α-orientation of both methyls at C-7 and C-8. This was further evidenced from the significant NOE interaction between H-4 and CH_3-7. On the basis of above findings the structure of kadsuphilin D (**2**) was established.

Kadsuphilin E (**3**) was also identified as an esterified benzocyclooctadiene lignan with an *S*-biphenyl configuration, on the basis of its IR, UV, CD, and NMR spectra. Its HR-ESI-MS (*m/z* 619.2150, $[M+Na]^+$) was consistent with the molecular formula $C_{32}H_{36}O_{11}$. The molecule of **3** possesses hydroxyl and ester moieties as revealed from the IR absorption bands at 3460 and 1744 cm^{-1} respectively. As in case of **1**, the ion peaks shown at *m/z* 536 $[M-AcOH]^+$ and 414 $[M-AcOH-$benzoic acid$]^+$ in the EI-MS spectrum together with the signals appearing at δ_H 1.58 (3H, s), and 7.27—7.48 (5H) in the ^{1}H-NMR spectrum indicated the presence of acetyl and benzoyl substituents, respectively, on the cyclooctadiene ring of **3**. Nevertheless, the ^{1}H- and ^{13}C-NMR data (Tables 1, 2) of **3** showed five methoxy signals and no methylene dioxy signals, indicating that **3** differ from **1** in the substituents at C-12 and C-14, being two methyl groups in **3** instead of one methylene dioxy group in **1**. It was found that the presence of three methoxy groups at C-12, C-13, and C-14 in **3** induced significant down field shift at C-14 ($\Delta\delta_C$ *ca.* 10 ppm) relative to that induced in **1**, **2** and **4** which possess a methylene dioxy group at C-12 and C-13 and a methoxyl group at C-14. Careful investigation of NOE correlations observed in the NOESY spectrum of **3** (Fig. 2) assigned the β-orientation of benzoyl and tertiary methyl groups at C-6 at C-7, respectively, while disclosed the α orientation of secondary methyl and acetyl groups at C-8 and C-9, respectively, the same as those found in case of **1**. Moreover, detailed investigations of the 2D (^{1}H–^{1}H COSY, HMQC, HMBC, and NOESY) spectra (Figs. 1, 2) established the structure of kadsuphilin E as **3**.

Kadsuphilin F (**4**) showed similar absorption maxima in the UV spectrum as those of **2**, in addition to strong negative cotton effect at λ_{max} 244 nm in the CD spectrum. Its molecular formula was established as $C_{29}H_{30}O_{10}$ by the HR-ESI-MS (*m/z* 561.1740, $[M+Na]^+$) and NMR data (Tables 1, 2). The presence of hydroxyl (IR 3446 cm^{-1}) and ester functionalities (IR 1717 cm^{-1}) were also indicated in **4**. The ion peaks displayed at *m/z* 520 and 398 in the EI-MS spectrum of **4** were thus suggested to be due to elimination of water, and benzoic acid and water, respectively. The ^{1}H- and ^{13}C-NMR spectral data of **4** and **2** (Tables 1, 2) were more or less the same concerning the biphenyl group and the cyclooctadinene moiety including their substituents. The only difference was in the replacement of an acetyl group in **2** with a benzoyl group (^{1}H-NMR: δ_H 7.26—7.44, 5H) in **4**. Moreover, the HMBC correlations (Fig. 1) observed from the tertiary methyl protons (δ_H 1.39, 3H, s) to C-9 (δ_C 85.9, CH), from H-9 (δ_H 5.84, 1H, s) to the biphenyl carbon C-11 (δ_C 107.5, CH) further proved the 9 position of benzoyl group in **4**. The NOE interactions observed between H-9 and H-11 revealed the β-orientation of H-9 and hence the presence of benzoyl ring on the α-disposition of the molecule. On the basis of above findings the structure of kadsuphilin F (**4**) was established.

Table 3. Antiplatelet Aggregation Activity (IC_{50}) of **1**—**5**

Compound	IC_{50} (μM)		
	PAF (5 nM)[a]	U46619 (1 μM)	AA (0.1 mM)
1	14.26±1.93	32.02±1.82	88.2±11.05
2—**5**	—[b]	—	—
Aspirin	[c]	[c]	30.5±5.7

a) PAF: platelet-activity factor, U46619: a thromboxane A2 agonist, and AA: arachidonic acid. *b*) Inactive: IC_{50} >100 μM. *c*) Not tested.

The isolated new compounds **1**—**4** were evaluated for antiplatelet aggregation activity. Preliminary pharmacological study of **1**—**4** revealed that kadsuphilin C (**1**) exhibited significant *in vitro* antiplatelet aggregation activity with IC_{50} at 14 μM by PAF assay (Table 3).[15,19]

Experimental

General Experimental Procedures Optical rotations were taken on a Jasco DIP-1000 digital polarimeter. CD spectra were measured on a JASCO J-810 spectropolarimeter. IR spectra were recorded on a Jasco FT-5300 infrared spectrophotometer. EI-MS and FAB-MS were obtained with a VG Quattro 5022 and JEOL JMS-SX 102 spectrometer. HR-ESI-MS spectra were recorded on a Finnigan MAT-95XL mass spectrometer. The NMR spectra were recorded on a Bruker AVANCE DPX300 FT-NMR at 300 MHz for ^{1}H and 75 MHz for ^{13}C or on a Varian Unity INOVA 500 FT-NMR at 500 MHz for ^{1}H and 125 MHz for ^{13}C, respectively, in $CDCl_3$ using TMS as internal standard.

Plant Material The aerial parts (stems) of *K. philippinensis* were collected at Green Island, Taiwan in September, 2002. A voucher sample (specimen code: TP 93-1) was deposited at the School of Pharmacy, College of Medicine, National Taiwan University, Taipei, Taiwan.

Extraction and Isolation The EtOAc soluble fraction (30 g) from the EtOH extract of aerial parts of *K. philippinensis* (1.1 kg, dry wt) was subjected to flash column chromatography on Silica gel, using *n*-hexane–EtOAc (stepwise, 100 : 1 to 0 : 100), acetone, and finally MeOH to afford 18 fractions.[13] Fraction 13 (1.3 g) eluted with *n*-hexane–EtOAc (1 : 1) was purified on Sephadex LH-20, using MeOH to separate a lignan mixture (570 mg) and fraction A (286 mg), respectively. A portion of fraction A (110 mg) was further purified by RP-18 HPLC using MeOH–H_2O (1 : 1) followed by preparative RP-18 TLC plates using MeOH–H_2O (3 : 7) to yield **1** (22 mg). The lignan mixture was primarily applied on silica gel flash column developed with *n*-hexane–EtOAc (stepwise, 100 : 1 to 0 : 100) to obtain fractions B (260 mg) and C (77 mg). A portion of fraction B (60 mg) was purified on preparative RP-18 TLC plates developed with MeOH–H_2O (3 : 7) to afford **5** (10 mg). The fraction C was separated on preparative silica TLC plates using *n*-hexane–acetone (3 : 1) to yield **3** (5.5 mg) and **4** (2 mg). Fraction 14 (26 mg) eluted with *n*-hexane–EtOAc (1 : 3) was chromatographed on preparative RP-18 TLC plates using MeOH–H_2O (3 : 7) to afford **2** (5 mg).

Kadsuphilin C (**1**): Yellowish white powder; $[\alpha]_D^{30}$ −5.6° (*c*=1.0,

CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 219 (4.76), 257 (3.96), 280 (3.78) nm; CD (c=0.1, MeOH) (mdeg) 213 (+4.77), 222 (−10.4), 244 (−11.9), 251 (+11.8) nm; IR (neat) ν_{max} 3564, 3450, 2933, 2359, 1744, 1721, 1645, 1455, 1372, 1254, 1224, 1109, 1043 cm^{-1}; EI-MS m/z 580 (0.5, $[M]^+$), 520 (3.6, $[M-AcOH]^+$), 449 (6.4), 398 (8.1, $[M-AcOH-$benzoic acid$]^+$), 355 (25.9), 343 (7.8), 315 (7.8), 105 (100); FAB-MS m/z 581 (0.5, $[M+H]^+$), 580 (0.9, $[M]^+$), 520 (1.9, $[M-AcOH]^+$), 399 (8.1, $[M-AcOH-$benzoic acid$+H]^+$), 355 (7.4), 307 (15.7), 154 (95.3), 136 (91.7), 105 (100); HR-ESI-MS m/z 581.2020 (Calcd for $C_{31}H_{33}O_{11}$, 581.2023), ^{1}H- and ^{13}C-NMR, see Tables 1 and 2, respectively.

Kadsuphilin D (**2**): Yellowish white powder; $[\alpha]_D^{30}$ +9.2° (c=0.2, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 215 (4.40), 258 (3.73), 285 (3.49) nm; CD (c=0.1, MeOH) (mdeg) 212 (+2.72), 255 (−7.93) nm; IR (neat) ν_{max} 3470, 2917, 2360, 1741, 1600, 1465, 1376, 1260, 1233, 1080, 1015 cm^{-1}; EI-MS m/z 458 (0.5, $[M-H_2O]^+$), 398 (0.2, $[M-H_2O-AcOH]^+$), 355 (1.9), 267 (5.6), 134 (29.8), 112 (16.9), 98 (53.9); FAB-MS m/z 471 (0.1, $[M+H]^+$), 458 (0.6, $[M-H_2O]^+$), 399 (0.9, $[M-H_2O-AcOH+H]^+$), 307 (7.8), 154 (100), 137 (67.5); HR-ESI-MS m/z 499.1583 (Calcd for $C_{24}H_{28}O_{10}Na$, 499.1580); ^{1}H- and ^{13}C-NMR, see Tables 1 and 2, respectively.

Kadsuphilin E (**3**): Yellowish white powder; $[\alpha]_D^{30}$ −59.7° (c=0.6, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 220 (5.00), 255 (4.81), 282 (3.93) nm; CD (c=0.1, MeOH) (medg) 220 (+9.7), 239 (−21.9), 268 (−1.87) nm; IR (neat) ν_{max} 3563, 3460, 2932, 2359, 1744, 1715, 1599, 1496, 1455, 1371, 1260, 1105, 1040 cm^{-1}; EI-MS m/z 596 (0.3, $[M]^+$), 536 (2.4, $[M-AcOH]^+$), 465 (1.9), 414 (0.9, $[M-AcOH-$benzoic acid$]^+$), 371 (3.9), 105 (100); HR-ESI-MS m/z 619.2150 (Calcd for $C_{32}H_{36}O_{11}Na$, 619.2155); ^{1}H- and ^{13}C-NMR, see Tables 1 and 2, respectively.

Kadsuphilin F (**4**): Yellowish white powder; $[\alpha]_D^{30}$ −21.6° (c=0.2, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 221 (3.97), 257 (3.04), 286 (3.00) nm; CD (c=0.1, MeOH) (medg) 225 (+6.07), 244 (−8.58) nm; IR (neat) ν_{max} 3446, 2934, 2359, 1717, 1610, 1458, 1368, 1260, 1110, 1071, 1050 cm^{-1}; EI-MS m/z 538 (0.4, $[M]^+$), 520 (0.9, $[M-H_2O]^+$), 398 (0.4, $[M-H_2O-$benzoic acid$]^+$), 368 (0.9), 355 (1.8), 105 (100); HR-ESI-MS m/z 561.1740 (Calcd for $C_{29}H_{30}O_{10}Na$, 561.1737); ^{1}H- and ^{13}C-NMR, see Tables 1 and 2, respectively.

Evaluation of Antiplatelet Aggregation Activity. Preparation of Rabbit Washed Platelet Suspension Washed platelets were obtained from rabbits as previously described.[15] Briefly, rabbit blood was collected from the marginal ear vein into tubes containing one-sixth volume of acid-citrate-dextrose as anticoagulant. The blood was centrifuged at 200×$\boldsymbol{g}$ for 15 min at room temperature. The platelet-rich plasma was mixed with 1/40 volume of EDTA (final concentration 5 μm) and re-centrifuged at 1000×$\boldsymbol{g}$ for 12 min. The supernatant was discarded and the platelet pellet was suspended in modified Ca^{2+}-free Tyrode's buffer (137 mM NaCl, 2.8 mM KCl, 2 mM $MgCl_2$, 0.33 mM NaH_2PO_4, 5 mM glucose, 10 μM HEPES) with 0.35% bovine serum albumin, heparin (50 unit/ml) and apyrase (1 unit/ml). Following incubation at 37 °C for 20 min, the washed platelet pellet was resuspended in Tyrode's buffer containing 1 mM Ca^{2+}. The platelet numbers were counted by hemacytometer and adjusted to 3.5×10^8 platelets/ml. To eliminate or minimize any possible effects of the solvent, the final concentration of the vehicle dimethyl sulfoxide (DMSO) in the platelet suspension was fixed at 0.5%.

Measurement of Platelet Aggregation Aggregation was measured by a turbidimetric method.[16] The PACKS-4 aggregrometer (Helena Laboratories, Beaumont, TX, U.S.A.), was used. Transmission of washed platelet suspension was assigned 0% aggregation, while transmission through Tyrode's buffer was assigned 100% aggregation. Platelets (0.5 μl) were pre-incubated with 2.5 μl of the vehicle DMSO (0.5%) to serve as control or saponins for 2 min and then stimulated with 2.5 μl of aggregation inducers such as AA (arachidonic acid), TXA_2 (Throboxane A_2), U46619 [5-Hydroxy-11α,9α-(epoxymethano)-prosta-5,13-dienoic acid] or PAF (platelet-activity factor). Aspirin was used as a standard compound for AA test.

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